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TABLE OF CONTENTS

CONGRESS KEYNOTE .............................................................. 1 - 5
KEYNOTE .............................................................................. 6 - 35
ORAL THEME A .................................................................... 36-78
ORAL THEME B .................................................................... 79-118
ORAL THEME C .................................................................... 119-146
ORAL THEME D .................................................................... 147-178
ORAL THEME E .................................................................... 179-182
POSTER THEME A ............................................................... 183-313
POSTER THEME B ............................................................... 314-397
POSTER THEME C ............................................................... 398-441
POSTER THEME D ............................................................... 442-529
POSTER THEME E ............................................................... 530-533

LIST OF ABSTRACTS

THEME A
Breeding, Genetics and Genomics
Agronomic traits
Arabidopsis
Association mapping-nested Bioinformatics
Cytology
DNA sequencing
Epigenetics
Gene cloning
Genetic diversity
Genetic transformation, (GMOs)
Genome analysis
Genome editing
Genotype by environment interactions
Germplasm resources
Hybrids & heterosis
Inheritance of traits
Interspecific hybridisation
Linkage analysis / linkage maps
Marker assisted selection
Metabolomics
Microspore culture / haploidy
Molecular marker technologies
Mutagenesis
Novel Traits
Other genetic related topics
Other species of Brassica, Crucifers, Resynthesized Brassica
Proteomics
QTL mapping
Seed coat colour / yellow seed
SNP markers
SSR markers
Transcriptomics

THEME B
Crop protection, biotic stress, biology of canola pathogens and insect pests
Aphids
Biological control
Black spot (Alternaria spp.)
Blackleg (Leptosphaeria maculans)
Clubroot (Plasmodiophora brassicae)
Flea beetle
Forecasting / modeling
Fungicides
Host-pathogen interaction
Insect pests
Insecticides
Integrated pest management
Light leaf spot (Pyrenopeziza brassicae)
Other insect pests
Pollen beetle
Pseudomonas
Seed treatment
Stem rot (Sclerotinia sclerotiorum)
Verticillium
Viruses
Weeds
White rust (Albugo candida)

THEME C
Seed chemistry, processing and utilization
Amino Acid
Analytical techniques
Anti-nutritional components
Antioxidant
Aquafeed
Biofuel / biodiesel
Cold Press
Dietary fibre
Energy
Eruic acid
Fatty acids
Feed quality and/or meal utilization
Food quality and nutrition
Functional components
Glucosinolates
High-oleic
Linolenic
Lipids
Meal
Napin
Oil
Phytosterol
Protein
Sinapin
Solvent extraction
Yellow seed

THEME D
Crop production, abiotic stress, environmental impact
Biomass
Climate change
Drought tolerance
Energy
Environmental impact
Fertilizer
Flower time
Greenhouse gas emission
Harvest techniques
Heat tolerance
Heterosis
Irrigation
Moisture stress / waterlogging tolerance

THEME E
Economics, policies and trade
Canola marketing and risk management
Climate change adaptation
Commodity promotion
Consumer demand
Disease and pest management
Global markets
GM and non-GM coexistence
Grain transportation
Intellectual property rights
International trade agreements
Low level presence
Non-tariff trade barriers
Partnerships in canola research
Regulation of transgenics and novel traits
Research funding

Nitrogen, ammonia, urea
Nutrient use efficiency
Organic production
Photosynthesis
Plant architecture, pods, leaf area
Pod shatter
Root biology
Salinity
Seed vigour
Seeding date / density
Semi-dwarf genotypes
Sulfur, sulphate
Sustainable production
Tillage / zero tillage
Volunteers
Weed Management
Winter hardiness
Yield traits, seed weight, etc.

Partnerships in canola research
Regulation of transgenics and novel traits
Research funding
The importance of canola from a global customer perspective

G. Crockett
M. Smith
US McDonald’s Corporation, USA

Rapeseed (Canola) Oil and McDonald’s Perspective

The presentation will provide a topline summary of McDonald’s global markets and their use of rapeseed oil as well as how McDonald’s projects plan to utilize rapeseed oil in the future. This includes consideration of customer preferences and the necessary characteristics of rapeseed oil in blends that enable restaurants to provide consistent quality foods that meet McDonald’s established sensory standards for color, flavor, and texture.

An overview of McDonald’s food evolution to remain relevant with today’s customers and how rapeseed oil fits into that evolution will also be provided.
Milestones on the road to the future

The milestones on the way to establishing the oilseed Brassicas as major crop species are many, with most having occurred since the 1960s. The oilseed Brassica crops (B. napus, B. rapa and B. juncea) are now established as the world’s third most important source of edible oil. Since 1960 the area of production in all major producing regions has grown exponentially with seed production increasing by 40 fold in Canada, 21 fold in Europe, and 13 fold in China. Seed and oil yield per hectare have also increased year over year. In addition, canola oil, in both its traditional and high oleic/low linolenic forms, can claim to be the world’s most nutritious oil. Moreover, canola quality meal is now considered the protein supplement of choice for dairy cows. The demand for Brassica oilseed and its products has provided producers with an alternative crop to cereals and other crop species. In Canada at least, the crop’s expansion has also established a growing, rural based, canola crushing industry.

Innovation and discovery are the basis of the crop’s success. Scientific advances have not been limited to genetics, the tools of detection and changes in how the crop is grown and managed have also been key to rapid development. Some of milestones will be highlighted such as the impact of gas chromatography, modified fatty acid composition, herbicides, haploid breeding, hybrids, NMR, NIR, gene and genome sequencing, gene transfer and marker assisted selection, to name a few.

However, the crop’s success has not been without evolving problems such as diseases, insects, weeds and the requirement for more intense management. Can we keep up with Mother Nature’s worldwide evolutionary resources? Nature is always ready to take advantage of the lush plate we place before canola’s multiplicity of hungry pests. The almost complete loss of Australia’s 1972 crop to blackleg or the devastation of the 2014 UK crop by flea beetles are reminders that we can’t let our guard down. Fortunately technology has armed us with an ever growing arsenal of tools, techniques and knowledge to fight such hazards.

We don’t know what the future may bring by way of new challenges, such as the identification of Verticillium longisporum, clubroot and the Swede midge on the Canadian prairies, or what new innovations may become available to protect or accelerate future advances. The rapid and revealing knowledge of the plant’s genetic makeup and the increasing effectiveness of marker assisted selection together with the potential internal and external use of RNAi’s and microbiome modification bode well for the future. The ability to image root growth and morphology in situ will add a new dimension to our understanding of plant growth and productivity. Aptomictic hybrids could reduce seed costs and overcome concerns of gene flow. Full resistance to shattering, introduction of yellow seeded hybrids and the elimination of secondary dormancy are clearly desired and attainable targets. In addition, the harnessing of large agronomy data sets and the use of unmanned aerial vehicles (UAV’s) could result in increased farm efficiencies.

As bright as the future may seem, it is critical that we find ways of countering misinformation while building public trust in scientific advances. One of the most effective and powerful tools at our disposal, gene transfer, lies largely idle due to a misinformed public and an onerous and expensive regulatory framework. If we are to fully benefit from our current and future knowledge this battle must be won.

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High throughput plant phenotyping approaches to improving yield potential, biomass, radiation and water use efficiency in grain crops

Plant Phenomics provides new opportunities for using non-destructive, high throughput tools to select germplasm with enhanced growth, biomass and nutrient use efficiency and determine the genes underpinning these important traits. In this presentation, the utility of a variety of tools based on proximal and remote sensing, is demonstrated for trait quantification and determination of genes and mechanisms in controlled environments and the field. In particular, examples of digital growth analysis for high resolution genetic analysis of nutrient use efficiency, chlorophyll fluorescence and hyperspectral reflectance for photosynthetic characterization, LIDAR for canopy architecture and radiation use efficiency optimisation and distributed canopy temperature sensors and aerial thermal imaging for water use efficiency, are shown for grain crops such as wheat and canola.

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The impact of genomics on *Brassica* genetics and breeding – a sequence level view of the triangle of U

**Background:** *Brassica* research has benefited greatly from the close relationship between the Brassica crops and the crucifer model Arabidopsis thaliana, the first plant species to have a fully sequenced and annotated genome (The Arabidopsis Genome Initiative 2000). The availability of the A. thaliana sequence allowed the rapid identification of candidate genes for traits of interest and through comparative mapping provided novel insights into the larger genomes of the Brassica crops (Wang et al. 2011; Town et al. 2006; Navabi et al. 2013). Yet, the genome of the weed is still an imperfect model for the crops, the current promise of access to whole genome sequences for each agronomically important Brassica species will revolutionise research and development of Brassica crops in the coming decade.

**Objectives:** In the 1940’s using cytogenetics U defined the unique relationship between six of the Brassica species, three diploids and three allopolyploids, formed from each possible pairwise hybridization. The objective is to understand at the sequence level how each of these species relates to one another and what has been the consequence of the interspecific hybridization events.

**Methods:** Each species that forms the triangle of U has now been sequenced, assembled and annotated (Wang et al. 2011; Parkin et al 2014; Chalhoub et al, 2014; Parkin, Sharpe et al, unpublished) All the genome sequences were generated using next generation sequencing technologies (Roche 454 and Illumina), using different combinations of libraries with varying insert sizes and relative genome coverage.

**Results and Conclusions:** The relative ease and cost of sequencing has allowed the impact of evolution and agronomic selection among the Brassica species to be studied at the DNA level. The genome sequences offer unprecedented insights into the impact of genome doubling on gene retention and correspondingly on trait divergence in polyploid genomes. Further the canola genome shows the impact of the high prevalence of illegitimate exchanges between the constituent genomes, with the selection of such events being responsible for some of canola’s distinguishing attributes. The B genome Brassica species appear excluded from this type of event with multiple rearrangements distinguishing this diploid genome from its relatives. The genomes provide not only insights into polyploid evolution but provide a foundation for the application of the next generation of breeding tools.

**References:**


Strategy and progresses upon rapeseed genetic improvement in Oil Crops Research Institute, CAAS

Consumption of vegetable oil in China is annually over 30 million tons, in which 65% is imported. Rapeseed oil accounts for 45% domestic production of vegetable oil in China. To assure the endurable supply, series of research plans, including the National Basic Research Program, the National Rapeseed Research Network Project, and Technology Innovation Project in CAAS etc., have been set up for rapeseed genetic improvement. Oil Crops Research Institute, one of the responsible institutions of these projects, is mainly focusing on the following activities:

1. Leading and joining the genome sequencing of three Brassica species, B. rapa, B. oleracea and B. napus. Genome-wide comparative analysis reveals the molecular characteristic of polyploidy evolutionary dominance in B. napus. Through interspecific hybridization between wild B. oleracea and B. rapa, some novel germplasms harboring desirable agronomic traits such as premature, high resistance on disease and lodging, have been created.

2. To elucidate maternal effects on seed oil content and to identify high oil-content gene loci. Several rapeseed lines with over 60% oil content (dry basis) have been developed through gene pyramiding.

3. For developing elite B. napus varieties with multiple resistance, germplasms resistant to shattering, club root, sclerotinia and abiotic stresses were identified, and non-transgenic herbicide-resistant source was introduced from Jiang Su Academy of Agricultural Sciences.

4. For high biomass and high yield breeding, the genetic basis of yield traits and related gene loci have been explored, and investigations on high photosynthetic efficiency, root system architecture and nutrient efficiency, and ideal plant architecture are on the way.

5. A novel pollination control system in rapeseed has been developed with Sinapis arvensis male sterility cytoplasm by somatic hybridization and the candidate mitochondrial genes were identified through mitochondrial genome sequencing.
Deciphering the post-neolithic *Brassica napus* oilseed genome: Subtile crosstalks between constituent homoeologous subgenomes generate diversity

Oilseed rape (*Brassica napus* L.), formed less than 7500 years ago by allopolyploidy between *B. rapa* and *B. oleracea*, rapidly became a successful crop. Together with more ancient and recurrent polyploidizations, this conferred an aggregate 72× genome multiplication since the origin of angiosperms and high gene content. We examined the *B. napus* genome and the consequences of its recent duplication. The most paleo-duplicated and youngest polyploid genome yet sequenced, *B. napus* has the highest gene density yet known. Recently joined in a common nucleus, its constituent An and Cn subgenomes are engaged in subtle structural, functional and epigenetic crosstalks, with abundant homoeologous exchanges playing a pivotal role in *B. napus* diversification, yet gene loss and expression divergence are just beginning. We showed that selection in *B. napus* oilseed types has accelerated loss of undesirable glucosinolate genes, while preserving the greatest known expansion of oil biosynthesis genes. These processes provide unique insights into early allopolyploid evolution and its interactions with crop domestication and improvement.

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Breeding oilseed mustard *Brassica juncea* using conventional and molecular approaches

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*Brassica juncea* is grown in around six million hectares of land in the north-western parts of India during the winter season. Mustard is well adapted to low water availability but suffers from a number of yield-limiting problems. Our research work is focused on the development of productive hybrids, quality breeding and disease resistance. There are two major gene pools in oleiferous types of *B. juncea* – the Indian gene pool (I) and the east European gene pool (EE). Hybrids between lines of the two gene pools are heterotic for yield. We have developed three hybrids – DMH-1 and DMH-4 based on CMS system 126-1 and hybrid DMH-11 using barnase – barstar system. Hybrids yield 20-30 per cent more than the national and regional checks. The east European and Indian gene pools have contrasting agronomic traits. However, transfer of oil and meal quality traits and disease resistance from east European to Indian lines leads to lowering of the yield of recipient lines due to linkage drag. We have carried out extensive mapping of quality traits, white rust resistance loci and quantitative traits in six different DH populations derived from I x I, I x EE and EE x EE crosses. Mapping work has used transcriptome based SNP markers and IP and SSR markers? Marker aided selection is allowing reduction in linkage drag. These efforts will in the near future lead to quality and disease resistant hybrids of mustard.
Omega-3: New fatty acids for the rapeseed industry

**Background:** Omega-3 long chain polyunsaturated fatty acids like EPA and DHA have critical roles in human health and development with studies indicating that deficiencies in these fatty acids can increase the risk or severity of cardiovascular and inflammatory diseases in particular. These fatty acids are predominantly sourced from fish and algal oils.

**Objectives:** In order to meet the increasing demand for these oils there is an urgent need for an alternative and sustainable source of long-chain omega-3 fatty acids. This talk will describe our genetic engineering efforts to produce a land-based source of DHA in our model species *Arabidopsis* through to *Camelina*, *Brassica juncea* and our target crop *Brassica napus*.

**Methods:** We used cutting-edge gene isolation, characterisation and screening methods to identify an optimal gene pathway for DHA production. These genes were screened in multiple species for activity before being combined into a single, eight-gene construct for DHA production. This was transformed in *Arabidopsis*, *Camelina sativa*, *Brassica juncea* and *Brassica napus* and the transgenic seed analysed.

**Results:** This talk will describe the progress that has been made in engineering DHA in plant seed. DHA levels that exceed the amount typically found in bulk fish oil have now been achieved in all four species. We will describe gene characterisation, construct designs, transgenic plants and seed oil fatty acid profiles obtained, as well as the progress of GM canola field trials.

**Conclusions:** A land-based, sustainable source of long-chain omega-3 fatty acids is an achievable goal to meet growing global demand.

**References:**
Dissecting the dimensions of complex traits for genomics-based breeding

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Background: The *Brassica napus* genome sequence enables deep insight into the complexities of the rapeseed genome. Screening of large, diverse populations with high-throughput comics profiling tools provides us with the opportunity to better understand the control of complex traits, tap novel genetic diversity and improve selection techniques for breeding.

Objectives: We are applying diverse methods including whole-genome resequencing, targeted sequence capture, transcriptome sequencing and genome-wide SNP profiling of large *B. napus* populations, representing diverse gene pools, to investigate genome structure in the context of complex trait regulation. Detailed information about copy number and presence-absence variation amongst genes and chromosome segments in the dynamic allopolyploid genomes of diverse *B. napus* germplasm provides a new dimension for understanding the origin and evolution of *B. napus*, and the selective forces that have continue to shape this young species into a highly successful crop. High-throughput screening of large breeding populations provides a basis for knowledge-based exploration of genetic diversity and genomic prediction of complex trait performance.

Methods: We investigate complex traits in large populations using approaches that integrate classical genetic mapping, genome-wide association studies, nested association mapping and dissection of linkage disequilibrium at trait-associated loci. By high-density genotyping of segregating populations, we can use parental genome sequences to reconstruct whole-genome sequences of large mapping populations based on recombination breakpoints, allowing us to trace haplotypes associated with traits of interest. Interpretation of trait regulation is supplemented by analyses of gene coexpression networks based on population transcriptomics. Detailed physiological and metabolic phenotypes help dissect complex traits and relate them to field performance in the context of multi-environmental performance and climate data.

Results: Examples will be given for omics-based approaches to investigate, dissect and predict complex traits like germination and seedling vigour, abiotic stress tolerance, nitrogen use efficiency, seed quality, heterosis and yield.
Seed oil biosynthesis in *Brassica napus*

The biosynthesis of *Brassica napus* seed oil, which is predominantly composed of triacylglycerol (TAG), involves metabolic events in the plastid and endoplasmic reticulum (ER). Fatty acids (FA) produced in the plastid eventually become esterified to form the cytoplasmic acyl-Coenzyme A (CoA) pool which is used as a source of acyl donor in TAG assembly in the ER. Canola-type *B. napus* seed oil is enriched in oleic acid (18:1\(\Delta^9\)) which is the major FA exported from the plastid. TAG biosynthesis involves the sequential acylation of the glycerol backbone and interplay with membrane metabolism in reactions which are predominantly catalyzed by membrane-bound enzymes. Formation of polyunsaturated fatty acids (PUFA), including linoleic (18:2\(\Delta^9,12\)) and α-linolenic acid (18:3\(\Delta^9,12,15\)), occurs in the ER and the introduction of PUFA into TAG relies on various acyl-editing mechanisms. Various metabolic engineering studies have shown that several biochemical reactions in carbon metabolism can be individually manipulated to increase seed oil content (Weselake et al., 2009, Biotechnol. Adv. 27: 866-878).

The majority of our research on TAG biosynthesis in canola-type *B. napus* has focused on membrane-bound type-1 diacylglycerol acyltransferase (DGAT\(_1\)) which catalyzes the acyl-CoA-dependent acylation of sn-1, 2-diacylglycerol (DAG) to produce TAG. Previously, we have shown that over-expression of a *B. napus* DGAT\(_1\) during seed development in *B. napus* can lead to enhanced seed oil content (Weselake et al., 2008, J. Exp. Bot. 59: 3543-3549). Metabolic control analysis indicated that the resulting increase in DGAT activity reduced the flux control associated with TAG assembly relative to FA biosynthesis.

The *B. napus* genome contains four closely related forms of DGAT\(_1\) genes which encode proteins differing mainly in amino acid sequence in the N-terminal region (Greer et al., 2015, Appl. Microbiol. Biotechnol. 99: 2243-2253). These DGAT\(_1\) isoforms can be divided into two clades with representatives of each clade encoded by genes in both the A and C genome. DGAT\(_1\) specificity assays conducted with recombinant forms of the enzymes produced in a mutant strain (H1246) of yeast (*Saccharomyces cerevisiae*) showed that each isoform was capable of utilizing a range of molecular species of acyl-CoA that are representative of FA found in canola oil. We have also used a directed evolution approach to generate several variants of isoform BnaC.DGAt1.a, which catalyzed enhanced TAG production in yeast (Siloto et al., 2009, Plant Physiol. Biochem. 47: 556-461). Recently, we were successful in solubilizing BnaC.DGAT1.a and purifying the recombinant isoform in an active form for the first time (Caldé et al., 2015, FEBS Lett. 589: 773-778). This advance sets the foundation for determining structure/function relationships in *B. napus* DGAT1 and may eventually lead to a rational approach for increasing the activity and altering the substrate selectivity properties of the enzyme.
Genetic and molecular dissection of complex traits related to oil production and yield stability in *Brassica napus*

Rapeseed is one of the most important oil crops in China. The annual average acreage and total production during 2011-2013 reached 7.4 million hectares and 14 million tons, respectively, amounting to a nine-fold increase of total production with only about 2.5-fold enlargement of growing lands compared with the rapeseed acreage and total production in 1970's. The development and extension of new cultivars with high yield have contributed a significant part to the steady increase of total rapeseed production in China. Since the discovery of Pol CMS, the utilization of heterosis has been a major factor for increased seed yield and stability. The growth of F1 hybrids now accounts for over 70% of the total growth area of rapeseed in China. Raising oil production potential by simultaneous improvement of seed yield and oil content has been the long-term goal for modern rapeseed breeding. The high oil production potential can only be realized in the cultivars with resistances to various bio- and abio-stresses, such as resistance to fungous pathogen *Sclerotinia sclerotiorum*, the most devastating rapeseed disease in China and other major production areas over the world. High oil production based on high seed yield and oil content and its stability are controlled by complex quantitative trait genes. A sustainable progress toward breeding higher yield and more stable cultivars will only be achieved by a better understanding of the genetic, genomic and molecular mechanisms underlying the complex traits. Studies of rapeseed genetics and breeding at Huazhong Agricultural University (HZAU), one of major rapeseed research institutions in China, has been focused on the genetic improvement of *Brassica napus* since 1950's. The major researches here cover genomics and evolution of *Brassica* species, trait genetics, germplasm enhancement and breeding for high yield double low cultivars. Since the last International Rapeseed Congress, studies exploring approaches to improve the yield potential and its stability in *Brassica* species are further promoted by applying genomics tools and knowledge in *Brassicas*. The current report will review the progresses in rapeseed genetic improvement of seed yield, oil content, disease resistance and other complex traits with case studies from HZAU, such as genomic dissection for various breeding populations, and identification of elite alleles and genes for yield potential and stability through genome wide association analysis and functional genomics.

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Keys to successful sclerotinia stem rot management in oilseed *Brassicas*

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**Background and Objectives:** Current management options in Australia against *Sclerotinia sclerotiorum*, the cause of Sclerotinia Stem Rot (SSR) in oilseed *Brassicas*, mainly rely on cultural and chemical controls that are often unreliable and can be cost prohibitive. Development of reliable and relevant methods of field screening diverse germplasm has been critical in the identification of high-level field resistance across diverse crucifer species. Recent ability to effectively characterize physiological specialization in pathogen populations has provided the first opportunity to not only monitor pathotype distributions, but to identify resistances against predominant pathotypes and to combine these resistances into future cultivars.

**Results and Conclusions:** Within *Brassica* species and interspecific breeding population studies and in other host screening studies, genotypes pathotype-dependent and some pathotype-independent in resistance expression have been identified. Pathotype-independent resistances are particularly important sources of resistance to exploit in developing new cultivars with effective resistance to SSR across multiple pathotypes. In addition to identifying high level host resistance to SSR, breeding populations of similar levels of resistance but narrow variation in the resistance range have also been identified. Such populations not only consistently display the level of resistance expected but also reflect genetic diversity of resistance sources needed to successfully develop new more-resistant cultivars. Significant progress in identifying appropriate host resistances against prevailing pathotypes makes successful management based on host resistance possible for Australia and elsewhere. Identification of distinct host resistance mechanisms and demonstration of separate genetic control for adult stem vs adult leaf resistances, are crucial to deployment of an effective array of resistances to manage SSR.

**References:**


Integrated pest management of insect pests of rapeseed

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Insects just love rapeseed! Surveys in the UK have shown the importance of the crop to a wide variety of invertebrates; 152 individual species and a further 50 groups were collected from the crop and identified to genus or higher taxonomic rank. The most abundant insects on the crop, perhaps unsurprisingly, were brassica specialist pests, but the crop also supports a great diversity of beneficial insects including the brassica specialist parasitoids of rapeseed pests and generalist predators such as ladybirds, lacewings and hoverflies. The diversity and abundance of invertebrates associated with the crop implies that, even when conventionally managed, the crop plays an important role in supporting populations of butterflies and pollinators, natural enemies of crop pests, and in maintaining food resources for farmland birds throughout the arable rotation. This potential could be harnessed further via use of Integrated Pest Management (IPM) approaches which minimise insecticide use.

IPM is an effective and environmentally sensitive approach to pest management that relies on a combination practices (including the judicious use of pesticides). There are four usual steps in IPM programmes:

1. Set action threshold
2. Monitor pest density & assess risk
3. Prevention – cultural methods e.g. crop rotation, use of pest-resistant cultivars; semiochemical e.g. pheromone repellents; habitat diversification e.g. intercropping, trap cropping
4. Control – mechanical (e.g. trapping); botanical; biological; conservation biocontrol (i.e. the encouragement of naturally-occurring enemies of crop pests to provide pest-regulation services in the crop); synthetic insecticides as a last resort.

The EU Sustainable Use of Pesticides Directive 2009/128 decrees that growers in Europe should use IPM wherever possible. But what tools are available now and what might be our options in the future?

In my talk I will introduce the major insect pests of rapeseed, detailing their life cycle and behaviours. I will then discuss the four steps of IPM programmes giving details and examples of each, with focus on my work on pollen beetles (Meligethes aeneus), cabbage seed weevils (Ceutorhynchus obstrictus) and cabbage stem flea beetles (Psylliodes chrysocephala). Action thresholds exist for most of the major insect pests of rapeseed but they vary widely between countries for the same pest. I ponder why this is and stress that, as action thresholds form the basis of IPM programmes, their determination should be well-grounded from good science. Effective monitoring and risk assessment tools are needed to facilitate working to action thresholds. Monitoring methods for most of the major pests of rapeseed are available but most of them are onerous and therefore less often used than they should be. I will describe some recent advances in monitoring and risk assessment for pollen beetle. Most preventative methods are at the development stages. I will present work on the application of understanding host-plant location processes to the development of pest tolerant cultivars, trap crops and repellents. Finally, I will detail the main natural enemies of rapeseed pests and describe work to augment current agri-environment schemes to deliver effective conservation biocontrol in the rapeseed crop.

I would like to dedicate this talk to Prof. Dr. Lloyd Dosdall (1952 - June 12, 2014).
Blackleg resistance in oilseed rape (*Brassica napus L.*) and strategies for developing protection against this disease

Stem canker (blackleg) caused by the fungus *Leptosphaeria maculans* (Phoma lingam Tode) is a major disease of *Brassica napus* worldwide, causing serious losses on crops in Europe, Australia and North America. A common and effective way to control this disease is the use of resistant cultivars. Two types of resistance have been described (1; 2) and used: (i) qualitative resistance controlled by specific single genes, which is effective from the seedling stage onwards and (ii) quantitative resistance, which is a partial, polygenic resistance mediated by Quantitative Trait Loci (QTL) and effective at the adult plant stage. Qualitative resistance can be quickly overcome by changes in the pathogen populations. The use of quantitative resistance, alone or in combination with qualitative resistance, was shown to be an effective way to get varieties with more durable resistance (3; 4). Both the diversity of genes involved in qualitative resistance and the potential diversity in genomic regions involved in quantitative resistance have been investigated. At least fifteen specific resistance genes have been described, of which two have recently been cloned and shown to be allelic (5). Genomic regions involved in quantitative resistance have been identified through linkage or association mapping. These studies showed that the complexity of this trait, with many homoeologous genomic regions involved, was related to the large number of duplications present in the *B. napus* genome (6). Optimal strategies for control of blackleg disease should take advantage of this diversity and take into account knowledge about the pathogen dispersal and adaptation ability to optimize deployment of resistant varieties in space and time.

References:


Strategies for control of extracellular pathogens of oilseed rape

Background and Results: Pathogens of oilseed rape (Brassica napus) may be classified as biotrophic (intracellular; Plasmodesmata brassicae, clubroot; Hyaloperonospora brassicae, downy mildew; Erysiphe cruciferarum, powdery mildew), hemibiotrophic (extracellular; Leptosphaeria species, phoma stem canker (blackleg); Pyrenopeziza brassicae, light leaf spot; Verticillium longisporum, verticillium) or necrotrophic (Alternaria brassicae, leaf and pod spot; Sclerotinia sclerotiorum, stem rot). This review will focus on short-term, medium-term and long-term strategies in Europe for control of diseases caused by the extracellular (apoplastic) pathogens. Short-term strategies include use of foliar fungicide sprays for control of phoma stem canker and light leaf spot. There are problems with insensitive to triazole fungicides in P. brassicae populations, Leptosphaeria biglobosa is less sensitive than L. maculans and many effective fungicides may be withdrawn as a result of EU legislation (Carter et al., 2014; Huang et al., 2011). Optimal control of both disease requires fungicide application in autumn, which can be guided by web-based forecasting schemes (http://www.rothamsted.ac.uk/phoma-leaf-spot-forecast; Stonard et al., 2011). Medium-term strategies include breeding for resistance against the causal pathogens of all three diseases (Boys et al., 2012; Brun et al., 2010; Verticillium ref). Such resistance breeding programmes can be accelerated by understanding the operation of resistance (R) genes against extracellular pathogens, postulated to involve Effector-Triggered Defence (ETD) mediated through receptor-like proteins (RLPs), by contrast with Effector-Triggered Immunity (ETI) that operates against the intracellular pathogens (Stotz et al., 2014). Such R genes may lose their effectiveness at elevated temperatures associated with global warming (Huang et al., 2006). As a long-term strategy, it is essential to assess potential impacts of climate change on the range and severity of epidemics of these diseases, to guide government and industry policy for climate change adaptation (Evans et al., 2008; Butterworth et al., 2010).

Conclusion: It is important to develop appropriate short-term, medium-term and long-term strategies to control oilseed rape diseases caused by extracellular pathogens.

References:


Verticillium ref
**Impacts of neonicotinoid use in oilseed rape and their mitigation**

In Europe the neonicotinoids clothianidin, imidacloprid and thiamethoxam have been used as seed treatment for more than ten years, including coating of oilseed rape. These actives have a comparably low toxicity to humans and other organisms such as birds. However, the safety of their use for bees is intensively discussed due to a high intrinsic toxicity to honeybees and comparably long persistence. On the other side long persistence and systemicity provide good control of soil and leaf-feeding pests. Honeybee toxicity has become more relevant for environmental regulations since severe bee incidents occurred during sowing of maize in 2008 caused by abrasion of clothianidin from treated seeds and drift of dust to adjacent areas. More than 12000 bee hives were damaged in Germany; some drift incidents also occurred in other European countries, the US and Canada. The exposure via dust drift during sowing had been neglected within risk assessment. After 2008 further research on other routes of exposure such as residues in guttation droplets were initiated. Risks due to exposure via residues in pollen and nectar were already considered when products containing these actives were first authorized, though residue detection was less effective at that time.

In 2013 the EU Commission suspended the use of these neonicotinoids for at least 2 years for crops which may be attractive for honeybees. Our latest research indicates that residues in guttation droplets are sufficient to kill single water collecting bees in several crops including rape but no effects on colonies were observed. Under German environmental conditions guttation provides no unacceptable risk of neonicotinoids to bees. Research in 2014 did not show any effect of residues in pollen and nectar on honeybees, bumblebees and solitary bees; though low max. values (>6 µg clothianidin/kg) were detected in pollen and nectar of winter oilseed rape (10 g clothianidin / kg seeds). Higher residues might occur in summer oilseed rape depending on treatment rates. In response to bee incidences in 2008 and its link to maize sowing of neonicotinoid-treated seeds, dust abrasion of seeds as well as dust drift during sowing were investigated. Dust abrasion of oilseed rape seeds showed that seeds treated in 2008 produced distinctly less abrasion compared to seeds treated before with further improvements in the following years. Drift experiments during sowing indicated varying quantities of neonicotinoids in adjacent crops originating from seed treatment with varying seed dust qualities. No effects on bees were detected if Heubach abrasion values of oilseed rape seeds per hectare were around 10 mg a.i. or lower. But the a.i. was still detectable up to 30 m from sowing. In general, negative effects on bees only occur if bees visit plants adjacent to the sowing. Bee safety can only be guaranteed by low dust abrasion and low contents of a.i. in dust.

No bee poisoning incidents were attributed to neonicotinoid seed treatment in oilseed rape in Germany in more than 10 years of use although almost all oilseed rape was treated with neonicotinoids.
Strategies and challenges in the management of clubroot disease of canola

**Background:** Clubroot is an important soilborne disease of crucifers caused by *Plasmodiophora brassicae*. It first emerged as an issue in the Canadian canola (*Brassica napus*) crop in 2003, when 12 clubroot-infested fields were identified in the province of Alberta. Annual surveys have since revealed that the disease is spreading at a fairly rapid rate, with nearly 1,900 confirmed infestations by 2014. The main mechanism of dispersal appears to be via the movement of *P. brassicae*-infested soil on farm and other equipment, although the movement of pathogen resting spores in windblown dust also has been documented (Strelkov & Hwang, 2014). Clubroot can cause significant yield and quality losses in susceptible crops, and the long-lived nature of the resting spores makes it difficult to control.

**Management:** Initially, clubroot management was focused on the sanitization of field equipment and long rotations out of canola in *P. brassicae*-infested fields, although neither strategy was widely implemented by farmers. The efficacy of various soil amendments and fungicides also was evaluated, but while some could significantly reduce clubroot severity, most were not cost effective for the large-scale canola production systems of western Canada (Hwang et al. 2014). Nonetheless, the fumigant metam sodium may have potential as a spot treatment to contain localized infection foci in areas where *P. brassicae* is still not established. Canola cultivars with excellent resistance to the predominant pathotypes of *P. brassicae* first became available in 2009-10 (Peng et al. 2014; Strelkov & Hwang 2014), and quickly came to be the most important clubroot management tool employed by farmers.

**Challenges:** *Plasmodiophora brassicae* continues to spread, with isolated infestations recently identified in the Canadian provinces of Saskatchewan and Manitoba, as well as in the neighboring American state of North Dakota (Chittem et al. 2014). As such, farmers in affected regions increasingly have relied on resistant canola cultivars, often growing them in very short rotations in heavily infested fields. This has placed tremendous selection pressure on pathogen populations, and in 2013 resistance was overcome in at least one field in Alberta. Further testing under greenhouse conditions showed that all canola varieties classified as clubroot resistant are susceptible to the strain of the pathogen from this field. Surveys in 2014 suggest that resistance has been overcome in several other fields, highlighting the continued vulnerability of the canola crop to *P. brassicae*.

**Conclusions:** Clubroot is a serious threat to canola. Genetic resistance to *P. brassicae* represents the most important management tool, but has been overcome in at least one field and likely others. This underscores the need for resistance stewardship and longer rotations out of canola where clubroot is an issue.

**References:**
Strelkov, S.E., S.F. Hwang. 2014

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Canola, successful science

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Canola is one of the great agricultural science success stories. Innovative traits and germplasm advancements have enabled a significant productivity gain making canola one of the largest and most valuable crops for producers and the food value-chain. Consumer trends continue to drive the need for more functional and cost effective food ingredients and productivity gains. The presentation will look across the value-chain at how food industry trends and the next generation of innovation will make canola an even more important crop to the global food industry.
Canola meal: Successes and opportunities to increase value

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Like canola oil, canola meal can be regarded as a success story. It is now the second most used protein meal in the world after soybean meal. It has found a premium market in its use as a feed ingredient for dairy cows. With increasing production of canola seed, the market for canola meal has continued to expand with no loss in value. Canola meal production in Canada increased from 2.5 million tonnes in 2008 to 4 million tonnes in 2014. The extra production has been exported, primarily to the US for dairy feed production. The relative value of canola meal to soybean meal in the US market during this period has stayed the same at 71%. It is a consistent and reliable protein source that is highly regarded around the world. Thanks are due to the founders and developers of canola for their dedicated work over the last 40 years.

Despite its success, there are still limitations and concerns about canola meal. It does not have the versatility of soybean meal. The protein content and overall nutrient digestibility is lower. It has found a steady market in the dairy, swine and aquaculture feed industries with increased growth potential in those markets but it does not have as wide an acceptance in poultry feeds. There are still some concerns with anti-nutrients, especially phytic acid, fibre and glucosinolate levels, which should all be reduced. There are opportunities to increase canola meal nutritional value by increasing protein content as well as improving amino acid and energy digestibility. Improvements can be made through plant breeding, processing and the use of feed enzyme technology with breeding changes seeming to be the most promising. Canola meal may never overtake soybean meal, but there are opportunities to increase its value so that it is an even more fearsome competitor.
Health actions of canola oil beyond cholesterol: The emerging belly fat connection

Although current dietary guidelines advise restricting the consumption of saturated and trans fats, considerable debate continues regarding the optimal dietary blend of polyunsaturated (PUFA) versus monounsaturated (MUFA) fatty acids. Recent data have shown potential weight control advantages of diets higher in MUFA, thus the purpose of this presentation is to review and evaluate emerging science on overall health benefits of MUFA rich oils, particularly canola oil. Results of review articles and recent clinical trial data suggest that MUFA possess lipid lowering abilities. Specifically, diets with DHA-rich canola result in better overall lipid lowering and favorable Framingham Risk Scores due consequent to larger impacts on TG levels and blood pressure, when compared with other PUFA and MUFA-rich oil blends. Moreover, results of a series of human intervention trials suggest that diets high in MUFA, including canola oil, improve adipose distribution and reduce belly fat, possibly due to effects on a cluster of lipid metabolism mediators termed fatty acid ethanolamines. As such, MUFA-rich canola oil appears to be a desirable option for replacement of other dietary fats and oils to protect against overweight and reduce cardiovascular disease risk in susceptible individuals.

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Development of new canola oils

Since its initial introduction into commercial production in 1993, high oleic canola oils have significantly impacted western Canadian agriculture. Over the past five years, up to 3,000,000 acres have been planted to high oleic canola varieties – roughly 15% of the total Canadian acreage. The high oleic canola acreage is incremental, and has not cannibalized any generic canola plantings. The best high oleic canola hybrid varieties rank with the best yielding generic canola hybrids and feature the best blackleg resistance available today. Essentially all these acres have been produced under closed loop with contracted growers benefiting from crop premiums above the market price for generic canola.

Since its introduction as a new to the world oil in 1994, high oleic canola oil today displaces ~1.4 billion pounds of partially hydrogenated oils annually. Reformulation by food processors and foodservice companies using high oleic canola oil effectively eliminates ~300,000,000 lb of trans fat and ~280,000,000 lb of saturated fat from public consumption every year. The nutritional improvement to public health, particularly in cardiovascular benefits, is huge. High oleic canola oil occupies the dominant position among all specialty oils in the food industry today. New entrants, e.g. high oleic soybean oil, are challenging this position and threaten to cannibalize the significant impact that high oleic canola oils have made to the entire Canadian canola industry.

As nutritional science, consumer needs and governmental regulations continue to evolve, new types of specialty canola oils will be needed to maintain the economic advantages created with the first generation high oleic canola oils. Such oils are expected to add additional incremental value to the industry. A view of market needs and potential canola based solutions will be reviewed, including agronomic requirements and food applications.

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Rapeseed proteins: Extraction technologies, functional properties and applications

Background: Rapeseed proteins - which make up about 20 to 25% of dry seed weight - possess high nutritional value as well as promising functional properties. In comparison to other oilseeds, rapeseed contains two major fractions of storage proteins with completely different properties, the 2S albumin napin with a molar weight of 12-17 kDa and the 12S globulin cruciferin with a molar weight of about 300 kDa.

Objectives: A new technology to produce pure rapeseed proteins, consisting of gentle oilseed processing, aqueous protein extraction, precipitation of cruciferin and expanded bed adsorption ion exchange chromatography for isolation of pure napin (or a new protein mix) will be presented and compared with already described processes.

Methods: The new technology was developed in laboratory and up-scaled into small pilot scale. In order to evaluate the resulting products the following functional properties were determined: protein content and purity, solubility, denaturation behavior, foaming capacity and foam stability, emulsifying and film formation properties.

Results: The new process allows to produce napin with a purity > 98%, cruciferin with a purity > 95% and, if desired, a new protein mix of about 56 - 57% napin and 43 - 44% cruciferin. Napin is 100% soluble, has exceptional thermal stability and is easy to process in O/W-emulsions which possess a very good stability. Cruciferin is characterized particularly by very good foaming properties.

Conclusions: Protein separation is reproducible and can be scaled-up. The resulting protein products possess interesting functional properties enabling a wide range of possible uses both in food and non food applications (cosmetics, biochemistry, pharmaceutical). Particularly, napin is comparable or even better than egg albumin and could therefore replace animal albumins, e.g. in vegan foods.

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Nutritive value of canola meal: The dietary fibre story

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As a consequence of the small size and high oil content of canola seed (42-45%), the resulting meal contains a relatively high proportion of dietary fibre with the neutral detergent fibre (NDF) and total dietary fibre (TDF) values being higher than those of soybean meal (SBM).

In addition to the fibre components of the seed, including non-starch polysaccharides (NSP), glycoprotein, and lignin with associated polyphenols, the dietary fibre content of canola meal (CM) could be increased further in the pre-press solvent extraction process since some screenings and other dockage are sometimes added back into the meal. As well, the processing conditions and temperatures used in the desolventizer-toaster may increase the dietary fibre content of the meal due to protein damage and the formation of lignin-like Maillard reaction products. Based on the recent surveys conducted in Canada, the content of NDF and TDF of CM averaged 29.6 and 38.0% DM, respectively, and ranged from 27.1 to 33.4% for NDF, and from 34.8 to 41.9% for TDF.

Various approaches have been undertaken in an attempt to reduce the fibre content of CM. These include breeding for yellow-seeded canola, dehulling or the use of microbial enzymes to enhance nutrient utilization by monogastric animals. Superior quality characteristics of newly developed yellow-seeded B. napus canola and canola-quality B. juncea mustard have been demonstrated. Although significantly lower in the dietary fibre contents, similar growth performance parameters in broiler chickens and turkeys to those of the conventional canola meal and SBM were observed when the diets were formulated based on digestible amino acids and available energy contents. This would indicate that all types of CM could effectively replace SBM in poultry rations, and that the development of low-fiber canola would result in quantitative changes as evidenced by increased oil, protein, and sucrose contents rather than qualitative changes due to decreased fiber content.

Another route in reducing fibre content is hull removal. When evaluating the meal from the tail-end dehulling process using sieving technology, a significant increase in protein content of the dehulled versus standard meal (from 36.8 to 42.0%) and a substantial reduction in the content of dietary fiber (from 30.0 to 21.4%) were noted. However, when fed to young broiler chickens and weaned pigs, no difference in growth performance was observed. Therefore, it could be concluded that canola fibre is simply a diluent and has minimal effect on nutrient utilization. This could be due to the fact that most of canola fibre is associated with the hull fraction and thus less water-soluble and biologically active. In addition, fibre components, including NSP and glycoproteins, may serve as substrates for feed enzymes and contribute to the release of additional energy (i.e., ~100 kcal/kg) from CM.

The exception to this would be meal overheating during pre-press solvent extraction process which may result in reduced digestibility of some amino acids. Based on our recent study with broiler chickens, the standardised ileal digestible amino acid contents ranged from 2.18 to 2.50% for arginine, 1.74 to 2.00% for lysine, 0.49 to 0.65% for methionine, and 1.00 to 1.38 for threonine. Such ranges of values are clear indication that the fraction of fibre deriving from amino acid damage would be an indication of low meal quality.
Towards deciphering genetic and physiological cues associated to nitrogen and water stress tolerance in oilseed rape

Winter oilseed rape is a very nitrogen-fertilizer consuming crop and is characterized by low nitrogen use efficiency (NUE). It is also very reactive to and penalized by water deficit insofar functional interactions occur in plants between N nutrition and water acquisition. A high proportion of absorbed N remains immobilized in senescent leaves and is returned to the soil failing to contribute to sink functioning and seed production. Improvement of N remobilization efficiency (NRE) during leaf senescence is likely to improve significantly the overall plant NUE, particularly in oilseed rape in which organic N recycling is rather inefficient. Water stress strongly impacts yield and water use efficiency is partly associated to efficient N and C recycling, allocation and partitioning. In order to decipher functional traits involved in nitrogen and water use efficiencies optimization, searching for genetic variation in physiological and molecular key determinants such as genes, enzymes and metabolites, we are developing a multi-disciplinary approach based on genetic, physiology and functional genomic strategies that will be described in the talk.

Recent years have mainly allowed the development of experimental systems and phenotyping tools as well as the exploration of the existing genetic material in trials conducted under controlled or field conditions. Genetic analysis is being developed and has led to the identification of QTL for oil yield, yield components and NUE parameters under different fertilization regimes. Some of the variables are also being acquired at key stages on a panel of genotypes to initiate association analysis.

At critical stages of resource allocation, metabolomic and transcriptomic fingerprints of source and sink tissues are performed during sequential senescence to highlight networks discriminating source leaf from sink leaf and during monocarpic senescence to compare leaf draining and seed filling under different fertilization regimes. Works are designed either under controlled or field conditions to permit discovering essential nodes of regulation and new candidates for both vegetative tissue NRE performance and seed oil accumulation. Efforts are also devoted to the development and the improvement of efficient phenotyping tools dedicated to NUE, WUE and leaf and root development.

The functional value of metabolism known to be induced under stress conditions is sought. These include the metabolic pathways involved in mobilizing glutamate and directed to glutamine, proline and gamma-aminobutyric acid (Gaba) production and utilization. Proteases and protease inhibitors are also being studied as key effectors of nitrogen remobilization whose regulation by stress is investigated. Besides these targeted investigations more comprehensive transcriptomic and metabolomic approaches are performed in oilseed rape under stress and in related species more or less adapted to adverse conditions with the prospect of discovering molecular markers of tolerance.

References


Confluence of data science and precision ag: Bringing digital agronomic insights to increase yield and minimize risk on the farm

Current unfettered access to relatively inexpensive data on a massive scale, advent of advanced sensor technologies, proliferation of high-end big data analytics, and availability of powerful seed genetics are ushering in globally the next wave of productivity and efficiency to our agricultural farms.

Demand to meet burgeoning global food and feed security needs under limited agricultural land and stretched input resource conditions, sustainably, requires new knowledge, insights, tools and practices that can illuminate and respond to the fast-changing landscape of the farms. Increasing need for long term soil security and climate-resilience while continuously boosting agricultural output is calling for adoption of digital ag tools that are coming into the marketplace at a very opportune time.

In this presentation, we seek to highlight how the confluence of big Data science and Precision Ag technologies could be harnessed to increase productivity and minimize risks on the farm through prospective digital insights derived from sensor-based measurements, data analytics, and powerful data science models.

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Next generation phenotyping for quantitative analyses of key productivity traits of crops: Challenges and opportunities for plant phenomics and plant breeding, from controlled environments to the field

It is widely accepted that continued development of both high-throughput and mechanistic, plant phenotyping methodologies are required to add value to genomic discovery and improve crop productivity by supporting plant breeding programs. We have developed a unique infrastructure for 2D, 3D and 4D quantitative analyses, which is currently applied to tackle research questions addressing the identification and selection of key shoot, root and seed traits of selected crops including rapeseed. This presentation provides highlights of our research, first focusing on an overview of state-of-the-art methodologies for non-invasive phenotyping under controlled environment and proximal or remote sensing in the field. Current challenges regarding data acquisition, analysis and interpretation will be discussed. Next, we will present case studies demonstrating the applicability of these methods to characterize genetic resources in various crop species as well as their phenotypic plasticity to the environment. The overarching research questions concentrate on assessing trait networks for improved acquisition and use efficiency of water and nutrients, nitrogen and phosphorous in particular. Emphasis will be given to rapeseed by introducing detailed studies of lateral root development in response to nitrogen limitation supporting association mapping efforts.

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Canola weed management systems – Mitigating weed resistance to herbicides

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Background: Weed management systems in canola (Brassica napus L.) have improved markedly since canola was first grown in Canada. Herbicidal weed control in canola is now relatively simple, but the repeated use of the same herbicide tools favours the selection of weeds that resist our most common herbicides and decreases the prospect for long-term, sustainable canola production.

Evolution of Weed Management in Canola: In the 1970s, when the first canola-quality B. napus and B. rapa varieties were developed in Canada, canola was usually seeded on land with low weed populations with pre-plant incorporated herbicides such as trifluralin, and later ethalfluralin, to control a good portion of the weed spectrum. Post-emergence graminicides were introduced in the 1980s and provided a high level of grassy weed control. Clopyralid was also introduced in the 1980s and controlled difficult species such as Cirsium arvense, Sonchus arvensis and Polygonum convolvulus. In 1990 ethametsulfuron was introduced to control weedy Brassica relatives to canola and was often tank-mixed with graminicides and clopyralid for full-spectrum weed control. Almost all of these herbicide options were rapidly abandoned when imidazolinone-, glyphosate- and glufosinate-tolerant canola were introduced in the mid-1990s. The latter herbicide systems provided a high level of weed control for the most important weeds in canola and continue to dominate weed management systems in canola today.

The Risk of Repeated Herbicide Use: While plenty of western Canada farmland harbours ALS-resistant (Group 2) weeds, much less land contains glyphosate-resistant (Group 9) weeds (only Kochia scoparia in western Canada); and, thus far, glufosinate-resistant weeds have not been detected here. However, whereas glyphosate- and glufosinate-tolerant canola have provided somewhat of a reprieve from Group 1 (ACCase)- and Group 2 (ALS)-resistance selection pressure, their overuse in high frequency canola rotations threaten additional weed resistance to glyphosate and new resistance to glufosinate.

Mitigating Weed Resistance to Herbicides: Rotating different effective herbicide modes of action and tank-mixing different effective herbicide modes of action delays selection of resistant weed populations. However, using alternatives to herbicides halts selection for weed resistance to herbicides. Combining optimal cultural practices (e.g. diverse crop rotations and higher crop seeding rates) against weeds and determining the utility and viability of harvest weed seed control techniques (e.g. Harrington seed destructor and chaff collection/baling) will provide growers with non-herbicidal weed control options that will prolong the life of valuable herbicide tools; relatively non-renewable resources.

References:
Genetics underlying variation in drought adaptation in *Brassica napus*

The long-term goal of my research is to provide detailed functional knowledge of mechanisms regulating drought responses and water use efficiency. I will report on results of using natural variation in drought tolerance among diverse lines of the important crop *Brassica napus*. This focuses on detailed physiological screens in segregating families to understand physiological mechanisms and identify causal polymorphisms underlying differences in drought adaptation. These findings will facilitate ongoing efforts to improve productivity of *B. napus* and other crop plants under drought and expand understanding of the evolution and physiology of drought adaptation and acclimation. My long term goal in *Brassica napus* is to understand the mechanisms underlying drought acclimation and adaptation at the molecular, physiological and population scale. These efforts will be facilitated by the close relationship to *Arabidopsis thaliana*, where existing functional data and molecular tools can be employed to dissect variation in *Brassica* spp.

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PHENOME, the French Plant Phenomic Network, a tool for rapeseed phenotyping

**Background:** Phenotyping has become the major limitation in programs aimed at building genotypes that maintain / increase crop performance under climate change and reduced inputs. The goal of Phenome is to provide France with an up-to-date, versatile, high-throughput infrastructure and suite of methods allowing characterization of panels of genotypes of different species under climate change scenarios.

**Methodology and facilities:** The Phenome consortium gathers 12 academic groups (mainly from INRA), two farmer-funded applied research organizations (ARVALIS and CETIOM), and subcontractors from public organizations specialized in sensor or imaging developments. It also indirectly involves, through research projects, the major European-based seed companies working in France.

**The five locations of Phenome involve:**

1. two platforms in controlled conditions, able to individually characterize 300 genotypes in a single experiment (1900 plants) with the possibility to measure individual plant growth, architecture (shoots and roots) and transpiration; ii) two field platforms with strong control of environmental conditions, in particular one free-air carbon enrichment (FACE) system, (800 individual plots). (iii) three field platforms with higher throughput (2000 individual plots). They are equipped with soil and climate sensors, and a ‘phenomobile’ designed by Phenome to capture functional images of each individual plot at high throughput. (iv)Two supporting “omic” platforms allow us to centralize and optimize high throughput metabolomic and structural measurements associated with the experiments in the phenotyping platforms. Several methodological projects are working to: i) improve our capacity to measure plant traits and environmental conditions with accurate and high throughput methods; ii) Organize phenotypic data originating from different nodes in a coordinated way iii) Handle very large datasets comprising data at different time scales and plant organization levels, and the interface between data collection and plant / crop models. Phenome will be an essential tool for (i) the academic community for original programs of genetics/genomics, (ii) private seed companies, which will have access to the infrastructure via collaborations and bilateral contracts, (iii) French SMEs involved in markets of phenotyping and precision agriculture.

**Rapeseed experimentations:** CETIOM has the lead on Dijon field Platform. 800 rapeseed plots designs and methodological trials were carried out the last two cropping seasons using drones flights for NDVI measurements for Biomass and LAI at different plant development stages, or flowering earliness with RGB acquisition. Comparisons with classical or pedestrian technics have been done to evaluate this new high throughout put technological opportunity. A new multicoptere drone and a Phenomobile V2 adapted to a wider number of plant species will be developed in the next two following years.

**Aknowledgements:** PHENOME, and RAPSODYN, the rapeseed projet, were supported by ANR in the frame work of “investissements d’avenir”
Biotechnology: A key driver in the
canadian canola industry; A farmer’s perspective

The application of biotechnology in Canola resulted in two major breakthroughs in growing the crop in Western Canada. The first was highly effective broad spectrum post emergent weed control (herbicide tolerance) which allowed for the crop to be grown under minimum tillage. The second breakthrough was the development of hybrid vigor which has resulted in substantially higher yields and stress tolerance. Both breakthroughs have combined for significantly expanded seeded acreage and higher total production which has manifested into a vibrant domestic crushing industry.

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Opportunities and challenges for canola in the next 25 years – Carbon and nitrogen fixation and science “fictation”

Over my career spanning about 25 years, I have witnessed dramatic changes in agriculture in western Canada – examples include reduced tillage seeding systems, engineered herbicide tolerance in canola, hybrid canola, short rotations and challenging new pests such as clubroot disease. I expect the next 25 years to be equally exciting. I am honored to give this keynote lecture on opportunities and challenges for canola. Drawing on my career experiences, I will narrow my focus to three topics: carbon and nitrogen fixation, and social perceptions of agricultural technology. I will briefly review the progress and potential for capturing the benefits of increasing atmospheric concentrations of the plant mega-nutrient, CO₂. Secondly, I will review progress in improving nitrogen use uptake, efficiency and biological fixation for brassica species. Given the interplay and feedback between plant C and N metabolism, there will need to be coordinated improvements in photosynthesis and N efficiency to achieve the best yield gains. Finally I will discuss the hurdles new technology faces in agriculture due to poor science knowledge of the general public, the ability of the internet to disperse information regardless of truth or fiction, reluctance of scientists to engage in social media on agricultural issues, and the poor public knowledge on risk perspectives versus benefit.
Rapeseed: Economic trends and prospects

The paper looks at economic aspects of rapeseed production and use on a global scale. The development of the rapeseed branch is assessed against the background of other agricultural branches, the global oilfruit branch and especially the “G4” (palm, soybeans, rapeseed, sunflower).

The rapeseed branch has developed very successfully. Since 1990, world production has almost tripled, caused both by an increase in rapeseed acreage (average: 3 percent per annum) and by an increase in rapeseed yields (average: 1.5 percent per annum). Within the oilfruit branch, only palm oil achieved higher growth rates. In contrast, grain acreage experienced almost no growth in recent decades.

Especially in the last decade (from 2000), the strong growth of the oilfruit branch was heavily pushed by the non-food segment. Between 2001 and 2011, the non-food use of vegetable oils („G4“) increased by 39 mio. t., while the food use increased only by 17 Mio. t. (feed use: +83 mio. t).

Meanwhile, global production of biodiesel has reached a level of about 27 bn. liters. This is equivalent to approximately 15 percent of global vegetable oil production, 1.5 percent of global diesel consumption and 0.15 percent of global energy consumption.

European producers are worried about possible changes in biofuel legislation which may severely deteriorate the economic prospect of rapeseed-derived biodiesel. Yet even in an extreme scenario with no EU-rapeseed-derived biodiesel at all, prospects for European rapeseed production would remain rather positive. As the global markets for vegetable oil are closely interrelated, excess rapeseed oil from the EU would find its way into the growing global markets for vegetable oil. In this scenario, the EU would become a net exporter of rapeseed. Even though this would result in a certain deterioration of EU farm gate-prices, on many EU locations farmers would regard rapeseed still profitable enough to keep it in their crop rotation.

In the global competition against soybeans, the position of rapeseed is negatively affected by the weak economic performance of rapeseed meal against soybean meal. If policy support for biodiesel would be cut back in future, the performance of the protein component might gain economic importance.

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EU-policy on intellectual property, new tools in plant breeding and access to plant genetic resources. Impact on innovation and competitiveness in rapeseed breeding and commercialization

Plant breeding consists in creating ever new combinations of best traits from different plants in new, innovative and improved plant varieties. It is therefore necessary for plant breeders to be able to start from the prior achievements of other plant breeders by using their varieties. They need access to plant genetic diversity. Having said this, a key issue for plant breeders worldwide is government policy for intellectual property protection (IP), access to and use of plant genetic resources (PGR) and new technologies.

Within the underlying conflict between protection of intellectual property rights on the one hand and the need for access to genetic diversity on the other hand, a balanced IP system is vital. Plant variety protection with its breeders’ exemption is such a balanced protection system whereas the patent system is much more restrictive. With their decisions in the so called broccoli and tomato cases, the European Patent Office extended the scope of patent protection to conventional breeding.

The EU regulation 511/2014 on the implementation of the Nagoya Protocol is shaping the legal framework under which access and use of plant genetic resources are allowed. Excessive obligations for documentation and the restriction of the breeders’ exemption are negatively affecting EU scientists and breeding companies.

The latest breeding methods like genome editing, cisgenesis or reverse breeding allow plant breeders to create new and better varieties faster and more precise. Unnecessary regulation and associated costs might impede the utilization of these breeding methods and with that innovation in plant breeding as such.
The plant with novel trait approach, its history and what it has meant for the commercialization of GM canola in Canada

The OECD was among the first to articulate some of the core principles for GMO environmental risk assessment in the Blue Book in 1986. In Canada, consultations with the scientific community endorsed the OECD principles as an approach to the environmental risk assessment of a GMO, then went further to say that the method used to produce a plant with a novel trait was not as important as the consequences of the introduction of a novel trait to a species. This input from scientists has resulted in the unique Canadian product based “plant with novel trait” (PNT) approach that despite the wider scope, effectively addresses regulatory issues such as how to deal with some of the emerging techniques for plant breeding that do not meet the regulatory definitions in more process based regulations yet may still result in plants with novel traits that a competent authority wishes to regulate. The Canadian approach to regulatory oversight has had a direct impact on the canola industry. Canada grows about 11.3 million acres of canola per year, primarily in western regions, making it the largest single producer country. Of that acreage, almost 95% is sown to herbicide tolerant lines, and a herbicide tolerant crop could be considered as a plant with a novel trait (PNT) and trigger regulation regardless of the whether it was produced using the methods of modern biotechnology or more traditional means such as mutation breeding. Science remains the foundation of regulatory oversight of PNTs in Canada and a recently published paper by Canadian regulators highlights the evolution in regulatory thinking (Schnell J. et al 2015) and steps towards modernization of the comparative safety assessment of PNTs

Jaimie Schnell, Marina Steele, Jordan Bean, Margaret Neuspiel, Cécile Girard, Nataliya Dormann, Cindy Pearson, Annie Savoie, Luc Bourbonnière, and Philip Macdonald

A comparative analysis of insertional effects in genetically engineered plants: considerations for pre-market assessments

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Designing canola’s future: The prospects and opportunities

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Canola is in many ways a designer crop, having been deliberately selected and bred for its oil and meal properties. Since that first step to designing the future, the biosciences, the industrial food system and farmers have successfully positioned canola as one of the healthiest, most flexible and most sustainable crops in the global agri-food system. This talk will review the roots of this transformation, examine the current opportunities and threats and assess the feasibility of continuing to meet and exceed the food, agronomic and industrial needs for the crop.
New population genomics re-capture lost genetic diversity in rapeseed

Background: Rapeseed (*Brassica napus*) is one of the most recently domesticated major crop species, and due to intensive breeding has become the most important oilseed crop in Europe. Modern varieties are based on a relative small subset of the available genetic diversity, because breeding progress suffers from low genetic diversity caused by severe selection bottlenecks in recent decades. Rapeseed is thus likely to respond strongly to programs aimed at selectively enhancing genetic variation for key economic input and output traits.

Objectives: A large consortium of breeders and academic institutions in Germany have established the flagship project "Pre-BreedYield: Precision breeding for yield gain in oilseed rape" aimed at enriching genetic diversity in the extremely narrow rapeseed gene pool using exotic and de novo germplasm resources.

Results: A very large Nested Association Mapping (NAM) population (N>2500) was generated by crossing 50 core accessions to an elite line. Furthermore, we used 1000 of these lines to generate experimental hybrids by crossing with a male-sterile tester genotype. All of the founder lines were re-sequenced at high depth to investigate structural genome variation, and the 2500 NAM lines were genotyped with a 60K Illumina Infinium SNP chip, allowing us to identify all recombination breakpoints in every line and impute its genome sequence from the parental sequence data. This unique genetic resource has been used in comprehensive phenotypic studies (e.g. field trials at 12 locations, deep phenotyping using new high throughput methods), enabling Genome Wide Association Studies (GWAS), Nested Association Mapping (NAM) and development of performance prediction models for numerous traits.

Conclusion: We established a highly interesting genomic platform for studying and using genetic diversity in an important global crop species and for dissecting and predicting complex traits.
Increasing the market value of canola through improved oil and meal quality traits

**Background:** Canola breeding programs in Australia have significantly enhanced canola as a commercially attractive oilseed crop. As current breeding programs tend to concentrate on production traits, such as increasing yield and oil content, as well as disease and insect resistance and drought tolerance, there has been minimal focus on oil and meal quality.

**Objectives:** This study aims to assess samples from the Australian National *Brassica* Germplasm Improvement Project (NBGIP), with a view to determining the suitability of this material for inclusion into future breeding lines to increase the quality of the Australian canola germplasm.

**Methods:** A total of 684 genotypes from the National *Brassica* Germplasm Improvement Program (NBGIP) grown over a two year period were used in this study. The NBGIP lines encompass a wide variety of germplasm from around the world. Some of the countries of origin that are included in the NBGIP lines are China, Taiwan, Russia, Ukraine, France and Australia. Samples from the National Variety Trial (NVT) canola program were also analysed to allow for comparison with the current Australian germplasm. A total of 165 samples from the NVT program were analysed. Oil, protein and glucosinolates content were measured in whole seed by NIR. Tocopherol content (method ISO 9936:2006 (E) and fatty acid composition (AOCS Ce 2-66) (AOCS,1998) were measured in the oil extracted from the seed. The meal component was analysed for sinapine content (Mailer et al, 1995)), neutral detergent fibre (AFIA Method 1.8A (a)) and acid detergent fibre (AFIA Method 1.9A (a)).

Dry matter digestibility (DMD) and dry organic matter digestibility (DOMD) contents were measured in meal based on the AFIA Method 1.7R (AFIA, 2011). Meal energies were determined for the different animal feeds based on standard calculations.

**Results:** Total tocopherols ranged from 536-1263 mg/kg oil in NBGIP canola while the range in NVT canola was 546-1178 mg/kg oil. The results from this study show that the metabolisable energy ranged from 8.9-12.5 MJ/kg for the NBGIP samples, and 8.7-11.3 MJ/kg in the NVT samples (in oil free, dry matter meal). Results showed that some of the genotypes in the NBGIP germplasm have relatively low NDF and ADF contents when compared with NVT and results from other studies.

**Conclusions:** The results from this study show that inclusion of some of the genotypes identified in the NGBIP germplasm into the Australian canola germplasm could have a positive impact on the quality of Australian canola oil and meal.

**References:**


Variation for pod shatter resistance in an international germplasm collection of *Brassica rapa*

**Background:** Dehiscence of mature pods or pod shatter is a natural mode of seed dispersal in the wild. However, domesticated crops like rapeseed show wide variation for shattering resistance. The crop losses due to shattering tend to vary in *B. rapa*, with some brown sarson genotypes tending to show substantial yield losses due to shattering. Very few attempts have been made to document variation for this trait in *B. rapa*. Documenting such a variation was considered important because *B. rapa* is A-genome donor for both *B. juncea* and *B. napus*. While *B. juncea* is in general resistant to pod shattering, *B. napus* is very prone to shattering with virtually little inherent variation. Identifying *B. rapa* genotypes with very high degree of resistance to pod shatter may be important for enhancing variation for this trait in *B. napus* germplasm.

**Objectives:** To document variation for pod shatter resistance in *B. rapa*.

**Methods:** A world wide germplasm collection comprising land races, historical and modern cultivars of *B. rapa* (83) was assayed for pod shatter resistance. To facilitate that, twenty five pods each were detached from the main racemes of each genotype. These were then kept in tarson tubes containing coarse silica gel granules to equilibrate moisture. The pods were oven dried at 70°C for 24 hours immediately before assessing pod strength. The relative resistance of each genotype to pod shatter was measured in terms of rupture energy (RE) using an improvised pendulum apparatus (Kadkol 2009) wherein the pendulum strikes the pod with a known force and records the energy absorbed to split it open. Molecular characterization was also carried out. For this, genic SSR markers were developed from sequence information of key shattering related genes like SHP 1, SHP 2, IND, FRUITFULL and NAC. These primers were used to amplify the genomic region of each candidate gene in an association mapping set (83 genotypes). Marker-trait associations were investigated using software TASSEL V 2.1.

**Results:** The test germplasm collection showed wide variation for pod shattering. The rupture energy varied from 2.3 (CN107763) to 9.5 mJ (LT 69). Bulk of genotypes figured in the rupture energy range of 3-5.5 mJ. Indian toria types in general were more shatter resistant than the brown sarson forms. DNA polymorphisms generated by genic SSRs were used for population structure analysis. The analysis divided global germplasm in three groups. Structure based grouping was not consistent with the grouping based on the rupture energy. Association mapping analysis suggested significant role of SHP 1 and SHP 2 in defining variation for pod shatter resistance. These loci and regulation mechanism may be of significant value for enhancing this trait in related *B. napus*.

**Conclusions:** Excellent variation exists for pod shatter resistance in *B. rapa* germplasm. Role of SHP 1 and SHP 2 in defining trait variation was emphasized.

**References:**
Deterrminate inflorescence: A key step towards architectural modification of *Brassica* oilseeds

**Background:** Oilseed *Brassicas* are naturally indeterminate. This growth habit results in competition between growing shoots and fruiting bodies for available metabolites, the fruiting bodies at lower portion of the inflorescence being at advantage. This growth habit generally results in less resources to the fruiting bodies at the terminal end which leads to no pod set or tip sterility. We report identification of determinate plant growth habit in *B. juncea*, *B. carinata* and its introgression from *B. carinata* into *B. napus*. Such a modification of crop architecture in *brassicas* is expected to enhance productivity.

**Objectives:** Establishing trait genetics and breeding value of determinate plant growth habit in *Brassica* allopolyploids.

**Methods:** Genetic and molecular studies were carried out using selected determinate and indeterminate forms. A set each of true breeding determinate genotypes of *B. juncea* (125), *B. napus* (79) and *B. carinata* (48) were evaluated in the replicated trials to establish breeding value of this novel germplasm.

**Results:** Plants with determinate plant growth habit with shoots terminating in pods, as of terminal flower mutant (TFL 1) in *Arabidopsis* (Alvarez et al 1992), were identified in progenies of resynthesized *B. juncea*. Such plants were also identified in *B. carinata* derived from the advanced generation progenies of intercross [(*B. juncea* x *B. carinata*)BC3 x *B. napus*]. Analyses of F1, F2 and F3 segregation revealed monogenic recessive inheritance for the trait in both *B. juncea* and *B. carinata*. In *B. juncea*, the gene for determinacy (Sdt1) was mapped to the linkage group B5 (Kaur and Banga, 2015). Determinate growth habit was introgressed into *B. napus* from *B. carinata*. Graphical genotyping of determinate *B. napus* types also revealed presence of B5 introgressions. The trait is stably introgressed in the three digenomics with excellent variation for key agronomic traits and no adverse association in terms of pod density, productivity or oil content was observed. Data for trait inheritance in *B. napus* will be presented.

**Conclusions:** Determinacy was under the control of single recessive gene and mapped to the linkage group B5 in *B. juncea*. Determinate progenies with high agronomic performance were identified.

**References:**
Laser microdissection and RNA sequencing of the *Brassica napus* – *Sclerotinia sclerotiorum* pathosystem

**Background:** The necrotrophic fungus, *Sclerotinia sclerotiorum*, causes widespread loss in crop yield and production each year including one of Canada’s most valuable agricultural systems – canola (*Brassica napus*). While the development of resistant and moderately tolerant lines are emerging, we still have yet to identify the genes and gene regulatory networks responsible for this host-pathogen interaction directly at the site of infection. Moreover, we know nothing about how this interaction is specified at the cellular level. Thus, understanding how the plant responds to this aggressive fungus should provide the information necessary to improve crop protection and performance.

**Objectives:** While our understanding of the host-pathogen interaction is becoming clearer, there is remarkably little information available for *Sclerotinia*, especially how canola perceives and responds to this pathogen directly at the site of infection. Thus, we have taken an integrative biological approach to understanding the genes and gene regulatory networks responsible for host pathogen interactions in universally susceptible and moderately tolerant lines of *Brassica napus* in whole leaf tissues as well as within the three tissue systems of the leaf directly at the site of infection.

**Methods:** We used whole leaf and laser microdissected leaf samples infected with *Sclerotinia* at 0 and 24 hours post inoculation for global RNA sequencing in the universally susceptible cultivar, Westar, and a moderately tolerant cultivar [Zhongyou821 (ZY821)]. Using the petal inoculation method, we aimed to investigate the plants defense response directly at the site of infection. We complement the global RNA profiling experiments with detailed anatomical studies of the plant-pathogen interaction at the light and electron levels and validated the sequencing data using qRT-PCR.

**Results:** We found large numbers of genes to be differentially expressed when the leaf is challenged with *Sclerotinia* using the petal inoculation method. Robust bioinformatics analyses including fuzzy K-means clustering and GO term enrichment show defense response molecules are rapidly activated at the cellular level directly at the site of infection in the moderately tolerant ZY821. These include processes associated with phytohormone signal transduction and cell wall metabolism. In addition to global changes in gene activity and the regulation of transcription factors and signaling molecules, tolerance to the devastating fungal pathogen in ZY821 is likely through differences in cell wall thickness and mesophyll abundance.

**Conclusions:** Our data provides a comprehensive transcriptome atlas of the canola-*Sclerotinia* pathosystem directly at the site of infection in the mature leaf. Data reveal large shifts in gene activity between the susceptible and moderately tolerant lines that are due to both genetic regulatory mechanisms and inherent structural architecture of the leaf tissue systems.

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Recent advances and future direction of canola, mustard and rapeseed breeding at the University of Idaho

**Background:** Few crops have adaptability to the Pacific Northwest (PNW) dry land growing condition, and that offer farmers rotation opportunities. Monoculture cereal production has resulted in a buildup of soil borne diseases and as a result cereal production has been reduced. Growers need alternative crops to rotate with cereals to: (1) reduce chemical inputs by breaking disease cycles; (2) improve profitability through reduced crop inputs and by product diversity; and (3) become more sustainable and internationally competitive. However, the lack of adapted cultivars was a major limitation to increased acreage of **Brassicaceae** crops. Our research is aimed at developing food quality (canola) and industrial quality (rapeseed) oil cultivars and condiment mustard cultivars that are highly adapted to the PNW (and other USA regions) that can be grown in crop rotation with small-grain cereals. In addition, the use of synthetic soil fumigants in the USA has negative environmental effects. Glucosinolate breakdown products in some **Brassica** species are highly allelopathic and have been shown to have nematocidal, bacteriological and pathological effects. Our research is aimed at developing **Brassica** cultivars that have high levels of specific glucosinolate types that could be used as biological soil fumigants.

**Objectives:** The overall objective of the research group is to develop superior **Brassicaceae** oilseed, forage, mustard, and soil fumigant cultivars that are highly adapted to a wide range of dry land and irrigated regions of the PNW and other USA regions.

**Methods:** A combination of traditional and novel breeding techniques has been used in our cultivar development program including: crossing followed by recurrent phenotypic selection, early generation selection and cross prediction, embryogenesis, interspecific and intergeneric hybridization, and more recently molecular markers, quantitative trait loci and genomic wide association studies.

**Results:** The Rapeseed, Canola and Mustard Breeding group at the University of Idaho began developing improved winter canola and rapeseed cultivars over 32 years ago. In 1992, the breeding team expanded cultivar development to include spring canola and spring rapeseed (**Brassica napus**), yellow mustard (**Sinapis alba**), and brown or Indian mustard (**B. juncea**). The latter two mustard species breeding efforts are directed towards condiment mustard cultivars and to develop 'designer glucosinolate' cultivars suitable for use as soil fumigants. Over the past 15 years the program has released 16 new varieties. This paper outlines the breeding objectives and procedures used in cultivar development. Also discussed is the future role of techniques to improve selection efficiency, including cross prediction, genomic wide association studies and marker assisted selection, wide crossing, and developing specialty industrial oils for bio-jet fuel feedstocks.

**Conclusions:** Cultivars released by this breeding effort has offered PNW growers flexibility and alternatives to include in crop rotations, be more environmentally sound, help to reduce crop inputs, improve profitability and sustainability, and make USA growers more competitive in international markets.

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Genetic diversity increases heterosis –
The Sprinter Brassica napus Project

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Background: Brassica napus cultivars are typically classified as either spring or winter flowering types. Winter type B. napus is predominant in Europe and China where it is planted in fall and flowers the following spring after a period of winter vernalization while spring type B. napus is planted and harvested in the same growing season in Canada and Australia. Due to these different growth habits and geographic isolation, winter types of B. napus have been found to be genetically distinct from spring types. It has been suggested by Tom Osborn and others that crossing spring flowering type B. napus containing winter type genetic backgrounds with spring B. napus may increase heterosis and produce higher-yielding hybrids.

Objectives: The goal of the Sprinter B. napus project was to broaden the genetic diversity of spring type B. napus by converting the flowering type of winter B. napus germplasm from winter to spring type through the introduction of fast flowering genes from rapid-cycling B. rapa.

Methods: Winter B. napus cultivars (e.g. Jetton, Darmor) were crossed with rapid-cycling B. rapa to generate F1 seeds. F1 plants were then backcrossed to their respective winter cultivars creating BC1 populations. All BC2-BC6-10 populations were generated by selecting the earliest flowering individuals from each BC population for backcrossing with original winter cultivars. Some early flowering BC plants were also crossed with a wide range of winter B.napus cultivars to develop spring type F1 and BC populations following the same strategy.

Results: The sprinter B. napus method demonstrated high spring type conversion rate resulting in at least one spring flowering type plant found out of 20 in all BC populations created from the conversion of more than 100 winter cultivars used in this project. Hybrids generated using the Sprinter B. napus conversion process were not statistically different from spring type B. napus hybrids for flowering and maturation time when grown under Canadian field conditions. In both 2013 and 2014 field trials, the higher yielding hybrid combinations were found through Sprinter B. napus project.

Conclusion: The Sprinter B. napus conversion process established an innovative, unexpectedly simple, efficient and reliable method to increase genetic diversity in spring type B. napus by converting winter B. napus to spring.

References:
Genomic insights into seed development in *Brassicas*

**Background:** Seed development represents an important phase in *Brassica* oil seed crop species. During this phase, developmental and metabolic programs are coordinated to produce the seed that contain the germline information and storage reserves. From the oil seed *Brassica* crop perspective, the qualitative and quantitative aspects of metabolites and especially the synthesis and deposition of fatty acids define the value of the seed. The advanced understanding of the making of *Brassica* seed and insights into the associated global genetic and metabolic programs are critical for improving oil seed *Brassica* crops.

**Objectives:** The goal of this study is to develop comprehensive systems level insights into molecular and biochemical programs coordinated with gene expression and metabolism – during seed development in *Brassica napus*.

**Methods:** The gene expression and metabolite profiling were performed in *Brassica napus* using microarray, RNA seq and LC/MS/MS based approaches.

**Results:** To explore the developmental and gene expression programs of *B napus* seed, we isolated seed components from fertilization to maturity and performed detailed developmental and gene expression studies. Analysis of large datasets generated using these approaches identified developmental and stage specific programs that are connected to gene expression and metabolic regulation during seed development. By combining this omic data with metabolite profiles, we constructed stage-specific metabolic sub-networks in *B napus* seeds. These analyses identified putative regulatory factors that control important seed traits. Functionality of some of these genes further confirmed their important regulatory roles in seed development, storage product synthesis and deposition.

**Conclusions:** Our integrated systems approach and studies using oil seed *Brassica* species *B napus* produced comprehensive datasets for seed development that include molecular atlas of gene expression. Integration of these datasets with metabolite profiles identified regulatory networks of seed development. Functional interrogation of key findings of this study revealed putative gene targets for improving quantitative and qualitative aspects of seeds in this important oil seed crop.

**References:**


Development of leading yield and blackleg resistant high oleic canola in Western Canada

**Background:** High Oleic canola has become an important source of income for Western Canadian farmers over the past 20 years. As a result, canola production acres have increased significantly over the past decade due to higher profitability, resulting in shorter canola rotations. The consequence of this shift in management practice has been an increased incidence of blackleg (*Leptosphaeria maculans*) disease, the most important disease of canola in Western Canada. In response, Cargill's global breeding program used innovative approaches to develop High Oleic canola hybrids exhibiting leading yield performance with very strong blackleg resistance. This unique combination of High Oleic canola with consistent yield performance and strong, durable blackleg resistance has been a key development to help Western Canadian canola producers achieve high financial returns.

**Objectives:** To demonstrate Cargill's innovative global breeding approach that consistently delivers High Oleic canola hybrids with industry leading yield and blackleg resistance.

**Methods:** Open pollinated breeding, hybrid breeding, genetic diversity, heterosis for grain yield, doubled haploid breeding, marker assisted breeding

**Results:** Over the previous 20 years, Cargill's High Oleic canola yields have consistently improved. Yield performance has progressed from 85% of 46A65 in 1996, to 140% of 46A65 in 2011. Currently yields of Cargill's High Oleic canola hybrid are equal to the industry leading commodity canola hybrids. Exceptional blackleg resistance has supported this high yield achievement.

**Conclusions:** Cargill's High Oleic canola program has provided a significant increase in farm income for west Canadian canola producers by combining specialty canola oil profiles, competitive high yields, all protected by the best blackleg resistance platform available.

**References:**
WCC/RRC data from Canola Council of Canada, CPT data from Canola Council of Canada.
Heterotic gene pool development in *Brassica napus*

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**Background:** Genetic diversity is a valued resource for breeders trying to exploit heterosis in *Brassica napus* L. Characterizing germplasm accessions into different heterotic gene pools is one of the first steps breeders should attempt when developing hybrid cultivars. Gene pool classification separates breeding material into distinct groups in order to focus experimental hybrid production on potential high heterotic parental combinations.

**Objectives:** The objectives of this study were: 1) to characterize *B. napus* genotypes using phenotypic and genotypic methods; 2) assign genotypes to heterotic breeding pools using multiple clustering methods; and 3) investigate the relationship between genetic distance and hybrid heterosis.

**Methods:** Twenty qualitative and quantitative phenotypic characteristics were used to create heterotic breeding pools based on Ward’s (Ward 1963) method of agglomerative clustering. Additionally, we compared to two genotyping methods, SRAP (Li and Qurios 2001) and GBS (Elshire et al, 2011) using the Nei (1972) standard genetic distance method and the Tamuri-Nei (1993) distance method. Both genotypic methods employed a neighbour joining clustering method (Saitou and Nei, 1987). Based on clustering distance, hybrid heterosis was compared to determine if genetic distance is an accurate predictor of high heterotic parental combinations.

**Results:** Despite using different clustering methods, phenotypic characterization produced similar hierarchical clusters compared to the clustering produced by SRAP (314 polymorphic markers) and GBS (80,005 bi-allelic SNPs) data. SRAP and GBS heterotic clustering was similar when compared with each other despite different distance methods and agreed with pedigree information. Genetic distance based on genotypic analysis was weakly, but positively correlated with hybrid heterosis.

**Conclusions:** Phenotypic and genotypic heterotic clustering was similar despite different methods and agreed with pedigree information. Genetic distance was positively correlated with hybrid heterosis ($R^2 = 0.18$); however, it lacks predictive power for high heterotic crosses using these specific distance/clustering techniques.

**References:**


Stability of field performance of *Brassica napus* L. spring canola hybrids with improved resistance to Sclerotinia stem rot in Canadian Prairies 2010-2014

**Background:** Sclerotinia stem rot (SSR) is an important disease of spring canola caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. Foliar fungicides can be applied to help manage this disease. The ability to manage Sclerotinia stem rot in canola using fungicides met with variable success until the registration of the first Pioneer Brand® hybrid (45S51) with field resistance in 2008. 45S51 was tested on a large scale vs. susceptible canola hybrid with and without fungicide treatment in 2008-2009, establishing thresholds of field performance (Falak et al, 2011). Further improved hybrids with field resistance to SSR (45S52, 46S53 and 45S54) were registered and released between 2010-2014, undergoing large scale trials across Western Canada with susceptible hybrid 45H29 used as a check.

**Objectives:** The objectives of this research were to quantify reduction of disease index, incidence and severity, while estimating stability of field performance of 45S52, 46S53 and 45S54 in various environments and geographies over the period 2010-2014.

**Methods:** SSR testing of 45S52, 46S53 and 45S54 was conducted from 2010-2014 in 321 large-scale grower managed locations. Susceptible hybrid 45H29 and three field resistant hybrids, 45S52 (2010-2012), 46S53 (2010-2011) and 45S54 (2011-2014) were planted in 1 ha blocks. Five samples of 50 individual plants from uniform parts of the field were rated for *Sclerotinia* damage. Data on disease incidence and disease severity were collected from which a disease index calculation SSFS (Sclerotinia sclerotiorum field severity) was derived. Only locations meeting threshold of 10% SSFS in 45H29 were used in this study. Disease pressure category was determined by SSFS value on 45H29 as follows: low (10%-15%), mid (15%-20%), high (20%-30%) and very high disease pressure (>30%).

**Results:** SSF data was generated at sixty six locations between 2010-2014. SSFS values ranged from 10%-56% with corresponding incidence ranging from 12%-82%.

The level of the overall disease reduction (SSFS) ranged from 67% on 45S54 under low disease pressure to 81.0% on 46S53 under high disease pressure. 46S53 was the most resistant product with overall SSFS reduction of 74.6% while 45S54 had the lowest level of protection (69.6%).

Disease incidence reduction was highest with low disease pressure and the least effective under very high pressure (61.6% vs. 50.6% for all hybrids). Disease severity reduction was increasing with the increase in disease pressure (27.5% for low vs. 46.6% for very high disease pressure).

**Conclusions:** In large scale on farm trials conducted over five years in Western Canada, a reduction of more than 65% in SSFS in 45S52, 46S53 and 45S54 vs. canola hybrid 45H29 was recorded.

Disease incidence reduction contributed more to the reduction in SSR under lower pressure environments, while disease severity reduction contributed more in higher pressure environments.

Field resistance was stable when exposed across years and geographies with diverse pathogen populations in Western Canada.

**References:**
A novel single-nucleotide mutation in a CLAVATA3 gene homologue controls a multilocular silique trait in Brassica rapa L.

**Background:** The silique of *B. rapa* is developed from the gynoecium, which normally consists of two carpels that are separated by a false septum, and thus has two locules (bilocular). Multilocular (more than two carpels) lines of *B. rapa* have been discovered in nature, and genetic analyses of multilocular traits in *B. rapa* have demonstrated that the number of locules is monogenically governed, with the allele for bilocular type showing completely dominance over the multilocular type. But its molecular mechanism remains unresolved.

**Objectives:** The trait of multilocular siliques of *Brassica* are considered advantageous because the multilocular type potentially produces more seeds per silique than the bilocular type, increasing the seed yield. Thus, the isolation and functional characterisation of the multilocular silique gene in *B. rapa* will provide valuable gene resource for both practical breeding and for molecular mechanism studies in *Brassica* crops.

**Methods:** The multilocular gene ml4 from *B. rapa* var. yellow sarson was isolated using a map-based cloning approach. Comparative sequence analysis of the candidate gene was conducted in the wild-type and mutant to reveal the caused variation in the number of carpels. Transgenic complementation and in vitro peptide assays studies were used to demonstrate the function of the candidate gene. Gene expression analysis detected the expression changes of key genes between the wild-type and mutant.

**Results:** The multilocular mutant exhibited enlargement of the shoot apical meristems (SAMS) during embryonic, vegetative and reproductive development. Multilocular mutant produced increasing numbers of floral organs, locules and seeds per silique, likely due to the enlarged SAMS. Histological analysis revealed that the extra locules were formed during the early stages in the developing gynoecium. The ML4 gene was isolated and determined that it encodes a small putative secreted peptide that is the putative orthologue of the *Arabidopsis* CLAVATA3 (CLV3) gene. Sequence analysis of two alleles revealed that the ml4 mutation was a novel C-to-T base transition that led to the substitution of Pro9 with Leu in the core CLE motif. Peptide assays and transgenic complementation studies demonstrated that the causal single-nucleotide substitution (C-to-T) was responsible for the formation of multilocular siliques in *B. rapa*. Expression analyses indicated that the putative negative pathway in the feedback loop between CLV3 and WUSCHEL was disrupted in multilocular plants.

**Conclusions:** A novel single-nucleotide substitution (C-to-T) in BrCLV3 is essential for the control of SAM size and numbers of the locules and seeds per silique. Expression analysis demonstrated that the putative negative pathway in the feedback loop involving CLV3 and WUS was disrupted in the ml4 mutant. These findings thus provide important information and insights into the molecular mechanism of multilocular silique formation in *Brassica* crops.

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Synthesis of a stable allohexaploid *Brassica*

**Background:** Breeding of *Brassica* oilseeds is expected to benefit greatly from resynthesis of existing allotetraploids through hybridization between extant diploid donors or through newly evolved concept of derived amphiploidy (Gupta et al. 2015). Resynthesis route not only helps to mobilize variation extant in diploid donors but also benefit from de novo variation resulting from the process of polyploidization and may offer novel avenues for phenotypic response through selection of new and useful genotypes (Rieseberg and Willis 2007). Attempts have also been made to carry the process forward by creating *Brassica* hexaploids. Synthetic hybrids can be made with relative ease (Chen et al. 2011) but theses hybrids are unstable: losing chromosomes in subsequent generations due to poor control of chromosome pairing behaviour. We report the synthesis of first *Brassica* hexaploid, that has now remained stable over three cycles of selfing.

**Objectives:** To produce a stable three-genome allohexaploid *Brassica* species (AABBCC; 2n=54). This was expected to allow bringing together genetic diversity present in the three progenitor species in a single hybrid crop to enhance crop productivity and the broaden adaptation niches of *Brassica* oilseeds.

**Methods:** Three-genome hybrids were generated from crosses between the one (B. rapa, B. nigra and B. oleracea) and two-genome (B. juncea, B. carinata, B. napus) species of *Brassicas*. Attempts were made to maximise the genetic diversity present in the final hexaploids. Chromosome doubling was induced through application of colchicine (0.2%) with 1% DMSO at four leaf stage. At least 100 pollen mother cells with well spread metaphase-I/diakinesis/anaphase-I were examined per plant for the determination of chromosome number and pairing behaviour.

**Results:** We synthesized a large number of interspecific combinations involving B. carinata x B. rapa (31) and B. juncea x B. oleracea (17). Hexaploidy could be confirmed in eight combinations, others are being confirmed. Of these two hexaploids, HexC1 and HexC2 could be carried forward to H3 generation. HexC1 showed consistent meiotic stability over three cycles of selfing. All the plants (running into several hundreds) in H2 and H3 generations were meiotically stable and showed normal 27II and 27-27 separation during anaphase-I. In contrast meiosis in HexC2 was aberrant and a high frequency of plants in H2 and H3 showed variable chromosome number. Both these hexaploids differ only for B. rapa parent with a common B. carinata parent. Implications of these studies in terms of existence of pairing regulator gene on A genome are being investigated as a component of Australia-India biotechnology fund.

**Conclusions:** A stable *Brassica* hexaploid has been synthesized. A genome may have a role in pairing regulation.

**References:**


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Homologous pairing control in 
*Brassica napus*

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**Background:** Homologous recombination during meiosis is essential for creating genetic diversity and faithful separation of the chromosomes to produce four haploid gametes. In polyploids such as *Brassica napus* the presence of both the A and C genomes requires a process to restrict chromosome pairing and recombination so that it occurs only between true homologues and not homoeologous chromosomes from the related genome. In wheat, which has a similar allopolyploid genome structure, homologous pairing is largely controlled by the Ph1 locus (Moore, 2014). Although it is possible that chromosomal rearrangements play a role in restricting pairing between homoeologues in *B. napus* there is growing evidence to suggest that *B. napus* may have evolved a similar genetic mechanism to wheat. Identification and manipulation of a pairing control locus in *B. napus* would be very valuable for crop improvement through the introgression of genes from wild relatives, and the subsequent restoration of genetic stability that would be required to maintain these improvements.

**Objectives:** Development of a reliable tool for monitoring chromosome pairing in *B. napus*, and the identification of loci/genes responsible for controlling faithful chromosome pairing in *B. napus*.

**Methods:** A DH population segregating for control of homologous chromosome pairing and recombination was developed from a cross between an adapted *B. napus* line and a resynthesized line. Individuals from this population were crossed with an unrelated cultivar to produce testcross populations that were phenotyped for homologous recombination using the *Brassica* 60K Illumina SNP array. The SNP array probe sequences were aligned to the *B. rapa* and *B. oleracea* genome assemblies to look for reciprocal allele gain/loss in homoeologous regions of the testcross individuals.

**Results:** Phenotyping of chromosome pairing has traditionally been done using cytology, but this is difficult in *Brassica* due to the similar size of the chromosomes and low level of distinctive banding patterns. Development of an accurate and reliable method for measuring homoeologous recombination using SNP markers can aid in the genetic mapping of genes/loci that influence chromosome pairing in polyploid *Brassica* species. Using the SNP array to analyse testcross populations we were able to measure homoeologous recombination using reciprocal gain/loss of A and C genome SNP alleles. The level of homologous pairing control for each DH individual was assigned based on the relative number of homoeologous recombination events in the 16 testcross individuals derived from each of 31 DH lines. These data were used to map a QTL that controls homologous recombination in *B. napus* to linkage group A9.

**Conclusions:** The identification of this pairing control locus is the first in *B. napus* allotetraploids. Further refinement of this locus and its precise mechanism for controlling meiotic recombination holds promise for improving *Brassica* crops through introgression of beneficial traits from wild relatives.

**References:**  
Genetic analysis for branch angle in *Brassica napus* L.

**Background:** Plant architecture, which refers to the spatial distribution of various parts of a plant, is an important agronomic trait that affects photosynthesis and seed yield (Diepenbrock 2000). Ideal plant architecture (ideotype) has the least competition among individuals, and it can influence photosynthesis and plant growth and finally contribute to the largest economic gain. Hence, breeding for ideotype is an effective way to improve rapeseed yield, especially for modern cultivation which requires high planting density.

**Objectives:** Branch angle, the angle between the branch and main stems, is an important agronomic trait of rapeseed architecture. Decrease of branch angle results in compact plant architecture, allowing more efficient light capture as planting density increased. The aims of study were to detect QTLs controlling branch angle for mining of elite alleles and to identify candidate genes involved in branch angle in rapeseed.

**Methods:** A natural population containing 143 cultivars and inbred lines from all over the world was grown in different environments and assessed for branch angle. Genome-wide single nucleotide polymorphism (SNP) of the lines were assayed with 60K *Brassica* Infinium® SNP array and association analysis was carried out to identify QTLs. At the same time, a segregated population on branch angle is constructed and used to map QTLs conferring branch angle by re-sequencing combined with bulk segregant analysis.

**Results:** Significant phenotypic variation was observed from 20 to 70 degree for branch angle among the 143 ecotypes. As a result, significant SNP loci that associate with branch angle were identified on chromosomes A02, A03, A07, C03, C05 and C07 by the MLM model of TASSEL 5.0, which jointly account for approximately 57% of the genotypic variation of branch angle. Among the key QTLs, peak SNPs were found to be near the key orthologue genes of BnaA.lazy and BnaC.lazy on A3 and C3 homologue genome blocks (Yoshihara et al., 2013). Besides Lazy orthologue genes, homologues of SPL14 and auxin-responsive GH3 gene of Arabidopsis thaliana were identified close to two clusters of SNPs on A7 and C7 chromosomes. Comparison of the results obtained from the association analysis with BSA QTL-seq, genomic hot spots relevant to branch angle will be selected. Further analysis on allele variation and functional confirmation is needed for understanding the genetic mechanism of branch angle in rapeseed.

**Conclusions:** This study identified multiple novel loci and refined the map locations of known loci related to candidate genes for branch angle. The associations provide a basis for further efforts to pinpoint causal variants and to clarify how the interaction of implicated genes affects branch angle in rapeseed.

**References:**

Genetic effects of yield-related traits and heterosis prediction by genome-wide SNP chip in *Brassica napus*

**Background:** The additive-dominance-epistatic (ADAA) model (Wu et al. 2006) can be used to predict various genetic effects, including interaction with environment. The newly developed *Brassica* 60K Infinium BeadChip Array is a very effective tool for SNP genotyping.

**Objectives:** To provide a theoretical basis for further improvement of *Brassica napus* yield, an ADAA model was used to analyze different genetic effects and correlations. To investigate the feasibility of heterosis prediction by SNP markers in rapeseed, the 60K SNP chip covering the whole genome of *Brassica napus* was used to estimate the genetic distance (GD) of elite parental lines of *B. napus*, and the correlation between GD and heterosis was analyzed for the guidance of hybrid development of rapeseed.

**Methods:** Forty-six F1 hybrids were produced using six maintainer lines and eight restorer lines of Polima cytoplasmic male sterility (CMS) in an incomplete diallel cross design. Ten yield-related traits of parents and derived F1 and F2 generations were planted under three different ecological environments (Wuhan, Hubei province; Zunyi, Guizhou province; and Chaohu, Anhui province) in the autumn of 2012. The 14 parental lines were genotyped by 60K SNP chip of *Brassicas*. GD based on SNP genotyping results was estimated with MEGA5.0 software. Correlation analysis between GD and heterosis was conducted by SAS9.1 statistical analysis software.

**Results:** The dominant effects of all yield-related traits were obviously greater than their additive effects and epistatic effects, suggesting that heterosis is important to improving the yield of rapeseed. Among three yield components, silique per plant (SPP) and seed per silique (SPS) both showed a significant negative correlation with thousand-seed weight (TSW), but silique density (SD) was genetically correlated with all three components of yield to a certain extent. GDS of the 14 parental lines ranged from 0.1883 to 0.8811, with an average of 0.5217. At genetic similarity of 0.65, the parental lines were divided into four groups. Except for number of effective primary branches, all other nine yield-related traits showed significant heterosis in F1 hybrids. Especially for plant height (PH), SPS, branch height (BH) and yield per plant (YPP), the average mid-parent heterosis were 6.83%, 5.19%, 7.85% and 20.78%, respectively. Significant positive correlation between heterosis and SNP estimated GD was detected for PH, BH and YPP.

**Conclusions:** SD is an important trait. The conflict among TSW, SPP and SPS, can be reconciled via selection of the genetic effect components of SD. GD estimated by genome-wide SNP makers has very significant positive correlation with heterosis for traits with high and universal heterosis, including plant yield. Thus, the genome-wide 60K SNP chip of *Brassicas* can be used to well predict heterosis in rapeseed.

**References:**
A Cys2/His2-type zinc-finger protein, BnLATE, enhances silique shattering resistance by negatively regulating lignin accumulation in the silique walls of *Brassica napus*

**Background:** Silique shattering resistance is one of the most important agricultural traits in oil crop breeding. Seed shedding from siliques prior to and during harvest causes devastating losses in oilseed yield. Lignin biosynthesis in the silique wall is thought to affect silique shattering resistance in oil crops.

**Methods:** Based on microarray expression analysis of two *Brassica napus* accessions, Zhongshuang 11 (ZS11) and 73290, which showed differences in silique shattering resistance (Huang et al., 2014), we identified and characterized *B. napus* Late Flowering (LATE), which encodes a Cys2/His2-type zinc-finger protein, and conducted ectopic transgenic over-expression of BnLATE under the double enhanced CaMV 35S promoter (D35S) in wild-type *Arabidopsis* plants.

**Results:** The D35S::BnLATE transgene resulted in a marked decrease in lignification in the replum, valve layer (carpel), and dehiscence zone. pBnLATE::GUS activity was strong in the yellowing silique wall of transgenic lines. Furthermore, the expression of BnLATE and the lignin content gradient in the silique wall of 73290, a *B. napus* silique shattering-resistant line, were similar to those in transgenic *Arabidopsis* lines expressing BnLATE. Transcriptome sequencing of the silique wall revealed that genes encoding peroxidases, which polymerize monolignols and lignin in the phenylpropanoid pathway, were down-regulated at least two-fold in the D35S::BnLATE transgenic lines. Comparative examination of pBnLATE::BnLATE transgenic lines with wild-type control showed that lignification in the carpel and dehiscence zone was remarkably decreased, as well as silique shattering-resistance, expression of peroxidase coding genes were very similar to that of D35S::BnLATE transgenic lines.

**Conclusions:** Our results suggest that BnLATE is a negative regulator of lignin biosynthesis in the yellowing silique wall. Through restraining the polymerization of monolignols and lignin, BnLATE promotes silique shattering resistance in *B. napus*.

**References:**

Genomic prediction of hybrid performance in canola (Brassica napus)

**Background:** Genomic selection (GS) is a modern breeding approach where genome-wide single-nucleotide polymorphism (SNP) marker profiles are used to estimate individual breeding values of untested genotypes (Heffner et al. 2009; Jannink et al. 2010). This novel biometrical approach is actively gaining currency in plant breeding for the prediction of hybrid performance and improvement of various complex traits (Lorenzana et al. 2009; Crossa et al. 2010). In GS, genome-wide markers are used that capture both large and small genetic effects and thus potentially account for a majority of the genetic variance for a given trait. In principle, no prior information on the effect of individual markers is needed.

**Objectives:** In this proof-of-concept study we aimed to test and refine genomic prediction techniques for a number of important traits in spring-type canola hybrids, based on genome-wide SNP profiles of parental lines. In the absence of strong heterotic pools we explored the effects of population substructure and training population size on genomic prediction accuracy within a large diversity panel of spring-type canola lines, used as pollinators of two divergent male-sterile maternal parents.

**Methods:** A total of 475 genetically diverse pollinator lines, representing adapted and novel gene pools for spring-type canola, were genotyped along with two diverse male-sterile testers using the 60K SNP Brassica Infinium genotyping array. The 950 F1 hybrid combinations between the pollinators and testers were evaluated for field emergence, days to flowering, lodging, oil yield and seed yield along with essential seed quality characters including oil and glucosinolate contents. Genomic prediction models for hybrid performance were developed using the ridge-regression best linear unbiased prediction (RR-BLUP) method (Whittaker et al. 2000; Meuwissen et al. 2001), both within the whole population as well as within individual or combined sub-populations.

**Results:** Genomic prediction accuracy ranged from 0.34 for emergence to 0.78 for oil content for predictions across the whole population, and from 0.28 to 0.74 within sub-populations and their combinations.

**Conclusions:** As expected, the prediction accuracy increased substantially with increased size of the training population. We applied the prediction models for stringent pre-selection of the best predicted hybrid combinations for each trait. Compared to the mean observed performance of all hybrid combinations, the mean performance of selected, genomic-predicted hybrids improved for all of the traits investigated. For high-value traits like oil yield and seed yield, hybrid performance prediction using genome-wide SNP markers shows considerable potential for pre-selection of promising hybrid combinations prior to resource-intensive field testing over multiple locations and years.

**References:**


Broadening of genetic diversity in spring canola (Brassica napus L.) using C-genome of B. oleracea var. capitata, B. oleracea var. albogabra and B. oleracea var. italica

**Background:** Canola (Brassica napus, AACC, 2n = 38) is one of the most important oilseed crops in Canada as well as in the world in terms of acreage and production. The genetic base of spring B. napus is quite narrow (Fu et al. 2010). Improving seed yield, other agronomic and seed quality traits in spring canola B. napus through breeding requires germplasm in the breeding programs with broad genetic base. Genetic diversity in spring B. napus canola can be broadened through introgression of genome component from the two parental species or other allied Brassica species (Bennett et al. 2012), or using the other forms of B. napus, such as winter and semi-winter types (Diers and Osborn 1994).

**Objectives:** The objective of this research is to widen genetic diversity in spring B. napus canola by use of three different variants of B. oleracea (CC, 2n=18).

**Methods:** A canola quality B. napus line was crossed to B. oleracea var. capitata (cv. Badger Shipper & Bindschadler), B. oleracea var. albogabra and B. oleracea var. italica (cv. Premium Crop). In vitro ovule culture technique was applied to produce F1 plants using B. napus as female. The F1 plants were self-pollinated to produce F2 population. Pedigree breeding method was applied with selection for different agronomic and seed quality traits such as, spring growth habit, erucic acid and glucosinolate content.

**Results:** Repeated selection over generations resulted canola quality euploid spring type B. napus families from these interspecific crosses. Flow cytometric analysis for nuclear DNA content revealed that most of the families were similar to the B. napus parent for nuclear DNA content.

**Conclusions:** Spring type B. napus canola can be developed from B. napus × B. oleracea interspecific crosses while using cabbage, broccoli and Chinese kale as the B. oleracea parent in the crosses.

**References:**
Induced sequence variations within life cycle genes of rapeseed and their impact on flowering time and hybrid yield

**Background:** Rapeseed (*Brassica napus* L.) is grown in different geographical regions of the world. It is adapted to different environments by modification of flowering time and requirement for cold. A broad variation exists from very early-flowering spring-type to late-flowering winter cultivars which only flower after exposure to an extended cold period.

**Objectives:** We aim to identify life cycle genes from *B. napus* and we are interested how different paralogs interact with each other. Furthermore, we are studying pleiotropic effects of flowering time genes.

**Methods:** We have established a mutant platform of rapeseed based on two EMS treated rapeseed populations. Mutants are identified by their genotype using the TILLING strategy.

**Results:** In *Arabidopsis thaliana*, the PEBP-domain genes *FLOWERING LOCUS-T (FT)* and *TERMINAL FLOWER-1 (TFL1)* are important integrators of different flowering pathways. Six *FT* and four *TFL1* paralogs have been identified in *B. napus*. We selected EMS mutants of the *B. napus* winter-type inbred line Express 617. In total, 103 mutant alleles have been determined for *BnC6FTb*, *BnC6FTa*, and *BnTFL1-2* paralogs. We chose three non-sense and fifteen missense mutant lines (M3) which were grown in the greenhouse. Although only two out of 6 *FT* paralogs were mutated, six out of eight *BnC6FTb* mutant lines flowered later as the control, whereas all five *BnC6FTa* mutant lines started flowering as the non-mutated parent. Mutations within the *BnTFL1-2* paralog had no large effects on flowering time but on yield components. *F1* hybrids between *BnTFL1-2* mutants and non-mutated parents had increased seed numbers and seed yield suggesting that heterozygous mutations in a *TFL1* paralog may impact heterosis in rapeseed.

**Conclusions:** We demonstrate that even single point-mutations in *BnFT* and *BnTFL1* paralogs have effects on flowering time despite the redundancy of the rapeseed genome. Moreover, our results suggest pleiotropic effects of *BnTFL1* paralogs beyond the regulation of flowering time.

** References:**


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High-resolution developmental transcriptome of the biofuel crop *Camelina sativa*: a valuable resource for functional genomics

**Background:** Due to a number of desirable agronomic attributes and a unique fatty acid profile of the seed oil that has applications in food, feed and biofuel industry, *Camelina sativa* is being embraced as a viable industrial bio-platform crop. Development of *C. sativa* as a sustainable bioenergy feedstock will require significant increase in crop productivity and improvement of oil composition for industrial applications. Genetic and genomic tools that aid in deciphering the complete complement of genes, their structure and organization, and the patterns of gene expression are the key to the successful advancement of this novel oilseed crop through future breeding of high-yielding cultivars with re-engineered oil composition.

**Objectives:** The recent completion of the reference genome sequence (Kagale et al., 2014, *Nature Communications* 5:3706 (2014)) marked an important milestone in *C. sativa* research. The objective of the present study was to generate a genome-wide developmental transcriptome map of *C. sativa* by RNA sequencing of tissue samples collected at major developmental stages during its life cycle.

**Methods:** We constructed and sequenced RNAseq libraries made from 12 different tissue samples collected in triplicates at four major developmental stages, including germination, vegetative growth, flower development and seed development. Using the Illumina HiSeq2000 platform, ~727 million 100 bp paired-end reads (73 GB) were generated, corresponding to an average of 61 million reads per tissue sample. After filtering low-quality, adapter contaminated, and short reads, high quality reads from each sample were aligned to the *C. sativa* reference genome using TopHat2, and expression levels were calculated using Cufflinks.

**Results:** We have generated a digital atlas of this comprehensive transcriptome resource (http://bar.utoronto.ca/~asher/efp_camelina/cgi-bin/efpWeb.cgi) which enables interactive visualization of expression data through a searchable database of electronic fluorescent pictographs. An analysis of this dataset supported expression of >90% of the annotated genes in *C. sativa* and provided a global overview of the complex architecture of temporal and spatial gene expression patterns active during development. A combination of gene-centric and network-based systems approaches allowed us to uncover transcriptional relationships between genes and tissues. It has further helped in the identification of tissue-specific expression signatures that highlight dynamic reprogramming of *C. sativa* transcriptome and reveals functional transitions occurring during vegetative and reproductive tissue development. A high quality census of transcription factors, analysis of alternative splicing and tissue-specific genome dominance provided insight into the transcriptional dynamics and sub-genome interplay among the well preserved triplicated repertoire of homoeologous loci.

**Conclusions:** We have generated an extensive and high quality expression map that covers a wide range of tissues and developmental stages in *C. sativa*. This comprehensive developmental transcriptome atlas in combination with the reference genome sequence provides a powerful resource for genomics research which can be leveraged to identify functional associations between genes and understand the regulatory networks underlying developmental processes. Generation of these landmark resources for Camelina has solidified Canada’s leadership position in this emerging oilseed crop.

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RNAseq based genome wide associative transcriptomics to delineate the genetic basis of trait/phenotype variation in *Brassica juncea*

**Background:** Genome wide association studies (GWAS) can efficiently delineate the regions of the genome contributing to a range of phenotypes, generally using populations of highly diverse lines. *Brassica juncea*, a member of the *Brassicaceae* family, constitutes an important oilseed crop globally. It is a recently formed natural allopolyploid between two diploid species, namely *B. rapa* (A sub-genome) and *B. nigra* (B sub-genome). Recent polyploids can impede the process of SNP discovery due to genetic complexity. To circumvent this, a novel associative transcriptomics approach has been utilized, which is based on the use of transcribed sequences to identify SNPs representing variation in gene sequences and gene expression (GEMS), and further correlate this variation with phenotypic traits (Bancroft et al. 2011; Harper et al. 2012).

**Objectives:** Identification of SNPs and GEMS associated with trait variation in *B. juncea* using GWAS.

**Methods:** STRUCTURE, phylogeny and principal co-ordinate (PC) analyses was performed on genotype-by-sequencing (GBS) data to study genetic diversity. Phenotyping of the selected accessions was carried out in two seasons using randomised block design in three replicates. Several desirable traits were evaluated, including agronomic, fatty acid profile and glucosinolate traits. Total RNA was extracted in three biological replicates from the leaf tissue (4th leaf stage) grown in greenhouse conditions. The Illumina Truseq RNA libraries were prepared and sequenced using Illumina HiScan SQ sequencing platform.

**Results:** Analyses of GBS data from 192 *B. juncea* germplasm lines showed high levels of genetic variation, as the top five PCs captured 33% of the variation and also allowed an estimate of linkage disequilibrium across the genome. A total of 48 *B. juncea* lines were selected representing genetic and phenotypic diversity among the 192 accessions. The discovery of sequence variation in the transcribed regions and transcriptome profiling analysis across 48 *B. juncea* lines is being carried out using *B. rapa* and *B. nigra* reference genomes. In addition, association analysis will be performed using SNPs and GEMS for the phenotyped traits.

**Conclusions:** Two distinct populations were identified among the *B. juncea* accessions, separating the Asian lines from others. This variation is being further dissected to capture SNP and gene expression changes underlying the observed wide spectrum of phenotypic diversity.

**References:**


Screening of genetic variation and QTL and association mapping for root developmental traits in oilseed rape (*Brassica napus L*)

**Background:** Variation in root architecture is essential for the adaptation of plants to target environments since it determines their efficiency in acquiring soil resources. Soil exploration by plant roots is a function of root growth and architecture. Various crop species and cultivars have different kinds of root systems and different capacities to penetrate into deeper soil layers in search of nutrients and water. Root vigor and architecture have a significant influence on the ability of the plant to access soil water; hence root traits play a key role in plant growth and ultimately yield (Shi et al., 2012). Despite the importance of the roots, few studies have systematically investigated the extent of genetic variation for root vigor and architecture in *Brassica napus*. The root system of oilseed rape is extremely plastic in both vertical and horizontal distribution depending on water supply.

**Objectives:** In this study, a digital root phenotyping system based on mini-rhizotrons was optimized for phenotyping root traits in rapeseed. Genetic variation in root architectural traits was evaluated through biparental QTL and association mapping in genetically diverse *B. napus* populations, to identify markers and germplasm for breeding of useful root traits.

**Methods:** Root architectural traits were studied in the Express 617 x V8 DH population (94 lines) and the ERANET-ASSYST *B. napus* diversity set (n=496), including winter, spring, and vegetable and swede type. Five root traits were selected for phenotypic analysis: primary root length (PRL), rate of primary root growth (RoG), lateral root length (LRL), lateral root number (LRN) and lateral root density (LRD). A gel-based in vitro rhizotron system was optimized to digitalize the root developmental parameters in large numbers of genotypes under controlled conditions. Seeds were surface sterilized by washing with 6% NaOCl and were sown on the plates containing growth medium (standard MS medium in Gelrite). Plates were then placed vertically in the growth chamber for seed germination. Plant root development was estimated at 3, 5 and 7 days after sowing by scanning with a flatbed scanner. Images of the growing root system were obtained by digitizing plates from the bottom and were analyzed by using image analysis software Image J NIH Images (Abramoff et al, 2004). On day 7, number and length of each visible secondary root were also recorded. Data from primary and lateral root length and number of lateral roots were used for quantitative trait locus (QTL) mapping and association analysis using genome-wide SNP data.

**Results:** The non-destructive gel based system facilitated the visualization of the root system in large *B. napus* population. The root system in rapeseed originates from a primary root and continues to develop secondary roots growing outward and downward to the tap root. A large variation, broad segregation and medium heritability of root architectural traits in the biparental population proved that these are quantitatively inherited traits controlled by multiple genes which give intimation to proceed for genetic improvement and selection of rapeseed lines with improved root system. In the bi-parental population, 11 QTL regions associated with root architectural traits, while in the *B. napus* diversity set 38 significant marker-trait associations were detected. These represent a first step towards marker assisted or genome-based selection, as well as for map-based gene discovery. Fine mapping of such chromosomal regions will help to determine candidate genes responsible for natural phenotypic variation of these traits.

**Conclusion:** Assessment of root traits under field conditions can be slow and expensive, and this gel-based phenotyping of *B. napus* facilitates the screening of large population for root traits. Identification of the genetic elements associated with root traits will provide grounds for the selection of genotypes with potentially improved abiotic stress tolerance and nutrient uptake efficiency.

**References:**


A genome-wide association study of plant height and primary branch number in Rapeseed (*Brassica napus*)

**Background:** Plant architecture of a crop play a highly important role for its agronomic performance. Plant height (PH) and number of primary branches (PB) are two of major factors affecting plant architecture in rapeseed (*Brassica napus*). The past studies have revealed that these two traits were controlled by multiple quantitative trait loci (QTL), however these QTL studies typically localizes QTLs to 10 to 20 cM intervals. In our previous study, we performed genotype analysis of an association panel with 472 accessions using a 60 k *Brassica* Infinium® SNP array, and the genome-wide association study (GWAS) successfully dissected the genetic architecture of seed weight and seed quality in *B. napus* (Li et al. 2014).

**Objectives:** To uncover the genetic bases of the PH and PB in rapeseed by GWAS approach, and to obtain information of DNA marker or gene for an ideal plant architecture by molecular design breeding.

**Methods:** The trials were conducted at the experimental farm in Wuhan, China. The 472 rapeseed accessions were grown following a randomized complete block design with three replications from 2011 to 2014. At maturity, five plants from the center row of each plot were used to investigate their PH and PB. Best linear unbiased predictors (BLUP) were estimated for each line for each trait. The TASSEL 5.0 was used for genome-wide association study with PCA model and PCA+K model.

**Results:** Seven QTLs on chromosome A3, A5, A7 and C7 were detected for PH. The QTL on the upper of A3 was repeatedly detected in 2 years, and the other QTLs were sensitive to environments. Except for the QTL on A5, the other QTLs were detected in previous studies based on linkage analysis. For PB, four QTLs on A3, A7, C5 and C7 were detected. The QTL on the upper of A3, about 1 Mb far from the QTL of PH, was repeatedly detected in 2 years and the other QTLs were vulnerable to environmental influences. QTLs on A3 and A7 were reported in previous studies, and the other two QTLs were novel. In the genomic regions around the GWAS peaks, some orthologous genes involved in flowering development and phytohormone biosynthesis and signaling were identified.

**Conclusions:** The present study dissected the genetic architecture of the PH and PB in rapeseed by GWAS approach. One QTL insensitive to environments for PH and PB was detected, respectively. Although most of the QTLs were detected only in single year, they were detected in previous studies based on linkage analysis. Since LD decay is very low in this panel, the detected QTLs in our study will be located in close proximity to the candidate genes controlling PH and PB and these tightly associated SNPs would be of significant benefit in a molecular design breeding approach to improve rapeseed plant architecture.

**References:**

Development of nested association mapping population in oilseed rape (Brassica napus L)

Background: Nested association mapping (NAM) is a technique firstly designed for dissecting the genetic architecture of complex traits in maize. It combines the advantages and eliminates the disadvantages of linkage analysis and association mapping for identifying quantitative trait loci. Using the maize NAM population, the genetic architectures of several complex traits including flowering time, leaf angle, plant height, kernel composition and disease resistance have been successfully dissected through joint-linkage analysis and genome-wide association study (GWAS).

Objectives: Understanding of the genetic architectures of agronomically important traits and identifying genes underlying these traits in oilseed rape are the bases for further improvement in breeding programs. Genetic mapping of quantitative traits have identified a number of loci controlling these traits using bi-parental populations. However the resolution using such populations is low due to limited recombination. The objective of this study is to develop a nested association mapping population for genetic dissection of complex traits in oilseed rape.

Methods: To develop a NAM population for dissecting the genetic architecture of agronomic important traits in oilseed rape, we chose Zhongshuang 11, one of the most successful commercial cultivars as the reference line due to its use in the public rapeseed sequencing project. Zhongshuang 11 was crossed to 22 diverse rapeseed inbred lines to create the F1 population. The F1 plants were then self-fertilized for six generations via single-seed descent (SSD).

Results: A NAM population contained 21 families were developed, with 120-200 recombinant inbred lines (RILs) per family and a total of 3200 RILs within the NAM population. The parental lines were sequenced at a minimum of 10 x coverage using the Illumina HiSeq2000 platform. A total of 3.5 M high-quality single-nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) were identified by aligning the short reads to the reference genome sequence. Each of the 3200 RILs was genotyped by sequencing (GBS) reduced representation libraries constructed by double digestion of genomic DNA using restriction enzymes SacI and MseI. Genetic linkage maps were constructed for the RIL families with a range of 4000 – 9000 SNPs.

Conclusions: The NAM population represents a new permanent resource for the rapeseed genetics community, which will be very useful for understanding the genetic architectures of complex traits.

References:
Asymmetrical genome evolution and its impact on trait formation in *Brassica* crops

**Background:** Genome polyploidization has provided significant sources of genetic variation for plant adaptive evolution and new species formation. However, the way in which molecular evolution of polyploid genomes builds up genetic architecture underlying speciation is unclear and its impact imposed on trait formation which comes from synergistic duplicate genes of polyploidy genomes is unknown.

**Objectives:** *Brassica* is an ideal model to address these questions. Here, we used *Arabidopsis thaliana* as an outgroup to conduct comparative genome analysis of newly sequenced *Brassica oleracea*, *B. rapa* and *B. napus*.

**Results:** We revealed multi-layered modes of asymmetrical interspecific and intraspecific genome evolution. Between parallel species *B. oleracea* and *B. rapa*, these layers include: asymmetrical gene retention rates, asymmetrical TE amplification, asymmetrical tandem duplication of genes and asymmetrical alternative splicing variants between the two sister species; Between subgenomes within species, they are: massive and asymmetrical subgenomic gene loss, great variations between paralogs at the DNA sequence level, expression differentiation of triplicated, α-duplicated and tandem duplicated genes across different tissues in the two diploid species, asymmetrical DNA sequence variation (Liu et al. Nature Communications 5:3930, 2014) and asymmetrical homeologous exchanges (Chalhoub et al. Science, 2014), asymmetrical epigenomes and asymmetrical recombination between the genomes A and C in *B. napus*. The epigenomes include small RNA, DNA methylation and histone modification. Further, we used association markers from a genome-wide association study of a large population to have revealed differences in detectable traits such as flowering time and oil content between syntenic regions of the subgenomes A and C.

**Conclusions:** These patterns provide new insight into genome evolution underlying speciation and trait formation and will underpin research in genetic improvement of these important crops.

**Reference:**

C. Tong 1
X. Ge 2
T.J. Yang 3
B. Chalhoub 4
R. Snowdon 1
Q. Pan 2
L. Liu 6
C. Dong 1
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Using the Illumina Infinium 60K array to identify species and genetic diversity in *Brassica* germplasm

**Background:** Breeders and researchers rely heavily on germplasm collections as an extremely valuable resource for crop improvement. However, germplasm sourced from these collections is sometimes misclassified on the basis of species due to errors in provision of resources to the germplasm bank (which accepts passport data for new accessions as provided) or other issues. This hinders effective use of these resources.

**Objectives:** We aim to demonstrate that high-throughput genotyping tools such as SNP arrays can quickly, efficiently and cheaply confirm species and characterise diversity in germplasm collections, and in particular differentiate between the closely related *Brassica* crop species and wild relatives.

**Methods:** We genotyped 180 *Brassicaceae* samples sourced from the Australian Grains Genebank using the Illumina Infinium *Brassica* 60K SNP array. Presence of the *Brassica* A and C genomes combined with principle components analysis clearly separated *B. rapa*, *B. oleracea*, *B. napus*, *B. carinata* and *B. juncea* samples into distinct species groups, and proved more effective than hierarchical clustering methods. Several samples were also validated using chromosome counts.

**Results:** Overall, 18% of samples (32/180) were classified as the wrong species. Of these 180 samples, 23/76 (30%) were supplied on the basis of suspected misclassification by the germplasm curator staff on the basis of phenotype and were in fact misclassified. Another 9/104 (9%) of the samples randomly sourced from the genebank without additional information were also found to be misclassified on the basis of species. Surprisingly, several individuals were also found to be the product of interspecific hybridisation events.

**Conclusions:** The SNP (Single Nucleotide Polymorphism) array proved effective at confirming species in the *Brassica* germplasm set, and also provided useful information related to genetic diversity. As similar high-throughput genotyping methods become available for additional crop species and cytodemes, this technology will comprise an efficient and cost-effective method to screen germplasm collections worldwide, facilitating use of these valuable resources by researchers and breeders.

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Genome-wide characterization of genetic diversity, population structure and linkage disequilibrium in *Brassica napus* L. germplasm

**Background:** Rapeseed/Canola (*Brassica napus* L.) is one of the most important oilseed crops cultivated in many parts of the world. North Dakota is the leader in canola acreage and production with over 83% of U.S. acreage and produces about 84% of all U.S. canola (1.02 million acres and 1.7 billion pounds with a value of $364 million – 5 yr. average from 2009-2013; USDA-NASS). It is crucial to study and preserve genetic diversity in canola since the diversity is the only source of resistance to different stresses as well as various agronomically important traits. Studies that describe the genetic variation in canola populations are limited in USA. The germplasm-based studies help to understand the genetic variation and marker-trait associations that can have applications for marker assisted selection.

**Objectives:** To assess the genetic diversity, population structure and linkage disequilibrium (LD) of canola core collection and its future utility in association mapping studies.

**Methods:** A total of 367 canola germplasms originated from 27 countries were genotyped using GBS Illumina pipeline. The GBS reads were mapped to the reference genome of *Brassica napus* (Chalhoub et al. 2014). LD (r2) within each of the 19 chromosomes was estimated between the markers using PROC allele in SAS 9.3. Population structure was assessed using multilocus data implemented in Structure (Pritchard et al 2000). Principal component analysis (PCA) was used to separate individuals based on axis of variation. PCA was calculated using smartpca program of the Eigenstrat software (Price et al. 2006). Neighbor joining tree for the 367 individuals with the subset of markers was generated in clustalX (Larkin et al. 2007).

**Results:** A total of 42,575 high quality polymorphic SNPs were identified and used to assess genetic diversity and population structure present in the 367 canola germplasms. Of these SNPs, 20,543 were found on genome A and 21,624 on genome C. The majority of the intrachromosomal LD values were less than 0.3 (99 percentile = 0.28) with a mean of 0.0 and a median of 0.006. Low level of LD is evident from the heatmaps developed for each of the individual chromosomes. Three subpopulations were estimated using a subset of 12,908 markers based on LD. The individuals of each of these subpopulations were belonging to all geographical types with no specific distribution.

**References:**


Development of core collection and trait-specific reference sets in Indian mustard [Brassica juncea (L.) Czern & Coss.] germplasm

Background: A total of 1836 Indian mustard germplasm accessions are being conserved as ex situ seed bank collection at ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, Rajasthan, India. The accessions were classified into a) exotic acquisitions; b) indigenous collections; c) advance breeding materials and d) other types. It is very important to efficiently study and use these germplasm resources in mustard crop breeding for sustainable gain in yield under different situations. Frankel and Brown (1984) proposed the idea of core collections, in which a limited set of accessions, with a minimum amount of repetitiveness, were chosen to represent a maximum genetic diversity of entire germplasm resources. Detailed research on a core collection can provide an effective way of characterizing the larger collection that it represents.

Objectives: The objective of our research was to characterize a base collection of 1836 Indian mustard accessions using 16 agro-morphological traits to identify trait-specific germplasm accessions for agronomic traits and to develop a core set of Indian mustard germplasm to enhance utilization of genetic resources in crop improvement programs and simplify their management.

Methods: The 1836 Indian mustard accessions together with five standard checks were evaluated for 16 agro-morphological traits in augmented block design. A number of diverse germplasm accessions with agronomically superior traits were identified based on multi year evaluation data. To develop a core set, the data were analyzed using Powercore software based on advanced M (maximization) strategy implemented through a modified heuristic algorithm (Kim et al., 2007). The statistical consistency between the core and entire collections is measured following the standard procedure to retain all characteristics for quantitative traits.

Results: In this study, a total of 134 diverse Indian mustard germplasm accessions with agronomically superior traits (earliness, short plant stature, long main raceme, more number of siliquae on main raceme, long siliqua, more seeds/ siliqua, bold seed size, high oil content and high harvest Index) were identified for use in breeding programme. Further, a core collection of Indian mustard consisting of 146 accessions was constructed from 1836 accessions by heuristic search based on 16 agro-morphological traits. The comparison of means, variances, frequency distribution etc., indicated that the core subset represents the entire collection. All the groups specified in the base collections viz., exotic acquisitions, indigenous collections, advance breeding materials and other types were represented in the core set.

Conclusions: The core collections have been shown to be efficient option for studies on genetic diversity, population structure, association mapping and targeted allele mining for agronomically important traits, including biotic and abiotic stress tolerance/resistance. The Indian mustard core set developed in this study is being evaluated extensively under the potential environments to identify the sources for agronomical traits and to use them in the Indian mustard improvement programs. The diversity represented in the core collection will therefore, be a guideline to the brassica breeders for a wider use of germplasm resources available in the genebank.

References:
Locating *Brassica* A and C genome centromeres by half-tetrad analysis

**Background:** Centromeres are essential for regular cell division, comprising a core functional domain where spindle fibres attach to pull apart sister chromatids or homologous chromosomes during mitosis and meiosis. Identifying precise centromere locations is important for genetic mapping experiments because centromeres suppress recombination. Locating centromeres through analysis of sequencing data can be difficult due to the presence of large tracts of repetitive DNA in centromeric regions. Active centromeres can also be difficult to distinguish from ancestral, derelict centromeres which retain a high degree of sequence similarity.

“Half-tetrad analysis” provides a complementary genetic approach for determining the positions of functional centromeres. Half-tetrad analysis involves genotyping experimental progeny derived from unreduced gametes (i.e., half of a meiotic tetrad) generated by failure of the first or second divisions to separate homologous (non-sister) chromosomes or sister chromatids, respectively. In unreduced gametes derived by first division restitution, heterozygosity is maximal at active centromeres, declining towards the telomeres due to recombination between sister and non-sister chromatids during the first meiotic division. In unreduced gametes derived by second division restitution, this trend is reversed. The position of functional centromeres is simply obtained by plotting marker heterozygosity levels across each chromosome.

**Objectives:** We set out to identify precise genetic locations of active centromeres in the *Brassica* A and C genomes.

**Methods:** Half-tetrads sampled by microspore culture of *Brassica* interspecific hybrids (genome configurations, AABC and CCAB; Nelson et al. 2009) were genotyped using the Illumina Infinium *Brassica* 60K array. Heterozygosity of polymorphic SNP markers was plotted against their physical locations on *Brassica* A and C chromosomes in the *B. napus* reference genome of Darmor (Chalhoub et al. 2014) in order to identify the genetic position of centromeres.

**Results:** Genetic positions of active centromeres were determined for all 19 A and C genome chromosomes, in some cases to <1 Mbp. C-genome positions were consistent with those previously reported by Parkin et al. (2014) but more finely resolved. Several large inversions in the Darmor sequence assembly (Chalhoub et al. 2014) were also detected over the centromere regions.

**Conclusions:** Our study links genetic mapping and physical genome sequences together for the first time to confirm the locations of active A-genome centromeres, and also provides higher resolution positioning of C-genome centromeres compared to those reported by Parkin et al. (2014). This combined physical and genetic information will facilitate further investigation of centromere structure and function in *Brassica* for basic research and breeding purposes.

**References:**

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Can a lonesome poor A genome of *Brassica napus* survive at diploid stage?

**Background:** There are two strategies available for understanding structural and/or functional modifications that took place during the stabilization of polyploid species. The first one allows assessment of events that arose immediately after the formation of a polyploid species by crossing and doubling its parental genomes in order to produce synthetic forms. The second one tries to elucidate the changes that occurred since the polyploid species was created by extracting in the polyploidy one of its parental genome.

**Objectives:** In the present study, we tried to identify the structural rearrangements that occurred since the origin (about 8000 years ago) of oilseed rape (*Brassica napus*, AACC, 2n=38), which is a natural hybrid between *B. rapa* (AA, 2n=20) and *B. oleracea* (CC, 2n=18). To that purpose, we produced an original plant material in which the *B. napus* A subgenome was extracted.

**Methods:** We used two methods to extract the diploid AA genome from *B. napus*. Firstly, AAc F1 interspecific hybrids (produced by crosses between *B. napus* var Darmor and *B. rapa*) were backcrossed three times to *B. napus*, and AAc plants were selected at each generation. Secondly, the initial AAc F1 hybrids were crossed to *B. rapa* and plants with AA genomes were selected for, selfed and also backcrossed to *B. napus*. After four cycles of such crossing, we selected AA plants with mainly the A genome of *B. napus*. Using the 60k SNP Illumina microarray and the sequence of *B. napus* genomes var Darmor, we assessed the genomic structure of the so far extracted *B. napus* A subgenome.

**Result:** We found that the backcrosses of AAc F1 interspecific hybrids to *B. napus* (first strategy) could not permit to eliminate the C chromosomes by selfing since the progenies were male sterile. The second strategy allowed production of AA plants with a regular meiosis. We expected more than 68% of Darmor A genome in this plant. To validate this assessment, genomic structure was established by SNP analysis using markers specific of A genome of Darmor and of the *B. rapa* variety used in the initial crosses. The homozygous or heterozygous stage of each marker physically anchored was determined. Additionally, CDarmor genome regions introduced by homeologous recombination were characterized.

**Conclusions:** From this original material, it will be possible to determine the comparative evolution of the A genome in a diploid and polyploid genetic background. The first data seem to indicate that rearrangements are too large and/or too frequent to obtain 100% A genome of *B. napus* at the diploid stage. However, functional analyses will allow identification of the rearrangement impacts.

**References:**

A. Pelé  
G. Trotoux  
F. Eber  
S. Nègre  
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M. Lodé  
M. Gillet  
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High-throughput multiplex cpDNA re-sequencing clarifies the genetic diversity and genetic relationships among *Brassica napus*, *Brassica rapa* and *Brassica oleracea*

**Background:** *Brassica napus* (rapeseed) is a recent allotetraploid plant that is cultivated as the second most important oilseed crop worldwide. The origin of *B. napus* and its genetic relationships with its diploid ancestor species still remain largely unresolved. The maternal origin of *B. napus* has been systemically investigated by means of the cpDNA diversities during the past decade. However, there are still significant controversies regarding the maternal origin and evolutionary mechanism of *B. napus*. To date, the expeditious and economical identification of the genome-wide cpDNA variants in a large population still remains rather difficult.

**Objectives:** The cpDNA based phylogenetic studies for rapeseed should be reinforced by employing the genome-wide cpDNA diversities in a large enough collection of *B. napus* and its relatives. The genetic relationships between *B. napus* and its diploid ancestor species should be finely determined to promote the utilization of elite alleles from other species.

**Methods:** A novel high-throughput pangenomic re-sequencing method has been developed, and it finely effectuates expeditious identification of the populational cpDNA variants. The cpDNA from a total of 488 worldwide *B. napus* accessions, 139 *B. rapa* accessions and 49 *B. oleracea* accessions were populationally re-sequenced using Illumina Solexa sequencing technologies. Their intra-specific cpDNA variants and their allelic frequencies were called genome-widely and further validated via Ecotilling analyses of the rpo region. A series of cpDNA variants based analysis were performed.

**Results:** The cpDNA of the current worldwide *B. napus* population comprises more than 400 variants (SNPs and short InDels) and maintains one predominant haplotype (*bncp1*). Whole-genome re-sequencing of the cpDNA of *bncp1* haplotype eliminated its direct inheritance from any of the *B. rapa* or *B. oleracea* species. The distribution of the polymorphism information content (PIC) values for each variant demonstrated that *B. napus* has a much lower cpDNA diversity than *B. rapa*. However, a vast majority of the wild and cultivated *B. oleracea* appeared to share one same distinct cpDNA haplotype, definitely contrasted to its wild relatives.

**Conclusions:** This finding suggests that the cpDNA of the three *Brassica* species are well differentiated. The originating mechanism of *Bncp1* haplotype needs to be further exhaustively explored in other *B. napus* relatives, and there is also a big possibility that it may result from the interactions between cpDNA mutations and the natural/artificial selection. These exhaustive cpDNA variation data would provide primary information for the cpDNA/chloroplast related researches.

**References:**


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Genome-wide association analyses identify novel loci for blackleg resistance in *Brassica napus*

**Background:** Blackleg caused by a fungal pathogen, *Leptosphaeria maculans* is a major threat to the canola industry worldwide. Understanding the genetic basis of natural variation for resistance to *L. maculans* will allow breeding of durable resistant varieties of canola. Although QTL mapping allowed identification of both qualitative and quantitative loci for resistance to *L. maculans* (Delourme et al. 2011; Raman et al. 2013), its utility is limited to specific populations. Genome-wide association analysis (GWA) enables us to mine a large number of alleles in a genetically diverse germplasm.

**Objective:** To investigate the extent of genetic variation and identify loci associated with resistance to *L. maculans* in two *B. napus* diversity sets representing Australian ‘National Brassica Germplasm Improvement Program’ (NBGIP, 188 genotypes) and BnASSYST (139 spring genotypes).

**Methods:** NBGIP accessions were evaluated for resistance under glasshouse conditions using single spore blackleg isolates and five different sources of stubble which were used to release blackleg ascospore showers onto canola seedlings. BnASSYST accessions were evaluated for field resistance at Wagga. All accessions were genotyped with DArTseq markers (Raman et al. 2014). Trait-SNP marker association analysis was performed using whole genome mapping approaches.

**Results:** NBGIP accessions displayed a wide range of genetic variation for resistance to *L. maculans*. However, a narrow genetic variation for field resistance was observed in the BnASSYST set. GWA analysis utilising ~25,000 SNP markers with MAF >0.05 detected significant associations (P <0.001, up to r² = 21%). Some of these loci corresponded to known genomic regions on chromosomes A2, A7/C6 and A10. Besides, novel genomic regions e.g. on A1, A3/C3, A4/C4, A5/C5, A9 and C8 were also identified, which need further validation. Loci localised on chromosomes A1, A2, A7 and A10 were confirmed in bi-parental populations derived from Australian DH populations using QTL mapping.

**Conclusion:** We identified genetic variation and loci associated with resistance to *L. maculans*. Future studies will reveal whether new loci are indeed novel.

**References:**
Construction of chromosome segment substitution lines in *Brassica napus* using resynthesized napus

**Background:** Development of chromosome segment substitution lines (CSSLs), in which each line carries a single or a few defined chromosome segment of donor genome and has a pure genetic background from a recurrent genotype, is a fundamental approach to conduct the QTL mapping in order to improve the mapping precision, to utilize the diverse wild genetic resources for crop utilization and to study the epistatic and additive gene interactions.

**Objectives:** In the present study, a set of chromosome segment substitution lines carrying overlapping chromosome segments of resynthesized *Brassica napus* PSA12 in a genetic background of DAS elite line (DAS_EL) has been constructed.

**Methods:** To develop the CSSLs, a set of 94 DH lines derived from DAS_EL x PSA12 were genotyped on illumina 6K SNP array and a high density linkage map consisting of 2500 SNP markers and spanning 3600 cM was used as a reference map. Introgression of donor genome in DH lines and backcrossing populations was visualized using CSSL finder software and the same software was used for selection of backcross individuals in each backcross generation. Selected DH lines were repeatedly backcrossed and genotypic selection was used in each backcross generation for selection of individual plants to represent ¼ chromosome of PSA12 genome. Infinium 6K SNP array and 230 genome-wide KASPar markers designed were used genotypic selection.

**Results:** The present results demonstrate the usefulness of genotypic selection in determination of introgression of donor genome. The substituted chromosome segments and the genetic background of individual line were more accurate as the reference linkage map used was in alignment with the physical map. ¼ chromosome substitution lines identified in this study are a valuable resource for QTL mapping, marker-assisted breeding and trait improvement.

**Conclusion:** Genotyping of ¼ CSSLs on high-density SNP arrays combined with re-sequencing is a powerful tool for large-scale gene discovery and can have a significant impact in trait improvement of canola.
Brassica oleracea as a model for epigenome analysis

**Background:** Domestication of *Brassica oleracea* has resulted in an array of starkly contrasting phenotypes that are familiar to us all as common vegetable crops. The tremendous amount of morphological variation present within *B. oleracea* is a testament to both the polyploid origin and flexibility of its gene expression. Numerous layers of information regulate gene expression, including the interaction among promoters, enhancers, repressors and the general transcription machinery. Superimposed onto these are additional regulatory mechanisms acting through epigenetic pathways involving DNA methylation, histone modifications and non-coding RNA molecules that together control the expression of complex traits. The recent development of a high-quality genome sequence for *B. oleracea* has heralded the beginning of the post-genomics era for this species and it also serves as a model for its close relatives *B. napus* and *B. carinata* (Parkin et al., 2014). DNA methylation is an important epigenetic mark that regulates gene expression but the information is not encoded in the primary DNA sequence.

**Objectives:** This study focuses on the detection of DNA methylation patterns in *B. oleracea* and its affect of gene expression. We describe the application of whole genome bisulfite sequencing (WGBS) and high-throughput transcript profiling (RNA-Seq) to characterize global DNA methylation and transcript abundance patterns. We discuss the bioinformatics challenges of working with an ancient polyploid crop species.

**Methods:** DNA methylation and gene expression levels were generated from selected *B. oleracea* genotypes using WGBS and RNA-seq respectively. A combination of publically available and custom bioinformatics methodologies are applied to these data to detect and quantify gene expression and epigenetic information that are interpreted within the context of available genome annotations.

**Results:** The methylome and gene expression pattern from *B. oleracea* genotypes exhibiting phenotypic variation in quantitative traits has been generated. Differences in gene expression and DNA methylation patterns were detected and the extent of the correlation among these data is presented and discussed.

**Conclusion:** Phenotypic variation is dependent on genetic variation. This variation arises from a combination of sequence variation and changes to the magnitude of gene expression. Here we present methodologies and bioinformatics pipelines designed for *Brassica* species to better understand how gene expression is regulated.

**References:**
Why sex and agriculture conflict: Evolutionary omics approaches to developing asexual (apomictic) seed production

An organism’s choice to reproduce with or without sex has long puzzled evolutionary biologists. Apomixis, a natural form of reproduction in plants whereby seeds are produced asexually, has evolved repeatedly from sexual ancestors in many taxa. Apomixis is of interest on a number of levels, ranging from population genetics to evolution, but also from an applied perspective, as it represents a disruptive technology which could significantly change agricultural practices (e.g. fixing heterosis in hybrid crops). The switch from sex to apomixis is hypothesized to result from deregulation of developmental pathways leading to sexual seed development, and the trigger for deregulation involves the global genomic effects of hybridization and polyploidy.

We study apomixis in wild plant populations, and use evolutionary theory to guide our experimental approaches. High-throughput methods are employed to understand population-level phenotypic (seed production) and genetic (polyploidy, genetic structure) variability. These data are then used to design targeted experiments, whereby candidate genes for apomixis are identified using tissue-specific “omics” methods in particular genotypes. These candidates are then used (1) in transformation experiments to attempt apomixis induction in sexual plants, and (2) in population-level studies to understand the origin and evolution of apomixis with respect to sexuality in natural populations.

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Breeding strategies for oilseed Brassica improvement in India

Brassica breeders aim to make simultaneous improvement of agronomic performance, disease resistance and quality traits through various breeding strategies. In India, up to 1970, mass and pure line selection was the main breeding methods which were used in breeding programme and 26 varieties were developed. After 1980, varieties developed through hybridization increased and 22 varieties were released in each of the 8th and 9th decade of 20th century. This number further increased to 41 during first decade of the 21st century. Simultaneously, 12 varieties had been developed through mutation breeding. Since the early 80's, systematic and vigorous recombinant breeding was followed and large number of varieties have been identified and released. A total of 145 varieties (Indian mustard-93; toria-16; yellow sarson-11; gobhi sarson-11; brown sarson-3; karan rai-4; taramira-6 and black mustard-1) of rapeseed mustard have been released after inception of AICRP-RM in 1967 till 2014. These include six hybrids. Rapeseed-mustard varieties having tolerance to biotic (white rust, Alternaria blight, powdery mildew) and abiotic stresses (salinity, high temperature) and quality traits have been recommended for specific growing conditions. In Brassica napus first commercial hybrid PGSH 51 based on tour CMS system was released by PAU, in 1994. Later on Hyola 401 based on pol CMS system was released by Private sector, Advanta, India, in 1997/2000. CCS HAU, Hisar have developed hybrids using ogura CMS system in B. juncea whereas, PAU, Ludhiana have developed hybrids in both B. juncea and B. napus are in advanced stage of evaluation at state and national level. In Indian mustard, sustained efforts resulted in the release of five CMS-based hybrids, among them, NRCHB 506 and DMH 1 were released in 2009 and Coral432 (PAC 432) in 2010.

First low erucic acid variety, Pusa Karishma of Indian mustard and first double low variety of Brassica napus, GSC 5 of gobhi sarson was released in 2004 and 2005, respectively. Presently, eight low erucic varieties have been released in Brassica juncea. In gobhi sarson (B.napus), 6 double low (canola quality) varieties have been released which are either at par or superior in performance than the non-canola varieties. Inter-specific hybrids were produced by fusing mesophyll protoplast of B. juncea and B. spinescens. In B. juncea by protoplast fusion of Moricandia arvensis with the fertility restoration function of this male sterile B. juncea by introrgression. Molecular markers such as RAPD, RFLP, AFLP and SSR have been used for improving selection efficiency and selecting plant genotypes with the desired combinations of traits. Markers linked with white rust resistance fatty acids, oil content, yellow seed colour and fertility restorations have been reported. Bar, Barnase and Barstar based herbicide resistance and genetic male sterility have been used in the development of experimental hybrids. Future challenges are efficient utilization of rapeseed-mustard genetic resources, genetic enhancement of heterosis in mustard and toria for further enhancing the yield potential and developing high yielding varieties/hybrids with improved oil and seed meal quality for food, feed and industrial uses using conventional as well as biotechnological approaches.

References:
Overexpression of BnLACS9 upgrades the biosynthesis of chlorophyll in *Brassica napus* 

**Background:** The BnLACS9 was isolated from developing rape embryos, its cDNA encoding a novel acyl-CoA synthase (Pongdontriand Hills, 2001). It activates free fatty acids and esterifies free fatty acids to acyl-CoAs, which is the substrate of lipid synthesis. In *Arabidopsis thaliana*, loss galactolipids mutant had no visible green tissues under Pi-sufficient conditions and accumulated chlorophyll to barely detectable levels (Kobayashi et al., 2013). Little was known between BnLACS9 and chlorophyll biosynthesis.

**Objectives:** Overexpression of BnLACS9 improved the chlorophyll content of the leaf, the biomass of the plants and the oil contents of the seeds. The objectives were to understand the mechanisms that BnLACS9 involved in the chlorophyll biosynthesis.

**Methods:** Subcellular localization and overexpression of BnLACS9 in tobacco and rape showed the BnLACS9 have the function of LACSSs. Expression profiling of the BnLACS9 in *Brassica napus* showed that it was mainly expressed in the young leaves and flowers of *Brassica napus*, where the lipids metabolism is exuberantly. In the plants of overexpression of BnLACS9, the number of the chloroplast grana lamellae and the content of chlorophyll of the leaves were both increased compared to the wide-type. The key lipids in the development of the thylakoids grana lamellae, MGDG and DGDG, were determined using TLC. The MGDG and DGDG contents of the leaf was increased significantly in the overexpression of BnLACS9 plants. Transcriptome data showed overexpression of BnLACS9 upgrades the pathway of acyl-CoA biosynthesis.

**Results:** Overexpression of BnLACS9 improved the contents of Acyl-CoA, which was the substrate of glycolipids. The increased acyl-CoAs upgraded the expression of the genes related to the glycolipids synthesis and led to MGDG and DGDG were increased, providing enough material for the formation of the chloroplast grana lamellae, which increased the number of chloroplast thylakoid grana lamella lead to increasing chlorophyll content. The result revealed that the BnLACS9 was a positive regulatory factor of glycolipids and chlorophyll synthesis.

**Conclusions:** BnLACS9 play an important roles in the biogenesis of the chlorophyll through regulate the formation of glycolipids which was part of the chloroplast. The net photosynthetic rate, the dry weight of the whole plant and the oil content of the seeds were significantly increased accompanied the increasing of the chlorophyll content by overexpression of BnLACS9.

**References:**


Extending the variability of insect resistance in rapeseed (B. napus L. var. oleifera Metzg.) by interspecific crossing with an application of immature embryo culture

**Background:** Interspecific hybridization is an important tool to transfer traits across species, so it is widely applied for improving of Brassica crops. The data resulting from our own research, as well as those available in the research literature shows, that an important problem to be solved is the resistance of oilseed rape (Brassica napus) to insects. In recent times, due to the prohibition on the use of certain pesticides in the EU emerged the problem of resistance to cabbage root fly (Delia radicum), aphids (Brevicoryne brassicae) and flea beetle (Phyllotreta cruciferae). Among species of Brassica genus, there are those which have resistance to the above-mentioned pests and can thus be used to introduce these characters to rape. Wild species of the genus Brassica, which show resistance to the above mentioned pests are, for example, B. fruticulosa, B. tournefortii and cultivated B. hirta.

**Material and Methods:** By the year 2014, the interspecific crosses were performed. The maternal forms were chosen oilseed rape cultivars and male-sterile line MS-8 and as pollinators were used the following species: B. tournefortii, B. fruticulosa, and B. hirta. All hybridization were performed with the application of an in vitro embryo culture according to the method described by Wojciechowski (1998). The immature embryos were isolated from young siliquae at different developmental stages i.e. heart and early and late torpedo, 14-19 days after pollination. The siliquae were surface sterilized by subsequent immersion for 1 minute at 70% and 99.8% ethanol and washed two times for 5 minutes in sterile water. Under the stereoscopic microscope the ovules were removed aseptically by cutting them lengthwise along the suture. The excided embryos were transferred to White (W) or Murashige & Skoog media and incubated at 26 °C ± 2 °C at 16 h light phase and 8 h dark phase. According to the way of regeneration, after 3 weeks the embryos were transferred onto fresh MS or MS medium modified by Keller (MSK). When the embryos had grown into plantable seedling, they were transferred for rooting on Nitsch & Nitsch (H3) medium. Rooted seedlings were transplanted directly in the soil and after 10 weeks of vernalization grown further in the glasshouse.

**Results:** The effectiveness of interspecific crosses varied widely depending on which species where used as pollinator. The lowest efficiency was observed in the combinations in which the pollinators were B. tournefortii or B. fruticulosa. In this case, there were no seeds set on the plant but in in vitro embryo culture the efficiency measured by the number of regenerated hybrid plants ranged from 30,0% to 76,19%.

**Conclusion:** Applying of in vitro embryo rescue method enable to obtain interspecific hybrids.

**References:**

**Aknowledgements:** The Authors thanks to the Ministry of Agriculture and Rural Development, Poland for financial support of task no. 54.
Development of *Alternaria* blight resistant Indian mustard (*Brassica juncea* L. Czern & Coss.) lines derived from *Brassica juncea x Brassica alba* through conventional and embryo rescue techniques

**Background:** *Brassica juncea* is an important oilseed crop, grown in tropical and subtropical regions of the world. An important source of oil and fat in human diet, it also serves as raw material for industry and trade. *Alternaria* leaf blight caused by *Alternaria brassicae* is the serious disease resulting in heavy yield losses in the country. *B. alba* possesses resistance to *A. brassicae*.

**Objectives:** Wide hybridization has been carried out for introgression of valuable traits from wild species into cultivated crops. Conventional breeding methods have failed to introgress this trait since the crosses are incompatible. Success in interspecific crosses can be achieved by employing biotechnological approaches. The present study was conducted to develop interspecific hybrids between *B. juncea* and *B. alba* through embryo rescue, successful establishment of F1 plants in the pots and subsequent rearing them to maturity.

**Methods:** Interspecific hybridization was carried out between *B. juncea* (cv. RH 30, RH 8812, RH 0270 and RH 0345) and *B. alba* using conventional plant breeding techniques. The 10-20 DAP siliquae were excised and developing ovules were cultured on modified MS media supplemented with different growth regulators. All the cultures were incubated at 25±1°C under 16h/8h light/dark photoperiod. After 3-4 weeks, germinated ovules were transferred to MS modified media for shoot elongation and rooting. Well grown plants were transferred to plastic pots containing sterilized sand:soil mixture. Well established interspecific plants were moved to greenhouse.

**Results:** Best germination response was observed in 20 DAP ovules on basal medium supplemented with Kinetin (2.5 mg/l) and casein hydrolysate (0.5 g/l) (for cv. RH 30 X *B. alba*) and MS + BAP (2.5 mg/l) + CH (0.5 mg/l) (for other crosses). Among different rooting media tried, maximum rooting response was on MS medium with IAA (0.5 mg/l) in all the hybrids. The regenerated hybrid plants were transferred to a mixture of sand:soil (1:1) ratio where 80 percent hybrid plants survived for crosses i.e. cv. RH 30 X *B. alba* and cv. RH 8812 X *B. alba*. In cv. RH 0270 X *B. alba* and cv. RH 0345 X *B. alba*, 75 and 67 percent survival was observed. The F1 plants had very few seeds and the plants with improved fertility and resistance to Alternaria blight were selected in following generations (F7) where hybrid plant’s characteristics were comparable to *B. juncea* plants. Thirteen *Brassica juncea x B. alba* in F7 advanced progenies were screened against *Alternaria* blight under artificial inoculation conditions. These were spray inoculated with pure culture of *A. brassicae* (105 conidial suspension/ml distilled water) at initiation of flowering and siliquae development stage.

**Conclusions:** Two advanced progenies of interspecific crosses i.e. RH 1372 (RH 0270 x *B. alba*) and RH 1378 (RH 8812 x *B. alba*) were found as promising rich donor source lines for *Alternaria* blight resistance. These will be utilized for developing *Alternaria* blight resistant cultivars.
Proteomic analysis of temperature sensitive male sterility SP2S in rapeseed

**Background:** Temperature-sensitive male sterility (TGMS) is important for utilization of the heterosis in two-line hybrid. SP2S is a new TGMS rapeseed bred by us from a spontaneous semi-sterile plant found in 2007. SP2S is sensitive to lower temperature, that is, treatment under cool condition (temperature<15°C) can revert the fertility of SP2S, and this trait followed two pair of recessive genes inheritance pattern.

**Objectives:** In order to deeply understand the expression of TGMS, we analyzed the floral bud proteome of SP2S and the fertile NIL SP2F with the objective of identifying differentially expressed proteins and their potential roles in male sterility.

**Methods:** We analyzed the proteomic profiles at two key developmental stages (Pollen-mother-cell meiosis and uninucleate microspore) by using 2-dimensional gel electrophoresis. Results: Total 780 spots and 28 well reproducible spots with 2-fold or higher differentially expression were detected. Twenty-three spots (9 spots at PMC meiosis stage and 14 at uninucleate microspore stage) were successfully analyzed by MALDITOF/TOF mass spectrometry and 27 proteins were identified by BLAST searching against UniProtKB databases. An elongation factor at PMC stage and 4 proteins at uninucleate stage (aconitate hydratase, triosephosphate isomerase, mRNA splicing factor, glutathione S-transferase) were up-regulated in SP2S. However, more proteins were absent or down-regulated, included those associated with amino acid metabolism (glutamine synthetase, L-O-methylthreonine resistant 1, and argininosuccinate lyase), cytoskeleton (Transationally-controlled tumor protein homolog, actin, and tubulin), RNA editing and modification (RNA methyltransferase, RNA recognition motif-containing protein, and pentatricopeptide repeat protein), photosynthesis (light-harvesting complex LHCB2:4 and phosphoribulokinase), synthesis and degradation of protein (asparagine-trnA ligase and AAA ATPase family), lipid metabolism (transfase involved in exine synthetic, flower-specific purple acid phosphatase, and lipoxygenase), oxidoreductase (coniferaldehyde/sinapaldehyde dehydrogenase and alcohol dehydrogenase), and defense (protein-tyrosine-phosphatase and alpha/beta-hydrolases), etc.

**Conclusions:** Disturbance on the expression of these proteins may disrupt the coordination of developmental and metabolic processes, resulting in defective tapetum and unviable microspores. This is the first proteomic investigation on temperature-sensitive male sterility in rapeseed, and the results provide new insights into molecular events associated with the male sterility.
Identification and analysis of BnaA.tsMs: A novel gene required for meiotic chromosomal organization in *Brassica napus*

**Background:** We discovered a newly bred thermo-sensitive dominant genic male sterility (TSDGMS) line TESA which originated from a spontaneous mutant of the inbred line TE5 in *Brassica napus*. The TESA exhibits ecotypic sensitivity, the fertility of TESA is normal at low temperature, and it will transform to completely sterility when temperature is higher than 20°C during florescence (Zeng et al., 2014). Based on the observation of morphological, the TESA mutant phenotype was observed at the PMC stage, in which the PMCs did not progress to tetrad production. We have cloned the gene from TESA, BnaA.tsMs, that is involved in sister chromatid cohesion and chromosome segregation the during male meiosis.

**Objectives:** To date, many dominant genic male sterility (DGMS) lines have been studied in *Brassica napus*. However, the abortion mechanism and gene functions of DGMS have been unclear. This research of abortion mechanism of TESA and function of BnaA.tsMs gene will greatly accelerate construction of new male sterility line in *Brassica napus*.

**Methods:** The BnaA.tsMs gene was cloned by map-based cloning from TESA. To perform observations of flower development, standard paraffin and plastic sections were generated. To obtain chromosome spreads, inflorescences were harvested and fixed in Carnoy’s solution (ethanol:glacial acetic, 3:1, v/v). Anthers containing PMCs undergoing meiosis were incubated with 3% cytohelicase, 3% pectolyase, and 3% cellulase in citric acid buffer for 90 min at 37°C and then counterstained with DAPI. FISH was performed in meiotic chromosome spreads by 45S rDNA from clone pTa71.

**Results:** In the BnaA.tsMs mutant allele, an L-to-F transition converts a Leu at position 281 to a Phe (L281F), causing thermo-sensitive dominant genic male sterility (TSDGMS). In TESA male meiosis, the classical steps of prophase were not observed; chromosomes did not undergo synapsis, and they formed 38 univalents instead of 19 bivalents. The 38 univalents generated an ordered metaphase plate and underwent an equational division. Then, the chromatids formed chromatin again, in the same manner as in S-phase, and stopped progression at telophase I.

**Conclusions:** We report the cloning of a new thermo-sensitive dominant genic male sterility gene in dicots that may cause defect of male meiosis. There is a long tradition of meiosis research in *S. cerevisiae*, animals and plants, but only rudimentary knowledge of the mechanisms is available in *Brassica napus*. Our findings present new perspectives for the application of thermo-sensitive dominant genic male sterility (TSDGMS) in *Brassica napus*.

**References:**

Creating a novel recurrent selection population in *Brassica napus* by massively introgressing subgenomic components from four oilseed *Brassica* species

**Background:** The three basic genomes, i.e., A, B and C in the *Brassica* U’s triangle, were differentiated into three sets of subgenomes, i.e. Ar/Aj/An, Bni/Bc/Bj and Co/Cc/Cn, respectively. To enlarge the genetic diversity and utilize heterosis for *Brassica napus* (AnAncnCn), a population of new-type *B. napus* (ArArCcCc) was developed with 72 accessions of *B. carinata* (BcBcCcCc) and 25 accessions of *B. rapa* (ArAr) as founders (Xiao et al. 2010). Since the population shown great polymorphic at Cc subgenome it was named as Poly-Cc population. Strong heterosis for seed yield was observed when the inbred lines generated from the Poly-Cc population were crossed with testers of traditional *B. napus* (unpublished data). However, to increase the heterosis potential and enable a durable heterosis utilization, further research should be conducted.

**Objective:** Add the polymorphic degree at Ar subgenome on the Poly-Cc population at first, and then, bring subgenomic specific genes from all of *Brassica* oilseed species, i.e. *B. rapa* (with Ar), *B. juncea* (with Aj), *B. carinata* (with Cc) and *B. napus* (with AnCn), to the population followed by recurrent selection.

**Methods:** Extensive genetic recombination was achieved through half random mating via dominant genic male sterile system. Population genetic analysis was conducted with software of PowerMarker 3.25, MEGA 4.0, STRUCTURE.

**Results:** A transition population of new-type *B. napus* polymorphic at Ar subgenome, named as Poly-Ar population, was constructed with 111 cultivars of *B. rapa* and 7 cultivars *B. carinata* as founder parents. The Poly-Ar population was integrated with the existed Poly-Cc population by random cross-pollination of both populations to the ArArCcCc plants with a character of dominant genic male sterility (DGMS). After five rounds of random mating and intensive selection in the integrated population which was polymorphic at both Ar and Cc subgenomes (named as Poly-ArCc population), significant genetic gains on the agronomic and seed qualitative traits were obtained. While genetic structure is being analyzed with molecular markers, the population was cross-pollinated with one hundred selected lines of *B. napus* with background of elite cultivars and exotic introgression of either Aj from *B. juncea* or Ar/Cc from *B. rapa* and *B. carinata*, respectively. Two rounds of extensively genetic recombination among the subgenomes of Ar/Aj/An/ and Cc/Cn in the population were achieved through the DGMS system, and followed by intensively recurrent selection. A pre-breeding program for oilseed rape based on the novel recurrent selection population and genomic knowledge and techniques will be presented in the conference.

**Conclusions:** A novel recurrent selection population of new-type *B. napus* diversified on Ar/Cc genome was developed, and extensive recombination among Ar/Aj/An/ and Cc/Cn subgenomes has been achieved in the population. An intensive phenotypic and genomic selection will be recurrently carried out with the population and unique lines are expected developed from a novel pre-breeding program.
Molecular and phenotypic diversity in *Alternaria* isolates in North-west regions of India and response of mutant doubled haploid *B. juncea* genotypes to *Alternaria* under artificial inoculation

Background: The genetic base for fungal diseases are limited; none of the cultivars of *B. juncea* (Bj) presently being grown in India are resistant/immune to *Alternaria* blight (AB).

Objective: To evaluate the diversity spectrum/genetic evolution in AB isolates for understanding host-pathogen interaction and to recover novel genetic source for fungal disease tolerance through haploid mutagenesis.

Methods: Single spore isolates of AB (32 *A. brassicae*, 20 *A. brassicicola*, 3 *A. alternata*) from 8 states of North West India, were evaluated for morphological, pathological, cultural, biochemical and molecular characteristics. The doubled haploid (DH) mutant progenies were derived from microspore mutagenesis through ENU/EMS in Bj var. Varuna/Pusa Bold. DI was evaluated (three leaves/plant) on a 0 to 5 scale (0- no symptoms, 0.5- 5%, 1.0- 10%, 1.5- 15%, 2.0 -20%, 3.0- 30%, 4.0- 40%, 5.0- 50%).

Results: RAPD analysis revealed a high level of genetic diversity for *A. brassicae* (57-78%), *A. brassicicola* (78-92%), *A. alternata* (89-100%) isolates. *A. brassicae* isolates clustered into four major clades (genetic diversity 0.45-0.65), *A. brassicicola* formed three clades (genetic diversity 0.51-0.76), and *A. alternata* isolates fell in the same clade (GS 0-0.20). Extensive variations were observed in isolates for the parameters studied, however, no correlation could be established among them. The DI for DH mutant progenies was 0.03-1.0 under epiphytotic field conditions and 1.3-3.3 by detached leaf method. Among 40 DH-M4 progenies tested in field (GBPUAT, Pantnagar) under artificial inoculation, four genotypes were moderately resistant (18-30% disease) at 75 DAS. Six genotypes showed 10-12% diseases at pod formation stage. Five lines had no stem infection two weeks before maturity.

Conclusions: The study indicated a non-specific relation between the isolates interacting and infecting with different species/varieties of *Brassica* spread over north/north-west regions of India, suggesting that the genes for resistance/tolerance for fungal pathogens may be present in mono-genome *Brassica* and that targeted mutagenesis may be useful for recovering such mutations. The selected DH lines form a valuable gene pool for developing Indian mustard with high tolerance to *Alternaria* blight.

References:

Canola protein nanoparticles: A promising delivery system for encapsulation of bioactive compounds

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Background: Encapsulation is an emerging technique to improve solubility, permeability and bioavailability of bioactive compounds after food processing and gastrointestinal digestion. Food proteins are generally recognized as safe (GRAS) biopolymers and have been attracted great attention for using as delivery materials due to versatile functionalities.

Objectives: We hypothesised that cruciferin, a major canola protein, due to good functional properties, high denaturation temperature and resistance to gastric enzymes, could be an appropriate carrier for delivery of bioactive compounds. Preparation and evaluation of encapsulating property of cruciferin nanoparticles were our objectives.

Methods: Cold gelation and combination with chitosan were used to prepare calcium-induced cruciferin particles (Cru/Ca) and cruciferin/chitosan particles (Cru/Cs). In addition to the size, zeta-potential, morphology, cell toxicity and cell uptake of the particles, their encapsulation properties were also studied using two water-soluble and -insoluble model compounds (brilliant blue and beta-carotene). The protection effects of the encapsulation systems were evaluated in the simulated gastrointestinal tract and also in a heat treatment.

Results: The prepared Cru/Ca and Cru/Cs particles were spherical in shape with an average size of ~ 207 and ~ 165 nm, respectively. Negatively-charged Cru/Ca particles (-33 mV) and positively-charged Cru/Cs particles (+20 mV) were stable for more than three weeks at 4°C. The prepared nanoparticles did not show any toxicity to Caco-2 cells after 24 hours of incubation at a concentration of 2.5 mg/mL. Confocal microscope images revealed that fluorescently-labelled particles were uptaken by Caco-2 cells after 6 h incubation. The model compounds were loaded in the particles at encapsulation efficiencies of 80-86%. In vitro release studies showed that both particles were resistant to simulated gastric fluid; while Cru/Ca particles released 70-90% of the model compounds in simulated intestinal fluid, Cru/Cs particles were resistant to intestinal conditions (released only 10-15% of the compounds). The encapsulation also significantly increased the stability of loaded beta-carotene in a heat treatment (75°C and 30 min) compared to not-encapsulated form.

Conclusions: Cruciferin has the ability to form nanoparticles for encapsulation of bioactive food compounds. For the first time, two types of nanoparticles were successfully developed from cruciferin; these particles were appropriate carriers for encapsulation of both hydrophilic and hydrophobic compounds. The cell uptake of the particles revealed that the carriers might also improve the absorption of less-soluble and/or less-permeable compounds in the intestine. This study demonstrated the potential use of cruciferin as a natural polymer for delivery of bioactive compounds in the gastrointestinal tract to target different sites of action.
Parasitoids of swede midge in Saskatchewan

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Background: The swede midge was recently accidentally introduced to North America, having been identified in 2000 in Ontario. The midge has had a major impact on canola in eastern Canada, leading even to temporary moratoria on canola production in some areas. Intensive surveys for natural enemies in eastern Canada revealed that swede midge seems free of parasitoid-induced mortality there. More recently swede midge has been found on the prairies, and there swede midge was observed to be attacked.

Objectives: To survey parts of Saskatchewan where swede midge occurs to determine which parasitoids are involved and what levels of mortality are caused by these species.

Methods: Fused canola flowers with midge larvae inside were collected at five locations in late July, and at nine locations in early September 2014. Flowers were placed on a moist mixture of sand and peat, and midges and parasitoids that emerged were counted. The soil was then kept for three months in cold for diapause, after which emerging insects were again counted.

Results: At least two species have been recovered so far, with combined levels of parasitism reaching over 20%. One species, an undescribed Gastrancistrus species in the family Pteromalidae, seems to attack swede midge larvae. Another, tentatively identified as Inostemma sp. (Platygastridae), likely attacks eggs.

Conclusions: Unlike in eastern Canada (Corlay et al. 2007), on the prairies swede midge is attacked by parasitoids. It may be possible to introduce these species to eastern Canada as biological control agents. However, more information is required about the wasps first, particularly with respect to host specificity.

References:
AvrLm7 and AvrLm3 frequency evolution in French Leptosphaeria maculans populations

Background: Phoma stem canker, caused by the fungus Leptosphaeria maculans, is mainly managed through the deployment of resistant varieties. Specific resistance genes (Rlm) are present in commercial varieties and the effectiveness of a given Rlm is a function of the frequency of the corresponding avirulence allele in field populations of the pathogen. Rlm genes exert a strong selection pressure on fungal populations, leading to the selection of virulent isolates and the breakdown of the resistance. However the durability of a given Rlm gene may vary, depending on the plant genetic background (1), the fitness cost linked to the loss of the avirulence gene (2) and agronomic practices (3).

Objectives: After the very rapid breakdown of Rlm1 in the 90’s in France, it was questioned whether all released Rlm genes could be overcame at the same speed. The Rlm7 resistance gene was introduced in commercial hybrids in France at a time when most (>99.5%) of the isolates possessed the avirulent allele AvrLm7 (4). This was an appropriate field situation to address this question.

Methods: The frequency of virulent isolates was monitored in populations of L. maculans at a national scale in 2000-2001, 2010, and 2013. From 8 to 20 sites were sown with varieties devoid of Rlm3 and Rlm7. Isolates were collected from individual leaf lesions from independent plants and phenotyped for their virulence profiles toward Rlm3 and Rlm7 using standard inoculation tests (4).

Results: A total of 1787, 577 and 1173 isolates were collected and phenotyped in 2000-2001, 2010 and 2013, respectively. While only one virulent isolates toward Rlm7 (avrLm7) was found in 2000-2001, their mean frequency reached 3.99% in 2010 and 19.8% in 2013. In 2013, the frequency of avrLm7 isolates varied from 0% (britany) to 45% (center region). Noticeably, all avrLm7 isolates (all sampling years) but 6 (2013 sampling) were avirulent toward Rlm3. Therefore, only 0.5% of the current French L. maculans populations can infect both Rlm3 and Rlm7 varieties.

Conclusions: Compared to the Rlm1 breakdown that happened in only 3 growing seasons in France, it took avrLm7 10 years to reach a mean frequency of virulent isolates of 20% at the national level. Large regional variations are however observed in relation to the intensity of oilseed rape cropping and Rlm7 use. This population survey also clearly confirms the negative interaction between the avirulence genes AvrLm3 and AvrLm7, which offers great perspectives for durable management of specific resistance genes in oilseed rape.

References:

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Resistance to blackleg disease: From pathogen perception to induction of defense

**Background:** Blackleg caused by the ascomycete fungus *Leptosphaeria maculans* is a common disease in most canola/rapeseed (*Brassica napus*) producing countries. After germination of *L. maculans* spores on cotyledons and leaves of young seedlings, hyphae enter and grow between the mesophyll cells. In the absence of race specific resistance (R) genes, the pathogen continues to grow causing tissue collapse and the development of lesions on cotyledons, leaves and at the base of the stem. To date 16 R genes against *L. maculans* from *Brassica* species have been identified but the nature of these genes has remained unknown until recently. Here we present cloning LepR3 and Rlm2, the first R genes against blackleg to be reported from *Brassica*. The components of the LepR3 and Rlm2 recognition complexes and their down stream signals will be discussed.

**Methodology:** Map-based cloning of LepR3 and Rlm2 was carried out with final complementation of the resistance phenotype produced by transgenic analysis. The *L. maculans* effector AvrLm2 was cloned by genome sequence comparison of 40 *L. maculans* isolates that differentiated for their virulence against Rlm2 plants. The genome of *L. maculans* reference isolate 00-100 was sequenced using Roche 454 and other *L. maculans* isolates were re-sequenced using Illumina technology.

**Results:** We applied map-based cloning approach to isolate LepR3 and Rlm2 resistance genes against blackleg, located within the same genomic interval on chromosome A10 of *B. napus*. LepR3 and Rlm2 are alleles of the same gene encoding for receptor-like proteins (RLP) (1, 2). In addition we discovered that the previously cloned *L. maculans* effector AvrLm1 is recognised by LepR3, revealing an example of a single Avr gene being recognised by two independent R genes (LepR3 and Rlm1). We also cloned *L. maculans* effector AvrLm2 as the pathogen Avr gene that triggers Rlm2-initiated defense response (3). Our investigation into the LepR3 and Rlm2 recognition complexes and downstream signalling pathways revealed that LepR3 and Rlm2 proteins interact with the *Arabidopsis thaliana* and *B. napus* receptor like protein kinase (RLK) SOBIR1. The results of our efforts to identify AvrLm1 target in *B. napus* and to define the gene expression profile of pathogen and host plant genes during infection will be presented.

**Conclusion:** Cloning of LepR3 and Rlm2 resistance genes against blackleg disease and identifying their corresponding Avr genes provide the first insight into the perception of *L. maculans* by its host plant and establish a model system to investigate the components of R gene complex for recognition of *L. maculans* effector proteins, their host targets and downstream signalling pathways.

**References:**


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Control of pyrethroid resistant pollen beetles – effect of thiacloprid and two pyrethroids on population dynamics

**Background:** Over a long time synthetic pyrethroids were used to control pollen beetles (*Meligethes aeneus F.*) in Germany. The extensive and indiscriminate use of this insecticide class resulted in a high selection pressure on the beetles, ensuing in the formation of resistance, which has spread over many European countries.

**Objectives:** Insecticide applications should reduce yield loss of bud feeding but in addition also the reproduction of the beetles to minimize the infestation pressure in following cultures, e.g. vegetable crops and oilseed rape in the following year. The aim of the study is to provide data for a control strategy for a sustainable reduction of the population density without influencing natural mortality caused by parasitization.

**Methods:** To test the effect of insecticides on the reproduction of pollen beetles field trials were carried out near Braunschweig in 2013 and 2014. The neonicotinoid Biscaya (a.i. thiacloprid) and the pyrethroid insecticides Karate Zeon (lambda-cyhalothrin) and Mavrik (tau-fluvalinat) were sprayed in replicated plots of 0.1 ha size at different BBCH growth stages of winter oilseed rape. In all plots the infestation of overwintered pollen beetles was observed. Additionally the number of eggs per bud and the number of larvae dropping to the ground for pupation was recorded and the new pollen beetle generation emerging from treated and untreated plots was trapped in photoelectors. The larvae were investigated for parasitization with the key larval parasitoids *Tersilochus heterocerus* and *Phradis* spp.

**Results:** In 2013 Biscaya reduced the number of overwintered pollen beetles until 3 DAA. Egg laying was significantly reduced by application of Biscaya. In addition there was a direct-lethal effect on L1-larvae when Biscaya was applied at BBCH 60/65. As a consequence of both (L1 mortality and egg laying) a reduced number of larvae and new generation pollen beetles hatched. In 2014 these effects of Biscaya on the population dynamic were confirmed. Application of the pyrethroid insecticide Karate Zeon had no effect on overwintered pollen beetles but resulted in an increase of the next generation beetles compared to control values. Mavrik reduced overwintered pollen beetle numbers more than Biscaya and had also an effect on population dynamic but to a lesser extent than Biscaya.

**Conclusions:** By a careful selection and termination of insecticides it seems possible in addition of controlling pest damage also to reduce pest pressure in the following season by influencing population dynamics of pollen beetle.

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Mapping of quantitative trait loci in Brassica napus lines from Pakistan and China providing resistance to Sclerotinia sclerotiorum

**Background:** Canola is one of several hundred crop species susceptible to the fungal pathogen *Sclerotinia sclerotiorum*. Partial resistance has been identified in a few plant species including some *Brassicas*. After screening of *B. napus* germplasm with isolates representative of the Canadian pathogen population, lines with quantitative resistance were identified originating from Pakistan, China, South Korea and Japan. These sources of resistance together with a reliable disease phenotyping method and high throughput genotyping has facilitated mapping of quantitative trait loci (QTL) conferring resistance against sclerotinia stem rot.

**Objective:** To map major QTLs conferring resistance to *Sclerotinia sclerotiorum* in *B. napus* germplasm and develop molecular markers to aid transfer of this resistance to canola.

**Methods:** Seven doubled haploid (DH) mapping populations segregating for sclerotinia resistance were developed from crosses with a susceptible line and each of three resistant lines, Zhongyou 821 (Zy821, China), PAK54 and PAK93 (Pakistan). All DH lines were genotyped with 6000 SNP and ~700 SSR markers. Linkage maps were constructed for each DH population and an integrated map was generated (Joinmap). DH plants at full flower were phenotyped for sclerotinia resistance by inoculating the main stem with mycelium of a single virulent *S. sclerotiorum* isolate, #321. Five disease measurements were used for QTL analysis (QTL Cartographer). Meta-analysis was used to align sclerotinia QTLs identified in each DH population as well as QTLs from the literature (BioMercator).

**Results and discussion:** A distinction was made between major and minor QTLs based on phenotypic variation explained (R2), LOD value and number of times each QTL was identified across mapping populations. QTLs were labelled with chromosome number (A1 to C9) and ancestral genomic block (A to X) (Schrantz et al. 2006). Major QTLs in PAK54 mapped to chromosome A3F, A6A, A9A, A10R and C1F, which were different from those in PAK93 on chromosome A5J, A7X, C2X and C6E. A total of eleven QTL were mapped in five DH populations derived from ZY821. Six of these QTLs were present in either PAK54 or PAK93. Most sclerotinia resistance loci mapped to blocks U, F and R. We are currently examining possible duplication of resistance loci resulting from allopolyploid in the *B. napus* genome. Meta-analysis showed that some of the QTLs reported in the literature mapped to our QTLs despite differences in disease phenotyping methods. Seeds of sclerotinia resistant lines from Pakistan, Japan and South Korea are available for cultivar development from Plant Gene Resources of Canada via the corresponding author under a material transfer agreement. Lines from Pakistan have high genetic diversity and would provide diversification in Canadian and Australian canola breeding programs in particular (Gyawali et al. 2013).

**References:**


Impact of the neonicotinoid insecticide thiamethoxam on the lifespan of the honeybee *Apis mellifera* L. in a large scale study conducted under natural conditions

**Background:** Thiamethoxam, active ingredient of Cruiser OSR®, is a neonicotinoid systemic insecticide used as seed dressing for oilseed rape protection against early season pests (Elbert et al., 2008). Its use on oilseed rape has been banned in France since 2012 after the release of a semi-field study showing that ingestion of a sublethal dose of thiamethoxam resulted in significant homing failure among honeybee foragers (Henry et al., 2012). To determine if those results could be generalized to natural conditions, we carried out a large scale experiment with free foraging honeybees.

**Objectives:** A two-year large-scale study was designed to measure via RFID technology whether honeybee survival could be impacted by exposure to thiamethoxam while foraging freely in a zone partially sowed with oilseed rape seed-dressed with Cruiser OSR®.

**Methods:** In 2013 and 2014, 10 hives were placed in a 150 square kilometers intensive cereal farming system area located near Niort in the West of France (the “Zone Atelier Plaine et Val de Sèvres” research facility). Then, in this area where regular farming practices remained unchanged, 153 ha and 135 ha of seed dressed oilseed rape were sown in 2013 and 2014 respectively. Each hive was settled at a certain distance of the treated oilseed rape fields in order to generate variation in thiamethoxam exposition pressure in the honeybees foraging area. In each hive, 5000 individuals were tagged with RFID microchips in order to monitor honeybees entering and leaving the hives. All oilseed rape fields, whether treated or untreated with thiamethoxam, were georeferenced in order to calculate a thiamethoxam exposure index. Data concerning population levels of adults and larval stages, food storage and diseases were collected in RFID hives to assess the general fitness of the colonies.

**Results:** The thiamethoxam exposure index was calculated on the basis of treated fields’ surface and distance from RFID hives. Statistical analyses were performed to assess the influence of this index on tagged honeybees’ lifespan. General healthcare of the colonies, population levels, food storage were studied according to thiamethoxam exposure intensity. Influence of the year of experiment and climatic conditions on the percentage of undetected honeybees was also assessed.

**Conclusions:** Our two-year study generated a huge amount of individual data. The performed analyses allowed us to investigate whether honeybee exposure to thiamethoxam via free foraging on oilseed rape crops protected with Cruiser OSR® has a significant impact on individual lifespan.

**References:**


Screening for resistance to black leg in winter canola germplasm adapted to the U.S. Southern Great Plains

**Background:** Black leg (*Leptosphaeria maculans*) is a widespread disease of winter canola in the southern Great Plains. Little is known about the reaction of locally adapted germplasm to black leg. Multi-locus resistance to canker development in the field is partially effective depending on the environment. Major-gene resistance, expressed as a hypersensitive response to leaf spot on cotyledons, is controlled by one or more resistance genes (RLm) that interact in a gene-for-gene manner with avirulence genes (Avr) in the pathogen.

**Objectives:** Entries were screened for field resistance to black leg. Avr alleles in isolates were assessed in order to identify prevalent races of the pathogen. The seedling reaction of germplasm to predominant races was determined to identify major-gene resistance.

**Methods:** Entries were screened from 2011-2013 by inoculating field plots with stubble collected from problem fields and evaluating canker severity after swathing. Avr alleles in local isolates (n=94) were identified by inoculating differentials with one or two Rlm genes or by PCR testing for Avr1, Avr4-7, and Avr6. Differentials included Westar (no Rlm), Quinta (Rlm1), Glacier (Rlm2, Rlm3), and Jet Neuf (Rlm4).

**Results:** In the field, disease incidence was high (50-100%), but canker severity (% stem girdling) averaged less than 50%. Most of the popular glyphosate-tolerant (Roundup Ready; RR) cultivars were more susceptible than conventional (non-glyphosate tolerant) cultivars and hybrids. In the local pathogen population, Avr1 was present in 37% of isolates, Avr2-3 was present in only 10% of isolates, while Avr4-7 and Avr6 were present in 100% of isolates. Based on limited screening of Jet Neuf (Rlm4), most isolates lack Avr4, and Avr7 is likely responsible for the positive PCRs for Avr4-7. A few isolates (n=3) had all Avr alleles (Avr1,2,3,4-7,6) while a few (n=6) only lacked Avr1 (Avr2,3,4-7,6). Most isolates had fewer Avr alleles and differed only in Avr1. Many isolates (n=32) were Avr1,4-7,6 and most (n=53) were Avr4-7,6. Except for DKW46-15 which was heterogeneous (nearly resistant), RR cultivars and hybrids were susceptible to the predominant races. Some conventional cultivars were resistant or heterogeneous (Kiowa, Sumner, and Wichita), and MH06E10 was resistant to all races. The conventional hybrids Rossini, Visby, Safron, Dimension, and DKSensei were also resistant to all races. The breeding lines KS4426, KS4428, and KS4564 were resistant or heterogeneous to all races. Most (62%) of the 55 entries were susceptible to all races.

**Conclusions:** Most local isolates of *L. maculans* can overcome Rlm1, Rlm2, Rlm3, and probably Rlm4. Rlm6 and Rlm7 should be highly effective. Most popular RR cultivars lack effective Rlm genes and also were the most susceptible in field trials. Several conventional cultivars and breeding lines had non-specific (heterogeneous) resistance to one or more races. Rlm7 is likely present in the conventional entries (n=6) with resistance to all races. There is a need to improve black leg resistance in locally adapted winter canola because most entries (62%) were susceptible to predominant races.

**References:**

Balesdent et al., 2005, Phytopathology 95:1061-1071.
Analysis of quantitative adult resistance to blackleg in canola

**Background:** Blackleg caused by *Leptosphaeria maculans* is a highly evolving fungal pathogen affecting the canola industry in Canada, Australia and Europe. Quantitative resistance (horizontal or non-race specific) that shows intermediate resistance can be less effective and difficult to identify compared to qualitative resistance by single dominant genes.

**Objective:** The objective of this study was to evaluate the effectiveness of a previously mapped intermediate blackleg resistance locus at the adult stage under greenhouse and field conditions.

**Methods:** Populations segregating for an intermediate resistance locus were planted and inoculated using cotyledon inoculation. All plants were genotyped using closely linked molecular markers. Blackleg disease symptoms were scored when the plants were fully mature using a 0 (no disease) – 5 (completely susceptible resulting in plant death) scale. Data for the adult stage included the percentage of plants that died before maturity and the disease scores at maturity for surviving plants. Scores for resistant plants ranged from 1 to 3 and susceptible scores ranged from 4 to 5. Near iso-genic lines (NILs) containing the intermediate blackleg resistance locus along with the parents and other controls were planted in a blackleg nursery at the Ian N. Morrison Research Farm Station in Carman, Manitoba. Disease scoring was done at maturity using a 0-5 scale.

**Results:** In the greenhouse, 182 plants were inoculated using a weak isolate. Of these plants, 101 did not harbour the intermediate resistance locus and 94% of the plants died before reaching maturity. Only 2% of these plants survived and received scores ranging from 2-3 (resistant) and 4% of the plants survived and received a score of 4 (severe infection). Of the 81 plants evaluated with the intermediate resistance locus, only 28% died at the early stages, 56% survived with scores 1-3 and 16% with a score of 4. In plants treated with a highly virulent isolate, 100% of the plants without the resistance locus died early in development while 47% of the plants with the resistance locus survived and were rated 1-3. Of the plants treated with another virulent isolate, 64% of plants without the resistance locus died during early development and 27% survived with ratings of 1-3 and 8% survived with a score of 4. Regarding plants with the resistance locus, 24% died during early development and 76% survived with ratings of 1-3. In the field, disease scores ranged from 0.8 to 3.3 (resistant to intermediate) for NILs with the intermediate resistance locus. However, the susceptible control, Westar had an average rating of 4.5 (most plants died before maturity). This demonstrates that the intermediate resistance locus performed well under severe blackleg disease pressure in the field. Because a mixture of isolates was used in the field evaluation, this suggests that the resistance locus confers horizontal resistance.

**Conclusion:** The results indicate that the intermediate resistance locus can confer resistance to multiple isolates in the greenhouse and field, suggesting that this resistance locus has excellent potential in blackleg management in western Canadian canola production regions.

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**Plasmodiophora brassicae** its genome and associated microbiome

**Background:** Clubroot caused by *Plasmodiophora brassicae*, is a widespread soil-borne disease with high economical impact on *Brassica* oil- and vegetable crops. *P. brassicae* is phylogenetically located among the Phytomyxids within the poorly known supergroup Rhizaria. Phytomyxids consist of two groups: the *Plasmodiophorids* which are parasites of plants and oomycetes, and the *Phagomyxids*, comprising pathogens of sea grass, diatoms, and brown algae.

**Objectives:** In order to enhance our biological understanding of this important plant pathogen we have sequenced its nuclear genome, the mitochondria and monitored the microbial communities associated to this disease in soil, rhizosphere and inside roots of oilseed rape.

**Methods:** Next generation sequencing technologies, along with bioinformatic softwares have been used to establish genome data enabling various comparative genomic analysis, and to elucidate microbial communities associated with healthy and diseased conditions.

**Results:** We will present the 25.5 Mb genome, its developmental stage-specific transcriptomes and the transcriptome of *Spongospora subterranea*, the closely related potato scab pathogen. The *P. brassicae* genome is reduced in metabolic pathways and harbors phytohormones contributing to its gall phenotype. Phylogenetic analysis points to its important role in deciphering evolutionary relationships and gene diversification of early eukaryotes, further demonstrated by its complex mitochondrial genome. A genome that is approximately three times larger than the mitochondria in *S. subterranea* (Gutiérrez et al. 2014). Different microbial communities dominate inside the roots of oilseed rape, its rhizosphere and in the soil of infested or healthy nature. For example, the two bacterial families *Pseudomonadaceae* and *Enterobacteriaceae* are expanded in the diseased clubroots. Details on the structure of the microbiota associated with the clubroot disease will be presented together with other data.

**Conclusions:** The eukaryotic Rhizaria, comprises several groups of uncultivable free-living protists such as Radiolarians, Foraminiferans and Gromiids, as well as the parasitic *Plasmodiophorids* and *Haplosporids*. Here, we provide genome and transcriptome data on two plant pathogenic *Plasmodiophorids*. Together with the microbial communities associated with the clubroot disease we now hold new knowledge useful in the design of new controlling measures against this widespread plant disease.

**References:**
Genetic resistance to blackleg in the Canadian banola (*B. napus*) germlasm and the breakdown of the dominant *Rlm3* by the *L. maculans* pathogen population

**Background:** *Leptosphaeria maculans* is the causal agent of blackleg (aka phoma stem canker), an economically important disease of *Brassica napus* (canola/oilseed rape). This pathogen has led to epidemics in France and Australia and is now a growing concern in the Canadian Canola (*Brassica napus*) industry where genetic resistance in major varieties has long provided an effective means of disease control. Both seedling resistance controlled by major resistance genes (*Rlm* genes) and adult plant resistance mediated by quantitative resistance (minor) genes to *L. maculans* have been identified in canola varieties. Tighter rotations and increased acreage driven by the economic returns of canola have led to the emergence of new virulent races. Major gene resistance to blackleg is analogous to the gene for gene interaction model whereby *Rlm* genes defend against isolates with corresponding *Avrlm* genes. However, host genetic resistance can be overcome with population shifts and the emergence of new races of the pathogen.

**Objectives:** The aim of this study was to identify the major seedling resistance genes carried in the Canadian canola (*B. napus*) germlasm as well as the predominant avirulence genes carried by the Canadian Blackleg pathogen population.

**Methods:** To identify host resistance to blackleg, a set of genetically characterized *L. maculans* isolates were employed to identify major resistance genes (*Rlm* genes) in 206 Canadian canola varieties/lines. 104 of these canola varieties/lines were further evaluated for adult plant resistance (APR) under controlled conditions. To identify pathogen race structure, 674 *L. maculans* isolates isolated from stubble collected in 2010 and 2011 across Western Canada were characterized at ten avirulence genes using a set of *Brassica* lines carrying known *Rlm* genes.

**Results:** On the host side, the results indicate that 85% of tested canola varieties/lines carry seedling resistance. However, except for *Rlm3* which was present in the majority of lines tested, the rest of *Rlm* genes were rarely detected. Adult plant resistance to blackleg was identified in 56% of tested varieties/lines. On the pathogen side, a total of 55 races were detected with two dominant races accounting for 45% of the pathogen population. *Avrlm6* and *Avrlm7* were the dominant avirulence genes and were present in >85% of isolates and *Avrlm3, Avrlm9*, and *AvrLepR2* were the least observable and present in <10% of isolates. The above results indicate the breakdown of *Rlm3* resistance in Western Canada most probably due to selection pressure in response to the presence of this single resistance gene in most varieties.

**Conclusions:** Genetic resistance to blackleg in the Canadian Canola germplasm is derived from a combination of adult plant resistance and a few seedling resistance genes with heavy reliance on *Rlm3*. Most of the blackleg pathogen population is now virulent on *Rlm3* and while there are many races, a few races account for the majority of the pathogen population. The gradual increase in blackleg disease incidence and severity in western Canadian disease surveys indicates the need to incorporate both adult plant resistance and diverse seedling resistance into commercial varieties. Management strategies such as pathogen monitoring and cultivar rotation are key factors in preventing significant yield loss over the long term.

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Molecular mapping of clubroot resistance in spring *Brassica napus* canola with resistance introgressed from the European winter canola cv. ‘Mendel’

**Background:** Clubroot disease caused by the soil-borne pathogen *Plasmodiophora brassicae* Woronin is a major threat to the production of *Brassica* crops worldwide. Breeding for resistance requires the identification of resistance sources and successful introgression of the resistance gene(s) into crop germplasm (for review, see Rahman et al. 2014). The European winter canola cv. ‘Mendel’ show resistance to many *P. brassicae* isolates including pathotypes 3 which is prevalent in Canada (Rahman et al. 2011).

**Objectives:** The identification and the genomic localization of molecular markers linked to clubroot disease resistance gene(s) in spring canola lines derived from the European winter *B. napus* cv. ‘Mendel’ will help to understand the mechanism of the resistance and also aid in the marker assisted breeding of this crop.

**Methods:** To introgress clubroot resistance into Canadian spring *B. napus* canola, crosses between Canadian spring canola and European winter canola cv. ‘Mendel’ were made and several resistant lines were developed through pedigree breeding. Two of the resistant lines were further crossed to the clubroot susceptible spring canola line A07-26NR and doubled haploid (DH) populations were produced from the F1’s. Simple sequence repeat (SSR) markers were used to identify markers linked to the resistance in two DH populations as well as in segregating F3 and F4 families derived from the original Canadian spring canola × ‘Mendel’ crosses.

**Results:** The inheritance and molecular mapping studies suggested that at least one dominant gene is involved in the control of clubroot resistance derived from the European winter *B. napus* cv. ‘Mendel’. The resistance gene was mapped to the A3 chromosome of *Brassica rapa*.

**Conclusions:** We identified two SSR markers linked to the clubroot resistance gene. Markers identified in this study can be used in marker assisted breeding as well as pyramiding of multiple clubroot resistance genes.

**References:**


Comparative genomics facilitate cloning of *Leptosphaeria maculans* avirulence gene *AvrLm2*

**Background:** Blackleg caused by *Leptosphaeria maculans* is a major disease of the oilseed crop *Brassica napus* (canola/oilseed rape) and other *Brassica* crops worldwide. Race identification of the pathogen through cloning and marker development of avirulence genes is a crucial step for the disease management. Map-based cloning has been successfully applied to clone five *L. maculans* avirulence genes. However, this approach is time consuming and has limitations such as incompatibility of desired parental isolates for crossing. With the advent of next generation sequencing and the availability of whole genome sequences of *L. maculans*, a combination of genetic mapping, high-throughput phenotyping and intraspecies comparative genomics can facilitate the identification of avirulence genes.

**Objectives:** Cloning *L. maculans AvrLm2* gene corresponding to the race specific *B. napus* resistance gene *Rlm2*.

**Methods:** *AvrLm2* was previously reported to be located within the *AvrLm1-AvrLm6* gene cluster of *L. maculans* genome (Fudal et al., 2007). To compare *AvrLm2* genomic interval, we re-sequenced *L. maculans* isolates using Illumina sequencing platform. Sequence reads for each isolates were mapped to the reference genome v23.1.3 using Bowtie2 and visualized using GBrowse 2.0. We then searched the *AvrLm1-AvrLm6* genome interval for SNP(s) (single nucleotide polymorphism).

**Results:** Three SNPs coincident with the *AvrLm2* phenotype were identified in the predicted effector gene *LmCys1*. Complementation of a virulent isolate with *LmCys1*, as the candidate *AvrLm2* allele, restored the avirulent phenotype on *Rlm2*-containing *B. napus* lines proving that the predicted effector gene is *AvrLm2*. Mutation analysis showed that only the non-synonymous changes of g397->A/c397 or g398->A398, both of which lead to a change of amino acid at Gly133, were responsible for the loss of *Rlm2*-mediated recognition specificity. Expression pattern of *AvrLm2* alleles (*Avr* and *avr*) were similar during infection and picked at 5 days after inoculation.

**Conclusions:** The cloning of *AvrLm2* described here provides an example for the rapid cloning of effector genes through comparative genomics and as an alternative or complementary approach to map-based cloning. *AvrLm2* encodes a small cysteine-rich protein with low similarity to other proteins in the public databases. Unlike other avirulence genes, *AvrLm2* resides in a small GC-rich island within an AT-rich isochore of the genome, and was never found completely deleted in virulent isolates.

**References:**
Spread of clubroot on canola in Canada, 2003-2014

Background: Clubroot caused by *Plasmodiophora brassicae* is an important disease in oilseed rape (*Brassica napus*) crops in Europe. However, it had not been reported on canola-quality *B. napus* on the Canadian prairies until 2003. The initial discovery in 2003 consisted of a small cluster of infested fields (Tewari et al. 2005).

Objectives: To summarize the spread of *P. brassicae* across the Canadian prairies, and contrast the rapid movement on the prairies with slow dispersal at a site in southern Ontario.

Methods: Surveys for clubroot on canola were initiated in 2004 in the province of Alberta. The initial surveys were relatively small, involving just 41 fields in 2004, 112 fields in 2005, and 250 fields in 2006. Since 2008, more than 400 commercial canola crops in central and southern Alberta were examined each year, and individual counties conducted additional surveys. Annual surveys of more than 100 canola crops in both Saskatchewan and Manitoba were conducted over the past six years. At the Muck Crops Research Station (MCRS) of the University of Guelph, located in the Holland Marsh in Ontario, one block is heavily infested with clubroot (90-100% severity), but susceptible hosts in adjacent blocks develop moderate or even no clubroot. In 2014, replicated trials of clubroot-susceptible brassica vegetables in a trial 50 m from the infested block and at two nearby research sites were rated for clubroot to assess the distribution of *P. brassicae* at sites that are accessed routinely by equipment and workers from the MCRS, with no sanitation measures to restrict transfer of inoculum.

Results: *P. brassicae* has spread across large areas of Alberta, with more than 1800 fields infested. Isolated fields with trace levels of clubroot have been identified in Saskatchewan and Manitoba, and severe clubroot has recently been reported in North Dakota (Chittem et al. 2014). In contrast, clubroot severity at the site adjacent to the infested block at the MCRS was < 50%, < 20% 1 km away, and 0% 4 km away, which indicates that spore movement is quite limited.

Conclusions: *P. brassicae* is spreading rapidly on the Canadian prairies via both short- and long-distance dispersal mechanisms. Pathogen spread at MCRS is much more limited, and more similar to spread in Germany, where there is little movement of inoculum of *P. brassicae* from region to region (Strehlow et al. 2014). This difference may be related, at least in part, to the size of the inoculum source (field size, field proximity, resting spore concentration; Gossen et al. 2013), patterns of movement of equipment, and for MCRS, possibly soil type.

References:
Genetic dissection of resistance to *Verticillium longisporum* in *Brassica* and *Arabidopsis*

*Verticillium longisporum* is a soil-borne fungus infecting cruciferous hosts. It penetrates host roots, grows towards the xylem and spreads within the xylem vessels to colonise the whole plant. Infected plants show a complex pattern of disease symptoms and developmental reactions, incl. premature seed ripening, chlorosis or stunting. *Brassica* species as well as *Arabidopsis thaliana* are hosts of *Verticillium longisporum*. The aim of our study is to identify genes controlling disease resistance parameters and implications of host development for pathogenesis and defense.

Genetic mapping in *Arabidopsis* and *Brassica alboglabra* revealed a number of QTL explaining the variation for different resistance parameters. In *B. alboglabra*, a closely related species of *B. oleracea*, two major and one minor QTL were found that explained significant proportions of the variation for the degree of fungal colonisation and/or AUDPC in F3 families. QTL for the control of different stunting parameters showed some overlap with QTL for AUDPC or colonisation. Two major QTL controlling differences in flowering time were identified on C8 and C9 that did not show interdependence to disease resistance. As for *B. alboglabra*, fungal shoot colonisation was the most reliable resistance parameter in repeated experiments with *Arabidopsis*. Resistance to *Verticillium*-induced stunting was inherited more independent from resistance to shoot colonisation than in *B. alboglabra*. QTL for colonisation resistance did show linkage with QTL that controlled development or with the morphological marker erecta. Fine-mapping of different disease parameters in the erecta region in near-isogenic lines revealed a close linkage of loci controlling either stunting or colonisation resistance. A tailor-made (tm) NIL was created that represented the colonization resistance QTL vec1 from the erecta region in a fixed genetic background. µ-array experiments revealed striking differences in gene expression patterns depending on the presence of the QTL and the inoculation. Candidate genes from the QTL region have been identified and cloned. Expression patterns revealed the involvement of different resistance pathways in vec1-controlled defense. Possible syntenies between known QTL for *Verticillium* resistance in *Brassica* and the candidate genes from *Arabidopsis* will be presented. Defense related hormones indicated that SA played a role in stunting resistance while JA contents seemed to play a role in colonization resistance.

*Verticillium* resistance is a complex, quantitative trait based on often subtle effects of genes that are related not only to defense responses. Further studies of transgenics expressing the cloned candidate genes will reveal their role in resistance to *V. longisporum* and will support the breeding for resistance in oilseed rape.

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Clubroot resistance breeding in canola through gene mapping and marker assisted selection

**Background:** Clubroot caused by an obligate biotrophic parasite, Plasmodiophora brassicae is one of the most economically important diseases of canola/rapeseed and other brassica vegetables in the world. The origin of clubroot disease is not clearly known, it appears as old as its host. Currently, clubroot disease is well spread in more than 60 Brassica crop growing countries. In Canada, clubroot disease is spreading rapidly in canola/rapeseed crop in the Prairie Provinces. Soil-borne and obligate biotroph nature of the pathogen as well as long term viability of resting spores pose big challenges in controlling clubroot disease through various management practices such as chemical control, biological control and other agronomic. Breeding clubroot resistant cultivars is therefore more reliable, cost-effective and environmentally sustainable. In various sources of clubroot resistance in Brassica species, European turnips are the best ones so far, which can be used for breeding clubroot resistant canola/rapeseed cultivars. To use the clubroot resistant sources effectively, it is necessary to map/fine map the resistance genes to develop molecular markers that can be used for marker assisted selection (MAS) in canola/rapeseed.

**Objectives:** Novel resistance genes in European turnips need to be mapped and molecular markers can be developed to transfer these resistance loci in canola/rapeseed cultivars through MAS.

**Methods:** In our clubroot research program, initially we identified the European clubroot differential (ECD) set having high levels of resistance to Canadian field isolates of *P. brassicae*. We developed segregating populations using all turnip accessions in the ECD set for gene mapping and molecular marker development.

**Results:** In total, three resistance loci have been identified and they represent all major loci in the ECD set. All near iso genic lines for all resistance loci have been developed and molecular markers closely linked to each clubroot resistance locus have been developed for MAS. These near iso genic lines are being used to investigate interactions of each locus with different pathotypes. Further, resynthesized *B. napus* lines developed to integrate these clubroot resistance loci from European turnips to Canadian canola and advance backcross progenies have been developed through MAS. Meanwhile, all clubroot resistance loci were introgressed in canola (*B. napus*) from progenitor species (*B. rapa*) and subsequent backcrossing. The results showed clubroot resistance loci were fully functional in canola.

**Conclusion:** In our clubroot research project, we mapped three novel clubroot resistance loci including eight haplotypes and introduced these eight haplotypes of clubroot resistance into canola. Molecular markers have been developed and used for MAS in canola/rapeseed breeding for clubroot resistance gene introgression. In addition, multiple clubroot resistance loci can be pyramid through MAS to develop high levels of clubroot resistance in canola/rapeseed.

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Identification of QTLs for resistance to *Sclerotinia sclerotiorum* and their Interactions with flowering time QTLs in *Brassica napus*

**Background:** Stem rot caused by *Sclerotinia sclerotiorum* is a major yield-limiting factor in canola production and its incidence in field closely associates with flowering time. There is an urgent need to develop molecular markers linked to resistance for breeding.

**Objectives:** This study is to develop molecular markers linked to *Sclerotinia* resistance.

**Methods:** This study used a *B. napus* RILs population that derived from a cross between 888-5 (a susceptible and early flowering line) × m083 (a resistant and later flowering line) to construct a high dense SNP genetic map through employing the *B. napus* 60K SNP Infinium iSelect HD BeadChip. Resistances to *S. sclerotiorum* were identified using three identification methods (Liu et al., 2005), of which, field methods was performed in multiple environments.

**Results:** The genetic map comprised of 9,278 SNPs, and spanned 4071cm of the *B. napus* genome. For phenotyping, resistance evaluation methods included stem inoculation with the fungal mycelial plugs from the edge of colonies cultured in PDA media, artificial field disease nursery identification (5 years) and natural infection tests in 3 locations, and Correlation coefficients among the three methods were significantly positive, and among multi-environments in field natural infection were also positive (P<0.01). For flowering time, phenotyping was done in 6 natural infection tests of 3 locations. The flowering time was negatively and significantly related with disease index in all the environments. QTL mapping was performed with two methods: single environment and multi-environment detections. A total of 31 QTLs for resistance were detected on A2, A3, A4, A7, A8, A9, A10, C2, C3, C6, C7 and C8 linkage groups, each explaining 6.14% to 42.61% of phenotypic variation. Of them, 12 QTLs were repeatedly found in multiple environments. Major QTL qSSE2-1, explaining 10.50%-27.30% of the phenotypic variation across the 8 environments, and showed independence of environment. Meanwhile, total 21 putative QTLs for flowering time were found on A2, A3, A9, A10, C1, C3 and C7 linkage groups, each explaining 6.13%-34.50% phenotypic variation. Among them, 7 QTLs were identified in more than one environment. Three major QTLs (qFT2-3, qFT2-4 and qFT2-1) were found in multiple environments, contributing 16.40%-34.50% of the phenotypic variation. And the major QTL qSSE2-1 for *S. sclerotiorum* resistance linked with a major QTL qFT2-1 of flowering time. In addition, one microsynteny of QTL regions between A2 and C2 was found. According to the direction of QTLs additive effect of this region, the susceptibility to *S. sclerotiorum* was linked to early flowering time.

**Conclusions:** The molecular markers for *Sclerotinia* resistance and flowering time and their linkage relationship will be of benefit to improving the efficiency of resistance breeding in oilseed rape.

**Reference:**
Leptosphaeria maculans – An aggressive OSR pathogen and air pollutant

Background: Blackleg is a damaging disease of oilseed rape worldwide, caused by Leptosphaeria maculans and L. biglobosa. The disease is initiated by airborne ascospores, released from fruiting bodies formed on infected stubble from previous seasons. Ascospores are discharged in conducive weather conditions, starting from late summer onwards in Europe or the following spring in North America. Leaf infections initiated by ascospores lead to endophytic colonization of petioles. The formation of severe lesions at stem bases and root collars leads to pre-mature seed ripening, lodging and yield losses. The release of ascospores greatly depends on weather conditions. Disease control is at its most efficient not later than two weeks after mass ascospore release. PCR methods enable the discrimination of L. maculans propagules from those of other fungal species, including L. biglobosa. Quantitative real-time PCR has facilitated the quantification of the number of spores of both species, which helps to control the disease. Recent advances in genome and proteome analyses are currently enabling us to extend our knowledge of the genes and proteins of L. maculans.

Objectives: The aim was to compare the ascospore release patterns of Leptosphaeria spp. and incidence of phoma stem canker disease of OSR in Poland and the UK, and elaborate forecasting models for ascospore release of (i) all species of Leptosphaeria, and (ii) L. maculans and L. biglobosa. We have also tested the hypothesis that airborne Leptosphaeria ascospores can partially explain respiratory problems encountered in the autumn (‘autumn asthma’).

Results: In the last 10 years, the highest concentrations of the ascospores of L. maculans and L. biglobosa measured at ground level reached ca. 300 s m⁻³ in Poland and 3350 s m⁻³ in the UK. To predict the date of the first sudden rise of ascospores of this species complex a comprehensive forecasting model, SimAsco, has been developed. The predictive quality of the model was evaluated using the dataset collected at 12 site-years in Poland (2007-2012). SimAsco proved to be effective, with an efficiency of 0.74. A forecasting model was also elaborated for Poland and the UK to monitor the fluctuations of ascospore concentrations of all species of Leptosphaeria in the air. In addition, based on detailed in silico analyses, we have demonstrated that L. maculans produces proteins with high identity and similarity to commonly-known aeroallergens of several other well-characterized moulds. There were 81 proteins exceeding 50% amino acid identity, which significantly exceeded the allergen amino acid sequence identity thresholds recommended by FAO/WHO for allergenic proteins in food. High concentrations of Leptosphaeria spp. ascospores in the autumn and the postulated allergenicity of their proteins make this fungal genus a possible ‘culprit’ contributing putatively to respiratory problems.

Conclusion: Leptosphaeria maculans is not only the aggressive pathogen of OSR, but it is also an air pollutant that produces numerous allergenic proteins
Brassica rapa genome survey for genes corresponding to B. juncea QTL for white rust resistance

Background: Brassica juncea (Indian mustard) is a major oilseed brassica with white rust one of the important disease in mustard growing areas of India. QTLs for white rust resistance were reported in B. juncea, B. rapa, B. napus and Arabidopsis thaliana, genes for resistance were identified only from A. thaliana. In B. juncea, QTLs showed monogenic inheritance and A. thaliana gene sequence based Intron Polymorphic markers were flanking the white rust resistance QTLs on ‘A’ genome of B. juncea (Punjabi et al, 2010). Availability of B. rapa genome sequence (A genome) provided the opportunity to study white rust resistance QTLs of B. juncea.

Objectives: In silico genome wide survey of B. rapa genome sequence for identification of resistance related genes corresponding to B. juncea QTL for white rust resistance.

Methods: QTL for complete resistance, AcB1-A5.1 was mapped earlier on chromosome A05 of B. juncea, spanning a distance of 6.4cm. The corresponding region to this QTL from A. thaliana genome was used to search similar regions in the B. rapa whole genome sequence, using BLASTN search tool available online at Brassica database (BRAD), similar search was performed with Phytozome portal. BLASTN search was performed by dividing the corresponding region of Arabidopsis into 15 blocks of 20 loci each. Protein domains were predicted for the candidate resistance related genes using Pfam and genes were grouped according to domain similarity.

Results: The QTL AcB1-A5.1 corresponded to a 703 kb region in A. thaliana genome, this region matched to homologous sequences on three chromosomes of B. rapa i.e A03, A04 and A05. The BLAST results of both Brassica database and phytozome were compared; phytozome gave better results with larger regions of homology. A 385kb region on chromosome A03, 634 kb on chromosome A04 and 871kb region on chromosome A05 of B. rapa genome sequence were found corresponding to A. thaliana region. The arrangement of homologous blocks on chromosome A05 was inverted while in chromosome A03 and A04, the arrangement was the same as in Arabidopsis. Total fourteen resistance related genes were identified on these three chromosomes, which were further divided into three classes based on protein domains.

Conclusion: Use of native genes into particular species provide less penalty on performance of agronomically high yielding varieties, also it is easy to transfer genes in same or related species using marker assisted breeding. Confirmation of identified genes will be useful in developing high performing B. juncea varieties with resistance to white rust.

References:
Epidemiology and management of Sclerotinia stem rot in canola

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Background: Canola (B. napus L.) is the major oilseed crop in Australia. Sclerotinia stem rot caused by Sclerotinia sclerotiorum (SSR) is a major disease affecting canola production in most of the canola growing regions in the world (Bradley et al. 2006, Turkington et al. 1993). In Australia, this disease has emerged as a serious problem over the last few years. Losses from SSR in Western Australia have been estimated at A$59 and 23 million in 2013 and 2014 seasons respectively. Various fungicide products are registered for the management of Sclerotinia in Australia, however, information is required under what conditions the fungicide usage is most cost-effective to curtail the disease.

Objectives: Three major objectives of the investigations were to determine the efficacy of fungicides, optimize the timing of foliar fungicide application and characterization of environmental conditions involved in the spore production and disease development.

Methods: Field trials were conducted in the canola growing regions of Western Australia during 2010-2014 to determine the efficacy of various fungicides and the optimum timing of application of foliar fungicide to manage Sclerotinia stem rot in canola. Fungicides were applied at various bloom stages at the recommended rates. Per cent petal infection was recorded each year. Weather parameters including rainfall, temperature and humidity were recorded from the nearest weather stations. Sclerotinia assessments were made on 50 plants per plot two weeks before harvest, and disease incidence was calculated. Plots were harvested for yield. Data were analysed by ANOVA or REML (Residual maximum likelihood) using Genstat release 16.

Results: Various fungicide products were effective in the control of Sclerotinia stem rot, however, yield benefits were achieved when fungicide spray applications were applied synchronizing with the timing of onset of disease epidemics. For example, in a year where spore release was delayed, late fungicide application applied at, or after, 50% bloom significantly reduced the Sclerotinia stem rot incidence and significantly improved the seed yield with highest gross margin compared to nil treatment. Very early spray at 6-7 leaf stage (prior to commencement of flowering) did not significantly increase yield and gave a negative change in gross margin. Trial data indicated that rainfall and relative humidity were the two key drivers in the development of Sclerotinia and threshold values were determined. This is a major breakthrough in determining the risk factors and consequently assisting growers in making fungicide spray decisions.

Conclusions: Timing of fungicide application should coincide with the onset of spore release but also taking into account whether subsequent seasonal conditions will be conducive for disease development. The role of weather conditions in disease development will be further discussed.

References:
Improved management of light leaf spot by understanding the structure of *Pyrenopeziza brassicaceae* populations

**Background:** Light leaf spot, caused by the fungal pathogen *Pyrenopeziza brassicaceae*, is currently the major disease problem in oilseed rape (*Brassica napus* L.) production in the UK and also affects vegetable *brassicas* such as cabbage, cauliflower and Brussels sprouts. The disease was considered a problem in Scotland and North England but has substantially increased in all parts of England over the last decade. Furthermore, oilseed rape breeders have seen more light leaf spot in the northern parts of Europe. Due to the polycyclic nature of the disease, the pathogen has the potential to adapt to an environment (McDonald & Linde, 2002). Effective control of light leaf spot to reduce yield and economic losses is difficult to achieve. Fungicide control of the disease in crops is difficult since fungicides must be applied when the pathogen is growing asymptomatically in plant tissues (Figueroa et al., 1994). Additionally, decreased sensitivity toazole fungicides has been reported (Carter et al., 2013). Exploiting plant resistance against the pathogen could help control the disease but current commercial cultivars show poor resistance (HGCA recommended lists, http://www.hgca.com/varieties). The structure of the pathogen population has previously been described as highly genetically diverse (Majer et al., 1998).

**Objective:** To determine the population structure and to study the host range of *Pyrenopeziza brassicaceae*.

**Methods:** Field assessments with 10 cultivars were done at five locations in England and one location in Scotland to discover possible shifts in pathogenicity towards specific cultivars. Furthermore, oilseed rape and vegetable brassicas were inoculated with *P. brassicaceae* to identify the host range and possible gene-for-gene interactions.

**Results:** Oilseed rape cultivars showed differences in susceptibility to *P. brassicaceae* at different locations. Cultivar differences were also recorded in in planta experiments.

**Conclusions:** The results suggest that different pathogen populations may be present at different locations. With increased information about pathogen populations regional advice for deployment of cultivars can be given to farmers for a more effective use of cultivar resistance.

**References:**
Genome wide association analysis and differential expression analysis of resistance to *Sclerotinia* stem rot in *Brassica napus*

**Background:** *Sclerotinia sclerotiorum* is a necrotrophic pathogen, which has no specific host and infects more than 400 plant species. *Brassica napus* is one of the most important oil crops in China, stem rot caused by *Sclerotinia sclerotiorum* is the major disease, leading to yield and quality loss of rapeseed. Resistance to *S. sclerotiorum* showed quantitative inheritance with additive effect and medium heritability, some QTL were found through QTL mapping.

**Objectives:** To understand the genetic mechanism of *S. sclerotiorum* resistance in *B. napus* and identify the candidate genes resistance to *S. sclerotiorum* through GWAS and transcriptome analysis.

**Methods:** Totally 347 *B. napus* were collected in the study and cultivated in southwest university of Beibei, Chongqing, China in 2012 and 2013, the *Sclerotinia* resistance of detached stem inoculation was evaluated according to Mei et al. (2012). SNP genotyping was performed using the *Brassica* 60K Illumina SNP array, and significant associated SNP loci were identified. In addition, transcriptome sequencing of stem in resistant and susceptible *B. napus* after inoculation with *S. sclerotiorum* was conducted.

**Results:** A total of 18 significant associations were identified for stem resistance on A8 and C6. SNPs on A8 were located in a 409 kb haplotype block and association signals detected on C6 were consistent with previous studies of QTL mapping. After *B. napus* infected by *S. sclerotiorum*, photosynthesis, glyoxalic acid and carbon metabolism were suppressed, while secondary metabolites, sulfur metabolism, especially GSH and glucosinolates were synthesized and immune system was activated, and these systems played an important role in defense response. Numerous ERF and WRKY genes were also found and mostly were up-regulated. Besides, specific genes related with jasmonic acid pathway, lignin biosynthesis, defense response, signal transduction and transcription factors responsible for stem resistance were found.

**Conclusions:** Combining the SNP-trait association and transcriptome sequencing results, 24 common genes were found, including a tau class glutathione S-transferase (GSTU) gene cluster. This study was useful for further gene identification and function analysis.
The importance of the low temperature threshold for clubroot development on canola

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Background: Many studies have been conducted to understand the relationship between temperature, infection by Plasmodiophora brassicae, and the development of clubroot. The focus has been on optimum temperatures, but research has also shown that seeding into cool soils can reduce symptoms, and cool temperatures prior to harvest suppress symptoms. A greater understanding of the low temperature threshold that prevents or inhibits clubroot development will improve disease forecasting and timing of seeding of crops and research trials.

Objective: To determine the relationship between temperature and clubroot symptom development, with emphasis on identifying the low temperature threshold.

Methods: Each year from 2011-2014, a field trial at the Muck Crops Research Station of the University of Guelph, Bradford ON was seeded to canola cv. InVigor 5030 at a site naturally infested with P. brassicae. The study was arranged in a randomized complete block design with four replicates. The treatments were seeding at 2-week intervals from early May until late June, to provide a wide range of temperature and soil moisture conditions. Plants were harvested 4 and 6 weeks after seeding and clubroot severity was assessed. Daily rainfall and mean soil and air temperatures were recorded. Correlation analysis and stepwise regression were used to determine the relationship between clubroot severity, and soil and air temperature, rainfall and degree days with a base of 12.5, 14 and 17 °C.

Results: Degree day accumulation (base 14 °C) in the first 2 or 3 weeks after seeding was the best indicator of clubroot incidence or severity at 6 weeks after seeding, especially where soil moisture was not limiting or excessive. Degree day calculations with a base threshold of 12.5 and 17 had lower correlation coefficients than day degrees with a 14 °C base. Air temperature was more closely correlated to clubroot development than soil temperature.

Conclusions: Degree day accumulation with a minimum threshold of 14 °C was best for predicting clubroot development. However, the number of days when mean temperatures are above this threshold may be a better indicator of clubroot development than either mean temperature or degree days. These results are consistent with controlled environment studies, which showed that primary infection of root hairs occurred at 10 °C, but that cortical infection did not, and that only low levels of cortical infection occurred at 15 °C (Sharma et al. 2011 a,b).

References
Growing winter oilseed rape without neonicotinoid seed treatments – The UK perspective

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Background: On 1 December 2013 a restriction adopted by the European Commission on the use of clothianidin, imidacloprid and thiamethoxam (neonicotinoid insecticides) came into force (EFSA, 2013). It addresses the use of pesticides in the treatment of crops attractive to bee pollinators and also for cereals. The restriction was adopted in order to protect, or at least to assess the impact of withdrawal, on bee pollinators. This paper gives an overview of work commissioned by AHDB Cereals and Oilseeds on the implications of the restriction on the neonicotinoids, focusing on crop protection of winter oilseed rape in the UK in the 2014/2015 cropping season.

Objectives:
• To confirm whether pyrethroid resistance is present in UK populations of cabbage stem flea beetle (CSFB)
• To assess the incidence and severity of CSFB in winter oilseed rape
• To assess CSFB larval pressures
• To determine the area of oilseed rape lost to CSFB

Methods: Twelve samples containing 289 beetles, spanning six counties were received, from growers with suspected resistance, and tested for the presence of the knock down resistance (kdr) mutation.

County level information on the incidence and severity of CSFB during the period 22-29 September 2014 was provided by a network of 23 local agronomists from ADAS. Assessments were based on approximately 32,000ha of winter oilseed rape crops, equivalent to 5% of the national area.

In February 2014, CSFB larval assessments are being carried out by ADAS in counties previously identified as having the highest proportion of oilseed rape crops above the adult CSFB treatment thresholds (HGCA, 2014).

Around 3,500 arable farmers received a winter planting survey in November 2014. It included questions to help assess the impact of the neonicotinoid restrictions on oilseed rape planting and cabbage stem flea beetle damage.

Results: CSFB resistance testing confirmed the kdr mutation was present. Enhanced metabolic resistance was also detected.

An estimated 3% (equivalent to 18,000ha), of the national area of winter oilseed rape had been lost by the end of September 2015 due to CSFB. Counties in the East of England were estimated to have been worst affected including Hampshire/Surrey where loses of up to 28% were estimated.

Larvae assessments and planting survey results will not be available until spring 2015.

Conclusions: During crop establishment pyrethroid insecticides were the only available option for controlling CSFB. However the confirmation of resistance to pyrethroids is likely to have created more serious issues in controlling this pest.

Crop losses were recorded mainly in the East of England. Here, many crops were drilled in late August/early September. A prolonged period of dry weather resulted in crops remaining at the cotyledon stage for longer. Optimum conditions during early stages of growth may give an untreated crop a better chance to grow away from CSFB attack.

References:
EFSA. 2013. Conclusion on pesticide peer review. EFSA Journal 2013; 11(1):3066-3068
HGCA. 2014. Cabbage stem flea beetle. Information Sheet 24. HGCA
Resistance of selected oilseed rape cultivars registered in Poland to stem canker of brassicas (Leptosphaeria ssp.)

**Background:** Among other fungal pathogens, oilseed rape in Poland is exposed to Leptosphaeria maculans and L. biglobosa, which cause stem canker of brassicas (blackleg). They are responsible for considerable yield loss of oilseed rape. High profitability of oilseed rape production has caused great development of breeding varieties with new characters, including resistance to some diseases with different genetic backgrounds. Every year new population and hybrid varieties are introduced to the market. The aim of this study was to characterize the resistance of winter oilseed rape cultivars to Phoma leaf spotting/stem canker in field conditions and to discriminate the species of Leptosphaeria by LAMP technique (Jedryczka et al. 2013).

**Material and Methods:** Screening of plant susceptibility/resistance was done in 2013 and 2014. The study aimed at comparing 13 (2013) to 44 (2014) cultivars of oilseed rape registered in Poland by the Central Station for Variety Testing (COBRU) or introduced to the candidate list due to high yield and the presence of Rlm7 resistance gene. The experiment was done in Dlon (N51°41’22,0”, E 17°04’23,0”) Wielkopolska (Great Poland) region. The determination of Leptosphaeria species was studied using Loop-mediated DNA amplification (LAMP) method. For this purpose leaf and stem samples were collected from three individual plants per variety.

**Results:** In both seasons, the cultivars with Rlm7 resistance gene showed significantly less symptoms, as compared to cultivars with no Rlm7. The resistance also differed significantly within cultivars with no Rlm gene. The isolates on leaves of cultivars without Rlm7 resistance gene belonged mainly to L. maculans (72%), whereas on cultivars harbouring this gene they were scarce (5%) and all belonged to L. biglobosa.

**Conclusion:** The pathogen population of fungi causing blackleg of oilseed rape in Poland is still composed of L. maculans and L. biglobosa. The population of L. maculans is avirulent on Rlm7 resistance gene, i.e. contains avrLm7 allele.

**References:**

**Acknowledgement:** Experimental work was funded by the Ministry of Agriculture and Rural Development of Poland, project number 54.
Suppression of clubroot of oilseed rape by soil amendments with different fertilizers

Background: Clubroot, caused by *Plasmodiophora brassicae*, has been one of the most destructive diseases of oilseed rape in Germany and recently has become a more frequent problem worldwide. Our previous field studies showed the number of very virulent pathotypes has increased over the past years and subsequently commercial resistant cultivars become susceptible (Zamani Noor, unpublished data). Therefore, understanding the type of pathotype of the pathogen may be useful for developing better strategies to study the disease epidemiology, which should lead to more effective control of the disease.

Objectives: Previous studies described that when lime or calcium cyanamide are applied to the infected soil, the soil became suppressive to clubroot (Hwang et al. 2011; Dixon 2012) Field trials with natural infection on three different locations in Germany were conducted in 2014 to investigate control strategies for improving resistance in susceptible and resistant cultivars by evaluating the effect of different fertilizers application at different times during the growing season.

Methods: Calcium cyanamide (300kg/ha; 50% calcium oxide) and burnt lime (150kg/ha) were applied to the soil surface one day prior to the sowing or when the oilseed rape plants had reached the growth stage BBCH 11-12. Soil moisture, temperature and soil pH at two different depths (15 and 30 cm) were measured once every week after sowing date. Clubroot disease incidence and severity were assessed visually for the development of root galls.

Results: The preliminary results showed clear differences between the treatments. Changing the time of application had significant impact (P ≤ 0.05) on the final severity of the disease. Relative to untreated control, clubroot incidence and severity were significantly lowered by application of fertilizer at later growth stages. In comparison with calcium cyanamide, burnt lime application has lower effect.

Conclusions: Clubroot is becoming to a major problem on oilseed rape fields in Germany. Preventing and controlling disease in contaminated fields is very difficult. Significant yield losses would result from disease development at early growth stages. At our study, application of calcium cyanamide after sowing has greatly reduced the disease severity and disease incidence.

References:


Influence of soil moisture and leafhopper feeding densities on phytoplasma titres, aster yellow symptoms and seed yield of hybrid canola

Background: Aster yellows (AY) caused major production losses to canola in western Canada in 2000, 2007 and 2012 (Olivier et al. 2011; Miller et al. 2013). The disease is caused by a phytoplasma vectored by the aster leafhopper, Macrosteles quadrilineatus. A five-point rating scale based on phyllody, virescence and presence of bladder-like pods was developed to assess the incidence and severity of AY symptoms in canola (Olivier et al., 2014). Symptoms appeared related to leafhopper densities and soil moisture.

Objectives: Investigate the effect of leafhopper feeding densities and soil moisture on the frequency and severity of AY symptoms and seed yield of hybrid canola.

Methods: Untreated hybrid canola was grown in dry soil (20-30% moisture content) and wet soil (70-100% moisture content). Plants at the early 2nd true-leaf stage were placed in cages (n = 4 plants/cage) and exposed to eight densities of AY-infected leafhoppers (n = 2 -16 adults/plant) for 10 hours. Numbers of leafhoppers feeding on each plant were recorded hourly. Plants were grown at 20°C under high light intensity (>400µmol/m²/s). The frequency and severity of AY symptoms were assessed after 6, 8 and 10 weeks using a five-point rating scale (Olivier et al., 2014). Droplet-digital PCR (Bahar et al., 2014) was done on the leaves, petioles, stems and roots of selected plants to quantify phytoplasma titres 8 weeks after infection. The remaining plants were grown to maturity and harvested to determine seed yield and 1000-seed weight. Analysis of variance and orthogonal contrasts for linear, quadratic and cubic trends were used to assess the effect of moisture and feeding densities on phytoplasma titres, AY symptoms, seed yield and 1000-seed weight.

Results and Conclusions: Soil moisture during infection had a pronounced effect on phytoplasma titres, incidence and severity of AY symptoms and subsequent seed yield of hybrid canola. Plants infected in dry soil had lower titres, fewer symptoms, less severe symptoms (AY rating 0-1) and higher seed yield than plants infected in wet soil. Titres, symptoms and yield in dry soil were not affected (P≥0.05) by leafhopper feeding densities. In contrast, phytoplasma titres and frequency/severity of AY symptoms in wet soil increased curvilinearly (linear and quadratic contrasts P≤0.001) as leafhopper feeding densities increased. The majority of plants exposed to feeding densities above 4 leafhoppers/plant had elevated phytoplasma titres in leaves, petioles and roots; severe AY symptoms (AY rating 3-5) and produced little or no seed. Relationships between feeding densities, phytoplasma levels, AY ratings and seed yield in dry and wet soil will be described.

References:
Early fungicide application reduces blackleg impact on canola only when cultivar resistance is broken and the disease pressure is high

**Background:** Although several fungicides are registered in Canada for control of blackleg ([*Leptosphaeria maculans* (Desm.) Ces. & de Not]) on canola (*Brassica napus*), foliar fungicide application generally is not considered necessary in western Canada, especially on resistant cultivars (Kutcher et al. 2013). In recent years, blackleg has been increasing in much of the Canadian prairies, a circumstance attributable likely to shortened crop rotations and shifts in pathogen race structure. It is not clear, however, whether fungicide treatments should be recommended if cultivar resistance is overcome by the pathogen.

**Objectives:** To assess the benefit of fungicide treatments in relation to application timing and host resistance based on multi-site and multi-year field trials across canola growing regions in western Canada.

**Methods:** Field plots were established at five locations in western Canada between 2011 and 2012. The susceptible cv. Westar was used to represent the worst-case scenario of resistance breakdown. Diseased canola residues from previous years were left in the plot area for pathogen inoculum. The fungicides Headline® (pyraclostrobin), Tilt® (propiconazole), Quadris® (azoxystrobin) and Quilt Xcel® (propiconazole + azoxystrobin) were applied at the 2-4 leaf stage individually, in a split application (Headline then Tilt and vice versa) at the 2-4 leaf and prior to bolting, and Headline alone just prior to bolting. Unsprayed plots were used as a check. The resistant (R) cultivar 45h29 and moderately resistant (MR) cultivar 43e01 were treated with Headline at the 2-4 leaf stage only as additional checks. At crop maturity, blackleg incidence and severity were assessed on 50 plants by examining cross-sections of lower stems and tap roots in each plot. Seed yield was recorded after harvest.

**Results:** Data from a total of 17 site-years showed varying levels of blackleg. When all site-years were analyzed together, all treatments, except Tilt applied at the 2-4 leaf stage or Headline applied prior to bolting, reduced blackleg and increased seed yield of Westar. When data were analyzed separately based on disease severity (DS: 0-5), the trend was the same for trials with moderate to high levels (DS>1.0) of disease (8 site-years). However, no difference was observed with disease incidence, severity or seed yield under low levels (DS<1.0) of disease (9 site-years). Headline often reduced the disease incidence and severity on MR and R cultivars but did not increase the yield substantially.

**Conclusions:** Early application of pyraclostrobin or azoxystrobin reduced the impact of blackleg on canola when cultivar was lack resistance and the disease pressure was high. Foliar fungicide treatment for blackleg was of little benefit for MR and R cultivars.

**Reference**

Integrated pest management of the rape winter stem weevil (*Ceutorhynchus picitarsis*) in France

**Background:** Rape winter stem weevil (*Ceutorhynchus picitarsis*) is a major pest for winter oilseed rape (WOSR) in France. Adults colonize fields in autumn to feed and lay eggs. Larvae are the harmful stage, since they grow in leaf stalks in winter and autumn and can migrate into the heart of the plant and destroy the terminal bud. Since 2009/2010, infestations have become very important in some French areas and despite repeated treatments, farmers are unable to control them. Several hypotheses can explain this situation, like re-infestation or resistant populations to pyrethroids.

**Objectives:** Since autumn 2011, CETIOM (The Technical Center for Oilseed Crops and Industrial Hemp) carried out studies to 1) understand the phenomenon, 2) develop new management strategies less dependent on insecticides.

**Methods:** For 3 years, CETIOM has monitored rape winter stem weevil autumn flights in untreated fields thanks to three kinds of traps. Coupled with this work, experiments were led to determine the more effective date of treatment, based on the egg laying dynamic. A monitoring was also performed to know if weevil populations were resistant to pyrethroids: insects were exposed in small bottles to several insecticide doses. Finally, we have been testing new strategies like associating frost-sensitive legume crops with WOSR or improving crop establishment. We also compared different varieties.

**Results:** In some experiments, we showed that there was little or no difference between plots, regardless of the date of treatment. Rape winter stem weevil arrivals were often spread out but this could not explain the lack of difference between plots in some experiments. The resistance monitoring showed that in these experiments, there were pyrethroid resistant rape winter stem weevil populations. Associating frost-sensitive legume crops with WOSR and improving crop establishment sometimes reduced weevil harmfulness.

**Conclusions:** In France, with the expansion of resistant populations to insecticides and the reduction of authorized chemicals, there is more than ever an urgent need to develop new management strategies which are less dependent on insecticides.

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Impact of neonicotinoids suspension in EU28

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Background: While the global number of managed honeybee colonies is increasing, there are still honeybee health challenges to solve. Research is under way to determine the cause of the Colony Collapse Disorder (CCD). The recent suspension of neonicotinoids in Europe in some crops attests to the growing concerns surrounding honey bee decline and the use of certain pesticides.

In 2012 a French-based study published in Science concluded that a small percentage of free-ranging honeybees whose brains were dosed with thiamethoxam at levels far in excess of field level doses became confused, failing to return to the hive.

The European Commission asked the European Food Safety Authority to study the safety of neonicotinoids. The study, published in January 2013, claimed that a high acute risk to honeybees was identified from exposure to neonicotinoids via dust drift from seed treatment uses in maize, oilseed rape and cereals. The study also claimed that an acute risk was identified from exposure via residues in nectar and/or pollen, but only at unrealistically high levels. On 29 April 2013, 15 of the 27 European Union Member States voted to restrict the use of imidacloprid, clothianidin and thiamethoxam for two years from 1 December 2013.

The economic impact of neonics suspension in the European Union: The Project Compass research report published by the Humboldt Forum for Food and Agriculture, an independent analysis, assessed the value of neonicotinoids in corn, sugar beet, oilseed rape, wheat, barley and sunflower across the EU economy. It was found that the neonicotinoids contribute to a total of 2.1 billion EUR p.a. to crop commodity market revenues, and that they improve production efficiency by 0.7 billion EUR p.a.

A partial suspension of neonicotinoids significantly reduces the competitiveness of EU production, reduces crop diversity and makes Europe more dependent on global imports of animal feed. The report forecasted that up to 50,000 full-time jobs could be lost, and growers would suffer an average income loss of up to about 5%.

Growers faced in the 2014 fall planting season challenges to establish the winter oilseed rape without neonicotinoids as seed treatment. In UK and Germany for instance, farmers had no choice but to return to older and less effective foliar insecticides to control important pests such as Cabbage stem flea beetles. The suspension will negatively impact the oilseed rape planted area due to the losses farmers face.

Looking into the future: The US Agriculture Department and the EPA concluded that neonicotinoids were significantly down the list of possible CCD contributors. They cited as primary drivers colony management, viruses, bacteria, poor nutrition, genetics and habitat loss. By far the biggest culprit—the report called it “the single most detrimental pest of honeybees”—was identified as the parasitic mite Varroa destructor.
The effect of fungicides on *Leptosphaeria biglobosa* and *L. maculans*, phoma stem canker severity and oilseed rape yield

**Background:** Phoma stem canker, a disease of oilseed rape (*Brassica napus*) caused by closely related pathogens *Leptosphaeria biglobosa* and *L. maculans*, is an economically important disease causing annual yield losses of approximately £1000M worldwide (Fitt et al., 2008). Both pathogens follow a monocylic disease cycle causing leaf spotting in autumn/winter and stem cankers in spring/summer. Severe cankers decrease transportation of water and nutrients to the developing seeds, resulting in reduced yield (Eckert et al., 2009). When colonising oilseed rape, *L. biglobosa* and *L. maculans* exist in close proximity on the leaf - competing for resources as they move down the main leaf vein and into the plant stem (Fitt et al., 2006). Fungicides are commonly used to decrease severity of phoma stem canker on oilseed rape. However, the efficacy and longevity of active chemicals is under threat from continuously evolving pathogen types (Carter et al., 2014).

**Objective:** To identify what effect commercially applied fungicides have on *L. biglobosa* and *L. maculans* interactions, phoma stem canker severity and oilseed rape yield

**Methods:** Winter oilseed rape field trials were done for three cropping season and phoma leaf spotting and phoma stem canker severity were assessed. Species composition in the stems of both upper stem lesions and basal stem cankers in the 2013/2014 cropping season was assessed using QPCR. The airspora for ispecies was monitored using a Burkard spore sampler, and species-specific DNA was quantified using QPCR. Fungicide sensitivity tests in vitro were done using fungicide amended agar plates at differing concentrations.

**Results:** The two pathogens differed greatly in their growth rates in vitro, with *L. biglobosa* growing much faster than *L. maculans*. The EC50 values show that *L. biglobosa* is significantly more tolerant of azole-amended media than *L. maculans*. Refinzar, containing active ingredients (a.i.) penthiopyrad and picoxystorbin, was as effective at controlling phoma leaf spotting and phoma stem canker in the field as Proline (a.i. prothioconazole).

**Conclusions:** Differing sensitivities to azole fungicides could be selecting *L. biglobosa* resulting in an epidemic where current fungicides do not fully control the disease. A combination of SDH I + QoI fungicides could be used to control epidemics caused by a *L. biglobosa*.

**References:**


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Pathogenic variability of *Sclerotinia sclerotiorum* isolates on *Brassica* differentials

**Background:** Sclerotinia rot (SR) caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is a major disease of oilseed *Brassica* all over the world. It is a ubiquitous, omnivorous, soil-borne phytopathogenic ascomycetes fungus capable of infecting more than 500 host species (Sharma et al. 2015). Infection occurs on leaves, stems and pods at different developmental stages, causing seed yield losses of up to 80%, as well as significant reductions in oil content and quality. Apart from favourable weather conditions and high soil moisture, germination of overwintered sclerotia, release, survival and germination of ascospores are important factors for the development of disease.

**Objectives:** Resistance in oilseed *Brassica* against the disease is lacking, only partial tolerance to *S. sclerotiorum* has been reported (Sharma et al. 2012). Keeping in view, the different *Brassica* differentials were challenged against 25 geographical isolates of *S. sclerotiorum* to confirm the variation among pathogen population and genetic difference in host species.

**Methods:** Twenty five geographical isolates of *S. sclerotiorum* causing SR of *Brassica* were collected during 2009-12 from 25 locations in 9 states of India and was maintained in vitro. An experiment was conducted to study the pathogenic variability during 2012-13 at ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur, India (77°27’E, 27°12’N; 178.13 m MSL). Nine *Brassica* differentials i.e. *B. juncea* (cv. Rohini) *B. carinata* (cv. Kiran) *B. rapa* var *toria* (cv. PT 303), *B. rapa* var yellow sarson (cv. NRCYS 5-2), *B. rapa* var Brown sarson (cv. KOS 1), *B. nigra* (cv. BN-1), *B. napus* (cv. GSC6), *Eruca sativa* (cv. T-27) and *B. alba* were used during the study. These were sown in two replications and 65-70 days after sowing, plants were inoculated with stem inoculation technique. 3-weeks after inoculation the observations on stem lesion length and per cent disease incidence were recorded.

**Results:** All the 25 different geographical isolates showed significant variation in stem lesion length (cm) and per cent disease incidence. Based on pathogenic variability the isolates can be grouped as highly virulent (SR-06 and SR-10), virulent (SR-01, SR-02, SR-04, SR-08, SR-12, SR-15 and SR-25), moderately virulent (SR-03, SR-07, SR-09, SR-11, SR-20, SR-21 and SR-24) and less virulent (SR-05, SR-13, SR-14, SR-18, SR-19, SR-22 and SR-23). Highly susceptible *Brassica* differential were all var of *B. rapa* and *E. sativa* while highly tolerant was *B. alba* (lesion size 0.5-1.9 cm).

**Conclusions:** Morphological variability and genetic diversity of different geographical isolates of *S. sclerotiorum* were already proved. The present study demonstrated existence of pathogenic variability among the geographical isolates which could be helpful to design resistance breeding for *S. sclerotiorum* in oilseed *Brassica*.

**References:**
Screening of germplasm resources for resistance to *Plasmodiophora brassicae* in *Brassica napus* L.

**Background:** Clubroot, induced by *Plasmodiophora brassicae*, endangers more than 100 species of plants, mainly in the tribe of *Brassicaceae*. The threat of clubroot has become more and more prominent to *Brassica* crops, including rapeseed (*Brassica napus* L) and *Brassica* vegetables in China (Liang Y, et al. 2001). In Sichuan Province, one of the biggest rapeseed producers in China, clubroot is especially spreading rapidly in recent years. The occurrence of the disease has quickly expanded from the western plain areas to the central and eastern hilly areas in the province, causing an increasingly heavy loss of yield and quality in rapeseed. However, resistant rapeseed varieties are lacking and other control practices could only exert limited effects on the disease (Liu Y et al. 2009). Breeding for resistant varieties to clubroot in *B. napus* L is imperative.

**Objectives:** Screening of clubroot resistant materials from the existing rapeseed varieties and resource materials can be a rapid way to battle with the clubroot disease and will facilitate the breeding for resistant varieties in *B. napus* L. (Liu Y et al. 2009; Fu M L et al. 2011). The major objectives of the present study are to know the status of resistant materials in our own resource breeding materials and to screen out clubroot-resistant materials for breeding of clubroot resistant varieties.

**Methods:** A total of 279 lines of *B. napus* L. from our breeding stock were selected to identify the resistance to clubroot (*P. brassicae*), including 160 pure lines and varieties collected from rapeseed institutions in China, 93 elite inbred lines and 26 resynthesized (RS) lines developed in our own Rapeseed Research Center. Spores of *P. brassicae* were isolated from root tumors of infected rapeseed plants (*B. napus* L) collected from the severely infested rapeseed fields in Dayi county on the plain area of Sichuan Province. The suspension of spores was adjusted to a density of 1 x10⁸ / ml. The inoculation to the rapeseed seedlings was made at the 2-true-leaf stage in greenhouse. The identification of disease incidence and severity index in the tested lines was done for 20 to 30 plants in each line, 50 days after inoculation. In order to confirm the results from the first primary tests, 54 selected lines comprising relatively highly resistant, moderately resistant, slightly resistant and susceptible lines were tested again with three replications by the same procedure. Classification of resistance grades of the tested lines was evaluated with a five-grade standard based on the disease severity indices: highly resistant (<30.0), moderately resistant (30 – 40), slightly resistant (40.0 – 50.0), susceptible (50.0 – 80.0), highly susceptible (> 80.0).

**Results:** In all the 279 lines of the first primary test, only one line (H484-2, RS line) was found to be very low in disease incidence (16.67%). The all others showed a high incidence ranging 85 to 100%. Ninety-seven percent of the tested lines showed an incidence of above 90%. The severity indices of all the lines ranged from 7.14 and 97.28. Only 2 lines (H484-2, RS line and D4TZ, progeny of German line X RS line) showed a low severity index of < 30.0, grouped in the highly-resistant category. Seven lines, including 2 canola-quality lines, showed relatively low severity indices between 40.0 and 50, grouped in the slightly resistant category. All the others showed high severity indices larger than 50.

In the 54 lines for the second test, the average incidences of infected plants ranged from 9.83% to 100%. About 57% of the lines showed an incidence of over 80%. The severity indices were between 4.66 and 79.88. Six lines were evaluated in the highly-resistant category, 4 lines in the moderately-resistant category, and 26 lines in the slightly-resistant category. Summarizing the two consecutive experiments, only two lines (H484-2, D4TZ) were confirmed to be consistently highly resistant, and 2 lines (H218-4, NLS-1) to be consistently slightly resistant to clubroot.

**Conclusions:** Based on the two tests, we concluded that in our resource materials of *B. napus* L., highly resistant materials to clubroot disease are in a dramatic shortage. Only 2 lines were confirmed to be highly resistant and 2 confirmed to be slightly resistant. Meanwhile, the occurrence and severity of clubroot disease are affected by many factors. All the lines should be further tested with different pathotypes of *P. brassicae* and under strictly controlled conditions. Especially, the materials with a severity index of less than 50 must be evaluated again, to procure the reliable highly resistant lines.

**References:**


Use of the effector-triggered defence concept to identify candidate resistance genes

Background: Effector-triggered defence (ETD) is a plant response against invading apoplastic fungal pathogens (Stotz et al., 2014). This defence response depends on recognition of apoplastic effectors by receptor-like proteins (RLPs). Based on the assumption that RLPs play a critical role in defence against apoplastic pathogens, new opportunities for disease resistance breeding have emerged. Oilseed rape is attacked by apoplastic pathogens, including *Leptosphaeria maculans* and *Pyrenopeziza brassicae*, which cause phoma stem canker and light leaf spot, respectively. The first RLPs cloned from oilseed rape operate against *L. maculans*.

Objectives: The main objective is to define the RLP complement of *Brassica napus* from its recently published genome. The distribution of RLP genes and other aspects of genomic information are used to determine their usefulness in predicting resistance genes operating against apoplastic pathogens of oilseed rape.

Methods: A protein motif-based search was used to define the RLP complement in *B. napus*. This information has been used to visualize all predicted RLPs on the 19 chromosomes of *B. napus*. This data will be statistically analysed to develop a tool to assist in resistance gene identification. To understand physical interactions between leucine-rich repeat (LRR) domains of resistance (R) proteins and corresponding effectors that are directly recognized by host receptors, computational molecular docking was used. Structures of complexes were predicted from the known structures of effectors and models of LRR domains. This will be relevant for interactions between *Rlm2* and *AvrLm2* and *Rlm4* and *AvrLm4*.

Results: The distribution of RLPs across the *B. napus* genome has been visualized. Like nucleotide-binding LRR receptors (NLRs) that operate against haustoria-forming filamentous pathogens, RLPs are clustered. The distributions of NLRs and RLPs differ in oilseed rape.

Conclusions: Based on the genomic analyses of RLPs, new tools will become available to accelerate cloning of R genes that control ETD. Genome-guided methods have already been used to identify R genes that encode cytoplasmic NLRs; these are important for effector-triggered immunity (ETI) against haustorial pathogens. More detailed information on specific amino acids in R proteins that interact with pathogen effectors can be derived from docking of interacting proteins.

References:
A hidden pathogen with uncommon properties – reviewing the state-of-the-art of *Verticillium longisporum* on oilseed rape

**Background:** *Verticillium longisporum* (VL) is a soilborne vascular pathogen of oilseed rape (*Brassica napus*) which causes premature ripening associated with potential significant yield losses. First reports on the disease date back to the 1960s in Sweden and the 1980s in North-East Germany, but more recently outbreaks of the disease were observed in the UK, France and Poland. In 2014, occurrence of VL has been confirmed for the first time in Canada (Manitoba). The pathogen is an amphihaploid hybrid of *Verticillium* and host-specific on *Brassicaceae*.

**Objective:** This research aims at elucidating the interaction of VL with oilseed rape, particularly focusing on differential resistance responses in *B. napus* lines and the damage potential of the disease in the field.

**Methods:** *B. napus* accessions and their parental lines have been screened in the greenhouse and field. Interactions on plants inoculated in the climate chamber or laboratory were studied on the phenotypic, histological (CLSM), transcriptomic and metabolomic (physiological and biochemical) level.

**Results:** Three different hybrids representing three genetically distinct lineages have been identified so far and were shown to differ in host specificity (Novakazi et al. 2015). Hyphae of VL attach to the roots in the root hair zone and penetrate the rhizodermis to colonize the root parenchyma intra- and intercellularly (Eynck et al. 2007). The lack of ROS generation or cell death responses by the plant during these early stages of interaction implies a biotrophic or even endophytic relationship. Following root invasion, the fungus remains strictly xylem-limited and colonizes the hypocotyl, where intense plant responses are triggered as expression of cultivar resistance (Eynck et al. 2009). Resistance derives from the C-genome of *B. napus* (AACC). Cultivar resistance is expressed in the hypocotyl and consists of fast and massive parenchymatic accumulation of cell wall-bound phenols, lignin and vessel occlusions, obviously halting the pathogen from further spread into the shoot. No drought stress parameters (stomatal conductance, transpiration rate, gas exchange, photosynthesis rate, proline content, leaf water content) are induced under VL infection. VL induces salicylic acid (SA) dependent genes PR-1 and PR-3 while the jasmonic acid/ethylene dependent PDF1.2 does not respond. VL induces strongly elevated levels of SA/SAG in xylem sap and stem parenchyma, which surprisingly correlate with susceptibility and disease severity. Increased levels of SA/SAG in diseased plants may indicate a fungus-induced rerouting of precursors from the phenylalanine/cinnamate pool towards SA synthesis depriving synthesis of CW-bound phenolics crucial for resistance. In contrast, SA deficient nahG transformed OSR plants exhibit a strongly elevated susceptibility to VL.

**Conclusions:** The findings so far demonstrate that VL is an atypical vascular pathogen in not inducing any wilt in its host. It is host-specific to *Brassicaceae* but may expand its host range by forming diverse pathotypes. Cultivar-specific resistance derives from the C-genome, is partial and quantitative, halting the fungus only at the hypocotyl interface. Resistance is dependent on CW-bound phenolics and lignin. A dual role of SA exists in basal and cultivar-specific resistance of *B. napus* to VL.

**References:**


DNA-based soil test a prerequisite for Swedish oilseed rape production

**Background:** Clubroot caused by *Plasmodiophora brassicae*, is recognised as a serious soil-borne disease in *Brassica* crops associated with appreciable yield losses. Disease outbreaks have in recent years caused problems in Sweden and worldwide in oilseed rape (OSR). Resistant cultivars of winter oilseed rape (WOR) are now available for the growers. Fast and reliable detection methods are a prerequisite for prediction of the infection potential of clubroot in field soils for implementing integrated management strategies in OSR production and crop development.

**Objectives:** Development and validation of a *P. brassicae*-specific real-time PCR assay, and to use this assay as a quantitative measure for direct detection of *P. brassicae* in naturally infested soil samples. Further, to combine these quantitative measures with methods for spatial prediction to assess the risk of disease development within fields.

**Methods:** A procedure using real-time PCR for the direct detection and quantification of *P. brassicae* in soil samples was developed and used for naturally and artificially infested soil samples containing different concentrations of *P. brassicae*. Bait-plants were used to validate the real-time PCR assay. The spatial distribution of *P. brassicae* DNA was determined and indicator kriging was used for spatial prediction to assess the probability of disease development within fields.

**Results:** Species-specific primers and a TaqMan fluorogenic probe were designed to amplify a small region of *P. brassicae* ribosomal DNA. The PCR assay was optimised to give high amplification efficiency and 3–4 copies of the target DNA sequence were detected. The inter-sample reproducibility was similar to, or higher than, that of assays for other pathogens quantified in soil samples. The detection limit in soil samples corresponded to 500 resting spores g⁻¹ soil and the estimated probability of clubroot based on an increased sampling density (n=40) showed an overall spatial trend in the variation of the amount of *P. brassicae* inoculums.

**Conclusions:** The integration of resistance as a management tool, along with other control measures, offers a robust management strategy. Soil tests are a prerequisite for growers and for successful field experimental activities. This rapid and sensible assay for predicting infection potential and distribution within fields for routine risk assessment is now commercially available in Sweden. The assay is used as guidance prior to seeding susceptible crops and as a routine for the Swedish Seed and Oilseed Growers Association for determining field experimental sites in farm fields. Data for interpreting soil tests are currently based on results from field tests of partly resistant cultivars of summer oilseed turnip rape. Activities are ongoing to carefully follow the reactions of resistant cultivars of winter oilseed rape at field level. Complementary knowledge of the prevalent pathotypes is required, since tolerance in resistant cultivars is observed and seems to vary in response to different pathotypes of clubroot.

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Overexpression polygalacturonase-inhibiting protein 2 of rice inhibits *Sclerotinia sclerotiorum* polygalacturonase necrotizing activities and improves resistance in transgenic *Brassica napus*

**Background:** Canola (*Brassica napus L.*) is an agriculturally and economically important crop in China, and its growth and yield are influenced by fungal pathogens. *Sclerotinia sclerotiorum* is a necrotrophic plant pathogen that causes stem rot disease in *B. napus*. *S. sclerotiorum* secretes several types of pectin-degrading enzymes, including pectin methylesterases, pectin lyases, and polygalacturonases (PGs). Polygalacturonase-inhibiting proteins (PGIPs) are typically leucine-rich repeat (LRR) proteins counteracted the action of PGs. PGIPs prevent cell wall degradation, therefore hamper the invasion process and the release of nutrients that is necessary for pathogen growth. Overexpression of *Atpgip1* and *Atpgip2* in *Arabidopsis* limits the colonization by *B. cinerea* and reduces disease symptoms. Overexpressing a pear pgip can increase PG-inhibitory activity and a decrease in susceptibility to *B. cinerea* in transgenic tomato and grapevine plants (Di Matteo A et al. 2006). In rice, *OsPGIP* was able to inhibit the PG of *Fusarium graminearum* (Lu et al. 2012). These findings suggest PGIPs are important players in plant innate immunity.

**Objective:** Overexpression of *OsPGIP2* in partial resistant *B. napus* 7-5, T45 and susceptible P61-5 material will improve resistance to *S. sclerotiorum*. We hope to discover the molecular mechanism of *OsPGIP2* interaction with *S. sclerotiorum* PGs (SsPGs).

**Methods:** *S. sclerotiorum* was cultured at 20 °C on potato dextrose agar. Mycelia plugs (8 mm diameter), excised from the edge of actively growing colonies and inoculated onto detached leaves and stem. Expression of SsPGs and *OsPGIP2* were induced by *Pichia pastoris* system. ROIs (Reactive Oxygen Intermediates) production and cell death were determined by DAB and Evans blue staining.

**Results:** The *OsPGIP2* overexpressed lines of three different materials exhibit a delay in the onset of symptoms upon *S. sclerotiorum* inoculation in seedling stage and adult stage, compared to their individual untransformed plants (WT). DAB and Evans blue staining suggest *OsPGIP2* lines have lower ROIs and less cell death than their WTs. *Pichia pastoris* system and in vitro analysis indicate *OsPGIP2* inhibits SsPG6 enzymatic activity. Defensive related genes involved in jasmonic acid and ethylene (JA/ET) or salicylic acid (SA) signaling pathway expression analysis with inoculation reveals that six genes (*BnCCR* *BnOPR* *BnLectin* *BnWRKY6* *BnSOT* and *BnPDF1.2*) show enhanced expression in transgenic lines derived from three individual materials. JA/ET signaling pathway in *OsPGIP2* transgenic lines is obviously activated by *S. sclerotiorum*.

**Conclusions:** The molecular mechanism of interaction between PG-*OsPGIP2* could be a help in understanding *OsPGIP2* can improve the resistance to *S. sclerotiorum*. Field protection assay should be estimated for transgenic lines derived from three materials. We will focus on the resistance pathways initiated with *OsPGIP2* concerning *Brassica napus* PGIPs.

**References:**


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Efficiency of major gene mediated resistance in *Brassica napus* to *Leptosphaeria maculans* in different regions of Germany

**Background:** Blackleg disease, caused by *Leptosphaeria maculans* (LM) is one of the most important fungal diseases in oilseed rape (OSR) production worldwide (Fitt et al. 2006). Genetic resistance is an important tool to control this disease. Seedling resistance is conferred by single major genes. Due to its sexual propagation, LM isolates evolving rapidly from avirulent to virulent strains on cultivars harboring major resistance genes. Therefore, resistance of OSR against LM conferred only by major resistance genes was often overcome and led to severe yield losses in the past (Rouxel et al. 2003; Sprague et al. 2006).

**Objectives:** The aim of this study was to determine the efficiency of major resistance genes to LM in different OSR growing regions in Germany by identifying the frequency of virulent isolates and determining the race spectra of LM.

**Methods:** We cultivated two OSR cultivars in fields throughout Germany from 2011 to 2014: i) ‘NK Bravour’ harboring no known major genes against LM (serving as trap crop) and ii) ‘Exocet’ harboring the efficient major gene *Rlm7* to observe resistance breakage in the field. In autumn and spring we collected true leaves with typical Phoma lesions to gain isolates of LM. Single pycnidia isolates were tested with a French and Canadian differential set through cotyledon inoculation for their virulence to different major genes. The differential set consisted of 10 OSR genotypes harboring the major genes *Rlm1, Rlm2, Rlm3, Rlm4, Rlm7, Rlm9* and *LepR1, LepR2* and *LepR3*. Thereby, the frequency of virulent isolates in a region was determined. Isolates showing the same complement with virulence alleles were grouped to the same race.

**Results:** The frequency of isolates being virulent to *Rlm1, Rlm2, Rlm3, Rlm4* and *Rlm9*, respectively, was above 85%. Conversely, the frequency of virulent isolates to Rlm7 was very low (< 5%). Interestingly, the frequencies of isolates being virulent to the major genes *LepR2* and *LepR3* showed a high variability between different regions, ranging from 35% to 100%. There was no isolate showing virulence to LepR1. Most isolates belonged to two races with a high virulence complexity.

**Conclusions:** Most tested major genes lost efficiency to LM. Only *Rlm7* and *LepR3* are still mediating resistance in OSR to PM in Germany. We assume that *Rlm7* may lose its efficiency with increasing deployment of this major gene in OSR in Germany.

**References:**


Mapping and SNP marker development in *Brassica* diploid species for breeding canola and mustard resistant to clubroot

**Background:** Clubroot disease, caused by *Plasmodiophora brassicae*, is an ever increasing problem in canola and mustard production in western Canada. A number of clubroot resistant (CR) canola (*Brassica napus*) hybrids are now available in Canada. However, there is some urgency about the erosion of CR in these hybrids with the emergence of a new pathotype. Identification of novel CR genes and pyramiding of these genes is the most effective approach to develop varieties with durable and broad spectrum resistance. However, the sources of resistance to clubroot in *B. napus* are very limited and no resistance was found in the mustard species *B. juncea* and *B. carinata*. Resistant germplasms highly resistant to clubroot were identified in the diploid *Brassica* species *B. rapa*, *B. oleracea* and *B. nigra* (Peng et al. 2014).

**Objectives:** Map CR genes from newly identified CR in diploid species using next generation sequencing technologies; develop SNP markers tightly linked to CR genes and transfer CR genes into canola *B. napus* and mustards *B. juncea* and *B. carinata*.

**Methods:** Segregating populations were made by crossing CR lines with susceptible lines. DNA sequencing or RNA-sequencing was performed with Illumina HiSeq or MiSeq platforms. Validation and genotyping of SNPs were carried out using the KASP method. Introgression of resistance to the amphidiploids was carried out using conventional breeding and molecular genetic approaches.

**Results:** Identification of CR genes was carried out in five *B. rapa* cultivars/breeding lines and five CR genes *Rcr1* to *Rcr5* were fine mapped. *Rcr1* was identified from bok choy, *Rcr2* from Chinese cabbage, *Rcr3* and *Rcr4* from canola and *Rcr5* from turnip. Four genes (*Rcr1, Rcr2, Rcr4* and *Rcr5*) were identified on *B. rapa* chromosome A03 and one (*Rcr3*) on A08. More than 30 robust SNP markers associated with the CR genes on A03 and four with the CR gene on A08 were developed. CR gene *Rcr6* was identified in *B. nigra* and mapped into *B. nigra* chromosome B3 corresponding to a region in *B. rapa* chromosome A08. In *B. oleracea*, one gene *Rcr7* was mapped on chromosome C7. Interspecific crosses with a *B. napus* canola line were performed using the CR donors in the diploid species and then back crossed with the *B. napus* canola line. Resistance to clubroot was confirmed in the introgressed *B. napus* lines by evaluation for CR and marker assisted selection. Amphidiploid lines in *B. napus*, *B. juncea* and *B. carinata* resistant to clubroot were resynthesized using CR lines from the respective diploid species.

**Conclusions:** Seven CR genes were mapped in all three *Brassica* diploid species and sexually transferred to the amphidiploids. Robust SNP markers tightly linked to each gene are available for marker assisted selection in canola and mustard breeding programs.

**References:**

Structural organization of lipid droplets in two rapeseed genotypes is linked with seed lipid content and oil extractability

**Background:** The worldwide oilseed production will face an increasing demand in the next decades due to a higher consumption for edible oil, the development of the biofuel industry, and the needs of molecules for green chemistry. Seed lipids due to their diversity constitute a unique renewable source for food, feed, energy and chemistry. They are stored in specialized organelles, lipid droplets (LDs), with specific structure conserved among living organisms, a neutral lipid core surrounded by a monolayer of phospholipids in which a variable number of proteins is embedded (Huang 1992). Longly considered as inert balls of fat, LDs are now considered as organelles, with complex protein and lipid compositions and their own dynamics (Beckman 2006). In a former work, we have suggested a link between seed oil extractability and LD stability (Jolivet et al 2013). Deciphering the molecular basis of such link could help to save energy used during oil rapeseed extraction.

**Objectives:** LD stability is a consequence of protein – phospholipid interactions, and is partly responsible for the difficulty to extract oil from rapeseed. The respective role of these two components was evaluated using two rapeseed genotypes to perform a detailed study of LD characteristics in mature seeds as well as throughout seed development.

**Methods:** Amber and Warzanwski accessions were chosen because their mature seeds differ (i) in crushing ability evaluated by a micro-pressing technique (Savoire et al 2010), (ii) in oil extraction yield and, (iii) in the stability of their purified LDs. LD morphology and size determination were investigated by microscopy, and pulsed-field gradient NMR (PFG-NMR). LD composition into triglycerides, phospholipids and proteins was compared between the two accessions.

**Results:** PFG-NMR and microscopy allowed revealing in situ a LD size difference between Amber and Warzanwski. Amber LDs were enriched with H-oleosins and steroleosins suggesting a better coverage of LD surface. Their phospholipid composition showed an increase in phosphatidylserine content facilitating lipid-protein interactions and a decrease of polyunsaturated species suggesting a more rigid structure.

**Conclusions:** PFG-NMR is a powerful non destructive method to characterize LDs in mature seeds. Differences found in composition of LD surfaces could explain uneven behaviours in Amber and Warzanwski seeds’ treatment.

**References:**
Jolivet P, Deruyffelaere C, Boulard C, Quinac A, Savoire R, Nesi N, Chardot T, 2013 Deciphering the structural organization of the oil bodies in the *Brassica napus* seed as a mean to improve the oil extraction yield. Industrial Crops and Products 44: 549-557
HPLC determination of bioactive compounds in canola oil for varietal screening

**Background:** Oil yield and oil stability in canola have been increased via successful breeding programs, but with the continuing growth of worldwide canola production, there is a need to improve the marketability of canola oil. Recent interest in the enhancement of potentially health-beneficial bioactive components present in the oil has developed (Ghazani et al., 2013; Szydlowska-Czerniak, 2011). Tocopherols, sterols and carotenoids are classes of bioactive components present in crude canola oil that can exhibit health-benefits when incorporated into the human diet. Methods to quantify these compounds in food matrices often involve rigorous sample preparation that minimises analyte degradation, resulting in time and cost expenditure (Azmir et al., 2013).

**Objectives:** To develop a robust HPLC method to simultaneously analyse canola oil samples for tocopherols, carotenoids, free sterols and esterified sterols, which reduces sample preparation and analysis time in comparison to previous techniques. To use this method to analyse a large population of Australian genotypes to determine genotype influence on the compounds of interest.

**Methodology:** A new method was developed using HPLC-MS/MS to simultaneously quantify tocopherols, carotenoids and sterols in both free and esterified forms in canola oil. DAD was used to monitor target wavelengths and provide a second level of quantification for some compounds. This method forms the foundation for a study to investigate the influence of canola variety on these trace analytes using 64 different canola genotypes grown in controlled trials in two regions in Australia. REML analysis of G x E effects will be performed to illustrate the degree of influence, providing useful information for breeding programs targeting genotypes with enhanced levels of these compounds.

**Results:** The newly developed method reduced sample preparation time, reducing the overall time and cost associated with analysis. The high selectivity of the method allowed the individual quantification of co-eluting sterol compounds that has prevented the use of normal phase chromatography in the past. Further application of this method could result in the calibration of a rapid NIR method for use in industry.

**Conclusions:** The developed method will be applied in further studies examining genotype influence and processing and storage parameters aided to enhance the bioactive components in oil.
Reduction of sinapine content in rapeseed (*Brassica napus L.*) by induced mutations in sinapine biosynthesis genes

**Background:** Sinapine is the most prominent antinutritive compound in the seeds of oilseed rape (*Brassica napus L*). A reduction in sinapine content could improve the quality of rapeseed meal as an animal feed and in food industry. As natural variation for seed sinapine content is limited in rapeseed, mutation screening is a reasonable approach to isolate low-sinapine genotypes.

**Objectives:** Our aim is to identify low-sinapine genotypes with loss-of-function mutations in sinapine synthesis genes. We try to study gene dosage and background effects in mutant combinations.

**Methods:** We screened a winter rapeseed EMS TILLING population for sinapine synthesis mutants. Single mutants were combined by crossing and phenotyped using HPLC and enzymatic analysis.

**Results:** In *Brassica napus* seeds, two paralogs of the sinapine synthesis genes SGT and REF1 are expressed. We identified and combined two stop codon mutants of the SGT and the REF1 paralogs and a stop and a splice site mutant of the REF1 paralogs by crossing and analyzed the segregating F2 offspring. Sinapine contents in the double mutants dropped dramatically by up to 71%. F3 seeds with two stop codon mutations in REF1 genes had the lowest sinapine contents (2.4 mg/g) as compared to the EMS control (7.5 mg/g). A REF1 splice site mutation did not result in a decrease in seed sinapine content probably due to incomplete splicing. Significant depletion of SGT enzyme activity in developing seeds proved loss-of-function of both gene copies and ruled out background effects. REF1 enzyme activities showed minor reductions and pointed at different substrate specificities of the paralogs and the presence of unspecific aldehyde dehydrogenases.

**Conclusions:** We demonstrate that only the combination of different knock-down mutations drastically alters the composition of a major secondary metabolite. The results cast new light on the activities of gene paralogs in a polyploid species.

**References:**
Fractionation of rapeseed from oil extraction to minor products

Starting with the composition of the seed, the presentation illustrates the breakdown of the seeds, mainly by mechanical processes.

The main product is still the oil. But since the demand for digestible vegetable proteins is increasing and different factors have a negative impact on the oil price, too, other by-products and new processes are becoming more interesting for oil producers and oil refining costs are more and more in focus.

Different steps will be illustrated: 1) Seed and pre-treatment, 2) De-oiling, 3) Oil and derivative processing, 4) Cake and flakes processing and finally there are some reflections on the by-products from both sides: 5) those from the oil fraction and those from the cake or expeller side.

The oil and the protein content, the hull thickness and therefore the fibre content, the sinapin and phytic acid content, the glycosinolate content etc. are not only given and fixed in the incoming raw material. All this can be influenced already with the first step: the pre-treatment and the kind of process used for the oil extraction.

Consequently, the oil refining starts between 10 ppm phosphorous or < 1000 ppm phosphorus in the oil depending on the extraction technique, but what is not in the extracted oil remains in the cake or expeller, meaning phosphorus, too. That influences the percentage of the substances in each fraction and ultimately the processes itself.

Sometimes the focus of the process is in the reduction of hulls and fibers in the cake used for feed without any reduction of the nativity of the proteins. Other research teams are looking for purified protein isolates with a high potential to adsorb water and/or oil or to be a good emulsifier. Research projects like SynRG (financed by FNR/BMELV) are supported in order to isolate minor components like polyphenols and to implement these in polymerization of dicarbonic acids made out of plant seed oils.

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Comparison of canolol content and antioxidant capacity of solvent-extracted oil from pretreated rapeseed with microwave

**Background:** To overcome the low oil extraction yield problem in cold pressing, it is advisable to seek new pretreatment instead of the usual thermal treatment of seeds before pressing. By using microwave radiation, a higher extraction yield can be obtained because the cell membrane is ruptured (Azadmard-Damirchi et al., 2010; Uquiche, Jeréz, & Ortíz, 2008; Yang et al., 2013). Although oil extraction yield by pressing from oilseed increased according to the application of microwave radiation, a part of oil still remained in the pressed-cake and they can be extracted afterwards by solvent. When pressing the oil from rapeseed, some of the phenolic compounds are transferred to the oil and most of them remain in the meal. However, there is a lack of information in the literature on the effect of pretreatment with microwave of rapeseed on total phenolic and canolol contents in oil from rapeseed pressed-cake extracted by solvent. Moreover, there is a lack of information in the literature on the comparison of total phenolic and canolol contents, and antioxidant capacity between pressed oils from rapeseed pretreated with microwave and solvent-extracted oils from pressed-cake, too.

**Objectives:** In this work, rapeseed is pretreated with microwave, then, the seed is pressed and the press-cake is extracted with solvent. The effect of conditioning time on the amount of total phenolic and canolol content of the obtained oil during the microwave pretreatment of rapeseed is studied. Also, 2, 2-diphenyl-1-picylhydrazyl (DPPH) and the ferric-reducing antioxidant power (FRAP) of both oil samples are monitored and the correlations are analysis between antioxidant capacities and phenolic content.

**Methods:** Rapeseed was treated with microwave under 800 W for 0, 1, 2, 3, 4, 5, 6, and 7 min at a frequency of 2,450 MHz, then, the seed was pressed and the press-cake was solvent extracted, and the influence of microwave pretreatment and extraction method on the content of total phenolic and canolol content, and antioxidant capacity of the pressed and solvent-extracted oil were evaluated. The influence of microwave pretreatment and extraction method on the content of total phenolic and canolol content, and antioxidant capacity of the pressed and solvent-extracted oil were evaluated. Canolol was synthesized according to the method described by Harbaum-Playda et al. (2010). The identification of Canolol was carried out on the basis of nuclear magnetic resonance (NMR) and mass spectrometry (MS). The MS used for analysis was a hybrid, triple quadrupole/linear ion trap mass spectrometer. The canolol content was quantified on the basis of the calibration curves using a chromatogram at 280 nm.

**Results:** The results indicated that the amounts of total phenolic and canolol present in the oil significantly increased and that their concentrations positively linear correlated with microwave time (r = 0.918 and 0.921) (p < 0.001). The contents of total phenolic and canolol of oil varied significantly (p < 0.01) depending on the extraction method besides microwave time, and total phenolic and canolol concentrations of the solvent-extracted oils were 13.20-32.28 mg/100 g and 29.66-163.85 µg/g. Also, the antioxidant capacities data obtained by the DPPH and FRAP procedures of oil significantly increased with microwave time (for the solvent-extracted oil, r = 0.928 and 0.959) (p < 0.001), and the DPPH and FRAP values of the solvent-extracted oil were 40.32-118.69 µmol TE/100 g and 232.70-445.14 µmol TE/100 g.

**Conclusions:** Microwave pretreatment of rapeseed benefited increasing the phenolic compounds and improving the antioxidant capacity of oil. Compared with the pressed oil, the solvent-extracted oil had the higher total phenolic and canolol content, and the antioxidant capacities determined by the DPPH and FRAP methods. However, the solvent-extracted oil had the higher acid value, peroxide value, lovibond color, and phospholipids content, and must be refined by degumming, decacidification, and bleaching.

**References:**


Bioactive compounds in canola meal

Background: The meal which remains after canola oil extraction is of relatively low value and is used mainly for animal feed (Alashi et al., 2014). This meal may have additional value in the pharmaceutical industry if potential health beneficial bioactive compounds with the ability to combat several modern day ailments could be identified.

Objectives: Canola meal extracts should be prepared using different solvents and characterized. Identification and characterization of protease inhibitors will be undertaken. In vitro antioxidant and bioactive properties of extracts were determined and activities would be further investigated through cellular assays.

Methods: All canola meal extracts (CMEs) were named according to solvent used for extraction. The antioxidant activities in all these extracts were determined by reagent based assay along with High pressure liquid chromatography (HPLC) and Liquid chromatography–mass spectrometry (LCMS) (Obied et al., 2013). Chromatography was used to purify protease inhibitors. All extracts were used in the anticancer assay based on topoisomerase inhibition, antidiabetic activity by dipeptidyl peptidase IV (DPP-IV) enzyme inhibition, antihypertensive activity by angiotensin converting enzyme (ACE) inhibition. Antilipase and cellular assay was used to determine potential antiobesity properties.

Results: The acetone and methanol extracts showed higher antioxidant. The extracts showed varying levels of both the topoisomerase-1 poisoning and inhibition activities which are indicators of anticancer properties. Acetone, butanol, and hexane extracts showed antiobesity activity, inhibiting adipocyte differentiation without causing cell toxicity. Butanol, acetone and water extracts showed high antidiabetic activity by inhibiting the enzyme DPP-IV.

Protease inhibitors (PIs) were also extracted from canola meal and purified to homogeneity from two different canola genotypes. Canola genotype-1 showed very strong antidiabetic activity compared with genotype-2. Water extracts the purified protease inhibitor from genotype-1 showed strong antihypertensive activity through the inhibition of angiotensin converting enzyme (ACE).

Conclusions: These potential bioactive and health-functional properties of canola meal extracts may increase the profitability for farmers, processors, food manufacturers, and the pharmaceutical industry.

References:
Digestibility of protein and energy increase with increasing protein content in rapeseed

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**Background:** Rapeseed is hitherto mainly produced because of its valuable oil content, the residual meal after oil extraction is mostly considered as a medium valued protein product. The amino acid composition of rapeseed is well balanced, but the digestibility of protein and energy is low compared with soybean meal. This is mainly caused by the high content of cell wall materials - non-starch polysaccharides (NSP) and lignin present in rapeseed. The hulls constitute about 28-30% of the dry matter (DM) in oil-free rapeseed meal and contain the highest proportions of NSP and lignin. Thus, removal of hulls results in an improved digestibility of the protein, but to date no commercial beneficial dehulling process has been developed (Jensen et al. 1995). The increasing demand for protein to feed the worlds growing population may put more focus on rapeseed protein.

**Objectives:** The objective of this study was to evaluate digestibility of protein and energy in defatted rapeseed meal from seed with varying protein content.

**Methods:** The rapeseed investigated comprised eight seed samples of double low spring rape (*Brassica napus L.*) obtained from Danisco Seed A/S, Holeby, Denmark and thirty seed samples of double low winter rape (*Brassica napus L.*) from DLF-Trifolium A/S, Store Heddinge, Denmark. The rapeseed samples were milled and defatted by diethyl ether, air dried and analysed for chemical composition by traditional methods.

The defatted rapeseed was autoclaved at 107°C for 20 min before diet formulation in order to inactivate myrosinase and improve palatability. Standard digestibility and N balance trials with rats were performed as previous described on each of the thirty-eight defatted seed samples. The diets were formulated with rapeseed meals as the sole source of dietary nitrogen (15 g N kg⁻¹). The remaining ingredients in the diet were maize starch, vitamins and minerals.

**Results and discussion:** Protein content varied from 17-26% of DM on whole seed basis, while oil content varied from 39-52% of DM and there was a significant negative linear correlation between protein and oil content (Y(% oil) = - 0.60 x (% protein) + 59.6(% oil); R² = 0.21; P < 0.001).

The digestibility experiments showed a positive correlation between protein content in the defatted meal and digestibility of protein and DM (P<0.001). Thus, 1 % increase in protein will increase digestibility of protein with 0.3% units and energy with 1% units.

**Conclusions:** The presented results shows that the nutritional value of rapeseed meal will increase with increasing protein content in the seeds. Thus from an nutritional point of view and in order to meet the growing demand for protein in the world more focus on protein content in future breeding programmes would be desirable.

**References:**
Standardized ileal digestibility of amino acids from two winter rapeseed products fed to broiler chickens

Background: Rapeseed products, which are valuable high-protein feedstuffs for poultry, have been the focus of extensive research recently.

Objectives: The aim of this study was to compare the standardized ileal digestibility of amino acids (SIAAD) from two winter rapeseed products fed to broilers.

Methods: Rapeseed cake and expeller from the rapeseeds of the Belarusian variety “Lider” were tested in this experiment. The methodology used in our study followed that proposed by Lemme et al. (2004). The experiment comprised 24 group cages with 12 birds per cage (a total of 270 birds). Three-week-old Ross 308 male broilers were assigned to 3 treatments with 8 replicates each. The main components of the experimental diets were rapeseed cake (treatment 1) and rapeseed expeller (treatment 2). Non-specific (basal) endogenous amino acid loss after feeding a N-free diet was determined in a separate group (treatment 3) of broilers. The obtained values provided a basis for the standardization of ileal digestibility coefficients. Until the end of week 3, male Ross 308 broilers received ad libitum a starter diet (commercial feed mixtures in meal form). After 7-day experimental feeding, all birds were sacrificed by cervical dislocation and digesta was immediately sampled from the distal two-thirds of the intestine section between Meckel's diverticulum and 2 cm anterior to the ileo-ceca-colonic junction. The intestine contents were pooled (within each pen), freeze-dried and subsequently analyzed for amino acid content. The calculations were conducted using the formulas proposed by Lemme et al. (2004).

Results: Rapeseed cake contained 32.0, 1.9, 0.8, 1.3, 2.2, 1.8, 0.6, 1.3, 1.4, 0.4, 1.6% and rapeseed expeller contained 33.8, 2.1, 0.8, 1.3, 2.3, 1.8, 0.7, 1.4, 1.5, 0.5, 1.7% of CP, Arg, Cys, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, respectively. The total glucosinolate content of rapeseed cake and expeller was 31.94 and 19.13 µmol/g, respectively. The following standardized amino acid digestibility coefficients were determined: rapeseed cake - 88.0, 73.5, 78.8, 83.4, 77.1, 87.9, 80.4, 77.7, 71.3, 74.1 and 76.9%, rapeseed expeller - 92.0, 83.4, 84.6, 88.3, 85.3, 92.0, 87.4, 85.3, 79.9, 81.1 and 83.3% for Arg, Cys, Ile, Leu, Lys, Met, Phe, Thr, Trp and Val, respectively.

Conclusions: Both rapeseed products were characterized by good AA digestibility, however the SIAAD coefficients of rapeseed expeller were higher than those obtained for rapeseed cake. The values from the present study are close to or slightly higher than those reported by Lemme et al. (2004) and Szczurek (2006) for broiler chickens fed diets containing rapeseed cake.

References:

Szczurek, W., 2009. Standardized ileal digestibility of amino acids from several cereal grains and protein-rich feedstuffs in broiler chickens at the age of 30 days.
Importance of linoleic acid in α-linolenic acid conversion to LC n-3 PUFA during fat substitution

Background: Arachidonic (AA, 20:4n-6), eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic (DHA, 22:6n-3) acids, synthesized by the liver from dietary linoleic (LA, 18:2n-6) and α-linolenic (ALA, 18:3n-3) acids, are predominant long-chain polyunsaturated fatty acids (LC PUFA) in plasma phospholipids (PL) (Rapoport 2013). Furthermore, the conversion of ALA to DHA may occur selectively in the brain. (Barcelo-Coblijn and Murphy 2009). In our rapeseed oil (RSO) studies (Seppänen-Laakso et al. 2002, 2010) ALA exhibited significant competitive effects by increasing LC n-3 PUFA and inhibiting LA conversion to AA.

Objectives: Individual fatty acid data (baseline, 3 and 6 weeks’ values) of the subjects (n=148) were taken from our previous studies, and the effects of plasma PL saturated fat and LA on LC PUFA were examined during fat substitution (nine groups including controls).

Methods: The groups changed butter to 1a) RSO (n=20, LA 24%, ALA 10%) or 1b) test margarine (n=23, LA 28%, ALA 3% with 18:1tr 16%), and margarine (LA 33%, ALA 2% with 18:1tr 8%) to RSO (2a, n=23) or olive oil (2b, n=23, LA 9%, ALA 1%). Two groups (3a, n=32) and a third group (3b, n=10) received also RSO, with daily dose of 16 ml (3.4 g LA and 1.8 g ALA). Further, a small group received soybean oil (4, n=6, LA 55%, ALA 7%).

Results: In RSO group 1a, 20% of the increases at 3-6 weeks, showed the highest DHA response for DHA-AE-EPA combination (average DHA rise 2%-units, range 1.6-3%-units), while lower response (1%-unit) was typical in AA-DHA profiles (28%) without EPA. Low DHA (±1%-unit) in group 2a was combined with EPA (28%), whereas in groups 3a-b increases in AA-DHA profile (24%) with no EPA were characteristic. In groups 1b, 2b and 4, unexpected rapid decreases in LC n-3 PUFA at 6 weeks (60%) were found.

Conclusions: The profiles well reflect the balancing steps between major LC PUFAs AA, DHA and EPA. Not only linoleic acid but also saturated fatty acids strongly affect the conversion of ALA to LC n-3 PUFA. Lack of EPA in AA-DHA combination, in turn, would suggest specific conversion for ALA.

References:
Seppänen-Laakso, T., I. Laakso, T. Lehtimäki et al., 2010. Elevated plasma fibrinogen caused by inadequate α-linolenic acid intake can be reduced by replacing fat with canola-type rapeseed oil. PLEFA 83: 45-54.
Genetic classification and diversity of yellow-seeded rapeseed (Brassica napus L.) accessions

**Background:** Yellow-seeded oilseed rape (B. napus) has thinner seed hull, less fiber, higher oil and protein content than its black-seeded counterpart so yellow seeded trait is valuable in oilseed rape breeding.

**Objectives:** We collected and developed various yellow-seeded accessions varying from pale to lemon, dotted yellow, greenish, reddish and yellowish brown colors in seed coat. In order to use these accessions in conventional and hybrid breeding, we selected some representative yellow-seeded accessions to perform genetic analysis.

**Method:** We investigated the allelism of seed coat color genes using the F1 and F2 populations of different crosses and studied genetic diversity using 48 SSR markers evenly distributed in A and C genome.

**Results:** Eleven yellow-seeded rapeseed accessions varying in seed coat colors from dotted yellow, greenish brown, reddish brown to yellowish brown were divided into five groups: group I containing Youyan10, C2V55, E718, and Armand-ys showing dominant inheritance in most crosses; group II including reddish brown Q33 and D615 showing incomplete dominance; group III consisting of 2006C, X2006, and 740C showing yellow seed coat as recessive trait in most cases; group IV having only yellowish brown Polo-ys and group V, one accession HY15. Most accessions in the last three groups showed a recessive trait of the seed coat colors while dominance or recessiveness of the seed coat colors in group I such as Youyan10 and group III such as 2006C varied when these accessions were crossed with different brown-seeded accessions. When Chinese varieties Yangyou4, Zheyou50, and Za77 were crossed with the yellow-seeded accessions of group I, all F1 plants had black seeds. On the other hand, when the European variety Solida and some Canadian spring varieties were crossed with the accessions in group III, all F1 plants had yellow seeds. Phylogenetic analysis based on SSR molecular markers showed that yellow-seeded accessions could be divided into eight subgroups, each of which were represented by 2006C and 740C, Youyan 10 system, Q33, Q4 from Shaaixi, Ramiro-ys from French, Armand-ys and Profit-ys selected from Canadian cultivars, and Polo-ys from Polish cultivar Polo. The phylogenetic tree generally agreed with the pedigrees and sources from breeders and originations.

**Conclusion:** The complex allelic and epistatic interactions among different yellow-seeded accessions could be attributed to genetic regulation networks and genome duplication in the evolution of allotetraploid B. napus. The phylogenetic analysis in our research provides some theoretical basis for the heterosis utilization of yellow-seeded oilseed rape.

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Monitor plan of rapeseed quality in China

**Background:** China is the largest rapeseed production country in the world (Zhang, 2007). In the past 15 years, the rapeseed breeding scientists bred a series of double-low rapeseed varieties. The yield and quality of rapeseed significantly improved.

**Objectives:** The aim of this study was to describe the change trend of quality of varieties and commodity rapeseed of double-low rapeseed during the past 15 years in China.

**Methods:** According to stratified random sampling method, more than 1000 samples of varieties and commodity rapeseed were collected from the main producing area every year. The oil content, glucosinolate and erucic acid were analyzed according to the National or International standard methods. The SAS and SPSS statistical tools were used to evaluate the changes of quality of these double-low rapeseed samples in China.

**Results:** In the past 15 years, the average oil content of rapeseed steadily increases and achieves exceeding 4% increase. The erucic acid contents of varieties and commodity rapeseed were 0.17% and 6.57%, which decrease by 94% and 60% respectively. The erucic acid contents of the new varieties approached to the international level. Meanwhile, the glucosinolate content of varieties and commodity rapeseed were 24.42mmol/g and 36.49mmol/g, which decrease by 31% and 44%, respectively. The rate of double-low rapeseed varieties and commodity rapeseed grew significantly, reaching to 99.53% and 71.19%, respectively.

**Conclusions:** In this study, the qualities and change trend of varieties and commodity rapeseed in China were summarized and analyzed. The qualities of both varieties and commodity rapeseed significantly increase in the past 15 years. Some of quality parameter of new varieties reaches to the international level.

**Reference:**
Domestication and molecular mechanism underlying yellow seed in *Brassica juncea (L.)* Czern & Coss

**Background:** Seed color is not only a morphological indicator, but also a major agronomical character associated with seed quality and seed dormancy. In *Brassica* species, yellow seed has thinner testa, less fiber, and more oil and protein than their black- or brown-seeded counterpart. Seed color is controlled by proanthocyanidins (PA) deposited in its testa. In *Brassica juncea*, seed color is controlled by duplicate loci mapped on the chromosomes A09 and B03.

**Objectives:** First, what genes do these loci encode in *B. juncea*? Then, how do these genes regulate seed color at the molecular level? Furthermore, how many alleles do these genes each have in *B. juncea*? When, where and how is *B. juncea* yellow seed domesticated? It is of great significance in science and breeding to elucidate these questions.

**Methods:** The segregating backcross populations were used for positional cloning of the genes for seed color. The yeast one hybrid (Y1H), RNA-seq, and restriction digest of amplified fragments for expression analysis were used to uncover the molecular mechanism underlying seed color. Allelic variation was analyzed in a collection of *B. juncea* accessions from over thirty countries. Genetic analysis and historical literature records were used to elucidate domestication of yellow seed.

**Results:** The cloned seed color genes mapped on the chromosome A09 or B03 both encode a bHLH transcription factor orthologous to *AtTT8* in *Arabidopsis thaliana*. The yellow-seeded parent Sichuan Yellow (S) has a 1275 bp inserted fragment between nt3047-nt3048 and a substituted base at nt 3046 of *BjuA09.TT8* and at nt2742 of *BjuB03.TT8* compared to the black-seeded parent Ziyeihe (Z). The mutated *Bju.TT8* genes were expressed in testa of S and its brown-seeded near-isogenic lines. However, TT8-regulated genes such as dihydroflavonol-4-reductase (DFr), leucoanthocyanidin dioxygenase (LDOX) and anthocyanidin reductase(ANR) for PA biosynthesis, were not expressed in S testa. The mutated *Bju.TT8* genes from S did not interacted in vitro with the promoters of both DFR genes from *B. juncea*. Analysis of variation in *Bju.TT8* of about 200 *B. juncea* accessions found three additional loss-of-function *BjuA09.TT8* alleles including two insertions and one deletion. The mutated *BjuA09,TT8* alleles occur in all subspecies of *B. juncea* although the mutated *BjuB03.TT8* allele occurs only in yellow-seeded mustard.

**Conclusions:** Concurrent mutations in both *BjuA09.TT8* and *BjuB03.TT8* genes are responsible for spontaneous yellow seed in *Brassica juncea*. The yellow seed is domesticated by artificial selection, most probably in China, after speciation of *B. juncea*. The mutated *Bju.TT8* genes, although transcribed, can not activate expression of downstream genes so that yellow seed lacks deposition of PA in its testa.

**References:**
Develop an extrusion-assisted extraction process to increase canola and industrial oilseed meal inclusion in feed

**Background:** Canola and rapeseed meal have been used as viable alternatives to soybean meal in feeds. However, there are anti-nutritional factors, such as glucosinolates and a higher content of fiber in meals, restricting their application at high inclusion levels in monogastric animals and poultry diets (Zhou et al. 2013). Additionally, lowering glucosinolates content in non-“double zero” oilseeds meal to levels typically found in canola meal would provide opportunities for these meals to be used in feeds, which makes biodiesel processors to be more competitive (Marillia et al. 2014). Moreover, glucosinolates is a natural preservative potentially used for pest control, and possible drug ingredients.

**Objectives:** The objective of this research was to develop an extrusion-assisted extraction process to (a) reduce glucosinolates content in canola and other meal; (b) increase digestibility of fibre; and (c) extract and recover glucosinolates as a natural preservative. This novel process is to replace the batch extraction process, enabling to carry out thermomechanical and chemical treatments in a continuous step.

**Methods:** A 40-mm twin-screw extruder (Century Extrusion, Traverse City, MI, US) was employed. Experiments were performed according to a factorial design with solvent, processing profile such as screw speed, solvent / solid ratio as the factors. The level of glucosinolates, crude fibre, and neutral detergent fibre in extrudates were measured, and compared with those in raw canola meal. The optimized process was selected by the most efficient combination of factors.

**Results:** The level of glucosinolates in extruded meal is lowered, and the fibre components in extrudates have been modified with a positive potential on the intake by animal. Extrusion assisted extraction process was validated.

**Conclusions:** The optimized process depicted the main advantages of this process such as reduced time, solvent, and reactant requirements; enhanced extraction yield and rheological properties; and high purity of the product. Further research is needed in terms of isolation of glucosinolates in liquid stream, and animal test.

**References:**
Volatile aroma compounds as markers for the assessment of the sensory quality of virgin cold-pressed rapeseed oil

Background: Virgin cold-pressed rapeseed oil becomes more and more attractive to the consumer as an alternative or in addition to virgin olive oil. The advantages of this type of oils compared to common refined edible oils are less processing, natural content of nutritionally important compounds, the intensive colour and the typical seed-like and nutty taste and smell. Although the process itself is simple with pressing of the rapeseed by a screw press and purification of the oil by filtration, sedimentation or centrifugation, the processing of high-quality virgin cold-pressed rapeseed oil is an art. Most important is the choice of the raw material that directly influences the quality of the oil. One important tool for the quality control of virgin cold-pressed rapeseed oil is the sensory assessment which is performed by at least 3 to 5 trained persons under standardized conditions. For rapeseed oil the method DGF-cII 1 (14), Appearance - Sensory assessment, can be used as a reliable, but personnel and time-consuming method with some uncertainties. A promising approach to support the sensory assessment of virgin cold-pressed rapeseed oil by analytical means is the profiling and characterization of volatile compounds in combination with statistical methods.

Objectives: The aim of the present work is to find analytical methods for the classification of virgin cold-pressed rapeseed oil in sensory good and bad oils as well as to find volatile compounds that are responsible for the typical aroma and specific off-flavours of the rapeseed oils, respectively.

Methods: The volatile compounds are extracted by dynamic headspace (DynHS), determined by gas-chromatography (GS) with FID and identified with MS detection. Aroma active compounds are detected by GC-olfactometry (GC-O) and matched with corresponding GC-MS peaks, while the identification is done by comparison with analyzed standard substances and with help of NIST-databases. Relationships between compounds and differences between samples on basis of the volatile compounds are investigated by statistical means.

Results: The profiles of volatile compounds from a sensory good rapeseed oil and a corresponding rapeseed oil with sensory defects such as fusty and musty are measured by DynHS-GC-MS and the aroma active compounds were identified by DynHS-GC-O. Additionally a dataset of 43 samples has been classified into two groups of sensory good and bad oils according to the sensory assessment of a panel group as basis for an automated analysis by statistical means. 64 volatile compounds have been detected on basis of DynHS-GC-O/MS data of which 41 compounds were described as aroma-active substances. 23 of all detected volatile compounds show significant differences in the peak intensities between sensory good and bad oils. Especially carboxylic acid esters occur with higher intensities in the sensory bad quality oils. Altogether 46 volatiles could be identified. A dataset of 41 of these identified volatile compounds together with the use of the Principle Component Analysis (PCA) results in a partial differentiation of sensory good and bad oils.

Conclusions: DynHS-GC in combination with statistical means can be a helpful tool to support the sensory analysis and assessment of virgin rapeseed oils. Further development is necessary.
Canolol enriched extract from heat-treated canola meal as an option to improve frying stability of high-oleic canola oil

Background: In comparison to other oilseeds rapeseed contains relatively high amounts of phenolic compounds, mainly derivatives of sinapic acid but during oil processing only small parts of the phenolic compounds go into the oil while most of the compounds remain in the press cake. During heating of the raw material sinapic acid is degraded by decarboxylation into the oil-soluble 2,6-dimethoxy-4-vinylphenol (vinylsyringol or canolol) which is described as a strong antioxidant in different systems.

Objectives: In the present work the effect of a 2.5% canolol-enriched extract obtained from the extraction of fluidized bed treated rapeseed meal with super-critical carbon dioxide should be investigated in a deep-fat frying study.

Methods: The extract was added to high-oleic rapeseed oil in amounts corresponding to 200, 500 and 750 mg/kg canolol and the effect on the formation of di- and polymer triacylglycerols, total polar compounds, secondary degradation products (anisidine value) and the iodine value during deep-fat frying was compared to commonly used antioxidants TBHQ (200 mg/kg) and rosemary extract (40 and 200 mg/kg) as well as a control without antioxidant.

Results: The canolol enriched extract showed a three times stronger effect on stabilizing the frying oil during processing than TBHQ or rosemary extract. The extract was also able to reduce the degradation of α- and γ-tocopherol during frying. The LD50-values for the degradation of tocopherols for the different amounts of added canolol enriched extracts ranged between 20.2 and 28.7 h for α-tocopherol and 15.8 and 19.6 h for γ-tocopherol, while the LD50-values for the other antioxidants were between 3.6 and 7.7 h, and 4.5 and 8.3 h, respectively. The canolol enriched extract showed a strong concentration-dependent performance with a better effect with a higher concentration.

Conclusions: The experiment showed that the addition of canolol enriched extract can be a promising possibility to enhance the frying performance of vegetable oils. Fluidized bed treatment of rapeseed meal is an interesting opportunity to induce the formation of canolol making rapeseed meal an interesting raw material for an antioxidant effective extract. This gives rapeseed meal an added value. Investigation on the composition of the extract as well as on antioxidant compounds besides canolol will be continued.
QTL analyses reveal pleiotropic effects of erucic acid and glucosinolate content on other seed quality traits in oilseed rape

**Background:** Traditional oilseed rape contains up to 60% erucic acid in the seed oil and about 90 µmoles of glucosinolates in the seeds. Through the conversion to canola quality oilseed rape, contents of those two constituents have been reduced to almost nil. This must have caused remarkable changes in the biosynthesis of other primary and secondary seed quality components. However, the biochemical consequences of the conversion to canola quality are not at all yet clear.

**Objectives:** To study the effect of conversion from traditional oilseed rape to canola quality on the contents of other primary and secondary seed components.

**Methods:** A number of DH populations segregating for erucic acid and glucosinolate content were analysed for their contents of other primary and secondary seed components (Amar et al. 2008; Schatzki et al. 2014, Suprianto 2014)

**Results:** Doubled haploid populations segregating for erucic acid and glucosinolate contents have been studied in field experiments and a number of pleiotropic effects of those two constituents have been detected in QTL analyses. Erucic acid content did not only influence oil content but also contents of sinapate and phytosterols (Amar et al. 2008). Glucosinolate content influenced seed storage protein composition (Schatzki et al. 2014) and seed fibre composition (Suprianto 2014).

**Conclusions:** The pleiotropic effects of erucic acid and glucosinolate content on other seed quality traits limits the application of NIRS calibrations and other analytical methods which are routinely used to screen Brassica genetic resources for valuable seed quality traits. Care must be taken to avoid detecting spurious genetic variation in genetic resources which may be caused by cross-correlation to erucic acid and/or glucosinolate content. The relevance of those results for further genetic improvements of seed quality traits in oilseed rape will be discussed.

**References:**
Cold pressed canola oil- applying terroir concepts to a commodity crop

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Background: Cold pressed canola oil processing is of increasing interest to canola growers as a value-added income stream. In partnership with the Manitoba Canola Growers Association, NuEats Food Innovation Inc has developed a scalable, low input pressing and clarification regime suitable for on-farm or small enterprise implementation.

Objective: To leverage increasing consumer and food service interest in the concept of ‘terroir’- commonly acknowledged as the set of special characteristics that the geography, geology and climate of a region, interacting with germplasm, expresses in the final product.

Methods: Multiyear samples of the same cultivar of canola grown in three distinct geographic regions in Manitoba were sourced, cleaned, crushed and bottled. The resulting cold press oil was evaluated for fatty acid profile, nutrition, appearance, cooking performance and sensory characteristics. Test marketing results were used to identify key attributes and pricing.

Results: Cold pressed canola oil samples demonstrated differences in fatty acid profile and sensory characteristics compared to traditional processed canola oil.

Conclusions: Regionally sourced cold pressed canola oil offers a value added opportunity for growers and is well suited to on farm enterprise. Acceptance was high with consumer as well as food service users.
Canola meal replacing wheat-DDGS as protein source for dairy cows

**Background:** Traditionally, dairy cow diets in western Canada contain canola meal (CM) as the principal source of protein because it is a high quality protein supplement (Hickling, 2008). On the other hand, growth of the ethanol industry using wheat as a feedstock has resulted in large quantities of wheat dried distillers grains with solubles (W-DDGS) being available as an alternative protein supplement. When compared with CM, W-DDGS contains less CP (37 vs. 42%) and is a poorer source of lysine (2.5 vs. 5% of CP; Maxin et al., 2013); thus, feeding W-DDGS in place of CM could potentially compromise cow performance due to a lysine deficiency. Also, dietary CP content can have major effects on ruminally-degradable protein (RDP) supply as the amount of dietary CP that is degraded in the rumen increases with dietary CP content.

**Objectives:** To determine the effects of feeding CM or W-DDGS in diets varying in CP content on ruminal N utilization, omasal flows, and milk production.

**Methods:** Eight multiparous Holstein cows were used in a replicated 4 × 4 Latin square design with 28-d periods. Treatments were: 1) source of protein (CM vs. W-DDGS); and 2) dietary CP content (15 vs. 17%). Feed intake, and milk production were measured during the last 8 d of each period. Omasal digesta flow was quantified using indigestible NDF, YbCl3 and Cr-EDTA as digesta markers, whereas ruminal microbial protein production was quantified using (15NH4)2SO4 as a microbial marker.

**Results:** DM intake and milk yield were unaffected by diet; however, numerically, cows fed CM produced 1 kg/d more milk when compared to cows fed W-DDGS. DM apparently digested in the rumen was greater in cows fed the high CP compared to those fed the low CP diet, with the difference in DM apparently digested in the rumen being greater in cows fed W-DDGS as compared to those fed CM (interaction, P = 0.02). RDP supply was greater in cows fed the high CP when compared to those fed the low CP diet when diets contained CM, whereas RDP supply was lower in cows fed the high CP when compared to those fed the low CP diet when diets contained W-DDGS (tendency for interaction, P = 0.08). RUP supply was greater in cows fed the low CP when compared to those fed the high CP diet when diets contained CM, whereas RUP supply was lower in cows fed the low CP when compared to those fed the high CP diet when diets contained W-DDGS (tendency for interaction, P = 0.06). Omasal flows of threonine and tryptophan were greater (P ≤ 0.03), whereas that of histidine and lysine tended (P ≤ 0.08) to be greater, in cows fed CM when compared to those fed W-DDGS.

**Conclusion:** When diets are formulated to contain 15 or 17% CP, CM or W-DDGS can support similar levels of milk production.

**References:**

Hickling, D. 2008. Pages 3–14 in Proc. 29th Western Nutrition Conference, University of Alberta, Edmonton, AB.

Molecular mapping and QTL for seed pigmentation and 19 genes of flavonoid pathway in *Brassica napus L.*

**Background:** Expression quantitative trait loci (eQTL) can detect the expression of a specific gene and the genotype at that gene’s locus, as well as evidence for clustered trans-eQTL that simultaneously regulates a large fraction of the transcriptome (Morley et al. 2004). In *B. napus*, the seed coat colour is determined by the phenolic compounds and procyanidins (Lepiniec et al. 2006; Qu et al. 2013). Dozens of genes involved in flavonoid biosynthesis pathway had been elucidated in Arabidopsis, and much more homologs had been found in *B. napus* (Chai et al. 2009; Chen et al. 2013). However, the function of these genes for *Brassica* yellow seed trait formation is still not well understood.

**Objectives:** This research focused on QTL identification responsible for four seed pigmentation and nineteen genes in flavonoid pathway to systematically elucidate the characteristics of flavonoid pathway, and provide the necessary information for seeking the key genes or regulation nodes controlled the yellow seed trait formation in *B. napus*.

**Methods:** We employed a sample of 94 recombinant inbred lines (RILs) from a population derived from a cross between black-seeded male parent cultivar Zhongyou 821 and yellow-seeded female parent line GH06. Major QTLs controlling four kinds of seed pigmentations were identified by genetic map construction. Then, transcript-level variation analysis was carried out on RNA from seeds of 30 days after flower (DAF) by qRT-PCR. Regarding as quantitative traits, the transcript levels of the flavonoid biosynthesis genes families were examined by QTL mapping method for eQTL detection. To confirm the candidate genes, sequences of association markers were used for BLASTN search in the BRAD and our local *B. napus* genome sequence database.

**Results:** A total of 57 QTLs for seed pigmentation and 75 eQTLs for nineteen genes were detected and distributed among 15 different linkage groups. Interestingly, 4 hotspot regions including 19 QTLs and 30 eQTLs were identified and distributed on the chromosome A03, A09 and C08, respectively. Besides, the most interesting hotspot in our study was the lower hotspot on chromosome A09, showed well synteny to genome sequences of *A. thaliana*, *A. lyrata* and *Brassica* relatives. A total of 8 transcription factors were identified in this region, three of them belongs to the flavonoid biosynthesis related MYB transcription factor family. Additionally, In trans-eQTL hotspot on chromosome A03, C08 and the upper A09, we identified 5, 1 and 10 transcription factors, respectively. Among these transcription factors, bZIP25, MYC1 and other function unclear transcription factors could be regarded as candidate genes in the trans-eQTL hotspots for further verification.

**Conclusions:** Molecular markers closely linked with these QTLs could be applied in marker-assisted selection of improving of feeding value of rapeseed. The functions of these candidate genes will provide insight into the molecular and biochemical mechanism of seed coat development in *Brassicaceae*, and elucidate the regulatory network underlying seed coat colour formation in *B. napus*.

**References:**


High inclusion levels of canola meal in laying hen diets

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Background: Canola meal (CM) inclusion levels in laying hen diets have traditionally been limited to a maximum level of 10% due to the presence of anti-nutritional factors, including glucosinolates, sinapine or tannins. The content of glucosinolates in canola meal has declined significantly over the years as a result of selection pressure by canola breeders. Based on the recent survey involving 11 Canadian crushing plants, the level of glucosinolates in CM averaged 3.9 μmol/g (Rogiewicz et al., 2012). Therefore, the rations for laying hens could now contain significantly more CM without causing any adverse effect on egg production or mortality due to hemorrhagic liver syndrome. In this context, Khajali and Slominski (2012) indicated that a dietary level of glucosinolates of 1.5 μmol/g would have no negative effect on laying hen performance.

Objective: To investigate the effect of different dietary levels of CM on egg production and egg quality parameters in laying hens.

Methods: A wheat/corn/SBM-based Control diet, and diets containing 4, 8, 12, 16 or 20% of CM were fed to 6 replicate cage units of 18 Lohmann LSL laying hens each per treatment throughout the 24-week study consisting of 2 phases and three 28-d periods in each phase. Diets were formulated to contain 17.0 and 16.4% of CP and 2,800 and 2,700 kcal/kg of metabolizable energy in Phase 1 and 2, respectively. Dietary glucosinolate content averaged 0.28, 0.52, 0.97, 1.30, and 1.49 μmol/g for the diets containing 4, 8, 12, 16 or 20% of CM, respectively. Hen-day production, egg mass, feed intake, and feed efficiency were determined three times in each phase at the end of a 28-d period. All eggs were weighed for 3 consecutive days in the middle of each period and 36 eggs per treatment were selected for egg quality measurements, including albumen height, Haugh units, specific gravity, yolk color, and egg shell elasticity and thickness.

Results: There were no significant differences in hen-day production, feed intake, feed efficiency, and mortality between dietary treatments (P>0.05). Egg weight was slightly higher (P<0.05) in the control group than in hens consuming 16 and 20% of CM in phase 1 (64.4 vs. 63.3 and 63.3g, respectively) and phase 2 (63.4 vs. 62.4 and 62.2 g, respectively) of the experiment. However, the egg mass was not affected as a result of the same or better hen-day egg production in hens consuming CM-containing diets. When compared with the control diet, different dietary levels of CM had no effect on egg and egg shell quality parameters.

Conclusion: It would appear evident that CM could replace SBM and used effectively in laying hen diets at the dietary level of 15-20%.

References:
Prospects of water-lean protein recovery from rapeseed press cake

**Background:** Production of protein-rich ingredients from rapeseed cold pressing residue has potential to bring additional value to the oil pressing industry. Commercialization of rapeseed protein production is, however, hindered by high investment and operational costs of the current water and energy-intensive processes. Rapeseed protein is generally extracted from the press cake with alkali or salt solution at 5-10% total solid content and recovered by acid precipitation. Although protein fractions with high purity can be obtained, introduction of salt into the protein fraction results in the need for additional washing steps. Instead of aiming at pure isolates, production of protein concentrates by simple, water-lean methods could improve the economics and sustainability of the concept without compromising technical properties of the product.

**Objectives:** The aim was to develop water-lean protein extraction and dry fractionation technologies for rapeseed press cake to improve technical and economic feasibility of rapeseed protein production.

**Methods:** Press cake from cold pressing of turnip rape (B. rapa) seeds was defatted by supercritical CO2 extraction and dry-milled. Two extraction processes were applied at increased total solid content to recover protein-rich fractions from the defatted press cake: 1) alkaline extraction followed by isoelectric precipitation and drying of the protein-rich precipitate, and 2) water extraction and drying of the protein-sugar extract. Carbohydrate-degrading enzymes were utilized to facilitate the recovery of protein extracts as described by Rommi et al. (2014). The effects of enzyme treatment and total solid content on the yield and production costs of protein-rich fractions were determined. As a water-free approach, protein content the defatted press cake was enriched by dry fractionation. The influence of particle size and operational factors on the separation of protein-rich kernel and fiber-rich hull particles by air classification were investigated.

**Results:** In order to extract over 50% of the protein from defatted rapeseed press cake at 20% solid content, alkaline pH or additional extraction rounds were needed. Carbohydrate-degrading enzymes disrupted the cell wall matrix, functioned well also at 40% solid content, and facilitated the recovery of protein extracts. Although enzyme-aided water extraction gave low protein yields at increased solid content, the resulting protein-sugar extracts possessed better dispersion stability than isoelectric protein precipitates from alkaline extraction. Raw material and energy formed the major costs of the evaluated extraction processes, and spray drying of the product and processing residues was indicated as the most energy-intensive phase. Air classification of defatted press cake produced light-colored, hull-free fractions with ca. 40-45% protein content.

**Conclusions:** Three water-lean concepts were developed to produce protein-rich fractions from rapeseed press cake. Due to significant drying costs in dilute processes, increasing the total solid content of extraction processes seems favorable despite reduced protein yield. Alkaline extraction and isoelectric precipitation is feasible at 20% solid content; however additional salt removal steps and technological functionality of the protein precipitates need to be considered. Enzyme-aided water extraction produces protein-sugar fractions with better dispersion stability, but the protein yield is compromised unless additional extraction rounds are used. Dry fractionation represents a promising alternative approach to be further developed to obtain fractions with higher protein content.

**References:**
Toasting and amino acid availability of rapeseed meal in pigs

**Background:** In oilmills, heat and steam in the desolventizer/toaster (DT) evaporate the hexane from the de-oiled rapeseed cake (RSC), thereby producing solvent-free rapeseed meal (RSM). A side effect is the degradation of glucosinolates (GSL), and an excessive heat treatment may lower the content of amino acids (AA) and their availability expressed as standardized ileal digestibility (SID).

**Objectives:** Variation of toasting in oil mills was to simulate by processing one batch of a defined rapeseed (RS) into 5 RSM, varying in their residence time (RT) in the DT. These RSM should be analyzed for GSL and AA and in pigs studied for the SID of AA.

**Methods:** In the DT of CREOL pilot plant, from the RSC soaked with hexane 4 RSM were produced under wet toasting conditions (WetTC) with increasing RT of 48, 64, 76, and 93 min. A fifth RSM representing 70 min mean RT was 60 min additionally processed (RSM 70+60), mainly by dry toasting conditions (DryTC). Six barrows (initial BW = 22 ± 1 kg), fitted with a T-cannula at the distal ileum, were allotted to a 5 × 6 row column design with 5 RSM-casein-cornstarch diets and 5 periods. The SID of AA in the RSM was determined as difference to the SID of casein AA.

**Results:** Increasing the RT reduced the GSL content – in RSM 70+60 by 90% of the GSL in RS defatted matter. Also the contents of lysine (inclusive its reactive part in guanidination) and cystine were diminished. The SID of most AA in RSM decreased linearly (P < 0.05) as the RT in the DT increased from 48 to 93 min – till 10 and 11%-units decrease for lysine and cystine SID (P < 0.05). The additional DryTC (RSM 70+60) resulted in SID values similar to RSM93.

**Conclusions:** For pigs an improved acceptance of longer heated RSM with very low GSL content is compromised by decreased content of limiting AA such as lysine and also by a lowered SID of most AA. However, a loss of AA due to longer toasting could be compensated by AA supplements. Considering further RSM quality criteria as protein solubility or fiber-fixed N, the toasting can be optimized. In conclusion, under the frequently used WetTC, the RT should not exceed 60 to 75 min.

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Recent advances in the development of commercial rapeseed/canola protein products

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Background: Currently, there are numerous drivers increasing the demand for vegetable proteins. In particular, the food industry is seeking nutritious, low allergen protein ingredients from sustainable and non-GMO sources. Canola proteins can meet all of these criteria and have long been of interest for use as food ingredients. However, preparation of commercially acceptable canola protein products has proven a challenge as canola seeds contain phenolics and other compounds that can negatively impact the sensory properties of the final protein products. It is of interest to advance current methods of protein extraction and purification to provide improved products and satisfy the current demand for alternative vegetable proteins.

Objectives: A first objective was to develop a commercially viable canola protein extraction and purification process to obtain isolates having improved flavour and colour profiles when compared to isolates obtained from traditional extraction and purification methods. The second objective was to ensure that the newly developed canola protein isolates possess functional and nutritional characteristics that address the requirements of food manufacturers.

Methods: Protein products were prepared from low-temperature desolventized canola meal. This specially prepared meal was extracted using a saline solution and the protein extract purified by a membrane process. The purified protein solution can either be dried to form a canola protein isolate or further processed by a dilution step to form a supernatant-derived canola protein isolate and a protein micellar mass-derived canola protein isolate.

Results: Canola protein isolates prepared from low temperature desolventized meal were found to be improved in colour and flavour over isolates made by previous methods. The purification process could be run to produce a protein isolate (trade name Nutratein™) comprised of a mixture of globulin (cruciferin) and albumin (napin) proteins. Alternatively, the purification process could be run to separate the globulins and albumins to produce a cruciferin-rich protein isolate (trade name Puratein®) and a napin-rich protein isolate (trade name Supertein™).

Nutratein™ has very good solubility across a broad pH range and has an excellent amino acid profile. It may be used to provide protein fortification in nutritional applications. Puratein® has excellent emulsifying, gelling and binding properties and may be used in applications such as dressings, meat analogues or baked goods. Supertein™ is highly soluble and has good foaming properties. It is valuable for use in applications such as beverages and aerated desserts. Supertein™ has a very high content of sulfur containing amino acids and so also may be particularly useful for functional foods.

Conclusions: A protein extraction and purification process involving low temperature desolventized meal, salt extraction, membrane and micelle technology was used to produce three canola protein isolates having colour and flavour properties improved over isolates made by past methods. The functional, nutritional and sensorial properties of these canola protein isolates open up new opportunities for use of canola proteins in food applications.
Genetic variation and inheritance of phytosterol and oil content in winter oilseed rape (Brassica napus L.)

Background: Phytosterols are natural constituents of vegetable oils with serum cholesterol lowering properties. Among vegetable oils, oilseed rape is ranked the second highest in phytosterol content after corn oil. Previous studies have shown that there is a close negative correlation between erucic acid and phytosterol content, explaining the inherently high phytosterol content observed in canola quality oilseed rape (Amar et al. 2008ab, 2009). On the other hand, highly contrasting phytosterol and oil contents were found in a collection of canola quality winter oilseed rape cultivars (Amar 2009) - “Sansibar” had the highest total phytosterol content (~480 mg 100 g-1 seed) and the lowest oil content (43%) while “Oase” had the lowest total phytosterol content (~360 mg 100 g-1 seed) and highest oil content (46%).

Objectives: To analyze the genetic variation and inheritance of phytosterol and oil content in the winter oilseed rape DH population “Sansibar” x “Oase”.

Methods: The DH population of 226 DH lines were tested at six environments in Germany and Sweden. A genetic map was constructed based on a total of 1642 markers, organized in 23 linkage groups, and covered a map length of 2350 cM with a mean marker interval of 2.0 cM. Phytosterols and fatty acids of the seed were quantified with gas-liquid chromatography. Seed oil content was determined with NIRS. QTL analysis was performed with multiple interval mapping. Identification of possible candidate genes underlying the QTL was performed by aligning the genetic map to the physical maps of Brassica rapa and Brassica oleracea.

Results: Broad-sense heritability estimates for all traits ranged from 0.84 to 0.90. Positive and highly significant correlations were observed between total phytosterol content and oil content ($r^2 = 0.24$), and between oil content and oleic acid ($r^2 = 0.48$). Between 1 and 6 QTL for phytosterols and fatty acids and six QTL for oil content were identified. With a good collinearity between genetic and physical map positions, candidate genes underlying major QTL ($R^2 \geq 25\%$) were identified: QTL for brassicasterol on A04 was colocalized with CYP710A1, QTL for campesterol:sitosterol ratio and 24-methyl:24-ethyl sterol ratio on A06 were colocalized with smt2, QTL for 18:1 and 18:3 on A01 were colocalized with FAD2 and QTL for 16:0 on A09 was colocalized with NATB.

Conclusions: Our results suggest that increasing both phytosterol and oil content is possible in canola winter oilseed rape. Major QTL corresponding to potential candidate genes could be useful for enhancing oil content and modifying the composition of phytosterols and fatty acids.

References:
Properties of napin from a scaled-up canola meal fractionation process

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Background: Seed storage proteins of crucifers are comprised of 12S cruciferin and 2S napin. Crucifer 2S proteins are reported to have diverse properties such as anti-microbial (Ye and Ng, 2009) and immunogenic (Barciszewski et al., 2000). Two polypeptide chains linked with four disulphide bonds (2 intra- and 2 inter-chain) compose the napin molecule (12-16 kDa) which is arranged as four alpha helices in the secondary structure. The scalable protein fractionation process developed by Agriculture and Agri-Food Canada allows obtaining napin in a highly purified form and the process is applicable to other crucifer oilseeds. Characterization of this napin protein and identification of its distinct properties enable directing this protein product towards suitable applications.

Objectives: Investigate physico-chemical and functional properties of napin protein product obtained from canola seed protein fractionation.

Methods: Commercial Brassica napus canola seeds (1 mT) were processed to obtain desolventized meal under low temperature. Meal protein fractionation was carried out at pilot scale according to Wanasundara & McIntosh (2013). Spray dried napin protein isolate (NPI) was used for investigating solubility, emulsifying, foaming, heat-induced gel formation, and mineral flocculating ability and compared with commercial whey protein isolate (WPI) where applicable.

Results: Selective solubility of napin proteins in low pH aqueous medium allowed their separation from rest of the canola meal proteins. Further concentration and separation of low molecular weight components generated a napin isolate having 93.5% protein on dbw, recovering ~15.6% of meal protein in the product. The spray dried NPI was light in weight and had bulk density of 0.19 g/mL compared to commercial whey protein isolate (0.39 g/mL). The solubility properties of NPI were comparable with the WPI between pH 4 and 10 exhibiting 83-95% solubility. Among the interfacial activities, air-water interface stabilization is a significant property that NPI can provide and gave 85-90% forming ability in the pH range of 4 to 10 which is not common among plant proteins. NPI showed comparatively less effective oil-water interface stabilization ability than WPI. Moisture-free napin gave initial thermal transition at 53 °C and then another at 156 °C. NPI formed very weak heat-induced gel networks. The ability of NPI to coagulate synthetic (1% w/v) clay solutions effectively by bringing down turbidity below 100 NTU could be a valuable property to use it as a flocculating agent.

Conclusions: Separation of napin from canola meal can be achieved by simple means. Obtaining napin from Brassica seed meal will enable to use these proteins in various useful applications based on their interesting properties.

References:
Adulteration detection and authentication of rapeseed oil based on chemometric methods and fatty acid profiles

Background: Edible oils are the most frequently counterfeited food (Moore et al. 2012). As the same as olive oil, rapeseed oils are also prone to be adulterated with the cheaper oil like soybean oil for economical reasons. Chemometric methods play an important role in the authentication identification and adulteration detection of edible oils. Since both the main edible oil and its adulterant are usually unknown, the traditional binary classification methods could not satisfy the requirement of adulteration.

Objectives: The more effective adulteration detection model should be built to quality inspection of rapeseed oil in practice. The aim of this study was to develop a robust model for authentication identification of rapeseed oils and determine the lowest detectable adulteration level (LDAL).

Methods: Random Forests (RF) and one-class partial least squares (OCPLS, Xu et al. 2011) were combined to identify the authenticity of the rapeseed oils by fatty acid profiles. Based on the previous studies (Zhang et al. 2014), 28 fatty acids were identified and quantified for rapeseed oils. Classification model was built by RF for rapeseed oil and other four kinds of edible oils. Subsequently, the OCPLS model was established. Moreover, fault oils adulterated with different levels of other edible oils were simulated by Monte-Carlo method and employed to determine the lowest detectable adulteration level of OCPLS classifier.

Results: The validation results that the RF could identify all of rapeseed oils and OCPLS classifier could completely detect the adulterated oils and are therefore employed to authenticity assessment. The LDAL of OCPLS model was determined as 12% for rapeseed oil.

Conclusions: In this study, RF and OCPLS were combined to identify the authenticity of rapeseed oil by fatty acid profiles. The LDAL of OCPLS model was determined by Monte-Carlo method. The built model is helpful in quality inspection of rapeseed oil for protecting the customers far from adulterated rapeseed oil.

References:
Genetic rescue of the lethality of \textit{Saccharomyces cerevisiae} mutants devoid of fatty acyltransferases responsible for the initial step of glycerolipid biosynthesis by an \textit{Arabidopsis thaliana} GPAT gene

**Background:** Polar glycerolipids are the primary building blocks of most membranes in living cells, and triacylglycerols (TAGs) are the most common storage form of neutral lipids serving as an energy or carbon source for a variety of cellular processes in many organisms. Despite the well-recognized importance of glycerol-3-phosphate acyltransferases (GAPTs) that catalyze the initial and committed step of glycerolipid biosynthesis, the nature or identity of the enzymes of plant origin is not well understood. Given that lipid metabolism is generally conserved between yeast and larger eukaryotes, genetic dissection of \textit{Arabidopsis} GAPTs in yeast could help unveil their functionality in glycerolipid biosynthesis, thereby moving a step closer to effective manipulation of oil biosynthesis in either seeds or vegetative plant tissues.

**Objectives:** We attempted to develop a robust genetic complementation system through leveraging synthetic lethality resulting from simultaneous deletion of GAT1 and GAT2 genes in yeast and to unravel the functions of the multigene family of \textit{Arabidopsis} GPATs using this system.

**Methods:** Standard yeast homologous recombination and transformation were employed to create the two double conditional knockout mutants gat1Δgat2Δ(\textit{GAL1}-GAT1) and gat1Δgat2Δ(\textit{GAL1}-GAT2). To replace yeast GAT1 or GAT2 gene in the corresponding double mutant by an \textit{Arabidopsis} GPAT gene, a shuttle vector carrying a LEU2 selection marker and an \textit{Arabidopsis} GPAT gene was introduced into the mutants. The negative selection of the strains with 5-fluoroorotic acid (5-FOA) was then performed to remove the pYES2-GAL1-GAT1 or pYES2-GAL1-GAT2 plasmid in which the URA3 gene functions in the conversion of the nontoxic 5-FOA compound to toxic 5-fluorouracil.

**Results:** Two conditional gat1Δgat2Δ(\textit{GAL1}-GAT1) and gat1Δgat2Δ(\textit{GAL1}-GAT2) mutants were generated. They cannot survive on 5-FOA medium, corroborating our previous finding that simultaneous inactivation of the GAT1 and GAT2 genes is lethal to yeast cells. In the presence of \textit{Arabidopsis} GPAT1, however, their growth defect on 5-FOA can be restored. The results strongly indicate that AtGPAT1 functions in the initial step of glycerolipid biosynthesis in a similar way to yeast GAT1 and GAT2. Furthermore, we created a novel complementation system based on the gat1Δgat2Δ(\textit{GAL1}-GPAT1) strain, which possesses high specificity and robustness for characterization of the putative GPAT genes.

**Conclusions:** \textit{Arabidopsis} GPAT1 behaves like yeast GAT1 and GAT2 genes with respect to involvement in mediating the initial step of glycerolipid biosynthesis. A novel genetic complementation system developed in our studies proves useful for functional dissection of a candidate GPAT gene from a eukaryote.

**References:**


Production of “functional Oil” rich in diglycerides and phytosterol esters with enzymatic transesterification

**Background:** Phytosterol esters (PEs), derived from phytosterols and inheriting all of the excellent properties of phytosterols, have a much greater solubility in oils and a lower melting point as compared to the corresponding phytosterols. Furthermore, the structural and metabolic characteristics of Dglycerides (DGs) compared with triglycerides appear to be responsible for suppression of body fat accumulation, body weight loss, and lower postprandial serum triglyceride levels. Recently, Ehud et al. pointed out that PEs mixed with dietary DGs could not only influence body weight but also prevent or reverse insulin resistance and hyperlipidemia; thus, they could be serve as functional ingredients for metabolic syndrome or diabetic sufferers (Ziv et al. 2009).

**Objectives:** The objective of this study is to develop a novel functional oil rich in both PEs and DGs with one-pot enzymatic esterification. A rapid and convenient method was proposed for the enzymatic transesterification of phytosterols with different vegetable oils to produce functional oils in one pot.

**Methods:** The esterification conditions were: phytosterols (50–150 mmol/L), triglycerides such as sunflower oil, corn oil, rapeseed oil, or linseed oil (80–640 mmol/L), lipase (10–40 mg/mL), and solvent (hexane, 10 mL) were added into an Erlenmeyer flask. The vial was placed in a shaking incubator at 45–60 °C with a shaking speed of 180 rpm for a certain time. The reaction bioconversion was monitored periodically by HPLC to confirm production.

**Results:** Four functional oils rich in both DGs and PEs with conversions >92.1% and controllable fatty acid composition were obtained under the optimized conditions. The prepared functional oil possessed low acid value (≤1.0 mgKOH/g), peroxide value (≤2.1 mmol/kg), and conjugated diene value (≤1.96 mmol/kg) and high diglyceride and phytosterol ester contents (≥10.4 and ≥20.2%, respectively).

**Conclusions:** A rapid and convenient esterification method using immobilized AYS@NKA as catalyst was developed to synthesize novel functional oils rich in both PEs and DGs in high yield under mild conditions. These findings could promote the wide application of the novel functional oils produced by the food grade process in different formulations of functional foods.

**References:**

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Identifying genes involved in nutrient uptake and utilisation in *Brassica napus*

**Background:** Oilseed rape (*Brassica napus L.*) is a crop of increasing importance, being the third largest source of vegetable oil globally (USDA, 2015). Demand for rapeseed oil in biodiesel is also strong (Berry et al., 2015). Processes involved in the uptake and utilisation of nutrients in oilseed rape are controlled by numerous traits including root architecture, membrane transport, root-to-shoot translocation etc. Due to the polyploid nature of oilseed rape, the genetic bases behind such traits are highly complex. Identifying genes that control these traits will enable them to be used in breeding strategies to improve nutrient use efficiency in crop plants.

**Objectives:** Use recently developed mapping techniques to identify key genes involved in the uptake and utilisation of nutrients (including Ca, Mg, and Zn) in a large diversity set of *Brassica napus* (ASSYST population, ~400 accessions; Bus et al. 2011, Körber et al., 2012). Characterise lines from the population that show extreme shoot nutrient concentrations and loci identified from mapping studies.

**Methods:** All plants from the ASSYST population plus controls were grown in polytunnels. Inductively coupled plasma-mass spectrometry (ICP-MS) analysis was used to measure elemental concentrations of 28 elements. These data were used to conduct a genome wide association analysis using SNPs and Gene Expression Markers (GEMs) to locate loci that correlated with elemental concentrations. Field studies were also conducted at two sites in which ~80 accessions were grown. Leaves from a subset of these plants were sampled at 6-8 leaf stage and analysed by ICP-MS.

**Results:** Leaf mineral element concentrations varied greatly between accessions with over 10-fold differences observed for many elements. Correlations between leaf element concentrations were also observed, including strong positive correlations between leaf Ca and Mg concentrations. Leaf mineral concentrations correlated positively between polytunnel- and field-grown plants. Early GWAS results identified several potential targets worthy of further study.

**Conclusions:** Correlations in mineral concentration between polytunnel and field grown plants indicate that high-throughput, low-cost polytunnel-based experiments are a useful way of assessing for differences between large numbers of accessions. The large range in observed mineral concentrations across elements and consistency in field demonstrates a genetic basis. This holds promise for GWAS and coupled with potential gene targets suggests the methods are well suited for use in determining the genetic basis behind nutrient uptake and distribution in oilseed rape. An understanding of such traits will enable greater control over them in breeding strategies.

**References:**


Intercropping frost-sensitive legume crops with winter oilseed rape reduces weed competition, insect damage, and improves nitrogen use efficiency

**Background:** Mixing plant species in agroecosystems is highlighted as an agroecological solution to reduce pesticides and fertilizers while maintaining profitability. In the French context, intercropping frost-sensitive legume crops with winter oilseed rape is potentially interesting and began to be implemented by farmers.

**Objectives:** In this study we aimed at measuring the services and disservices of this intercrop with three different legume mixtures, in terms of growth and yield for rapeseed, ground cover of weeds in autumn and damage caused by rape winter stem weevil.

**Methods:** The experiment was carried out at four sites in France from 2011 to 2014. Winter oilseed rape (Brassica napus) - subsequently referred to as 'WOSR' - was grown as sole crop and intercrop with three different legume mixtures: (i) faba bean (Vicia faba) + lentil (Lens culinaris) - subsequently referred to as 'FL'; (ii) grass pea (Lathyrus sativus) + fenugreek (Trigonella foenum-graecum) + lentil - subsequently referred to as 'GFL'; and (iii) purple vetch (Vicia benghalensis) + common vetch (Vicia sativa) + berseem clover (Trifolium alexandrinum) – subsequently referred to as 'VVt'. The experimental plots (120 to 200 m²) were set up in a randomized block design with WOSR, WOSR+FL, WOSR+GFL, WOSR+VVt as treatments with three replicates. In each trial, sole WOSR was managed according to local agriculture guidelines and conditions. The management of intercrops differed from sole WOSR in terms of nitrogen fertilization rates which were reduced by 30 kg ha⁻¹.

**Results:** We showed higher total aerial dry weights and total aerial nitrogen contents in the intercrops compared to sole winter oilseed rape in November. The companion plants contributed to the control of weeds and the mitigation of rape winter stem weevil damage, notably through the increase in the total aerial weight. In spring, after destruction of the companion plants, the intercrops had partially compensated a reduction in the N fertilization rate (-30 kg per hectare) in terms of aerial nitrogen content in rapeseed, with no consequences on the yield which was maintained or even increased. There were probably other interactions such as an improvement in rapeseed root exploration. The consequences were an increase in the nitrogen use efficiency in intercrops. The intercrop with faba bean and lentil showed the best results in terms of autumn growth, weed control, reduction in rape winter stem weevil damage, and rapeseed N content in spring and yield.

**Conclusions:** Intercropping frost-sensitive legume crops with winter oilseed rape is thus a promising way to reconcile yield and reduction in pesticides and fertilizer use and perhaps to benefit more widely to the cropping system.
Gene flow depends on the initial transgene location in the crop: 
Brassica napus-Raphanus raphanistrum model

Background: One of the main concerns for the development of herbicide tolerant oilseed rape varieties (B. napus, AACC, 2n=4x=38) is to prevent the introduction of the gene conferring herbicide tolerance in the genome of its related weeds. One of the most likely candidates is wild radish (Raphanus raphanistrum, RrRr, 2n=18), for which intergeneric hybrids can be formed in the fields, as well as progenies after pollination by wild radish. In advanced generations, it has been observed that all the herbicide tolerant wild radish plants present at least the oilseed rape additional chromosome carrying the transgene indicating an absence of recombination between the genomes. However, all these results were obtained from few transgenic lines grown in the presence of herbicide selection pressure and it is still unknown if the initial transgene location may have an impact on its transfer (by recombination) in the wild radish genome.

Objectives: We assessed whether introgressions can occur between oilseed rape and wild radish chromosomes and whether they depend on the initial location in the oilseed rape genome.

Methods: Plants of the fifth generation (G5) were obtained by open pollination under field conditions of F1 intergeneric B. napus-R. raphanistrum hybrids with wild radish. They generally presented a chromosome number close to 18 as wild radish. A representative sample of 307 plants among the 1626 observed were analysed using molecular markers specific to the parental oilseed rape varieties and absent from the wild radish population. The counts of the markers occurrence were assumed to follow the mixture of binomial distributions. The components of the mixture correspond to the different probabilities of introgression. Model-based clustering approach was proposed in order to assign the mixture component to each marker. Cytogenetic and molecular analyses were performed in the progeny of plants representative of different types of introgression.

Results: Molecular markers specific to oilseed rape with a frequency ranging from 0 to 0.27 were found in G5 plants and they were assigned to four classes. The assignment of the 105 analysed markers in the different classes gave the following results: 1 hotspot region that includes 2 adjacent markers (class IV), 4 medium spot genomic regions with 8 adjacent markers (class III), 11 low spot genomic regions with 20 markers (class II) and all the other ones belonging to the class I.

Detailed analyses of the plant progenies representative of the classes II to IV indicated that oilseed rape regions could be stably introduced in wild radish chromosomes but with a complex introgression and different rate of segregation.

Conclusion: Our results revealed that oilseed genetic material could be introduced in wild radish chromosomes but that some genetic regions can be more easily introgressed than others. The new technology allowing targeting transgene insertion could find new applications by choosing genomic regions with the lower probability of introgression in genome of weeds to prevent gene flow.
Pinolene-based compounds to reduce oilseed rape drought-induced yield losses

Background: Oilseed rape (Canola, Brassica napus L.) shows strong yield decreases when water stress periods occur at the reproductive stages: the total seed production can be affected up to 50% (Champolivier and Merrien, 1996). Whereas the breeding for a drought tolerant oilseed rape variety seems far to be achieved, the need to meet suitable yield under water deficit conditions force to study further agronomic techniques to achieve the purpose. Nearly fifty years ago, film-forming antitranspirants were considered a promising agronomic tool to preserve water in plants and thus avoid yield decreases under water deficit: their effectiveness was limited as their stomata-blocking property was strongly related to a drastic decrease in CO₂ assimilation (Kettlewell, 2014). More recently, they have been shown to be effective in improving yield under drought conditions when applied at the most drought-sensitive stage (Kettlewell, 2014).

Objectives: Evaluate the effectiveness of antitranspirant treatments in avoiding heavy yield losses of droughted oilseed rape. Quantify the effect of the treatments on relevant physiological traits and yield.

Methods: Winter oilseed rape (cv. Excalibur) plants were grown in 5 L pots and in glasshouse conditions after a 10-week vernalization period. Stress was applied at BBCH GS 6.0 until GS 6.9 (flowering stage) by removing the automatic watering. The film-forming antitranspirants (Poly-1-p and Di-1-p menthene) were sprayed on the adaxial surface of the leaves with an automatic pot sprayer at 1 l/ha dose rate in 130 l/ha water volume, 1 m/s speed, 3 bar pressure spray conditions. During the stress application plant gas exchange, stomatal conductance (Gs), relative water content (RWC), leaf water potential (LWP) and leaf temperature by thermal imaging (Lt) were collected. After the stress imposition the plants were re-watered to pot capacity. At complete maturity, plants were harvested and the yield components were evaluated.

Results: The results showed improvements in most of the physiological traits assessed: Gs was significantly decreased by the treatments (p<0.001) accompanied with a significant increase in CO₂ assimilated by droughted-sprayed plants compared to the un-sprayed, leading to a strong increase in water use efficiency. On the contrary the well watered-treated plants showed suppression in both Gs and CO₂ assimilation. LWP and RWC were significantly increased, proving the effectiveness of the treatments in improving plants water status under drought conditions. Thermal imaging analysis showed a significant increase in Lt under water stress conditions (p<0.001): the two compounds demonstrate different ability on blocking stomata with a stronger increase in Lt of the Di-1-p menthene compared to the Poly-1-p menthene treated plants. Gs and Lt were significantly correlated (p<0.001). Yield components were statistically increased by the film-forming treatments with a significant increase in pods per plant and seeds per plant.

Conclusions: Despite the common idea that a film forming antitranspirant treatment suppresses CO₂ assimilation and fixation leading to a decrease in photosynthetic efficiency and thus productivity, we demonstrate that applying film-forming compounds at 1l/ha over the most drought-sensitive stage on oilseed rape could be effective in avoiding drought-induced yield losses.

References:
Environmental life cycle assessment of rapeseed production in France within a public LCI-database of agricultural products

**Background:** The program AGRIBALYSE® was an initiative launched by the French authorities (ADEME) in order to create a public Life Cycle Inventory (LCI) database of French agricultural products. Two main assets of the program are a harmonized methodological framework for the production of the main crops cultivated in France, and collective validations at different stages of the LCI calculation (Koch and Salou 2013). Its outputs give keys to evaluate environmental impacts of agricultural practices.

**Objectives:** The Life Cycle Assessment (LCA) results obtained for the French rapeseed crop, at a national level, are discussed here. A focus was made on four impact indicators being energy demand, GHG emissions, Acidification, and eutrophication (ILCD recommendations and CML 2001), while identifying the most contributing steps during the crop life cycle. These results were compared to previous published data at an international scale (Ecoinvent) or within national projects.

**Methods:** The LCA methodology was applied, following the ILCD recommendations. The boundaries of our studied system were from cradle to farm gate; all up-stream processes (input production) were included but post-harvest operations were excluded. Data collected were obtained from national statistics adjusted with experts’ judgment. Our functional unit is one kilogram of harvested rapeseed, in order to assess the environmental impacts of food products. However the functional unit used during the data collection was one hectare of rapeseed crop and results per kilogram were assessed using the average yield. Both units are used in this article to better describe the multi-functionality of agriculture. Considering the weight of field emissions on environmental impacts, the models calculating emissions in soil, water and air were preferably adapted to the French context. When possible, special developments were made to estimate more precisely emissions like nitrate leaching.

**Results:** The main contributors to the selected environmental impacts were field emissions (N2O in air for Global Warming, NH3 in air for Acidification, NO3- nitrate in water for Marine Eutrophication). Experimental data, from a French network (the NOGAS project), showed that the direct emissions calculated with the tier 1 IPCC method (2006) tended to be overestimated: the calculated N2O emissions of rapeseed crops were 2.2 times higher than the measured emissions. The assessment of the effect of improved practices, such as organic fertilization or the introduction of legume crops in the rotation, showed some improvements on environmental impacts but it quickly reached its limits due to the use of models that were too simple to simulate all their environmental benefits.

**Conclusions:** These LCA results are currently available, in a version 1.1, and will be updated during 2015, with a V1.2. During the AGRIBALYSE® project, regarding data and models available, best choices were made to evaluate at a national scale French agricultural productions and to produce valid LCI references. Nevertheless, remaining work needs to be done to develop less simple models in order to better account for improved agricultural practices. Finally, these results represent a step forward to share with the agricultural sector in order to promote environmental evaluation and good farming practices.

**References:**
Koch P., Salou T., 2013. AGRIBALYSE®. Methodology V1.1 – Ed ADEME, Angers, France
Improving nitrogen-use efficiency in oilseed rape (Brassica napus)

**Background:** Nitrogen (N) fertilisers are used widely to improve oilseed rape yields. However, N is an expensive input and it is the biggest variable cost for winter oilseed rape production. Significant genetic variation in N-use efficiency (NUE) has been reported among oilseed rape genotypes (Berry et al. 2010) which can potentially be exploited by plant breeders. The aim of this study is to develop and test a field-based physiological model of N-use efficiency (NUE), defined as the product of N uptake and utilisation, in oilseed rape. This model will be used to identify novel traits for use in breeding strategies to improve NUE and inform the optimal use of N fertilisers.

**Methods:** A crop growth model has been developed which describes uptake and utilisation of N in oilseed rape and to relate this to yield. The model consists of modules for early leaf development, stem extension to mid-flowering, pod development and seed filling. It predicts dry matter production and development based on N uptake and partitioning. Radiation uptake and leaf senescence are used to calculate daily seed biomass accumulation based on dry matter. The model was based initially on literature data (Berry et al., 2006), which has now been updated. Addition of a sulphur (S) parameter to the model will be made when new data is available.

Data to calibrate and test the model are currently being collected from new field experiments. Genotypic variation in NUE is being studied among 84 genotypes of oilseed rape at two UK locations, for two years (2014-15, and 2015-16). The 2014-15 trials are being carried out at Bessingby (Yorkshire) on a chalky clay loam, and at Deeping (Peterborough) on a clay loam. Both experiments comprise two N application rates, a low (60 kg N ha⁻¹) and a high (300 kg N ha⁻¹) rate, applied as split-dressings of ammonium nitrate (34.5% N). A split-plot alpha design is being used, with N as the main plot factor and variety as the sub plot. The genotypes are a mix of elite commercial cultivars, genotypes from a diversity panel suited to associative transcriptomics (Harper et al., 2012), and genotypes previously identified with high and low NUE (Berry et al., 2010). Preliminary growth data from 2014-15 will be reported. The uptake and distribution of N and S at key growth stages will subsequently be analysed.

**Results:** The dynamics of oilseed rape yields in relation to N availability have been predicted well using the model. Sensitivity tests of the model parameters have been conducted by compiling N response curves using a wide range N fertiliser rates (0-480 kg N ha⁻¹). This has allowed thorough analysis of how varietal differences affect the model and assessment of the physiological relationship to the field. The model will be validated using data from 2014-15 field experiments.

**References:**
Enabling european farmers to grow oilseed rape sustainably

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Oilseed rape is an important crop in Europe and since 1990 the harvested area continues to expand1 mostly due to an increased demand for vegetable oil for human consumption and industrial purposes. Maintaining this growth sustainably on a finite amount of land requires a holistic approach to crop production, combining good agronomic practices with crop protection systems and support tools. BASF’s commitment to sustainable oilseed rape production is reflected in innovative solutions like its modelling tool, AgBalance, and plant protection systems such as the Clearfield® Production System and AgCelence®.

AgBalance analyzes all segments of the value creation pipeline from agricultural inputs, production, processing, and retail, ending with the consumer. This tool uses a well-established statistical modelling system2 to evaluate ecological, economic and social impacts, and compares the impact of decisions to improve sustainability in agriculture. AgBalance is currently used by stakeholders in 15 EU countries, and is a key tool in the evaluation of efficient solutions for reducing greenhouse gas emissions during the oilseed rape production process for biofuels, as well, for the assessment of winter oilseed rape (WORS) production systems in Germany.

One of the assessed WORS production systems is the Clearfield Production System which combines elite herbicide tolerant hybrid varieties with regionally tailored herbicides (HRAC group B) for season long control of broadleaf and grassy weeds, resulting in increased yields. In Europe, the combination of imazamox-based herbicides in combination with e.g. metazachlor (HRAC group K3) provides a cross spectrum herbicide with a dual mode of action which avoids herbicide resistance development. Clearfield oilseed rape is internationally recognized as non-GMO, and therefore can be freely grown and traded. Through collaboration with the vast majority of oilseed rape breeders, a broad portfolio of herbicide tolerant spring and winter hybrids have been launched and are in continuous development, including varieties intended for specialty markets, such as high oleic low linolenic (HOLL).

An increase in oilseed rape acres in Europe resulting in narrower crop rotations will favour disease development, such as Phoma lingam and Sclerotinia sclerotiorum. For this reason, BASF is offering a number of tools to help the farmer better understand the diseases that affect their crop and enable them to adopt the best crop management practices for sustainable crop production. BASF’s AgCelence plant health products have been carefully developed to provide disease control coupled with improved plant health. Pictor® and Eflor® each have a combination of two active ingredients and have been developed for in flowering control of Sclerotinia and Altenaria. Caryx®, a combination plant growth regulator and fungicide, and Alteno®, a combination of two actives, are products developed for autumn and spring disease control which also improve winterhardiness, root enhancement, plant architecture and lodging control in the spring.

To ensure the high yield potential of today’s oilseed rape varieties, BASF, through broad innovative solutions, strategic partnerships, and a holistic method for assessing agricultural sustainability, has brought and will continue to deliver a number of key products to the European marketplace in the areas of plant health and weed management.

References:

1 FAOSTAT. 2011.
Genetic control of seed germination and vigour in rapeseed (*Brassica napus*)

**Background:** Rapid and uniform seed germination is a crucial prerequisite for the achievement of high and stable yield levels in rapeseed production. Furthermore, enhanced germination facilitates a reduced time span between sowing and emergence and thus a gain in growing time. For breeding of vigorous cultivars with fast and uniform field emergence it is important to understand the genetic factors contributing to adequate germination performance and seedling growth.

**Objectives:** The genetic control of seed germination and vigour was studied in diverse *Brassica napus* materials using different methodical approaches. Linkage analysis was carried out in a segregating doubled-haploid winter oilseed rape population, and genome-wide association studies were performed in a genetic diversity panel to define genomic regions harboring promising genes affecting seed germination and early seedling growth. Furthermore a systems biological approach was performed to identify regulatory networks affecting germination performance and seedling vigour.

**Methods:** Linkage analysis was performed in a bi-parental winter oilseed rape population (n=250) and a winter type diversity panel (n=248). All lines were genotyped with the Illumina® Brassica 60k SNP array. Seeds from different production environments were subjected to extensive automated in vitro phenotyping of germination related traits, such as germination speed, absolute germination rate and radicle elongation rate. Marker sequences were mapped onto the *B. napus* Darmor-bzh reference genome. Candidate genes within trait-associated regions were identified by functional annotation followed by gene ontology analysis. Weighted gene co-expression network analysis (WGCNA) was used to explore co-expression networks involved in seed germination and early seedling development.

**Results:** The data obtained underscore the high dependency of seed germination and seedling growth on environmental factors, but nevertheless reveal substantial potential for improvement by genomic based breeding. Several candidate genes could be identified within genomic regions associated with germination speed, absolute germination rate, radicle growth and thousand seed weight. A number of promising candidates could be validated by sequencing based transcriptome analysis or physiological experiments.

**Conclusions:** Large-scale automated phenotyping revealed broad phenotypic variability for germination performance. Marker-trait associations and candidate genes provide a solid basis for the establishment of reliable genomic selection tools for improved seed germination and seed vigour in rapeseed.
Increasing canola yields under climate variability through genetic engineering of heat and drought stress tolerance

Canola crop productivity is ultimately defined by its yield. Recent agronomic and economic studies indicate that yield losses are most significantly attributable to unfavourable environmental conditions such as those imposed by drought and heat, and those conditions can occur throughout the growing season. The effects of these stresses are particularly damaging to canola yield when they coincide with the transition to flowering in Canola. Exposure to either drought or heat stress independently has a significant negative impact on crop yield and this effect is compounded when these stresses occur in combination. Therefore, improvement of dual stress tolerance to heat and drought in crop plants has become a top priority to stabilize yields in canola varieties. Aiming to solve this problem, we employed unique forward genetic screens and a range of genomic approaches to discover and characterize genes that are involved in the regulation of heat and drought tolerance. In particular, we have identified and completed the functional analysis of a subset of target genes that constitute a novel transcriptional regulatory cascade that controls the plant’s responses to these combined stresses. In the laboratory conditions, Arabidopsis and Canola plants with mis-sense expression of these regulatory genes were able to tolerate higher temperature or drought treatment beyond their control lines. More importantly, these plants produced higher seed yield than their controls when both stresses were applied simultaneously. The dual stress tolerance and yield enhancement properties of these transgenic plants were further confirmed in large-scale, multiple season and location Canola field trials. These results represent a significant breakthrough in canola crop improvement. Technologies derived from this research could enable canola farmers around the world to obtain higher yield and productivity over variable and adverse environmental conditions.

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Nested association mapping for drought tolerance

Background: *Brassica napus* is the most important oilseed crop in Europe and second one worldwide. Due to the climate change, it is foreseen, that drought events will take place more often in many areas of the world, like middle Europe. So it is important to ensure high yield and quality even under unfavorable conditions like drought.

Objectives: The main goal of the project is to dissect complex traits, like drought tolerance, based on a double haploid nested association mapping population (BnNAM-DH). This population should be phenotyped for drought tolerance in a pot experiment under semi controlled conditions. Before running the association mapping, preliminary analyses have to be done: calculation of the population structure or the linkage disequilibrium in the population. For the association mapping itself, a fitted mixed linear model has to be developed. Furthermore, epistatic effects and haplotypes will be be calculated.

Methods: For the construction of BnNAM-DH, 21 genetic diverse parents were crossed to one elite line. 210 genotypes of the population (10 genotypes per family) were grown in a pot experiment with two treatments: 1. well watered and 2. water scarcity for 28 days after flowering. Phenotyping was done for two years in 2013 and 2014. During growing season, flowering time and plant length were scored. After harvest yield per plant and yield related traits and seed quality traits were estimated. Genotyping was carried out using the 60K *Brassica* Infinium® SNP array. LD and population structure were calculated using the statistical software R. Association analysis for identification of QTL is performed using a mixed model approach with conditional analysis (forward/backward selection), cross validation and haplotype blocks. Also epistatic effects are calculated.

Results: Analysis of linkage disequilibrium revealed differences of linkage disequilibrium among the chromosomes. Population structure was calculated using principle component analysis. The first two principle components explain a small portion of the genetic variance (≈ 10%). The fitted mixed model is able to detect significant markers, with and without treatment interaction. For the first year, 12 significant marker by treatment interactions could be found after conditional analysis. Threshold was set at a LOD-score of 3. Several significant markers are located near to candidate genes, for the relevant trait.

Conclusions: The nested association mapping population in addition to a well fitted model is a useful tool to dissect complex traits and to understand the genetic basis behind each individual trait. Through extra features like cross validation and conditional analysis, it is possible to define a really small genomic region for the QTL. This makes it easier and faster in future, to find the corresponding functional single gene for each trait being analyzed.

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Assessing QTLs for yield and their stability under limiting nitrogen supply using a wide field trial network

Background: Rapeseed is a major oil crop with a relatively low Nitrogen Use Efficiency making its production highly dependent on N inputs. Improve yield and guarantee yield stability under low N input are new issues for rapeseed breeders in Europe. This addresses the question of the stability of yield genetic determinants under various nutrition regimes.

Objectives: The objectives of this work were to identify the genomic regions associated with yield in rapeseed and to assess their stability under contrasted environmental conditions, including N contrasted conditions.

Methods: A field trial network of seven locations was defined across France to represent the diversity of pedo-climatic rapeseed growing areas. For each location two contrasted N regimes were carried out (N+ optimal and N- low). This design allowed the field characterization of plant material during six years for linkage disequilibrium-linkage analysis purposes. Yield and yield-related traits were scored and N-responsive traits were calculated as N-/N+ and ∆N/N+. The plant material consisted in a diversity set of 93 accessions and two doubled-haploid populations that were all densely genotyped with the Illumina® Brassica 60k SNP array.

Results: N contrasted conditions were obtained across our field trial network. Very few genotype x N interactions were detected and a great stability of the QTLs was assessed between the N conditions. On the contrary, strong genotype x site interactions were found with most of the QTLs specific to one site only. This work came up with the detection of several QTL regions of interest with a particular dense one on the A5 linkage group with QTL stable across sites and N conditions. Candidate genes underlying this region were identified by functional annotation and gene ontology analysis.

Conclusions: A field phenotyping network was set up in the frame of the French project RAPSODYN (www.rapsodyn.fr/en) and validated for contrasting N conditions. Promising QTLs were detected and are currently transferred to elite lines and hybrids.
Differences on photosynthesis between leaf and silique of winter oilseed rape (*Brassica napus L.*) to potassium deficiency

**Background:** Leaf and silique are two main photosynthetically active organs of winter oilseed rape (*Brassica napus L.*), both of which play key roles in vegetative architecture and yield formation (Bennett et al., 2011). As one of the essential plant elements, the potassium (K) uptake of oilseed rape is substantial. Potassium is involved in many physiological processes, such as photosynthesis. Differ from leaf, silique is a non-foliar photosynthetically reproductive organ. It may result in distinct physiological and photosynthetic properties, along with their different responses to K deficiency.

**Objectives:** Changes on physiological and photosynthetic properties of silique under K deficiency were always neglected. Thus, the objectives were to analysis the differences on photosynthesis between leaf and silique to K deficiency, which will facilitate a better understanding of photosynthesis of oilseed rape and improve seed yield according to optimal potassium fertilizer management.

**Methods:** Measurement of gas exchange parameters was carried out on leaf and silique during their steady stage of photosynthetic function at field condition with K sufficient supply treatment (+K) and K deficiency treatment (-K). Light- and CO2-response curves, combined with imaging-PAM analysis were applied to reveal the distinguishing response characteristics and chlorophyll fluorescence of leaf and silique to K deficiency.

**Results:** The results showed that leaf K concentration was lower than silique, and under K deficiency, it decreased more than silique. Net photosynthetic rate of leaf was three times higher than that of silique. K deficiency significantly decreased the net photosynthetic rate; the average reduction rate was 28.0% and 26.2% for leaf and silique, respectively. The light compensation (LCP) and saturation (LSP) point of silique were higher than those of leaf. In contrast to the +K treatment, the LCP values of those two organs were significantly increased, however, the LSP values were declined in K-deficient organs. The reduction of the light utilization capacity of leaf was more than that of silique under K deficiency. Silique performed a higher capacity of CO2 utilization. The CO2 compensation point (CCP) of silique was lower than that of leaf; however, the CO2 saturation point (CSP) of silique was higher than leaf. K deficiency mainly increased the CCP values of those two organs. Furthermore, imaging-PAM analysis indicated that minimum fluorescence (F0) of silique was considerably lower than leaf. But the maximum quantum yield of PSII (Fv/Fm) and non-photochemical quenching (NPQ) of silique was higher than those of leaf. Greater heterogeneity emerged in leaf, especially the mesophyll near leaf margin under K deficiency.

**Conclusions:** Leaf organs equipped with the stronger photosynthetic capacity, however, pod could make the better of high light intensities and low internal CO2 conditions. Potassium deficiency imposed constraints on CO2 assimilation of two organs, yet leaf was more sensitive. Optimal K supply improved utilization efficiency of high light intensities and low CO2 concentration.

**References:**
Developing a non-destructive method for assessing canola genotypic differences in tolerance to heat and drought stresses

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Background: Canola is a crop sensitive to drought and heat stresses that usually occur with different frequency and duration almost every summer during canola flowering in Canada and elsewhere in the world. The performance of any crop depends strongly on its root architecture and function. Canola roots may play a central role in overcoming the constraints to growth and development imposed by heat, drought and other environment-induced stresses. An electrical capacitance method was reported to be sensitive in delineating stressed plants from healthy plants in hydroponic or sand-based growth media, but investigation is needed to test its suitability for use under field or potted soil conditions.

Objectives: A controlled study was conducted to (i) examine canola genotypic differences in root electrical capacitance, (ii) determine if the measured differences in root electrical capacitance can be used to delineate responses of canola genotypes to heat and drought stresses, and (iii) develop an electrical capacitance method to screen for root biomass and root-related traits.

Methods: The experiment was arranged in a split-plot design with temperature regime in the main-plot units, and combinations of genotype, drought, and growth regulator treatments in the subplots. The seeds were sown in plastic pots filled with soil mixtures (clay loam/peat moss and vermiculates). There were two treatments: high (27/17 °C) and normal (23/17 °C). Two water levels (sub-plot), 85% (i.e., control) and 45% SWC were applied to the plants. At 31 DAS, electrical capacitance, biomass of stem, leaf and root from each pot were determined.

Results: Compared to the control, high temperature and drought stresses significantly suppressed root and aboveground biomass, with large differences in measured parameters among canola genotypes. Changes in root electrical capacitance values reflected the responses of canola genotypes to the stress factors. High temperature and drought stress had a significant synergistic/interaction effect on decreasing root biomass and root capacitance. There was a linear relationship between electrical capacitance and root biomass, with larger R2 values (P < 0.01 n=63) in stress-tolerant genotypes across high temperature and drought stresses.

Conclusions: Our results indicate that drought stress severely restricted growth and dry matter accumulation in all genotypes, but there was an interaction between genotype and heat stress on canola biomass. Measuring electrical capacitance in soil-based pot study is a promising tool to estimate root biomass in examining canola genotypic differences in response to high temperature and drought stresses.

References:
Management of oilseed rape (OSR) volunteers to secure low alpha-linolenic acid content in High Oleic Low Linolenic (HOLL) OSR crop

**Background:** HOLL winter oilseed rape (WOSR) has high oleic acid content (C18:1, >75%) and low alpha-linolenic acid content (C18:3, <3.5%). HOLL oil is therefore heat-stable and can be used for frying without hydrogenation. The presence of conventional WOSR volunteers from previous crops can significantly increase C18:3 content, with a critical threshold density of two volunteers m-2 (Baux et al. 2011).

**Objectives:** To consolidate the HOLL market, it is important to secure oil quality, and therefore to limit volunteer density. We tested different management strategies to control volunteers, and analyzed the consequences on oil quality.

**Methods:** A field trial was carried out for harvest season 2013 in Agroscope, Changins. An imidazolinone-tolerant (IT, Clearfield® technology) HOLL variety was sown at a rate of 50 seeds m-2, together with a conventional WOSR variety (25 seeds m-2) to simulate volunteers. Six herbicide treatments were applied in fall 2012: i) control (no-herbicide), ii) pre-emergence herbicide (Devrinol® Top, 345 g/l napropamide, 30 g/l clomazone), iii) Cleranda® (17.5 g/l imazamox, 375 g/l metazachlor, IMI), at early post-emergence (CD 11), iv) at standard post-emergence (CD 12-13), v) at late post-emergence (CD 16), and vi) in two split ½ dose applications (CD 11 and 14). Two other field trials took place in 2013. First an IT-HOLL variety was sown with an increasing density of conventional volunteers (0, 1, 2, 5, 10 or 25 seeds m-2) and two herbicide treatments (pre- or post-emergence, as before). Secondly, two imidazolinone-sensitive HOLL varieties (hybrid or OP-line) were sown with an increasing conventional volunteer density (0, 1, 2 or 5 seeds m-2). In 2015, three methods to control weeds and volunteers (pre-, post-emergence herbicides, or mechanical treatment) have been integrated using an IT-HOLL variety in a long-term experiment comparing plough or superficial tillage since 1967. In all experiments, we assessed volunteers, weeds and crop density, as well as yield and oil quality.

**Results:** The Clearfield® system totally eliminated the volunteers, independently of the initial density and the method of application and hence secured HOLL oil quality (C18:3 <3%). Linolenic acid content of the OP-line variety increased significantly with two volunteers m-2. The hybrid variety was less affected by the presence of volunteers (no significant effect of two volunteers m-2), had a lower C18:3 content and a higher yield potential. Our results show that superficial soil tillage allows a 47% reduction of volunteers as compared to ploughing. In fall 2014, mechanical treatment and IMI-herbicide application were efficient to reduce volunteer density.

**Conclusions:** We identified three efficient strategies to guarantee low C18:3 content in HOLL WOSR. Varieties with high competitive ability and yield potential are less affected by the presence of volunteers. Mechanical weed control and adapted soil tillage can secure low volunteer densities. The Clearfield® system can efficiently eliminate conventional volunteers. However, there is currently a tradeoff between IMI-tolerance and variety competitiveness in terms of yield and oil quality.

**References:**

Effects of N fertilization on the carbon footprints of canola and mustard under diverse environments

Background: Canola and mustard plants require large amounts of nitrogen fertilizers to be applied in order to increase their seed yield and improve seed quality. However, nitrogen fertilization is one of the major contributors to greenhouse gas emissions in crop production. It is unknown how nitrogen fertilizer can be managed in canola and mustard to optimizing crop productivity while, at the same time, minimizing carbon emission from the crop production.

Objectives: This study was conducted to (i) provide a quantitative estimate of total greenhouse gas emissions and the carbon footprint of canola and mustard grown on the semi-arid northern Great Plains and (ii) determine the effects of environments and N fertilizer rates on the carbon footprint of canola and mustard.

Methods: Five oilseed crops, napus canola, rapa canola, juncea canola, juncea mustard, and alba mustard, were grown under the N rates of 0, 25, 50, 100, 150, 200, and 250 kg N ha⁻¹ at eight environmental sites (location x year combinations) in Saskatchewan. Those sites represent the major oilseed production ecoregions in western Canada. Straw and root decomposition and various production inputs were used to calculate greenhouse gas emissions and the carbon footprints.

Results: On average, emissions from N fertilization were 9 times the emission from the use of pesticides, and 11 times that of various farming operations. Straw and root decomposition emitted 120 kg CO₂ eq ha⁻¹, contributing 10% to the total emission. Emissions from the production, transportation, storage, and delivery of N fertilizer to farm gates accounted for 42% of the total greenhouse gas emissions, and the direct and indirect emission from the application of N fertilizer in oilseed production added another 31% to the total emission. Carbon footprint increased slightly as N rates increased from 0 to 50 kg N ha⁻¹; but as N rates increased from 50 to 250 kg N ha⁻¹, carbon footprint increased substantially for all five oilseed crops evaluated. Oilseeds grown at the more humid Melfort site emitted 1355 kg CO₂ eq ha⁻¹, 30% greater than emissions at the drier sites of Scott and Swift Current. Oilseeds grown at Melfort had a carbon footprint of 0.52 kg CO₂ eq kg⁻¹ of oilseed, 45% greater than that at Scott (0.45 kg CO₂ eq kg⁻¹ of oilseed) and 25% greater than that at Swift Current (0.45 kg CO₂ eq kg⁻¹ of oilseed).

Conclusions: Carbon footprint of oilseeds was a function of the rate of N fertilization applied to the crop, and the magnitude of this effect varied with environments. Environmental variation contributed 10% to the variation in carbon footprint. Nitrogen manufacture and application together contributed as high as 74% of the total emission accumulated during the course of the crop production. Key to lower carbon footprint in oilseed production is to improve N management practices.
Legumes in rotation affect hybrid canola and malting barley

**Background:** The high and unpredictable cost of fertilizer nitrogen (N) in western Canada has generated interest in alternative N sources. Legumes produce N through fixation, and may increase soil residual and mineralizable N, thus reducing the need for fertilizer N in subsequent crops. Hybrid canola (*Brassica napus* L.) has a high N requirement for optimum yield, but knowledge of the rotational effects of legumes on canola is limited.

**Objective:** The objective was to determine the effects of legume and non-legume preceding crops on yield, quality and net revenue of canola grown the following year and malting barley (*Hordeum vulgare* L.) grown after canola.

**Methods:** Field pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medik.), faba bean (*Vicia faba* L.), canola and wheat (*Triticum aestivum* L.) harvested for grain, and faba bean grown as a green manure were direct-seeded at seven locations in western Canada in 2009. Canola was seeded in 2010 and barley in 2011, with fertilizer N applied at 0, 30, 60, 90 and 120 kg/ha.

**Results:** On average, all legumes, except faba bean harvested for grain, produced higher canola and barley yields compared to when wheat was the preceding crop. Faba bean green manure produced the highest yields, while canola on canola produced the lowest canola yield. The legumes had little or no negative effect on percent canola oil or barley protein. Yields of both crops increased with increasing N rate, but percent canola oil decreased, and barley protein increased. The study showed that basing N management on a target yield, 25% less fertilizer N was required to maintain a given canola or barley yield when canola and then barley followed field pea or lentil rather than wheat. An economic analysis indicated that over the entire 3-yr crop sequence, lentil and field pea harvested for grain provided the greatest net revenue. Although the faba bean green manure produced the highest yields, this was insufficient to compensate for the loss of crop revenue and resulted in the lowest overall net return.

**Conclusion:** The results indicate that growing lentil or field pea for grain prior to hybrid canola can improve canola yield, subsequent barley yield and overall net revenue without negatively affecting canola oil or malting barley protein.

**References:**


Indirect N₂O emissions: Model-based quantification of N leaching and NH₃ emissions in OSR fertilized with mineral and organic fertilizers

Background: Emissions of nitrous oxide (N₂O) from arable land are a major contribution to the global GHG balance. To quantify their amount, direct and indirect N₂O emissions have to be considered. Direct N₂O emissions can be measured directly at the place of origin. Indirect N₂O emissions result from displaced reactive nitrogen compounds like ammonia or nitrate. They account for one third of the total global agricultural N₂O source and approximately two thirds of the uncertainty in the total source [1]. Fertilizing OSR with organic fertilizer may lead to high amounts of ammonia volatilization. The high N content in the plant residues enables a fast mineralization, which can cause N leaching after OSR harvest.

Objective: Using a model-based N balance approach, indirect N₂O emissions should be quantified by simulating ammonia emission and nitrogen leaching.

Methods: Starting in autumn 2012, a field experiment including a crop rotation (OSR, wheat, barley) was carried out two years in the northern part of Germany. In spring OSR was fertilized with 180 kg N, either as mineral (CAN) or organic (180 kg NH₄-N) fertilizer. Wheat and barley were fertilized with CAN (220 kg N and 200 kg N respectively). NH₃ emissions were detected after organic fertilizing events by Draeger tube measurement technique [2]. Direct N₂O emissions were measured weekly using manual chambers. Soil mineral N (SMN) samples were taken three times per growing season.

To quantify indirect N₂O emissions we used measured data, a dynamic simulation model to calculate N leaching and a semi-empirical model for NH₃ volatilization. SMN data at the beginning of the experiment were used as initial model data. Fertilization (experimental data) and modelled mineralization were used as system input, whereas modelled N uptake by plants, nitrogen leaching and gaseous N emissions (N₂O, NH₃ (measured); NOₓ, N₂ (modelled)) were regarded as system output. Finally we calculated indirect N₂O emissions using corresponding IPCC TIER 1 emission factors.

Results: In 2013 and 2014 we measured 45 and 40 kg NH₃-N per ha total NH₃ emissions, respectively. This results in calculated indirect N₂O emissions of about 0.45 kg N per ha and 0.4 kg N per ha, respectively, originating only from ammonia volatilization. That means, for example, indirect N₂O emissions in a range of up to 64% of the measured direct N₂O emissions in 2014. In autumn 2013, a SMN value of 25-30 kg N ha⁻¹ in a depth of 60-90 cm under wheat following OSR gives indication of a high N leaching potential which leads to considerable indirect N₂O emissions.

Conclusion: Fertilizing OSR with organic fertilizer leads to relatively high amounts of indirect N₂O originating from ammonia compared with measured direct N₂O emissions. High SMN values after OSR potentially lead to high N leaching.

References:
All together now – comparative analysis of genome-wide associations for flowering time, plant height and seed yield in winter-type *Brassica napus*

**Background:** Several studies have documented colocalization of QTL for flowering time, plant height and seed yield in *Brassica napus*. Interestingly, flowering genes like Bna.FT and Bna.TFL1 have been found to determine seed traits, and similar results have been found in *Arabidopsis thaliana* and *Brassica rapa*. In this study we tested the hypotheses (1) that climatic adaptation may be orchestrated by some general adaptation loci and (2) that some of those loci may be part of the flowering network.

**Objectives:** We studied flowering time as a measure of reproductive adaptation, plant height as a biomass parameter and seed yield as a measure of reproductive success. Through a genome-wide association study (GWAS) we aimed to identify chromosome regions potentially responsible for the simultaneous regulation of two or three traits. Genes underlying such regions are candidates to be overall adaptation loci and may represent important breeding targets.

**Methods:** A total of 140 European winter-type *B. napus* inbred lines were genotyped with 21,623 unique, single-locus single-nucleotide polymorphism (SNP) markers using the *Brassica* 60K-SNP Illumina® Infinium consortium array. Phenotypic associations were calculated over the years 2010-2012 for flowering time, plant height and seed yield in 3 locations in Germany using a mixed model with PC-adjustment. Gene ontology enrichment analysis was performed using the R package “Gostats” and the web-based platform REVIGO.

**Results:** We identified 68 cross-trait regions with potential adaptive value. Within these regions, *B. napus* orthologs for a number of candidate adaptation genes were detected, including central circadian clock components like CIRCADIAN CLOCK-ASSOCIATED 1 (Bna.CCA1) and TIME FOR COFFEE (Bna.TIC) along with the important flowering-time regulators FLOWERING LOCUS T (Bna.FT) and FRUITFUL (Bna.FUL). Gene ontology (GO) enrichment analysis of candidate regions revealed strong enrichment for response to environmental factors like abiotic, biotic or endogenous stimuli, hypersmotic response or response to heat. Moreover, there was enrichment for biosynthesis of both small and large molecules, (e.g. flavonoids), immune system processes and macromolecule methylation.

**Conclusions:** Our results provide a valuable framework to further improve the adaptability and yield stability of this recent allopolyploid crop under changing environments. By performing the study in adapted germplasm representing the broader gene pool of modern winter oilseed rape, we demonstrate the existence of considerable untapped potential for exploitation in breeding.
Characterization of morphophysiological responses associated with water stress tolerance in *Brassica napus*

**Background:** Adaptation to moisture stress is a complex outcome of constitutive or induced responses to the external stimuli. *Oleiferous Brassicas* are frequently subjected to moisture stress as these crops are cultivated primarily on light textured soils under low moisture conditions in many parts of the world, including India and Australia. Yield losses can be heavy if drought stress coincides with the reproductive growth (Lawlor and Cornic 2002). Identifying genetic variation for drought tolerance is critical as climate changes and anthropogenic activities are projected to further limit water availability in the southern hemisphere.

**Objectives:** Genetic variations for tolerance to water stress are yet to be adequately documented in *Brassicas*. Availability of international germplasm under longstanding ACIAR/GRDC funded projects allowed undertaking elaborate studies to quantify drought tolerance in canola *B. napus* and to identify associated morpho-physiological traits.

**Methods:** An assembled set of 30 genotypes was first field evaluated for drought tolerance under three irrigation modules: i) no irrigation ii) one irrigation (45 days after sowing) and iii) two irrigations (45 and 85 DAS). Based on the initial evaluation, 12 genotypes were further investigated for another two years, for photosynthesis and leaf traits (90DAS), water potential, SPAD, RWC (periodically), chlorophyll fluorescence, and root traits (100DAS), stomatal frequency/size (120DAS), and biochemical estimations (90DAS). Drought susceptibility/tolerance/efficiency were also worked out using standard protocols.

**Results:** Moisture restriction reduced stomatal conductance (Cs), transpiration (Tr) and consequently, photosynthesis (Pn). Cv. Tarcolla registered lowest Pn (4.8umolm-2s-1), suggesting low Cs (0.110molm-2s-1) and Tr (2.57molm-2s-1).Stomatal frequency and size were maximum in GSC6. Garnet had longest roots while cv. Karro showed maximum root area. Damage to PSII was maximum in cv. Karro (0.668) and least in cv. Ruby (0.728) under restricted moisture. Photochemical efficiency was comparable in Opal and RT-057. Leaf area/plant was lowest in Ruby and highest in EC609303. SPAD chlorophyll, RWC and water potential were high during vegetative phase and declined thereafter. Moisture availability improved LA by 33.8% and 53.8%. Water deficit up regulated sugars and proline in the elite genotypes and decreased seed storage products. Variations existed in drought susceptibility index (DSI) for growth and yield components. DSI computed on moisture stress and one irrigation for SY (0.05-2.07, mean 0.979) and DTE1 (69.9-99.3% mean 85.7%) was higher than calculated on two irrigations DSI2 (0.140-1.94, mean 0.968) and DTE2 (54.6-96.6% mean 77.1%). Overall, Cv. Opal and RT-057 (DSI ≤0.53, DTE ≥87.0%, DTE≥0.513) showed maximum tolerance to moisture restriction.

**Conclusions:** Test genotypes showed varied responses to moisture restriction. Cv. Opal and RT-057 showed maximum drought tolerance. Elite genotypes had higher Pn, lower damage to PSII, greater root length, RWC, sugars, proline lower DSI and higher DTE. Pn coupled with fluorescence, RWC along with DSI and DTE appeared reliable to monitor genetic variation for responses to moisture stress.

**References:**

Effect of S deprivation on osmotic potential components and N metabolism in oilseed rape leaves: identification of a new early indicator

**Background:** Compared to other plant species such as cereals and like many Brassicaceae, winter oilseed rape (Brassica napus) requires a relatively large input of mineral nutrients such as sulfur (S). As a consequence, oilseed rape is especially sensitive to S limitation. Identification of early sulfur deficiency indicators is of prime importance for Brassica napus which yield and the nutritional quality of seeds are negatively affected by S deficiency (McGrath and Zhao, 1996; D’hooghe et al., 2014).

**Objectives:** S is mostly stored as sulfate in leaf cell vacuoles and can be mobilized during S deficiency. So, this study investigated the impact of S deprivation on leaf osmotic potential in order to identify the mineral and/or organic compounds that contribute osmotically during sulfate mobilization. The objective was to identify early events during S deprivation, well before growth reduction and with kinetics close to the induction of sulfate transporters that could be potentially usable under field conditions as indicators of S nutrition levels.

**Methods:** Brassica napus were grown at vegetative stage during four weeks and then were exposed to S deprivation for 28 days (Control: 508.7 µM sulfate vs S deprivation: 8.7 µM sulfate). Plant samples were harvested kinetically. Each plant was sampled as emerged leaves, corresponding to leaves present at the beginning of treatments application (at d0) and new emerging leaves which correspond to leaves appearing during treatments. Plant samples were analyzed for osmotic potential, water content, mineral and organic solute contents, total S and N contents, 15N-nitrate uptake, nitrate reductase activity and transcript levels of sulfate and nitrate transporters.

**Results:** Brassica napus revealed two response periods to S deprivation. The first one occurred during the first 13 days during which plant growth was maintained as a result of vacuolar sulfate mobilization. In the meantime, leaf osmotic potential of S-deprived plants remained similar to control plants despite a reduction in the sulfate osmotic contribution, which was fully compensated by an increase in nitrate, phosphate and chloride accumulation. The second response occurred after 13 days of S deprivation with a significant reduction in growth, leaf osmotic potential, nitrate uptake and nitrate reductase activity, whereas amino acids and nitrate were accumulated.

**Conclusions:** This analysis of S deprivation suggested that a ([chloride]+[nitrate]+[phosphate]) :[sulfate] ratio could provide a relevant indicator of S deficiency, modified nearly as early as the over-expression of genes encoding sulfate tonoplastic or plasmalemmal transporters, with the added advantage that it can be easily quantified under field conditions.

**References:**

Genetic variation for nitrogen use efficiency in oilseed rape (*Brassica napus* L.)

**Background:** Oilseed rape (*Brassica napus* L.) is the most important oil crop worldwide after perennial oil palm and soybean. For oilseed rape production nitrogen (N) is the plant nutrient that has to be fertilized in the highest quantities, and meeting future human demand for oil yield will also require N fertilization. At the same time, N that is not taken up by the vegetation can escape from the agricultural production system, potentially causing severe damage in other ecosystems. Examples include nitrate leaching into groundwater, nitrogen run off into rivers, lakes and oceans, and losses of volatile NOx and ammonia into the atmosphere. Therefore, increasing nitrogen use efficiency is becoming a major global megatrend in agricultural production, in particular for crops used as a biofuel source. Besides more exact fertilizer applications coupled to plant nitrogen demand, the use of genetic diversity to breed for N use efficiency (NUE) is a promising strategy towards a more sustainable agriculture.

**Objectives:** Although breeding progress for resistance and quality traits is well described, there is still little information about plant traits that can be considered as key drivers of increased nitrogen use efficiency. In this project we are comparing winter oilseed rape varieties from different years of market release in order to identify NUE-related traits underlying breeding success that can be used to select for improved N efficiency in ongoing breeding programs.

**Methods:** Independent experiments were used to assess genetic variation for NUE:

1) During the 2012-2013 growing season a set of 30 highly diverse *B. napus* genotypes, including older and more recent varieties and resynthesized lines, was investigated in Mitscherlich pots at a high and low N level. By separating plant biomass at flowering (BBCH 67-69) and seed maturity into leaves, stems and pods the trait interrelationship for N uptake (NupE) and utilization efficiency (Nute) was studied in detail.

2) During the 2014-2015 growing season a set of 30 elite winter type oilseed rape varieties, including older and modern hybrids and lines were cultivated in the field at a high (220 kg N ha⁻¹) and a reduced (120 kg N ha⁻¹) N fertilization. These were subject to non-destructive and destructive nitrogen measurements during spring.

**Results:** The results give first insights into the ability of elite genotypes to acquire N during vegetative growth stages, into differences between inbred and hybrid cultivars, and into breeding progress in European winter oilseed rape. Furthermore, the pot experiments indicated a huge phenotypic variation for NupE until flowering within diverse *B. napus* materials, based on different N quantities in plant organs. While the genotypes also differ in their Nute during the generative phase, both trait complexes seem to be completely independent inherited. Therefore, our results suggest a great potential to improve total NUE.

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Strategies for Improving Winter Survival in U.S. Southern Great Plains Winter Canola

**Background:** Winter canola cultivar evaluation began in the late 1980s in the southern Great Plains of the United States. Initially, European cultivars were introduced into the region, but winter survival was poor because the materials were un-adapted to the harsh climatic conditions. In 1993, Kansas State University (K-State) started a winter canola breeding program and began coordinating the National Winter Canola Variety Trial (NWCVT). In the beginning, acreage growth did not exceed a few thousand hectares. However, in recent years, renewed efforts by public and private entities have led to a greater interest and dramatic acreage increases. An estimated 125,000 hectares were seeded in the southern Great Plains in fall of 2014.

Enhanced winter survival has been observed in the NWCVT over the past 22 years because of breeding efforts and refined production practices. Although adaptability has improved, work remains as producers face the effects of variable temperatures, drought, and climate change. In the 2013/14 and 2014/15 growing seasons, winter survival was a significant issue in many U.S. locations because of extreme cold temperatures, excessive fall growth, and a short winter acclimation period.

**Objectives:** Genetics and production management play critical roles in enabling a winter canola crop to survive winter weather conditions. Comparisons between top K-State cultivars, industry standards, and new winter canola cultivars will be made. Traits, including the semi-dwarfing trait and prostrate growth habit, will also be examined. The effects of seeding date and density will be evaluated in studies conducted in Kansas.

**Methods:** Head-to-head comparisons for winter survival and yield will be made between new cultivars and commercial checks across NWCVT sites in the southern Great Plains. Fall plant stand, spring vigor, winter survival, and yield will be assessed in the seeding density studies. A three-year planting management study designed as a cultivar by production practice factorial will be summarized (Assefa et al., 2014).

**Results:** Local and international canola cultivars show substantial improvement in winter survival compared with the check cultivars Sitro and Wichita. KS4549, an experimental cultivar from the K-State canola breeding program, offers enhanced winter survival and possesses a prostrate growth habit. Lower seeding rates reduce in-row plant-to-plant competition, resulting in greater winter survival than higher seeding density.

**Conclusions:** Adjustments to production practices may be necessary to enhance winter survival. New winter hardy cultivars from K-State will be released within the next two to three years. Cultivars possessing the semi-dwarfing trait have greater winter hardiness than the first hybrids introduced in the region. With the recent licensing of the OGURA-INRA hybrid breeding system, opportunities exist to incorporate winter survival traits from K-State germplasm into hybrid parent lines to be licensed by industry.

**References:**
Phenological and physiological traits to explore G×E interaction in canola in south-western Australia

**Background:** While genotype (G) × environment (E) interaction (G×E) complicates broad crop adaptation, understanding its causes facilitates breeding for specific adaptation. Knowledge of phenology in the adaptation of canola to different environments and the contribution of phenotypic traits to yield under these environments is necessary to explore the G×E interaction and enable breeders to select specific traits for a targeted environment.

**Objectives:** The major objective of this study is to i) evaluate the significance of the G×E interactions on seed yield and investigate whether there is a repeatable G×E interaction pattern which may lead to the identification of canola growing mega-environments; ii) identify the phenological and phenotypic traits that can explore the G×E interaction for targeted breeding.

**Methods:** A multiple-environment trial (MET) with a large number of genotypes over four year (2006-2009) was used to partition G×E interactions and investigate the role of phenology in canola adaptation to the environment. Another two year (2009 and 2010) detailed study was used to quantify the impact of phenological and physiological traits on yield in the low and high rainfall environments.

**Results:** Two mega-environments (ME) were identified. ME1 combines > 330 mm seasonal rainfall with a cooler, longer post-anthesis growing period. ME2 is more terminally drought-prone, with higher temperatures and < 300 mm rainfall, resulting in a short growing season. There were significant crossover yield responses to environment changes: the medium flowering genotypes produced significantly higher yield than the early flowering genotypes in ME1 but yielded poorly in ME2, and vice versa. Principle component analysis in two contrasting years confirmed that yield was positively correlated to flowering time in the high rainfall year and negatively in the drought year. Yield was highly correlated with increased biomass at vegetative stage and maturity, plant height, crop growth rate (CGR) during the linear growth period, leaf mass per unit area (LMA), pod number per unit area, and water soluble carbohydrate stored in stem in the high rainfall environment. In contrast, yield showed weaker or no correlation to any of these traits in the drought year. The key outcome of this G×E interaction study is the importance of phenology to the adaptation of canola and different role of phenotypic traits in the low and high rainfall environments. It is suggested that breeding for specific adaptation to each mega-environment should be targeted with a strategy focusing on drought and heat tolerance in ME2 and high biomass in ME1. In ME1, traits associated with achieving high biomass, including higher LMA, greater CGR, early vigour, and mid-late flowering could be used as selection criteria to improve yield. In the low rainfall environment, early flowering allows canola to escape drought and ensures enough water for grain filling that improves harvest index.

**Conclusion:** Canola genotypes showed significant G×E interactions in yield response to the environment. This interaction can be explored by target breeding through matching phenology to rainfall and growing season length and selecting traits contributing to the difference in biomass and HI.
Understanding the biochemical basis of abiotic stress induced lipid pathway adjustments

**Background:** Modification of membrane glycerolipid profile is an important part of plant metabolic adaptation to unfavorable environmental conditions. Profound changes of fatty acid desaturation in the lipid bilayer occur under temperature stress. Similarly, proportions of glycerolipid classes in membrane systems alter extensively under conditions of nutrient limitation. When plants encounter phosphorus starvation, for example, the quantity of phospholipid decreases, whereas the level of galactolipid species increases. These metabolic changes are associated with rebalancing of glycerolipid pathways located in the chloroplast and the cytosolic ER membrane systems. How such metabolic coordination is mechanistically modulated, and more importantly, what metabolic factors are involved remain to be fully explored.

**Objectives:** We were interested in identifying key biochemical junctures and metabolic factors influencing membrane lipid adaptation to abiotic stresses.

**Methods:** 1D and 2D TLC were standard techniques used for lipid separation, and gas chromatography was employed for fatty acid composition analysis. Lipidomic profiling through ESI-MS/MS analysis was performed at the Kansas Lipidomic Research Center. RNAseq were conducted on the Illumina HiSeq2000 at the National Research Council, Aquatic and Crop Resource Development (ACRD)-Saskatoon, Canada. Transgenic *Brassica* plants were generated through agrobacterium-mediated transformation.

**Results:** In a plant cell, glycerolipid synthesis relies on two major pathways located in the chloroplast and the endoplasmic reticulum (ER), known as the prokaryotic and eukaryotic pathway, respectively. Through comprehensive lipid profiling and lipidomics analysis, we show that the chloroplast prokaryotic pathway is up-regulated in response to cold stress, while high temperature promotes the ER eukaryotic pathway. Furthermore, under heat stress, C34 diacylglycerol (DAG) moieties of 16:0/C18 (sn-1/ sn-2) generated by the ER pathway are preferentially transported to the chloroplast over that of C36 (C18/C18) DAG, leading to a reduction in the overall capacity of fatty acyl desaturation in membrane lipids (Li et al., 2015). Through introducing a feedback-resistant Gly-3-P dehydrogenase gene (gpsAFR) from *Escherichia coli*, we generated *Brassica* transgenic plants with an augmented chloroplast lipid pathway (Shen et al., 2010). Significantly, these gpsAFR transgenic plants have a higher input from the chloroplast lipid pathway, resulting in a lipid phenotype resembling that of plants under phosphorus starvation. We will discuss the potential of such a transgenic approach in improving crop phosphorus utilization efficiency.

**Conclusions:** Our study highlights the significance of glycerolipid pathway coordination and the utility of lipid metabolism genes in improving plant adaptation to abiotic stress

**References:**


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Sustainability & carbon footprint of canola production in Western Canada

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The biofuel markets in some regions of the world such as Europe, the United States and California apply qualifying sustainability criteria to the biofuel feedstock production. A common component of the sustainability criteria is the carbon footprint for producing the feedstock. The published carbon footprints of canola production exhibit significant variation and range from 334 to 979 kg CO₂eq/tonne. Carbon footprints are known to be sensitive to spatial and temporal influences. This work is the first to be based on actual data collected from Canadian canola producers and it produces emission results one third lower than those reported by Shrestha, two thirds lower than those reported by Gan a decade earlier, and lower than the default value in the RED in Europe and the values used by CARB for the LCFS system. The lower footprint is the result of several differences in production practices including an increase in reduced and no till agriculture, and a reduction in summerfallow. Some of the ecozones of western Canada also exhibit relatively low N₂O emissions which is a function of the low levels of natural precipitation.
Comparison between Canadian canola harvest and export surveys

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Canola is the major oilseed crop in Canada, in 2014 the canola production was 15.496 million metric tonnes, the second highest production recorded in Canada – 17.876 million metric tonnes in 2013 – with a 5 year average production of 14.332 million tonnes. Every year, about 50% of this production is exported mainly towards Japan, China, and Mexico.

The Oilseeds section of the Grain Research laboratory reports canola quality data based on an annual voluntary harvest survey of western Canadian canola involving producers, grain companies and oilseed crushing companies. Quality parameters such as oil, protein, chlorophyll, glucosinolates, free fatty acids and the fatty acid composition are analyzed and reported. At the same time, monthly exports are monitored and the same testing is done on the individual Canadian canola shipments leaving the Canadian ports.

The results suggested that overall harvest survey data can help to predict the exports quality for oil, protein, free fatty acid and fatty acid composition. However, chlorophyll data of the harvest survey and export chlorophyll data showed large differences. Factors such as dockage and green seeds counts contributed to the differences between the two surveys for chlorophyll.
Identifying climatic limitation to oilseed rape yield in Switzerland

Background: Climate plays an important role in agriculture. Crops are submitted to various stresses such as water stress, heat stress, frost, that may decrease yield or quality. The aim of this work was to determine the most important climatic limiting factors of oilseed rape yield in Switzerland, in order to better understand yield variation among location and years.

Methods: We adopted a knowledge- and data-based climate suitability evaluation approach, where several climate indices estimated over relevant phenological phases are used to quantify climate-yield relationships. Four phases were calculated dynamically based on Habekotté (1997) phenology model: emergence, vegetative growth, anthesis, and seed filling. For each phase, response functions to climatic indices were pre-defined based on literature and expert knowledge, and automatically refined based on observed data as described for maize by Holzkämper et al. (2013). The chosen indices were maximum temperature, minimum temperature (below 0°C, or below -12°C for the vegetative growth phase), photothermal quotient, length of the phase and water availability. A different weight was attributed to each phase in order to account for the fact that climatic stresses can have different impacts on yield depending on the growth stage. The maximum yield, chosen as the best yield observed in Switzerland in the last 20 years, was set to 6.6t/ha. For each phase, only the strongest limitation was taken into account. The approach was applied to oilseed rape (Brassica napus L.) production in Switzerland and compared to yield data gathered from the main oilseed production regions in Switzerland.

Results: Comparison with independent yield data showed a good agreement of estimated crop-specific climatic suitability with scaled yields. The calculation of phase weight showed that the flowering phase was the most sensitive to climatic stresses, followed by seed filling (anthesis to maturity phase).

Analysis of climatic limitations on two locations in Switzerland showed that a low radiation, resulting in low photothermal quotient was the most important limiting factor for both phases, with more impact during flowering. This parameter is highly variable among years, but only smaller differences could be registered among locations in Switzerland, resulting in greater yield variability among years than among locations.

This approach gave a good estimation of oilseed rape yield variation in Switzerland. Further possible applications of this work could be either to map best climatic areas for oilseed rape growth, or even to adapt variety choice to regional climatic limitations.

References:
Holzkämper et al., 2013. Identifying climatic limitations to grain maize yield potentials using a suitability evaluation approach. Agricultural and forest meteorology. 168:149-159
Enhancing production and productivity of oilseed Brassica: Research need for 21st century

**Background:** India is one of the largest cultivators of oilseeds crop. Per capita consumption of vegetable oils has increased from 6.2 kg/year in 1986-87 to 14.2 kg/year during 2012-13. This eventually pushes the demand for oil significantly.

**Objectives:** Rapeseed-mustard is the third most important source of edible oil after soybean and oil palm. In India, it is commonly referred to as Sarson. It is an important cooking medium and dietary fat of the majority of northern, north-western, central, eastern and north-eastern states of India. It is also the most common medium of pickling and food preservation. Domestic production of edible oil has remained almost stagnant during last five years. Oilseed production in the country is facing several challenges related to biotic as well as abiotic stresses, natural resources, climate change and fragmented land holdings.

**Methods:** The projected annual requirement of total vegetable oil by 2025 A.D. is 27 m tonnes with the production target of 14.03 mt tonnes to meet enhanced per capita consumption 16.98 kg/year. This is challenging, nevertheless it is possible to achieve the goal by adopting vertical and horizontal growth. Immediate research need for vertical growth would conventional breeding with emphasis on sustainability, genetic engineering of through exploitation of available genetic variability. Heterosis breeding should be the major focus. Furthermore, augmentation or identification of trait specific germplasms, pre breeding and genetic enhancement, allele mining, proteomics, marker assisted breeding and gene pyramiding would facilitate better exploitation of the available gene pools in order to overcome the production constraints. Reducing the yield gap and additional area under cultivation are the viable approaches for horizontal growth. An estimate area of 1.08 mha could be brought under rapeseed-mustard cultivation from Eastern Uttar Pradesh, Bihar, West Bengal, NEH region, Madhya Pradesh, Jharkhand, Odisha and Chattisgarh where the fields remain fallow after rice cultivation. Non traditional area such as Karnataka, Southern Rajasthan and Vidarbha region of Maharashtra could contribute about 0.3 mha area for rapeseed-mustard cultivation.

**Conclusion:** It is possible to minimise the demand and supply gap by proper technology interventions for improving the yield. Also, bringing non-traditional area for cultivation will assist in achieving the target.

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Global change adaptation: What future for OSR crops and products? A foresight study for oilseed chains at 2030 horizon

**Background:** The French oils and proteins organizations wonder about the future of their productions, particularly OSR, at 15-year horizon. The evolution of the world is accelerating with a population rising from 7 billion today to 7.9 to 8.9 in 2030. The climate change effects on agriculture are largely subject to assumptions, also future mitigation/adaptation policies. Lifestyles and diets are changing, often richer in meat. The development of non-food uses is questioned by the changes in food needs. The flow of production and trade of oils and proteins evolve, particularly in Asia (China’s soybean imports, massive palm oil production in Indonesia-Malaysia). Some scientific and technical achievements are questioned, such as biotechnologies.

**Methods:** A panel of experts worked according to the French school of foresight methods: system's description, retrospective study, hypotheses’ formulation and aggregation of assumptions in 4 macro-scenarios on global and European context (then quantified) that have no predictive value when taken alone, but which together form a framework for understanding the issues and challenges, helping in strategic options' evaluation.

**Results:**
Scenario 1: “towards chaos”: international economic and political crisis, tensions on food due to high population, stagnating agricultural yields and impoverishment. The major challenge is to produce vegetable protein for human consumption. Policies aim at restraining price volatility while maintaining food security and standards of living.

Scenario 2: “Regional policies and bilateralism”: in the absence of international consensus, countries including Europe implement unilateral policies for climate change mitigation, leading to a latent protectionism. Veganism increases in Europe while consumption of animal protein progresses in China and Africa. Europe is strengthening its protein self-sufficiency and non-GMO policies, and exports top quality end-products.

Scenario 3: “trust”: international cooperation and standardization to prevent climate change: “green growth” scenario favored by a moderate population, a dynamic agricultural production, marked by animal protein consumption’s growth and by energy transition policies. Global demand of vegetable protein for feed explodes in a very open and competitive market.

Scenario 4: “cooperation forced by climatic and food tensions”: climate change puts agricultural production under pressure in a context of high population growth, leading to international cooperation. The main challenges are reaching sustainable food production and changing eating patterns. Policies focus on climate change, food security, and resource efficiency.

**Conclusions:** Massive development of palm oil production is expected in addition to oil from soybeans, whose production is supported by protein requirements. Without sanitary disasters on oil palm plantations, all scenarios highlight oil surplus and high plant proteins deficiency. Non-food uses of oils could therefore have a key role. The deficit of protein is a strong trend but needs to be qualified with regard to demands for human nutrition and food/feed industries.

The future OSR competitiveness is questioned particularly on three aspects: crop’s efficiency regarding resources (nitrogen, energy…), ability to better use the protein fraction in animal feed and to valorize it as human food, development of new industrial processes and uses of the oil fraction.

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Godet M., Durance P. Strategic foresight for corporate and regional development. Dunod, 2011
The use of precision gene editing to develop new non-transgenic traits in canola

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Background: New techniques continue to be discovered to develop desirable traits in plants. Different geographies and markets do not accept transgenic traits so alternative technologies must be developed to provide farmers with new and desired traits. Precision gene editing is a rapidly growing field which allows for specific targeted changes to be made in the DNA of a target organism. Gene editing can be done in a way to avoid the introduction of foreign genetic material and as such, will develop non-transgenic plants that are acceptable in those geographies and markets which prefer non-transgenic plants.

Methods and Results: RTDS™ (Rapid Trait Development System) developed by Cibus is a gene editing technology which can be used to target precise changes in genes of interest. Herbicide tolerance (HT) in both canola and flax were targets for the technology. Robust cell culture systems were developed to allow RTDS converted cells to be identified and regenerated into plants. Molecular screening confirmed the presence of the targeted changes and plants with the HT targeted change(s) were regenerated and are in various stages of commercial development.

Conclusions: Gene editing as demonstrated in applying RTDS to both canola and flax is a powerful new technique to develop traits in plants that are non-transgenic and can develop commercially valuable crops that fit into geographies and markets where other traits are currently not accepted.
Towards a Canadian government model policy for management of low-level presence of genetically modified crops in imported grain, food and feed

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Governments as well as public and private institutions around the world are actively seeking ways to increase agricultural productivity and make other useful improvements to crops. In support of these efforts, it is expected that the number and variety of genetically modified (GM) products commercialized will continue to increase. Once a GM crop is authorized for commercial use in a foreign jurisdiction, trace amounts of that crop may become mixed with other varieties of the same crop or other crops in that jurisdiction. This can happen during the cultivation, harvest, transportation, and storage of the GM crop. Even when best management practices are strictly followed, it is often difficult to prevent this from occurring. As a result, a GM crop that is not approved in the importing jurisdiction may unintentionally be present at low levels in the grain, food or feed products exported to that jurisdiction. This is what is called low-level presence (LLP).

Under the Canadian regulatory framework, the presence of an unauthorized GM crop in Canada constitutes non-compliance. The Government of Canada is developing a model that provides a more predictable, pragmatic approach to managing LLP in imported grain, food and feed.

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Under the Canadian regulatory framework, the presence of an unauthorized GM crop in Canada constitutes non-compliance. The Government of Canada is developing a model that provides a more predictable, pragmatic approach to managing LLP in imported grain, food and feed.
Comprehensive linkages between SNP and various important agronomic traits in rapeseed via QTL analysis and whole-genome evaluation

**Background:** Considerable phenotypes in TN DH population of *Brassica napus* were evaluated across different environments for eight years for up to 46 traits involving plant development, seed yield and yield components, seed quality, and stresses. QTL accounting for most of the traits has been separately mapped in the TN genetic map with different versions which were constructed mainly with RFLP and PCR-based markers (Qiu et al., 2006; Long et al., 2007; Shi et al., 2009; Feng et al., 2012; Jiang et al., 2014). High throughput markers, e.g. the Infinium chip of 60K SNP, are prevalent and being increasingly used in rapeseed research and breeding procedures. The newly published genome sequence of ‘Darmor’ in *B. napus* enables the 60K SNP markers to be anchored on particular physical position, associated to specific genome sequences and genes genome-widely, and, further on, associated with important traits.

**Objectives:** Develop a new version of TN DH map based on the 60K SNP markers and assign the markers with genetic value for comprehensive traits by QTL analysis and whole genome evaluation.

**Methods:** Genetic map was constructed with JoinMap4. QTL were evaluated by WinQTLCart v2.5. Meta-analysis was conducted for integrating the QTL accounting for different traits across environments. Genomic selection model was constructed with rrBLUP.

**Results:** A new version of the map spanning 2077 cM with 2041 loci including 1705 SNP-bin was constructed with TN DH population genotyped by 13,000 polymorphic SNP markers. Over two thousand QTL were detected from the population for the total of 46 traits. After integrating the redundant QTL either repeatedly detected from different environments or re-counted by total and components of the chemicals for glucosinolates and tocopherol, 1348 QTL for 27 traits were obtained and were further integrated into 530 elementary QTL for seed yield based on the trait correlation coefficient. The elementary QTL spanned 2.69 cM on average, equivalent to about 693 kb of the genome sequence, was anchored by at least one unique SNP-bin marker. Genome selection models have been constructed with all of the 2041 markers located on the TN genetic map. The prediction accuracy (R2) varied very much, from 0.57 to 0.96 according to the complexity of the traits. Adjusting the genetic value for the markers located in the interval of QTL seems having little effect on the prediction accuracy. The significance of the elementary QTL identified and the genomic selection models developed from the TN population for rapeseed breeding will be discussed.

**Conclusions:** 530 elementary QTL, most of which were pleiotropic, accounting for more than twenty important traits in *B. napus* across environments has been anchored with markers from the 60K Infinium SNP array on TN DH genetic map. The genetic effects for the traits for each of 2041 markers located on the map have been evaluated with genome selection models. The results would provide considerable information and novel insights for rapeseed breeding.

**References:**


Economic impact of rapeseed-mustard cultivation on farmers in India

India is the largest producer of oilseeds in the world and accounts for about 14 per cent of the global oilseeds area, 7% of the total vegetable oils production, and 10% of the total edible oils consumption. In 2013-14, the total oilseed cultivated area, production and the total edible oil production, under the nine oilseeds crops in India, were 27.25 million hectares, 29.38 million metric tones (mmt), and 7.45 mmt, respectively, with an average productivity of 1077 kg ha⁻¹. Recent change in the Indian edible oil consumption is impacting on the oilseeds cropping pattern, imports, exports, and inter-state trade. Currently, India’s annual consumption is about 17.5 mmt, which has increased gradually at a compounded annual growth rate of 4.6% over the previous decades. The growth in per capita consumption is attributable to both rising income levels, and living standards. Rapeseed-mustard is the biggest domestic oil bearing oilseed, and being grown in diverse agro-climatic conditions ranging from north-eastern/north-western hills to down south under irrigated/ rainfed, timely/late sown and mixed cropping. During 1950-60, the average contribution of rapeseed-mustard to the total oilseed area and production was 19% and 16% respectively, with 391 kg ha⁻¹ as compare to 485 of total oilseed productivity. With the sincere efforts of farmers and researchers the rapeseed-mustard production got quantum jump and contributes 24% area and 26% production of total oilseed during last decade. The average productivity of rapeseed-mustard has also increased to 1127 kg ha⁻¹ as compare to total oilseeds 1040 kg ha⁻¹. Though, rapeseed-mustard ranks second in terms of production, however, due to more oil content (35-45%), it ranks first in oil yield among all oilseeds crops. The major rapeseed-mustard producing Indian states are Rajasthan, Uttar Pradesh, Madhya Pradesh, Haryana, Gujarat and West Bengal accounted for nearly 86.5% area and 91.4% production. For sustaining the production of rapeseed-mustard, and make it more profitable to the farmer’s Government of India announces the minimum support price (MSP) every year. During last 15 year the rapeseed-mustard MSP increased with a 7% Compound Annual Growth Rate and reached to 3100/- per quintal in 2014-15. Once the oil is extracted, the remaining part of the seed is used to produce rapeseed-mustard meal, an important source of feeding for Buffalo, cows and poultry which are the major constituents of integrated farming and also the source of income of farming community. Rapeseed-mustard seed meal contains about 10% of oil is the major feed for animals as nutritional supply. The livelihood security of a multitude of stakeholders is dependent on the crop. India has an average (2011-13) yield of 1206 kg ha⁻¹ as against the world average 1916 kg ha⁻¹, which indicates a large gap of 59%. However, the yield gap is much lower has recorded in Haryana with the highest yield of 1869 kg ha⁻¹ and Gujarat of 1579 kg ha⁻¹ states. In India, data indicated about 80% yield gap over state yield. Such yield gap could be minimized by better adoption of available technologies for crop cultivation.
Canadian canola: The opportunities and challenges in international markets

Today, over 50% of the vegetable oils consumed by Canadians are produced from Canadian-grown and processed canola. However, this represents only 10% of the total crop grown each year, so the rest has to be exported. Canada produces a little more than 30% of the world’s annual supply of canola quality rapeseed but is responsible for roughly 70% of its global trade. Predictable access to international markets is critical to the success of Canadian canola. At the same time, consumer understanding and acceptance of canola oil as a versatile, healthy oil and the acceptance of canola meal by the animal feed industry as a quality source of protein is paramount to further success of Canadian canola.

In the five most recent years starting 2010, Canada produced an average of 14.9 million tonnes of canola seed per year. In the same five year period Canada annually exported an average of 8.1 million tonnes of canola seed, 2.4 million tonnes of canola oil and 3.2 million tonnes of canola meal to markets around the globe.

As standards of living in many of the world’s developing nations are increasing, there is the opportunity for growth in the canola industry. Everybody needs some fat – to provide energy, essential fatty acids, and help absorb fat-soluble vitamins. The National Academy of Science Dietary Reference Intakes, developed by Canadian and American nutrition experts, recommends that fat provide between 20% and 35% of total energy intake. But some fats are healthier than others.

Canola is the healthy solution. Canola oil is low in saturated fat and free of trans fat, high in poly and monounsaturated fat, contains no cholesterol and is a good source of vitamins E and K. Plus canola oil is very versatile and suitable for a variety of different cooking applications so it’s easy to use every day. It has a neutral flavour that doesn’t overpower other flavours. It remains liquid and free-flowing at refrigerator temperatures and its high smoke point makes it ideal for cooking methods that require high heat.

The opportunities for canola are great and will be enhanced by reducing trade restrictions, enhancing feed industry acceptance and increasing consumer awareness.
Thresholds for profitable genomic innovation: A Canadian canola case study

As an innovation makes its journey from idea to commercialized product, it faces a series of thresholds that act as decision points to proceed or discontinue. While many of these thresholds address features of the actual innovation itself, such as, prototype design and cost of production, some of the thresholds are external to the innovation. Thresholds of this nature can be regulatory costs, regulatory timing and intellectual property costs. Taken in combination, all of these thresholds have the ability to determine whether the innovation is commercialized or falls into the valley of death. This presentation models research and development and commercialization costs for genetically modified canola to test for variable sensitivities. This demonstrates how the internal rate of return plays a crucial role for firms as they determine whether to cross each of the thresholds.

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Challenges and opportunities for canola production in Brazil and Paraguay

Background: Canola is a typical oilseed of latitudes between 35º and 55ºN in temperate environments. Available areas for canola production in Brazil and Paraguay are situated at latitudes below 33ºS, and the aim is to grow this crop during the fall-winter months, in two-crops-a-year grain production systems. Research and production in Brazil started (1974) in the subtropical Southern states, with rainfall above 1,500 mm distributed during all months of the year. In this region, and in Paraguay, canola is subject to high moisture and frosts during the reproductive stages, a period of shorter days than those of most production and cultivar development regions of the world. Frost incidence (around 30 days a year) and severity (up to -10°C) increase from the lower to the higher altitudes of the production areas (250 to 1,100 m).

Objective: Provide an overview of the challenges and potential for canola production in Brazil and Paraguay.

Results: Production is based only on spring type hybrids of Brassica napus L. var. oleifera, with resistance to specific blackleg pathogenicity group incited by the fungi Leptosphaeria maculans (Desm.) Ces. & De Not.. GMO cultivars are not used due to the widespread adoption of Glyphosate-resistant soybean in these rotation systems. Currently, the hybrids developed in Australia are the best available alternative. Long term screening in the target growing regions is required to identify possible sources of germplasm with tolerance to severe frosts, and certain diseases associated with high humidity environments, such as those incited by Xanthomonas spp. bacteria, Sclerotinia spp., and Alternaria spp. fungi. Long term screening in the target growing regions is required to identify more suitable cultivars to the specific requirements of each of the diverse cropping regions. Research and some commercial production has shown that by employing low day-length sensitivity hybrids, canola production is also viable in tropical savannas with altitude above 600 m. Growing area and production peaked at 59,100 ha in Brazil (2011), and at 83,000 ha in Paraguay (2012), with average grain yields around 1,600 kg ha⁻¹. Many farmers achieve grain yields above 2,200 kg ha⁻¹ up to a top yield of 3,200 kg ha⁻¹, and the cropped area is likely to increase. Brazil has 37 million hectares of land under grain production, where soybean and maize are produced in the summer. Canola can become a cropping alternative on about 17 million hectares of under-utilised land in tropical environments, and in subtropical regions were it is possible to grow two crops every year, optimizing investment in land, machinery and other available resources.

Conclusions: Development of canola cultivars and management technologies suitable for subtropical and tropical grain production environments can be decisive for a major expansion of this oilseed’s cropping area to non-traditional regions of the world. Increases in canola production could expand human consumption of its oil in domestic markets and meet part of the requests of companies interested in sourcing large amounts of canola oil for biodiesel production in Europe.
Evaluation of rapeseed-mustard genotypes from Australia, India and China for agronomic and biochemical traits

**Background:** The rapeseed-mustard varieties being grown in Australia, India and China do not conform to ‘canola’ quality and/or is susceptible to fungal diseases.

**Objectives:** Breeding good agronomy canola quality *B. juncea*/*B. napus* free of fungal diseases by combining selected genotypes through ACIAr support.

**Methods:** Australian, Indian and Chinese cultivars/breeding lines of *B. juncea* (95) and *B. napus* (155) were evaluated at TERI research field (NCR, India). DI was evaluated (three leaves/plant) on a 0-5 scale (0–no symptoms, 0.5-£5%, 1.0-£10%, 1.5-£15%, 2.0-£20%, 3.0-£30%, 4.0-£40%, 5.0-£50%). Fatty acids, glucosinolates and oil content were estimated by GC, HPLC/Elisa, and NMR/NIR respectively.

**Results:** All *B. napus* accessions had low incidence (5-10%) of white rust (WR)/Alternaria blight (AB). Indian *B. juncea* recorded 5-50% WR/AB, while exotic accessions had 0-10% WR/ 5-10% AB. This could be due to different host pathogen susceptibility of exotic accessions to Indian isolates. Exotic *B. juncea* showed high incidence of bacterial wilt (10-40 plants/genotype) and *Sclerotinia* stem rot (5-20 plants/genotype), except JN028 and JN033 that were free from both. Indian cultivars had high erucic/high glucosinolate. Promising *B. napus* accessions with 68-71% oleic acid are OscarAg Outback, ZY007, ZY009, Rivette and TERI(oo)r9903. *B. juncea* genotypes CBJ002, BJ3, JM06006, JR042 and JN031 had 50-63% oleic acid, and JN004, JN010, JR042 and Rohini had 39-40% oil content. Promising accessions- Pusa Bold (PB)/PCR-7 (good agronomy), GZ5 (double low, yellow seed), GPZ (double low, good agronomy) from India; JR049 (double low), JR042 (oil content 40%), JN028, JN033 (free from Sclerotinia/bacterial wilt) from Australia; and CBJ003 (long pods) from China were used for intra-specific hybridization.

**Conclusions:** The useful variability in BC2/BC3 generations derived from intra-specific hybridization are: palmitic acid 2.46 (GZ5/PB) to 5.13 (GPZ/JR042)%; oleic acid 13.29 (GZ5/PB) to 46.81 (GPZ/CBJ003); linoleic acid 17.9 (GZ5/PB) to 37.75 (PB/JR042); linolenic acid 7.01 (GZ5/PB) to 18.75 (GZ5/JO009); erucic acid <2 to 34.04 (PCR7/JR049)%. Four populations derived from hybridization of GZ5 and three from GPZ with exotic genotypes have double low traits needing further agronomic improvement. The near double low populations from Pusa Bold/JR042 are promising with good agronomy.

**References:**
Characterization of a *Brassica napus* doubled haploid population derived from a winter by spring cross

A doubled-haploid (DH) population containing 115 lines was derived from a cross between a European winter growth habit *Brassica napus* cultivar and an Australian spring growth habit *Brassica napus* cultivar. Important traits in this population that are segregating include the vernalization requirement, as well as flowering and maturity. Even spring-like lines in this population, where vernalization is not required, tend to show later maturity than that of the spring checks. The current study aims to understand whether the traits for early flowering and maturity, as well as enhanced cold tolerance can be selected for together. It is hypothesized that individuals will be found that have cold tolerance as well as early flowering and maturity.

Three major areas of research have been undertaken: 1) evaluation of cold tolerance in a field setting, 2) evaluation of cold germination in a laboratory setting, and 3) characterization of the agronomic traits of this population including flowering times and maturity. Analysis of the agronomic and cold tolerance data together suggests that while there are few examples in this particular population, it is possible to combine enhanced cold tolerance derived from winter germplasm with early maturity in a spring growth habit. Early maturity in a winter background is desired due to improved agronomic performance in hybrid combinations. The combination of enhanced cold tolerance, lack of vernalization requirement and early maturity in an otherwise winter-type background represents a step forward in germplasm development within *Brassica napus*.

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Extraction of Brassica monogenomic from digenomic species: Reversing evolutionary pathway

**Background:** Resynthesis of natural allotetraploids is a common procedure to create novel variation as well as to infer structural and/or functional modifications in the participating genomes as a consequence of the process of polyploidization following interspecific hybridization and subsequent chromosome doubling. A large number of such examples are available in crop Brassicas. Uncoupling of the diploid components of a polyploidy is another avenue which have been sought but never adequately investigated. We report successful extraction of B.rapadiploid from Brassica juncea, a natural hybrid between B.rapa and B.nigra.

**Objectives:** To extract B.rapafrom a natural B junceaallotetraploid.

**Methods:** An interspecific hybrid was first produced by hybridizing B.juncea with B.nigra followed by cochiploidy to produce an allohexaploid. This was followed by three cycles of selfing and selection for reduced genome size using flow cytometry(Dolezelet al.2007). In every generation plants resembling B rapa, with lower chromosome number were retained for next cycle of selfing. GISH was performed as per (Schwarzacher & Heslop-Harrison,2000) to demonstrate the genomic constitution of the extracted B rapa.

**Results:** Crossing B. juncea with B. nigra followed by chromosome doubling produced an allohexaploid (AABBBD;2n=52) combination with B genome in tetrasomic dose. The allohexaploid plant was partially fertile but some seeds were produced following selfing. Plants in S1 progeny were queried for the genome size based on flow cytometric analysis. S1 select were selfed to obtain progeny for the next cycle of selection. After three cycles of selfing and selection, plants with genome size similar to B.rapa were retained for detailed meiotic analysis. We could identify one plant with 2n=22 (11II). Genomic in situ hybridization using B.nigra probe confirmed disomic addition of one B genome chromosome pair. Remaining 10II were confirmed to be from complete set of A genome. This plant was selfed to raise S4 generation. Plants with B. rapa phenotype and euploid chromosome number of 2n=20 could be identified. Molecular characterization of these plants using a set of chloroplast, mitochondrial and nuclear SSRs confirmed their distinctness from natural forms of B. rapa. Interestingly the extracted B. rapa plants seemed phenologically closer to winter forms of B. rapa as compared to spring types.

**Conclusions:** The novel crossing scheme coupled with molecular cytogenetic techniques helped to derive B. rapa (10II) from allotetraploid B. juncea (18II), a valuable resource for future genome analysis studies.

**References:**
A novel procedure to resynthesize *Brassica napus* from related allotetraploids

**Background:** Rapeseed (*Brassica napus* L.; AACC, 2n = 38) is an allopolyploid that arose through natural hybridization between *B. rapa* (AA, 2n = 20) and *B. oleracea* (CC, 2n = 18). Its narrow genetic base is implicit in dual bottlenecks of polyploidy and domestication. Intensive plant breeding activities, especially for canola quality may have further eroded the variation for traits of high breeding interest. We have developed a new method of derived amphiploidy. This procedure envisaged an alternate pathway to polyploidy by sourcing desired diploid genomes from related and intensively bred *Brassica* digenomics (Banga and Kaur 2009). Efficacy of this method was demonstrated through recreation of *B. juncea* by combining ‘A’ genome from *B. napus* and ‘B’ genome from *B. carinata* (Gupta et al. 2015). We report an extension of this procedure for resynthesis of *B. napus* through hybridization between *B. juncea* (2n=36; AABB) and *B. carinata* (2n=34; BBCC).

**Objectives:** To explore an alternate pathway for *B. napus* resynthesis by sourcing desired diploid genomes from related and intensively bred *Brassica* digenomics.

**Methods:** *B. napus* (AACC) was resynthesized by hybridization between two related digenomic species *B. juncea* (AABB) and *B. carinata* (BBCC). This was facilitated by spontaneous chromosome doubling in two hybrid (ABBC) plants and elimination of extra ‘B’ genome chromosomes following several cycles of selfing and selection for fertile plants with *B. napus* morphology in subsequent generations of selfed selects.

**Results:** Twenty-five progenies with varying degree of resemblance to natural *B. napus* were assayed for cytogenetic stability and genetic diversity. Plants of only six progenies showed predominant occurrence of 19 bivalents (2n=38) and equal distribution of chromosomes at anaphase as in natural *B. napus*. Genotyping with ‘B’ genome specific primers and genomic in situ hybridization (GISH) indicated introgression of ‘B’ genome segments. Genotyping with ‘A’ and ‘C’ genome specific primers confirmed genetic identity of six derived *B. napus* plants with natural *B. napus*.

**Conclusions:** We report resynthesis of *Brassica napus* by combining ‘A’ and ‘C’ genome from *B. juncea* and *B. carinata*, respectively. Study also documents ‘B’ genome introgression into resynthesized *B. napus*.

**References:**
Molecular analysis of yellow-seeded winter rapeseed (B. napus L. var. oleifera)

**Background:** Rapeseed meal is a very important by-product remaining after oil extraction from seeds and containing over 40% of protein in a dry matter. However, the utilization of rapeseed meal in feeding of non-ruminants, especially of poultry, is limited by the antinutritive products of glucosinolate breakdown, also in seeds of canola quality, as well as by high fibre content. The reduction of fibre content is possible by development of yellow-seeded genotypes of rapeseed, as the trait is associated with reduced seed coat thickness.

**Objectives:** Identification of quantitative trait loci (QTL) for seed colour and fibre content in Brassica napus.

**Methods:** In order to identify major QTLs contributing to reduced seed coat and yellow colour of seeds as well as fibre content two mapping populations were developed. They consisted of the offspring of reciprocal crosses between two black-seeded and two yellow-seeded doubled haploid lines (DH) of winter oilseed rape. The first population included 91 and the second one 103 DH lines. The phenotype traits of plants from both populations were analyzed during five vegetation periods in two environments. Molecular characteristics was conducted using RAPD, AFLP and STR primers. Some of them were described in literature as generating markers linked to the seed colour (Somers et al. 2001; Sabharwal et al. 2004, Yan et al. 2007).

**Results:** For both populations, 1061 products of amplification were obtained, whereas 448 of them were polymorphic. For the first mapping population 22 linkage groups were obtained and for the second one 26 groups. 163 polymorphic amplification products were included into the linkage groups; 28 of them were linked to different phenotypic traits and 6 were linked to seed colour.

**Conclusion:** From the obtained 22 and 26 linkage groups 19 will be located on the B. napus chromosomes and the residual small groups will be properly linked when the constructed genetic map will be supplemented with larger number of markers.

**References:**


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Identification of QTL controlling glucosinolate contents in leaf, stem and seed in a *B. napus* DH-population

**Background:** The development of low erucic acid varieties and subsequently the introduction of low seed glucosinolate (GSL) content through the polish cultivar ‘Bronowski’ were two milestones in the breeding of *B. napus* as oil crop. While the inheritance of seed GSL content has been studied well due to its importance, only a few quantitative trait loci (QTL) mapping studies with the focus on GSL contents in vegetative tissues exist (Feng et al 2012).

**Objectives:** We used a *B. napus* DH-Population derived from the cross of the resynthesized line ‘L16’ and the cultivar ‘Express’ to identify regions in the genome which are possibly involved in GSL metabolism.

**Results:** In a field trial we measured individual GSLs in leaf, stems and seeds in order to investigate the tissue-specificity of GSL-QTL. Applying Composite interval mapping we found a total of 115 QTL related to individual or groups of GSL. Of these 49 QTL influenced GSL traits of seeds, 31 QTL leaf GSL traits and 35 QTL stem GSL. Most of the seed QTL were mapped in linkage groups (number of QTL) A03 (13), C02 (11) and C09 (12). Linkage groups A03 and C09 also had the highest number of QTL in vegetative tissue (A03 15 and C09 14 QTL). Comparison of the QTL positions by trait between tissues generally showed more overlapping regions between leaf and stem than between seed and leaf/stem. Nevertheless, some of the mapped QTL were trait specific and detected only in leaf, stem or seed. The phenotypic correlation for the sum of GSL contents was high (> 0.94) between stem and leaf, but lower (about 0.5) between vegetative tissues and seeds.

**Conclusions:** The GSL content and composition of leaves and stem is mainly controlled by the same QTL. The QTL for seed GSL are partly the same, but many seed QTL are tissue specific.

**References:**

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Genetic and omics resources for integrated Brassica researches at the Institute for Genetics Environment and Plant Protection (IGEPP), Rennes France

Brassica research programs at IGEPP aim at i) studying the dynamics of polyploid genomes and the regulation of homologous and homoeologous recombination ii) identifying resistance genetic factors and deciphering the plant response to different bioagressor infections and iii) studying the determinants that guarantee the stability of grain yield (quantity and quality) under low nitrogen input and water shortage conditions. To address these different questions, integrated approaches are developed based on the exploitation of the genetic diversity of B. napus, B. oleracea and B. rapa species through the development of specific genetic and genomic resources and on the use of core facilities of different platforms.

Genetic resources: IGEPP hosts the French Biological Resource Center “BraCySol” (site web). “BraCySol” collections gathered 1000 B. napus accessions (oilseed rape, fodder rape,...) and 1200 B. oleracea accessions (mainly French landraces): cauliflower, kale, cabbages... “BraCySol” seeds are available on request. Specific research material was developed and can be shared through collaborations:

• diversity sets (150 to 200 inbred lines) dedicated to the analysis of disease resistance and abiotic stress response by genome-wide association studies,
• seven doubled-haploid segregating populations for linkage analysis,
• a nested-Association Mapping populations constituted of 15 crosses of 200 RILs each (out of which 8 are public)
• synthetic and semi-synthetic forms derived from different B.rapa and B.oleracea accessions as well as monosomic addition lines (AA+C chromosomes)
• one tilling population

Genomic resources: Our main genomic resources consisted in i) the seven genetic maps (96 to 356 individuals per DH segregating population) and an integrated map with 47000 markers anchored to the sequences of Darmor-bzh, Chifu-401, TO1000 and Arabidopsis thaliana including the Brassica subgenomes and the ancestral Brassicaceae blocks), ii) the characterization of the diversity sets using the 60K Infinium array, as well as iii) the resequencing of various oilseed rape accessions.

Plant cytogenetic platform: Our group has an expertise on cytogenetic analysis in mitosis and meiosis. The establishment of the meiotic behaviour allows determination of the pairing frequency in metaphase 1 between homologous and homoeologous chromosomes in B. napus haploid or diploid and in interspecific hybrids. The analysis can be completed by observation either at the genome scale through Genomic In Situ Hybridization with DNA of related species or with BAC specific of C genome or at the chromosome level through Fluorescent In Situ Hybridization with BAC specific of different chromosomes.

Metabolomic platform: Our metabolomics platform is dedicated to plant metabolomics, with a special expertise on Brassica species. Our set of chromatographic tools (LC-MS/MS, LC-UV, GC-FID and GC-MS) gives access to a wide set of profiling analyses, including primary and secondary metabolism, phytohormones, and untargeted metabolomics.
Identification of ideal tester genotype(s) in Indian mustard for screening of aphid infestation tolerant germplasm adapted to south-western region of Punjab province in India

Background: Indian mustard (Brassica juncea (L.) Czern & Coss) commonly known as raya, is confined to south-western region of province having about 30,000 ha under cultivation in Punjab, a north-western province of India. This crop gave maximum yield potential when planted from 10-25th October, but sowing of crop is delayed about one month due to prevailing cropping system of this region, which imposes serious impact of both biotic stresses as well as abiotic stresses. Among the biotic stresses, Aphid (Lipaphis erysimi Kalt.) causes about 10-90% losses in yield in India to Brassica crops depending upon severity of damage and crop stage (Singh, 2005). Cultivation of raya in Punjab particularly under delayed sowing is facing above biotic stresses thus emphasizing the need to develop resistant/tolerant germplasm for sustainable productivity.

Objectives: The objective of the present study were to use the GGE and AMMI biplot approaches to determine the performance of lines and their crosses under diverse environments to examine the combining abilities (GCA) and heterotic pattern (SCA) of the lines and identify the best tester(s) having acumen to select lines with good GCA for aphid infestation on raya under diverse growing conditions for sustainable productivity.

Methods: PBR 91, PBR 97, PBR 210, PBR 357, RL 1359, RLC 1, IC 255435, IC 255439, IC 266917 and IC 266933 were crossed in diallel fashion to generate 45 F1 crosses. These F1 crosses including parents were grown under optimum, delayed sowing and brackish water conditions for two years 2011-12 and 2012-13. The aphid population counts were made at weekly intervals from 10 cm terminal portion of central shoot and 3 leaves (one each from top, center and bottom) on each of 10 plants every F1 population.

Results: Significant differences among hybrids across environments were observed for aphid infestation. The GGE biplot explained 88.8% of the total variability GCA effects across the environment. Similarly, AMMI biplot captured 71.5% of the non-additive portion of the variability for this trait. Based on the visualization of AMMI biplot three F1 hybrids viz., PBR 91 x IC266917, RL 1359 x IC 266933 and PBR 357 x PBR 97 showed heterotic (non-additive) effects for tolerance to aphid infestation. While GGE biplot showed that PBR 91 and PBR 357 had high GCA effects for aphid infestation tolerance can be utilized to generate genotypes having tolerance to aphid infestation.

Conclusion: Biplots visualization revealed that IC 266917 and PBR 91 were good tester for wide adaptability and hence are also ideal tester(s) as they have power to discriminate GCA of germplasm possessing tolerance to aphid infestation under diverse agro-climatic condition in south-western region of Punjab province for sustainable productivity of this crop.

References:
Association of SNP markers and chlorophyll fluorescence parameters with low temperature tolerance in multiple *Brassica* species

**Background:** The Canadian Prairies is the major growing region for canola in North America with an average annual production of 12 million tonnes (Council of Canada, 2008). Although Canadian canola production has increased in recent years, it has largely been due to increased acreage. A new goal for the industry is to improve yield of the Canadian crop by 50% to continue to increase total production. A major factor affecting spring canola production in Canada is frost during seedling development in the late spring, resulting in significant yield reduction (Canola Council of Canada, 2008). Efficient tools to screen canola lines would enable breeders and producers to develop and select canola varieties that would perform better under low soil temperature and spring frost conditions.

**Objectives:** The objectives were to 1) determine the effect of low temperature on seed germination and seedling performance of a diverse set of *Brassica napus* and *B. rapa* lines, and 2) conduct an association study to identify SNP markers linked to alleles for improved cold tolerance.

**Methods:** Untreated field-grown seeds of 169 genotypes, including *B. napus*, *B. rapa*, *B. juncea*, and *B. oleracea* were sown in pots containing field soil and placed in Conviron TC80 germination chambers (Conviron, Winnipeg, Canada). Seed quality was visually assessed prior to planting. In each pot, 50 seeds of each genotype were sown at a seeding depth of 1 cm. Emergence was assessed at 5, 10 and 15°C. In a seedling performance experiment, seedlings at the cotyledon stage were freeze-shocked at -5°C for 75 minutes. Frost injury was visually assessed as well as being inferred from the measurement of the chlorophyll fluorescence parameter \(\frac{FV}{FM} = \frac{Fm - Fo}{Fm}\). DNA of all genotypes were hybridized and scanned using the 60K *Brassica* Illumina® Infinium® array according to the manufacturer’s instructions. We used the software STRUCTURE (Pritchard et al. 2000) to identify underlying populations among the lines and this was confirmed through PCA analysis. Genome-wide association analyses is being carried out with TASSEL (Bradbury et al, 2007).

**Results:** The time to onset of emergence, 50% (T50) emergence, and maximum (T≥90%) emergence was positively correlated with temperature. Onset of emergence began within 2 to 4 days of seeding for all genotypes at 15°C. At 5°C there was significant variability in seedling emergence among genotypes. As temperature decreased, smaller-seeded genotypes tended to emerge slightly faster than larger-seeded genotypes. However, a few larger-seeded genotypes emerged slightly faster than smaller-seeded genotypes. Chlorophyll fluorescence was highly correlated with the cold tolerance of the genotypes. The \(FV/FM\) parameter showed higher correlation with plant survival than any other parameters such as \(FV/FO\), \(Fm\), and \(Fv\), suggesting that \(FV/FM\) could be used to assess cold hardiness in spring canola varieties. Out of the 47,304 SNPs arrayed, a total of 36,079 SNPs were retained for the purposes of association analysis. Of these, 6, 416 high quality SNPs evenly distributed across the genome have been selected for characterizing the population structure, relationship among the lines and for potential association with \(FV/FM\).

**Conclusions:** The study demonstrated that the chlorophyll fluorescence method can be used in combination with molecular markers to achieve efficient gains for low temperature tolerance. With the availability of tools to screen large breeding populations for low temperature tolerance, it may be feasible for deployment in the canola breeding programs for selection.

**References:**


In vitro culture of isolated microspores and plant regeneration of white mustard syn. yellow mustard (Sinapis alba syn. Brassica hirta)

**Background:** White mustard syn. yellow mustard (Sinapis alba L. syn. Brassica hirta) is open-pollinated, an annual plant of the family Brassicaceae. Sinapis alba as an oil plant is of growing economic importance due to the multilateral uses for example: seeds for food production, as green manure crops, phytosanitary crops and valuable honey plant and other. White mustard among Brassicaceae is the most resistant to drought occurring in Poland late spring and summer. The seeds of traditional varieties of white mustard, including var. Nakielśka, is characterized by high erucic acid content in oil (35-45%) and high content of glucosinolates especially sinalbine (approx. 160 µmol/g-1), which remain in the meal extracted. In contrast, white mustard var. Bamberka, has lower than 1,2% erucic acid, but a similar content of glucosinolates as the traditional varieties. But the, first in Poland, double-low white mustard var. Warta is characterized by lower than 1,2 % of erucic acid in oil and 15 µmol g-1glucosinolates content and without sinalbine (Krzymański, 2014).

**Objectives:** We report preliminary results concerning the develop of efficient and reliable isolated microspore culture methodology for three different types of white mustard: traditional, with low erucic acid content and double low quality.

**Methods:** While in our previous studies observed a negative impact on Sinapis alba microspores rich in mineral and organic compounds NLN medium (Lichter, 1982) we have used KBS medium (Leelavathi et al. 1984). Microspores of white mustard were isolated from anthers which were cultured on solid medium KBS after exposure to low temperatures 4°C. The first divisions of microspores were observed after a few days of isolation from anthers. Over the next two weeks were recorded multicellular divisions and then forming the embryos. Visible to the naked eyes embryos were transferred to fresh KBS liquid medium and cultured at light. Green embryos were planted on stable MS medium. After a few weeks, were observed the formation of shoot primordia. Developed shoots on MS medium with kinin were transferred to rooting MS medium with IBA (Klóska et al. 2012). Androgenic plants of white mustard were planted to the soil and their further development took place in green house till the seed were collected.

**Conclusions:** The method of obtaining of white mustard androgenic plants from isolated microspore culture has been developed.

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**References:**

Development of an engineered male-sterile line 15A in *Brassica napus*

**Background:** High heterosis has been found in rapeseed (*Brassica napus*). Many approaches to utilizing heterosis including cytoplasmic male sterility and chemically induced male sterility have been established. However, these accepted approaches each have drawbacks. It is worth developing a new way to utilization of heterosis in rapeseed.

**Objectives:** This study is to develop a new rapeseed male sterile line by genetic transformation and evaluate utility of the resultant male sterile line.

**Results:** The gene barnase causing male sterility was introduced into the double-low rapeseed cultivar Xiangyou 15 by genetic transformation. The resultant male-sterile transformant was backcrossed to Xiangyou 15 for successive generations and a genetically stable male sterile line 15A has been bred. This male sterile line 15A is completely male sterility and its sterility is not influenced by air temperature. Observations showed that male fertility segregation fitted to the expected 1 fertile: 1 sterile in field. Fifty percent plants were killed after spray of 1% PPT at the seedling stage. The surviving plants were observed at the flowering stage and about 2% surviving individuals male-fertile. This implies that the surviving plants contain a single linked barnase-bar copy. There are no differences in characters including the number of flower buds, siliques per plant between 15A and its original parent Xiangyou 15 except male sterility. The male sterile line 15A has smaller flowers and completely deteriorated stamens at the beginning of flowering stage. However, its stamens are not completely deteriorated and aborted pollen grains can stain blue by methylene blue squash from the middle of flowering stage forward on. The male sterile line 15A, whether open pollinated or hand pollinated, sets seeds normally.

**Conclusions:** A engineered male sterile line has been bred in rapeseed successfully. This new male sterile line is better than traditional cytoplasmic or chemically induced male sterile lines because of its stability in male sterility and its linkage to herbicide resistance.

**References:**

Application of In vitro vernalization in winter canola doubled haploid production

Background: In vitro vernalization has been studied in several plant species including oilseed rape or canola (Brassica napus L). Shi et al. (2004) reported that in vitro vernalized doubled haploid plants of semi-winter oilseed rape had plant flowering frequencies comparable to that observed with the normal vernalization protocol.

Objectives: We studied in vitro vernalization with nine winter-type European canola doubled haploid populations in comparison to regular artificial vernalization using a cold room.

Methods: Regenerated plants from microspore culture were vernalized in solid culture medium.

Results: In vitro vernalization gave flowering frequencies and seed production similar to regular artificial vernalization. The application of in vitro vernalization shortened the doubled haploid production cycle by six weeks, potentially increasing flexibility and efficiency in breeding applications.

Conclusions: We developed an effective in vitro vernalization procedure in winter canola doubled haploid production.

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Extra-large seed germplasms of *Brassica napus* created through microspore culture

**Background:** Rapeseed yield per unit area is determined by two main components, the number of seeds per unit area and the seed weight (Berry et al. 2006; Shi et al. 2009). Thousand seed weight (TSW) is one of major yield components of rapeseed (*Brassica napus* L.). Thus, creation of germplasm with high TSW of rapeseed make a great significance for promoting the yield per unit area.

**Objectives:** Here, an extra-large seed germplasm GM01, with the biggest TSW 8.68g, was obtained through isolated microspore culture. Furthermore, we investigated the stability of TSW, observed and evaluated the morphological traits especially flora organ and agricultural characteristics of GM01.

**Methods:** The three-way cross was made from H8 (TSW of 3.6g), a Yunnan spring early-maturing rapeseed variety, “Legency” (TSW of 3.8g), a Canadian canola variety, and “020010” (TSW of 4.6g), a semi-winter late-maturing rapeseed variety. After that, microspores of F1 of the three-way cross was used to culture to obtain doubled hyploid lines (DH). The TSWs of DH were tested in Kunming, Yunnan. The DH with the highest TSW, GM01, was selected out to conduct the performance of the material in multiple sites for years. In detail, the fresh weight and diameter of flower bud, the diameter of pedicel, the length and width of petal, the fresh weight and length of ovary, and the morphological traits of stigma were investigated.

**Results:** One hundred and forty eight doubled hyploid lines(DH) were obtained from plantlets regenerating from microspore culture. For those DH, the TSWs of 53 lines were above 5.0g, among which the TSW of GM01 amounted to 8.68g. Furthermore, the TSW of GM01 was relatively stable with the variation only 10-15% among the multi-location field trials from 2007 to 2013. Compared with H8 and Legency, GM01 had larger flower organs, which were with the diameter (4.07±0.17cm), fresh weight (7.73±0.59g), larger flowers whose petal width was 0.96±0.02cm, thicker pedicel (0.82±0.08mm), bigger siliques whose length were 12.11±0.86cm and width (5.98±0.49cm), and the biggest seeds with the diameter (2.62±0.74mm). The DH lines of GM01 had less branches, siliques per plant, and seeds per siliques on quality respect, however, the glucosinolate and erucic acid contents were critically high, up to 130-140 mol/g and 30-40%, respectively.

**Conclusions:** One new higher TSW germplasm was obtained from the F1 DH of three-way cross by microspore culture. The material could blossom and bear fruits normally, to the most important, it showed considerable stability in multiple sites for years. However, the glucosinolate and erucic acid contents were much higher than that of their parents.

**References:**

Variation and inheritance of mucilage content in yellow mustard (*Sinapis alba* L.)

**Background:** Mucilage in yellow mustard seed is an important quality trait and functional food ingredient. Mucilage contributes to the consistency of prepared mustard products and has potential as a food gum additive (Cui et al., 1994). Therefore, development of yellow mustard cultivars with different mucilage contents is desired by the food industry. However, limited information is available on mucilage variation and inheritance in this crop.

**Objectives:** The objectives of the present study were to study the variation and inheritance of mucilage amount and viscosity in yellow mustard.

**Methods:** Mucilage was extracted from whole seeds and the amount was determined as a percentage of seed weight (Cui and Mazza, 1996). The mucilage viscosity of the samples was calculated as the viscosity of extract minus the viscosity of water and then multiplied by the volume of water and finally divided by the weight of the seed (cS*ml/g seed) (Raney and Rakow, 1999). The inbred line Y1494 has a high mucilage amount of 6.9 g/100g seeds and high viscosity of 139 cS*ml/g seed, while Y1497 has a low mucilage amount of 4.1g/100g seeds and low viscosity of 4.1 cS*ml/g seed. To study the inheritance of mucilage amount and viscosity, Y1494 was crossed with Y1497 to produce F1 seeds. The F1 seeds borne on the F1 plants were used to study the inheritance of mucilage in yellow mustard.

**Results:** The yellow mustard inbred lines exhibited great variation in mucilage amount and viscosity ranging from 2.3 to 6.5 g/100g seeds and from 12 to 184 cS*ml/g seed, respectively. The mucilage viscosity was positively correlated with the mucilage amount in yellow mustard (r = 0.90). Mucilage was controlled by the maternal genotype. The F2 seeds borne on the F1 plants averaged 5.8g/100g seeds in mucilage amount and 84.8cS*ml/g seed in viscosity, suggesting the high mucilage amount and viscosity were partially dominant over the low. The mucilage amount and viscosity of the F2 seeds borne on the F1 plants showed continuous distribution ranging from 1.3 to 6.2 g/100 g·seed and 12 to 242 cS*ml/g seed, respectively. QTL mapping on mucilage amount and viscosity is underway.

**Conclusions:** Yellow mustard comprises extensive variation in mucilage. Preliminary genetic studies suggest that mucilage seems like a multigenic quantitative trait.

**References:**


Hybrid breeding using the improved Ogura cms system in *Brassica juncea*

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**Background:** Hybrid breeding strategy has been used to enhance yield in *Brassica juncea* (AABB, 2n=36). Hybrid varieties based on the Ogura cms, Moricandia cms and 126-1 cms systems, respectively, have been reported in this species (Archana et al. 2012; Sodhi et al. 2006; Yao et al. 2012 and Canadian Food Inspection Agency, plant variety database). However, the hybrid variety 45J10 developed using the Ogura cms system had problems such as enlarged roots due to linkage drag. Agriculture and Agri-Food Canada obtained the *B. juncea* Ogura cms A line and restorer (R) line (RfoRfo) from INRA in 2003. Our molecular analysis indicated that the *B. juncea* R line (RfoRfo) carried a large radish introgression segment with the Rfo gene and the radish markers SchO3, SchA14, SG34, BolJon and PGInt as observed in the unimproved *B. napus* R line RRH1 (Primard-Brisset et al. 2005). In consequence, the R line has reduced male and female fertility as well as poor agronomic performance due to linkage drag. Improved *B. juncea* Ogura cms restorer (R) line VR4441 (RfoRfo) with only two radish markers SchH3 and BolJon has been successfully developed via marker-assisted selection in combination with the increased recombination frequencies involving the Rfo gene in resynthesized *B. juncea* germplasm (Tian et al. 2014).

**Objectives:** 1) Development of improved condiment and canola *B. juncea* Ogura cms R lines (RfoRfo) using the *B. juncea* R line VR4441 (RfoRfo) as restorer gene (Rfo) source; 2) Production and agronomic evaluation of test-cross hybrid based on the improved *B. juncea* Ogura cms hybrid system.

**Results:** Improved condiment and canola *B. juncea* Ogura cms R lines (RfoRfo) were developed using VR4441 as the Rfo gene source via pedigree breeding. The improved R lines (RfoRfo) had strong growth vigour compared with the checks (the elite canola *B. juncea* line 5607 and condiment *B. juncea* variety Cutlass) in the two-replicated field nursery in 2014. Test-cross hybrid O2152 (Rforf) between the oriental mustard variety “Cutlass” Ogura cms line (rfrf) and the improved R line VR4445 (RfoRfo) was produced in an isolation tent in 2013. The agronomic performance of the hybrid O2152 was evaluated in four-replicated yield trials in four locations (Saskatoon, Scott, Swift Current and Lethbridge) in western Canada in 2014. On average, O2152 yielded statistically significant higher (17.4%) than the check variety Cutlass.

Currently, heterozygous restorer lines (Rforf) are used as donor genotypes to produce doubled haploid (DH) Ogura cms R lines (RfoRfo) in condiment and canola *B. juncea*. Preliminary result indicates that the microspores carrying the Rfo gene is more embryogenic than those Rfo-negative ones in *B. juncea*. For instance, 150 plants were obtained using the canola *B. juncea* heterozygous restorer line C2532 (Rforf) as donor genotype, of which 122 (78.5%) plants were Rfo-positive.

**Conclusion:** The development of improved R line VR4441 (RfoRfo) has made the Ogura cms hybrid system fully functional in *B. juncea*. We can use the improved Ogura cms system to develop high yielding hybrid varieties in both condiment and canola *B. juncea*.

**References:**


Development and genetic studies of inbred lines with different linoleic acid contents in yellow mustard 
(*Sinapis alba L.*)

**Background:** Yellow mustard (*Sinapis alba L.*) is cultivated as an important condiment crop in the world. It is an obligate outcrossing species due to its sporophytic self-incompatibility. Open-pollinated population varieties of yellow mustard comprise great genetic variation. Different erucic and linolenic acid variants have been identified among the inbred lines developed via pedigree breeding in yellow mustard (Cheng et al., 2012). Genetic studies indicated that the erucic acid content is conditioned by multiple alleles of the *FAE1* locus, while two gene loci are responsible for the linolenic acid variation in this crop (Javidfar and Cheng, 2013; Zeng and Cheng, 2014; Tian et al., 2014).

**Objectives:** 1) Development of inbred lines with different linoleic acid (C18:2) contents; 2) Genetics studies and QTL mapping of C18:2 content in yellow mustard.

**Methods:** Inbred lines with different C18:2 contents were developed via pedigree breeding. The intron length polymorphism markers were used for construction of the regional linkage map of linoleic acid gene. QTL analysis of the C18:2 content was performed using the interval mapping method of MapQTL 6.0 (Van Ooijen, 2009).

**Results:** The inbred line Y1798 has a low C18:2 content (average: 4.7%), while line Y1801 contains a high C18:2 (average: 27.5%). Inheritance studies indicate a continuous distribution of the C18:2 content in the F2 population of Y1798 × Y1801. Two QTLS for C18:2 content were identified and mapped, respectively, to the linkage groups Sal01 and Sal02 in the reference map (Javidfar and Cheng, 2013). The first QTL explaining 39.0% of the C18:2 variation, was mapped to the linkage group Sal01. The second QTL was located in the linkage group Sal02 and responsible for 37.0% of the C18:2 variation. Together, the two QTLS accounted for 76.0% of the total C18:2 phenotypic variation.

**Conclusions:** Two linoleic acid variants have been developed in yellow mustard. QTL mapping indicated that the two QTLS located on the linkage groups Sal01 and Sal02, respectively, are largely responsible for the phenotypic variation of C18:2 content in this species.

**References:**


Background: Chinese hybrid rapeseed acreage is the largest country in the world, and most of the cytoplasmic male sterile hybrid comes from Pol (Pol CMS).

Objectives: Due to the cytoplasm relatively simple, it is urgent to create a new type of canola cytoplasmic male sterility. This study is by using unique Chinese Xinjiang wild rape (*Sinapis arvensis* L.) and Xiangyou15 (*Brassica napus* L.) to make the new cytoplasmic male sterile line.

Methods: By using bud pollination between Xinjiang wild rape (*Sinapis arvensis* L.) as female and *B. napus* varieties Xiangyou 15 as male parent. The hybrids obtained. F1 is highly sterile. The Xiangyou 15 self line 02-902 get backcross. The sterile plant rate of descendant is 43.33%. Since then, sterile plant and Xiang you 15 self line repeated backcrossing. The sterile plant rate in 2007 is 53% -67%, in 2010 sterile plant rate remained at 100%. Then by two backcross eventually bred sterile high fertility stable CMS 1193A and corresponding maintainer 1193B.

Results: 1. CMS1193A basic characteristics: CMS1193A belong to *Brassica napus* weak-winterness cytoplasmic male sterile line, CMS and maintainer line hybrid offspring complete sterility and stability. Bagging has strong self-incompatibility. After bagging and artificial pollination, seed normal, outcrossing normal seed rate of about 85%. Petals large, overlapping, stamens degradation, anthers triangular, pistil normal; petal length 13.2mm, petal width 9.3mm, an area of about 122.8mm2, pistil length 8.5mm, long stamens long 5.2mm, short stamens long 4.3mm. Height 172 cm or so, once effective branches 8, 9 or so secondary branches, number of pods per plant is about 340, 18 grains per pod, grain weight 3.6 grams. Double low quality. Resistance to stem rot. 2. Restorers found: 2012 summer lines 12 (91) are all normal fertile plants, and sterile hybrids fertile full recovery rate of 100%. And the heterosis is significant. Test cross found its extensive restoration sources.

Conclusions: This study is by using unique Chinese Xinjiang wild rape (*Sinapis arvensis* L.) and cultivars Xiangyou 15 (*Brassica napus* L.) to make the new CMS line.

References:
Guan Chunyun. Comparative studies of inheritable character in Xinjiang wild rape and *Sinapis arvensis* L. Acta Agronomica Sinica 22‐2‐214-219 1996 Li Xun, Guan Chunyun. Studies on cytogenetics of Xinjiang wild rape. 2. Analysis on morphological characteristics of chromosomes isozymes of peroxidase@mitochondrial DNA. Acta Genetics Sinica 22(6):470-477,1995
Identification of *Brassica napus* sources of resistance against pathogenicity groups 3 and 4 of *Leptosphaeria maculans*

**Background:** Blackleg caused by the fungus *Leptosphaeria maculans* is a serious disease of canola (*Brassica napus* L.) worldwide. In North Dakota, *L. maculans* populations have recently shifted their virulence profile from one dominated by strains of pathogenicity group (PG) 2 to one where the dominant group is PG-4. PG-2 strains cannot infect cultivars that have resistance genes *Rlm2* and/or *Rlm3* while the latter infects plants carrying *Rlm1, Rlm2* and/or *Rlm3* (del Río Mendoza et al., 2012; Marino, 2011; Nepal et al., 2014). Strains of PG-3, which are capable of infecting cultivars with resistance genes *Rlm2* and/or *Rlm3*, but are not that effective against *Rlm1*, constitute the second most prevalent group (Nepal et al., 2014). The recent occurrence of blackleg outbreaks that were caused by strains of these new groups (del Río Mendoza et al., 2012) adds a sense of urgency to the identification of effective sources of resistance against them.

**Objectives:** Identify *B. napus* plant introductions with effective levels of resistance against *L. maculans* strains of pathogenicity groups 3 and 4.

**Methods:** The screening approach consisted of two stages; in the first stage seedlings were evaluated in greenhouse conditions using the cotyledon inoculation method described by Nepal et al. (2014). Materials that advanced to the second stage were evaluated at the adult stage under field conditions. In the first stage, 579 *B. napus* plant introduction materials were evaluated using a mixture of five strains of *L. maculans* belonging to PG-4. All accessions were evaluated in replicated trials conducted at least two times. The materials that entered the second stage were inoculated with laboratory and field produced inoculum in 2014 and will be evaluated again in 2015. Disease severity at the adult plant stage was conducted before harvest by estimating the proportion of internal crown tissues visibly affected by the disease.

**Results:** Sixty plant introductions were considered resistant or moderately resistant to PG-4 isolates in greenhouse trials. Of the 21 accessions evaluated in field conditions, nine of them had significantly less disease (P < 0.01) than cultivar Westar and the two commercial controls used. The average severity for these accessions was < 10% compared to 81% of cultivar Westar and 26% of the commercial controls. The first stage of the screening of materials for reaction to PG-3 is under way.

**Conclusions:** *B. napus* plant introductions with high levels of resistance against *L. maculans* strains belonging to PG-4 have been identified and are currently being evaluated for their reaction to strains of PG-3. Doubled haploids will be developed from crosses involving some of these materials.

**References:**


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Marker assisted selection of Ogu-INRA cms system in NS rapeseed

**Background:** At the end of the 20th century, trends in rapeseed breeding changed direction from developing and using varieties to developing hybrids. This change in breeding programs was enabled by introduction of male sterility (cms) systems in rapeseed. At the Institute of Field and Vegetable Crops there is an ongoing breeding program with the goal of developing NS cms and fertility restorer (Rf) lines and hybrids (Marjanović-Jeromela et al. 2014). Ogu-INRA was chosen as a source of cms gene and R-2000 as a source of Rfo gene. It takes 6 years to develop rapeseed inbred lines; however, use of molecular markers can facilitate and accelerate this process.

**Objectives:** The aim of this study was to test the efficiency of markers linked to Ogu-INRA cms and Rfo genes (Sigareva and Earle 1997; Primard-Brisset et al. 2005) in NS experimental breeding material. This is especially important in testing experimental hybrids since some deviations in F1 expected segregation ratios were observed in the past.

**Methods:** Analyzed plant material included different experimental Rf and cms lines, and F1. DNA was extracted by use of modified CTAB method (Permingeat et al. 1998). Extracted DNA was used for amplification with BolJon and CMS primers in reaction (Dimitrijević et al. 2010). Obtained profiles were analyzed by BIO-CAPT V.97 program (Vilber Lourmat, France).

**Results:** Most of the obtained profiles corresponded to the ones amplified by Sigareva and Earle (1997) and Primard-Brisset et al. (2005). However, some deviations from expected profiles were observed. These will further be examined.

**Conclusions:** Preliminary results of this study showed that tested markers could be prosperous in facilitating NS rapeseed breeding programs and thus accelerating the process of developing not only, cms and Rf inbred lines, but also hybrids.

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**References:**


Fine mapping a major early flowering QTL QFTC2-1 by high through sequencing combine with genetic mapping in rapeseed (Brassica napus L.)

Background: In rapeseed, proper timing of flowering is a crucial determinant for adaption to different cultivation area and cropping seasons, also is an immediate indicator of the productivity. In South multiple cropping area of China, the rapeseed - rice -rice or rapeseed - rice or rapeseed -cotton rotation systems are rather pervasively. In those rotation systems, rapeseed with early maturity is one of the important limiting factor. So it is necessary to find and use key flowering time genes to breed early maturity rapeseed. Two rapeseed NIL lines R11 and R15 performed different flowering time were selected in pedigree breeding program. R11 usually blossomed 7-15 days earlier than R15 in winter rapeseed area while 6-12 days earlier in spring rapeseed area.

Objectives: Preliminary genetic analysis suggested that different flowering in the NILs was controlled by a major QTL. The objective of this study was to fine map and clone it, while using it in early maturity breeding was our final goal.

Methods: A F2 population was generated after self-pollinating F1 from R11 and R15. Flowering time data was recorded as the first flower blossomed and two extreme DNA pools, early flowering pool and late pool were constructed, respectively. Libraries of two pools and parents were prepared and then sequenced by an Illumina Solexa Hiseq2000 machine. Sequences analysis by BWA(Li and Durbin 2009) and GATK(McKenna et al., 2010) software to identify early flowering QTL. SSR markers were developed in QTL region by referring to the Darmor-bzh reference genome and an extreme early flowering population from F2 were scanned to confirm and fine map this QTL.

Results: A major early flowering QTL, QFTC2-1 was identified on the end of chromosome C2 after analyzing the sequences of two pools combine with parents. To confirm the early flowering QTL detected by high through sequencing, 40 linked markers were developed and a population containing 323 extreme early flowering plants was scanned. Finally, QFTC2-1 was fine mapped to a DNA region of approximate 95 kb. Now, we preliminary identified a gene that is homologous Arabidopsis genes involved in the flowering time pathway as candidate gene.

Conclusions: In this study, we fine mapped QFTC2-1 to a 95 kb DNA region and identified a candidate gene by high through sequencing combine with genetic mapping. This method took advantage of the high-through whole genome re-sequencing and bulked-segregant analysis (BSA) and genetic mapping. The results might be useful for cloning QFTC2-1 and using it in early maturity breeding by MAS.

References:
AP2/EREBP transcriptional factors responsible for cold stress in rapeseed (Brassica napus L.) revealed by dynamic transcriptome analysis

Background: Changes in temperature significantly affect the development of plants. Brassica napus L. is one of the ideal crops for studying cold tolerance capacity. Although certain progress has been made in studies of cold-tolerant genes and related transcriptional factors, a comprehensive and dynamic research on the cold-tolerant network and AP2 transcriptional factors regulation from the perspective of the has not yet been reported.

Objective: A complete transcriptome data responsible for cold stress was obtained for comprehensive understanding of the regulatory mechanism of AP2-EREBP transcription factors, and providing an effective reference for further development of cold-tolerant genes.

Methods: Transcriptome high-throughput sequencing method was applied in this paper to conduct temporal and spatial dynamic analyses for the entire transcript of rapeseed subjected to 4°C low temperature stress for 2, 6, 12, and 24 h. All the Arabidopsis AP2 domain of each treatment was used as a query to search the AP2/ERF gene domains in B. napus in the genome database using BLAST. We subsequently obtained the AP2/ERF genes for each species. The physical distribution of AP2/ERF genes on chromosomes was drawn by PERL scripts based on gene position in the genome. Phylogenetic and molecular evolutionary analyses were conducted using MEGAS. The dynamic and reliability of those transcription factors were further validated by RT-PCR.

Result: A total of 15,316 differentially expressed genes were identified, among which 451 AP2-EREBP transcription factors were discovered after comparing with the complete genome of rapeseed that was located in different chromosomes. After cluster analysis, these transcription factors were found to belong to five subfamilies, namely, DREB, ERF, AP2, RAV, and soloist.

Conclusion: A complete genome dynamic analysis was conducted on the stress-response gene and transcription factor regulation of rapeseed under cold stress. We obtained favorable transcriptome data for the comprehensive understanding of the regulatory mechanism of AP2-EREBP transcription factors, thereby providing an effective reference for further development of cold-tolerant genes and promoting the cold-tolerant capacity of plants.

References:
Cloning, sequence analysis of EsPL gene from Eruca sativa Mill and its plant expression vector construction

**Background:** Eruca sativa Mill has a sporophytic self-incompatibility reproduction system. Genetically stable self-incompatible (SI) and some self-compatible (SC) mutant species have also been identified in this crop. Cloning of SC genes has important significance in biology and breeding research. To isolate the genes associated with the self-compatibility of Eruca sativa Mill, we screened differential gene expression in SC and SI of Eruca sativa Mill by differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) in previous study. The pectate lyase gene EST was obtained.

**Objectives:** Experiments were performed to clone the full-length cDNA of Eruca sativa Mill pectate lyase gene (EsPL).

**Methods:** The full-length cDNA of EsPL was obtained by the technology of rapid amplification of cDNA ends (RACE). The cDNA sequence and the deduced amino acid sequence were analyzed by bioinformatics method. The expression vector of Eruca sativa Mill EsPL gene were constructed successful. The expression of EsPL before and after flowering in SI and SC were determined by real-time PCR.

**Results:** In this study, a novel gene, EsPL, from the anther of Eruca sativa Mill was isolated and characterized. The full-length cDNA was 1657bp, containing a 1371bp opening reading frame (ORF). The deduced protein was 456 amino acids with molecular weight 51.179kD and isoelectric point 9.42. The sequence alignment demonstrated that EsPL was high identity with other PL proteins from other species. So it was named the EsPL. Phylogram tree showes that there were closest evolutionary relationship of Brassica napus, Brassica rape and Eruca sativa Mill gene. Analysis by real-time PCR indicated that EsPL gene expression levels in SC anther is significant higher than SI anther after flowering. The expression vector of Eruca sativa Mill EsPL gene were constructed successful, which laid the foundation for further study the function of this gene.

**Conclusions:** The EsPL gene was cloned from the anther of Eruca sativa Mill and the analysis by real-time PCR suggested that EsPL played an important role in SI and SC Characteristics of regulation of Eruca sativa Mill.

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The BRASSINAM population, a new tool for investigating agronomic traits in Brassica napus L.

Background: A key component in the identification of genetic factors underlying agronomic traits relies on the availability of genetic material, such as mapping populations or panels of lines segregating for the desired trait. In winter oilseed rape (Brassica napus L.), several bi-parental populations have been developed for linkage analyses and at least one association panel is publicly available (ASSYST project, Bus et al, 2011). However, these resources have their drawbacks, notably in terms of power, resolution or allele frequency. Recently, multi-parental population schemes combining benefits from both linkage and association mapping approaches have been developed in crop plants, such as the maize NAM population (Yu et al 2008) or the wheat MAGIC population (Huang et al 2012).

Objectives: The BRASSINAM project aimed at creating a new genetic resource for rapeseed that will allow powerful analysis of genetic determinism of quantitative agronomic traits. This population should be of particular interest for both breeders and scientists. It will combine wide genetic diversity with adaptation to high-throughput and large scale phenotyping under European conditions.

Methods: The parents of the population were selected among a B. napus diversity set of 280 accessions that represented the main genetic pools. The diversity set was screened using 76 SSRs. Parents were selected and crossed with the French-adapted line AVISO. RILs were produced for each cross up to the F6 stage, were genotyped with 9,500 SNP and observed in nursery trials in 2 sites in 2014-15 to assess adaptation to French growing conditions.

Results: A set of 15 parents (12 WOSR, 3SOSR) was selected based on both genetic diversity as well as agronomic knowledge acquired by breeders. The population has been advanced to the F6 stage with around 200 individuals per cross. Field observations at F3 stage have allowed to assess winter hardiness of the SOSRxAVISO material in order to keep 200 adapted RIL for each population. Field observation at F6 stages such as flowering time allowed to identify potential subsets within the population to be used or avoided for particular traits. The genotyping data allowed a first run at association analyses with the traits observed, giving preliminary insights into the power and resolution that could be expected from this population. Finally, seeds are now available to potential collaboration.

Conclusions: This population represents a tremendous resource for further genetic studies and now needs to be exploited. Seeds are now available to potential collaborations. Within the frame of the Rapsodyn project, the parental lines and AVISO were re-sequenced in order to provide a high resolution allele scan of the population and a genetic map will be produced for the population. Imputation strategies will be investigated to further strengthen the resolution of the association analyses that could be performed with this material. Moreover, agronomic data will be obtained to assess performance under normal or N stressed conditions.

References:
Use of rutabaga (*Brassica napus* var. *napobrassica*) for the improvement of Canadian spring canola (*Brassica napus*)

Spring-type oilseed *Brassica napus* L., commonly known as canola, has become the cornerstone of agricultural production in western Canada with total acreage seeded increasing in each production year over the past two decades. However, the narrow genetic base of spring *B. napus* canola coupled with ever-increasing acres has led to emergence of clubroot disease, caused by *Plasmodiophora brassicae*, on the canola production areas. *Brassica napus* var. *napobrassica*, or Rutabaga, is a biennial fodder-type *Brassica* species that has potential to serve as not only a source of genetic diversity for *B. napus*, but provide strong resistance to *P. brassicae* pathotypes prevalent in the canola fields in western Canada. A F2 derived population of Rutabaga-BF × A07-26NR and a 3-way cross derived population of (A07-45NR ×Rutabaga-BF) × A07-26NR were evaluated for different agronomic and seed quality traits including resistance to *P. brassicae* pathotypes prevalent in western Canada. Results demonstrated that Rutabaga has the potential to broaden genetic diversity in spring type *B. napus*, as well as provide resistance to *P. brassicae* pathotypes.
Novel interspecific hybrid populations to investigate the problem of meiotic stability in *Brassica* synthetics

**Background:** *Brassica napus* (oilseed rape, 2n = AACC), *B. carinata* (Ethiopian mustard, 2n = BBCC) and *B. juncea* (Indian mustard, 2n = AABB) are all allopolyploid, with two genomes each resulting from ancestral hybridisation events between diploid species *B. rapa* (Chinese cabbage, turnip; 2n = AA), *B. nigra* (black mustard; 2n = BB) and *B. oleracea* (cabbage, cauliflower, broccoli; 2n = CC). However, attempts to recreate these ancestral hybridization events to produce novel allopolyploid genotypes from the current-day diploid progenitor species have been mostly unsuccessful, particularly in the case of synthetic oilseed rape. Resynthesised *B. napus* “falls apart” due to meiotic instability, which causes loss of chromosomes and chromosome segments and hence loss of critical genetic information and fertility over generations.

**Objectives:** We aim to isolate genetic and genomic factors contributing to fertility and subsequently meiotic stability in the three *Brassica* allopolyploid crop species.

**Methods:** Novel interspecific hybrid populations were generated by pairwise crossing between different genotypes of *B. juncea*, *B. napus* and *B. carinata* in order to produce populations segregating for A, B and C genome alleles and whole chromosomes. First generation hybrids had genome compositions AAbc, bbAc, ccAb, AAbcc and AAbbcc. Self-pollination and selection over generations for increased fertility as measured by self-pollinated seed set was undertaken. Molecular genotyping and cytogenetics approaches were used to track A, B and C genome allele inheritance, and self-pollinated seed set and pollen viability data were collected.

**Results:** Many hybrid genotypes were sterile, producing no self-pollinated seeds or even viable pollen, particularly in the first generation. However, several different genetic and genomic hybrid combinations also showed greatly increased fertility over subsequent self-pollinated generations. Involvement of unreduced gametes in early generations of *B. napus* x *B. juncea* and (*B. napus* x *B. carinata*) x *B. juncea* hybrid crosses appeared to greatly increase the chance of restoring fertility in later hybrid generations. Assessment of meiotic stability through allelic inheritance and cytogenetics and attribution to specific genetic and genomic factors is underway.

**Conclusions:** These interspecific hybrid populations will provide a valuable tool with which to identify key genetic and genomic factors responsible for meiotic stability in *Brassica* allopolyploids. Understanding the mechanisms underlying the process of hybrid species formation in *Brassica* will facilitate resynthesis of oilseed rape and the development of novel crop types in this genus.
Genetic variation and inheritance of epicotyl elongation before winter and its correlation with winter hardiness and vernalization requirement

Background: Epicotyl elongation before winter is considered as one critical component of the complex trait winter hardiness in winter oilseed rape (Kole et al. 2002). Thereby, genotypes with an enhanced epicotyl elongation before winter are most likely affected by strong frost. At present, little is known about the genetic variation and inheritance of this trait in winter oilseed rape and its correlation to other traits, e.g. vernalization requirement and flowering time.

Objective: The present work has been conducted to study the variation and inheritance of epicotyl elongation in a DH population derived from the cross of the two winter oilseed rape cultivars Sansibar x Oase in three different environments.

Methods: The DH population consisting of n=224 lines of the cross Sansibar x Oase were tested in three different environments in Germany: (a) in field experiments sown in August, (b) in field experiments sown in spring and (c) in greenhouse experiments without vernalization. Epicotyl length was measured before winter (a) and three months after sowing (b, c). Results obtained from replicated experiments were used for ANOVA and means over replicated experiments were used for correlation and QTL analysis.

Results: In all three experiments large and significant genetic variation was found for epicotyl length. Heritability for epicotyl length was high in all environments (91% to 94%). In the spring sown experiments 54% of the DH lines had a shoot length longer than 70 cm and produced flower buds, indicating a low vernalization requirement. Spearman rank correlation between the phenotypic mean values of the different environments were low (winter: spring r=0.14*; spring: greenhouse r=0.39* and winter: greenhouse r=0.12*), indicating that in different environments different genetic loci are involved in the expression of the trait "epicotyl length".

Conclusion: The majority of the DH lines appear to have a low vernalization requirement. This seems not to be related to frost tolerance, because frost damage was neither observed during the present field experiments nor during earlier field testing. Low correlation between phenotypic mean values of the different test environments excludes the possibility for early and simplified selection e.g. in greenhouse experiments.

References:
Expression of oleic acid character in different generations of rapeseed

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Background: During the course of present investigation law of inheritance for oleic acid character in rapeseed was studied. One high oleic acid line- 04-863(80.5%) and a low oleic acid line-04-1020 (developed by self-pollination of Xiangyou No.15 with 62.7% oleic acid) were used.

Objectives: The character of oleic acid is quantitative character and the expression of oleic acid character is related to the environment.

Methods: Measuring continuous variation of oleic acid character in rapeseed; Measuring broad sense heritability of oleic acid character in rapeseed; goodness of fit test for separate proportion of oleic acid character in rapeseed.

Results: The results are as follows: (1) Oleic acid character in rapeseed is a quantitative character, which can not be distinguished distinctly; (2) Oleic acid content in F1 is in between both the parents but female parent has more influence on it. If high oleic acid line-04-863 is used as female parent, the distribution of oleic acid content ( =73.41) is reflected to female. If low oleic acid content line-04-1020 is used as female parent, the F1 oleic acid content ( =67.75) is also reflected to female parent; (3) The oleic acid contents in both the parents and F1 are susceptible to environmental changes, as they show phenotypic differences and in one of their genotypes respectively; (4)F2 generation shows wide-ranging separation which is due to both genotypic separation and environmental Change. The measurement of phenotypic difference is studied by variance (V) and standard deviation (S). It has been recorded that the degree of variation departs from mean and standard deviation in F2 generation is larger than other generations, P1, P2 and F1, that is V=52.81 and S=7.28 in F2; (5) The distribution of variance in P1, P2, F1 and F2 shows nearly normal distribution. It is extremity type in a few and middle type in great many; (6) One major pair of gene is necessary to control rapeseed oleic acid character. This is due to separation of oleic acid character which shows a ratio of 3:1 i.e. law of segregation of Mendel, and by backcross ratio 1:1. The heritability of oleic acid is 69-71%, which is 69-71% due to the influence of heredity difference and 31-29% by environmental influence.

Conclusions: The above results show that the difference between quantitative and qualitative characters is not absolutely relative.

References:
Identification of QTLs for antioxidants in relation to Alternaria Blight in 
Brassica juncea L. Czern & Coss.

**Background:** Plants possess a series of enzymatic and non-enzymatic detoxification systems to prevent damage caused by accumulation of reactive oxygen species (ROS) in plant cells due to adverse environmental conditions and pathogen attack. These include: superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), Phenylalanine ammonia lyase (PAL), carotenoids (CAR), phenols, proline (PRO) and glutathione (GSH). Little information is available on the genetic and molecular aspects of these enzymatic and non-enzymatic antioxidants.

**Objectives:** To develop a QTL map for various antioxidants in relation to alternaria blight and high temperature stress.

**Methods:** A population comprising 53 F6 RILs, developed from a cross between an Indian variety, RL-1359, and European mustard line, NUDH-YJ-04 was used for the study (Gupta et al. 2014). POD, PAL, total phenols, O-hydroxy phenols (O-OHPhenols), PRO, CAR, GSH, alternaria blight severity and seedling survival were estimated by using their standardized methods. QTL mapping was performed by regression based composite interval mapping using WinQTL Cartographer v2.5_011 (Wang et al. 2012). It was graphically displayed using MapChart Version 2.1 (Voorips 2002).

**Results:** Extensive phenotypic variation was recorded for different biochemicals under question. Alternaria blight severity varied from 33.24% to 76.67% with mean disease severity of 51.26%. The per cent seedling survival under high temperature stress depicted a range of 0 to 100% with mean value of 67.58%. Alternaria blight had significant positive correlation with POD and proline; negative with PAL, o-OHPhenols and GSH whereas no significant correlation was observed between total phenols and severity of alternaria blight. A significant positive correlation has also been registered for survival percentage of seedlings under high temperature stress with proline and negative with GSH. A total of 13 quantitative trait loci (QTL) (ALTB1T-2; PAL-2; T-PHENOL-2; GSH-3; PRO-1; CAR-1; POD-1 and O-O HPHENOL-1) were detected and assigned to different loci in linkage groups with LOD score values ranging from 3.1-10.8 and R2 value of 16-48%.

**Conclusions:** Significant QTLs impacting variation for T-phenol (A1, A9), PAL(A1, B2), GSH (A2, A5, A6), PRO(A3), POD(A9), o-OHPhENOL(A10), CAR(A7) and ALTB1T(B7,B8) could be located respectively. As limited information is available for tagging of various biochemicals studied, our study can act as skeleton for detailed analysis of genetic control of these biochemicals.

**References**


Map-based cloning of a major quantitative trait locus (QTL), qSS.C9, for the number of seeds per silique (NSS) in oilseed rapa (Brassica napus L.)

**Background:** As a complex agronomic trait, seed yield of a rapeseed plant is multiplicatively determined by the number of seeds per silique (NSS), the number of siliques per plant and seed weight. It is well known that NSS is a typical quantitative trait like the other two yield components; however, the genes and the putative molecular mechanism underlying the natural variation of NSS remain unknown thus far.

**Objectives:** Our previous study mapped a major QTL (qSS.C9) controlling NSS on the B. napus C9 chromosome (Zhang et al. 2012). Comparative genomics showed that the closest flanking markers delimited qSS.C9 to a syntenic region of 911 kb on the C9 chromosome of Brassica oleracea genome. We expected to isolate qSS.C9 and primarily reveal the underlying molecular mechanism of how it controls the natural variation of NSS.

**Methods:** Near-isogenic lines (NILs) segregating at qSS.C9 were prepared by backcrossing a low-NSS parent HZ396 (SS, 11.0 ± 2.6) to a high-NSS parent Y106 (SS, 27.3 ± 3.7) for three times. We also constructed a new bacterial artificial chromosome (BAC) clone library from B. napus lines with high NSS and explored a map-based cloning strategy to identify the target BAC clone covering the candidate gene. Transgenic complementation and RNAi experiments were adopted to confirm the target gene of qSS.C9. The ovule development and cell plate formation in female meiosis were respectively observed by CLSM and callose staining.

**Results:** We identified a BAC clone covering part of the candidate region and got partial sequences of it which shows obviously sequence variation with the B. napus reference genome. Based on the sequence of this BAC clone, we analyzed the target sequences between NIL(Y106) and NIL(HZ396), and found obvious variations in two candidate genes (qSS.C9a and qSS.C9b). Genetic transformation of a 5.3-kb genomic fragment covering the predicted qSS.C9a from NIL(Y106) into NIL(HZ396) could significantly increase NSS in T0 and T1 generations, while knockdown the expression of qSS.C9a in a line with high NSS resulted in extremely low NSS, proving that qSS.C9a is the target gene of qSS.C9. Expressional and cytological analyses showed that qSS.C9a plays an exclusive role in regulation of cell cycle progression during the megaspore meiosis stage.

**Conclusions:** qSS.C9a functions as a positive regulator of NSS by promoting the normal progression of female meiosis in rapeseed. The functional characterization of qSS.C9 represents the first step toward unraveling the molecular mechanism controlling natural variation of NSS in rice and provides a potential locus for yield improvement in rapeseed.

**References:**
The male-sterile gene \textit{BnRfb} from 9012AB disrupts the tapetum degeneration and tetrad release both in \textit{Brassica napus} and \textit{Arabidopsis}

**Background:** A genic male sterility (GMS) line, 9012AB, has been used as an efficient pollination control system in China for hybrid production. Genetic analysis showed that \textit{BnRfb} alone can cause the male-sterile phenotype in 9012A which can be temporarily maintained by \textit{BnRfc} while recovered by \textit{BnRfa} or \textit{BnMs3} (Dong et al. 2012). Though we previously mapped the \textit{BnRf} locus to a 13.8-kb DNA fragment of a \textit{BnRfc}-containing bacterial artificial chromosome (BAC) clone on the \textit{B. napus} A7 chromosome (Xie et al. 2012), marker assay showed that great sequence variations may exist in the flanking regions of the three alleles.

**Objective:** An efficient approach should be adopted to obtain the sequences around the \textit{BnRf} locus, and the target gene of \textit{BnRf} should be narrowed down to a reasonable region. We expected to reveal the allelic variations between different alleles of \textit{BnRf} and how \textit{BnRfb} cause male-sterility fertility in this study.

**Methods:** A bulked \textit{B. napus} bacterial artificial chromosome (BAC) clone library containing both the \textit{BnRfa} and \textit{BnRfb} locus were prepared. A map-based cloning strategy was adopted for the identification of \textit{BnRf} by integrating the genetic maps from different mapping populations. The target BAC clones covering the candidate genes were screened by PCR method. The wide-type \textit{Arabidopsis} plants was used for transgenic complementation assay. The cytological observation of developmental anthers was comparatively observed between the male-fertile and male-sterile \textit{Arabidopsis} and \textit{B. napus} plants.

**Results:** Sequence comparison found around a 61-kb DNA fragment insertion in \textit{BnRfa} or \textit{BnRfb} candidate region relative to \textit{BnRfc}. \textit{BnRfa} and \textit{BnRfb} were further mapped to a 40.9-kb region of the insertion fragment including eight predicted open reading frames (ORFs). Genetic transformation showed that G14 both from the \textit{BnRfa}- and \textit{BnRfb}-containing BAC clones can cause the similar male-sterility in \textit{Arabidopsis} as in 9012A, and the resulting male-sterility can be reversed by introduction of the restorer gene \textit{BnMs3}, demonstrating G14 as the target of \textit{BnRfb}.

**Conclusion:** We cloned the male-sterile gene \textit{BnRfb} from 9012AB. The expression of \textit{BnRfb} in \textit{Arabidopsis} negatively regulated some vital genes responsible for tapetum degeneration and resulted in the delay of tapetum PCD and developmental arrest of tetrads. The true \textit{BnRfa} is actually not allelic to \textit{BnRfb} but corresponds to another tightly associated gene in the 40.9-kb candidate region, while \textit{BnRfc} is indeed a deleted nonfunctional allele. These findings laid a solid foundation for elucidating the molecular mechanism underlying the sterility maintenance and restoration in 9012AB.

**Reference:**


BnMs5 causes the male-sterility phenotype in rapeseed (Brassica napus L.) by suppressing the early event of meiosis

**Background:** Male sterility has been used as one of the most important pollination control systems in rapeseed. A spontaneous mutation in Rs1046AB which was transferred from Y13A could be used as a possible alternative to the extensively used Polima cytoplasmic male sterility (Pol CMS) in China. Previously genetic and molecular marker assays have showed that the male fertility of this genetic male sterility (GMS) line is controlled by a locus assigned as BnMs5 with three different alleles: BnMs5a (restorer type), BnMs5b (male-sterile type) and BnMs5c (normal male-fertile type) (Lu et al., 2013).

**Objectives:** Previous study has delimited BnMs5 to a 21kb region on a Brassica napus BAC clone JBN034L06 which contains six predicted functional genes (Lu et al., 2013). The aim of this study was to clone BnMs5 and identify the mechanism of BnMs5 in fertility regulation in Brassica napus.

**Methods:** A bacterial artificial chromosome (BAC) library with in the BnMs5 locus was constructed and screened to identify BACs with BnMs5a and BnMs5b. A genetic complementation experiment was performed to confirm the candidates for BnMs5a. The sequences of the three BnMs5 alleles were compared and the functions of BnMs5 were verified by qRT-PCR, GUS assays, RNA in situ hybridization and protein localization.

**Results:** We identified the target BAC clones covering the candidate region of BnMs5a and BnMs5b, respectively. Sequence comparison revealed that obvious variations exist between the candidate regions of the three alleles. From the six candidate genes, we found the introduction of one gene from the restorer line DH195 into a male-sterile line could completely restore the male-sterility phenotype in T0 and T1 generations, and the fertility conversion was cosegregated with the transgenic events. These results proved that this gene is the target gene of BnMs5.

**Conclusion:** We characterized a novel nucleus-localized gene BnMs5 by a map-based cloning strategy. The results here showed further insights into the molecular control of BnMs5 in meiosis and anther development. This work represents major progresses towards final understanding of the mechanism of BnMs5 and how three alleles acts in a dominance or recessiveness manner to condition the fertility conversion in Rs1046A.

**Reference:**

Comparative transcriptome analysis reveals carbohydrate and lipid metabolism blocks in *Brassica napus L.* male sterility induced by the chemical hybridization agent monosulfuron ester sodium

**Background:** Chemical hybridization agent (CHA) inducing male sterility is a widely used tool for seed production in crop heterosis utilization. We previously discovered that monosulfuron ester sodium (MES), an acetolactate synthase (ALS) inhibitor of the herbicide sulfonyleurea family, can induce rapeseed (*Brassica napus L.*) male sterility at approximately 1% concentration required for its herbicidal activity.

**Objectives:** To find some clues to the mechanism of MES inducing male sterility.

**Methods:** The ultrastructural cytology observations, comparative transcriptome analysis, and physiological analysis on carbohydrate content were carried out in leaves and anthers at different developmental stages between MES-treated and mock-treated rapeseed plants.

**Results:** Cytological analysis revealed that the plastid ultrastructure was abnormal in pollen mother cells and tapetal cells in male sterility anthers induced by MES treatment, with less material accumulation in it. However, starch granules were observed in chloroplastids of the epidermis cells in male sterility anthers. Comparative transcriptome analysis identified 1501 differentially expressed transcripts (DETs) in leaves and anthers at different developmental stages, most of these DETs being localized in plastid and mitochondrion. Transcripts involved in metabolism, especially in carbohydrate and lipid metabolism, and cellular transport were differentially expressed. Pathway visualization showed that the tightly regulated gene network for metabolism was reprogrammed to response to MES treatment. The results of cytological observation and transcriptome analysis in MES-treated rapeseed plants were mirrored by carbohydrate content analysis. MES treatment led to decrease in soluble sugars content in leaves and early stage buds, but increase in soluble sugars content and decrease in starch content in middle stage buds.

**Conclusions:** Our integrative results suggested that carbohydrate and lipid metabolism was influenced by CHA-MES treatment during rapeseed anther development, which might responsible for low concentration MES specifically inducing male sterility. A simple action model of CHA-MES inducing male sterility in *B. napus* was proposed. These results will help us to understand the mechanism of MES inducing male sterility at low concentration, and might provide some potential targets for developing new male sterility inducing CHAs and for genetic manipulation in rapeseed breeding.

**References:**


Heterotic grouping and the heterotic pattern among Chinese rapeseed accessions (*Brassica napus* L.)

**Background:** Heterotic groups and patterns are extremely important in hybrid breeding. However, to date, there have been limited reports on the heterotic patterns within Chinese semi-winter rapeseed, which is extremely important for Chinese rapeseed hybrid breeding.

**Methods:** Nine elite inbreds widely used in Chinese rapeseed hybrid breeding programs were crossed in a diallel mating design to develop 36 hybrids. These hybrids and their parents were evaluated for two successive years in Northern China. Five different methods, which were based on specific combining ability (SCA) effects, SCA-Yang’s effects, molecular markers, heterotic group specific and general combining ability (HSGCA), and heterotic grouping based on GCA of multiple traits (HGCAMT), were compared for their ability to classify the tested inbreds into heterotic groups.

**Results:** With regard to grouping of inbreds, breeding efficiency, and cross-mean yield variation explained by the cross types, the SCA method was the most promising one, followed by the SCA-Yang’s and molecular marker methods. Using the SCA method, three testers (8D129, 8C343, and 8D153) and opposing heterotic groups [(8C108, 8C189, and 8D129), (8C343, 8C360, and 8E001), and (8C272, 8D153, and 8E019)] were identified across environments. Chinese southern and northern rapeseed lines formed a different heterotic group. Four out-yielding crosses, 8D129 × 8E001 (high-check heterosis, 29.52%), 8C343 × 8D129 (24.76%), 8C189 × 8C272 (23.98%), and 8C272 × 8C343 (22.95%), were identified as ideal hybrids for further extensive testing in multi-location trials and are promoted for adoption and commercialization in China.

**Objectives:** To assess the genetic diversity and the combining ability of the nine rapeseed breeding lines in China; to compare the five different methods, SCA method, SCA-Yang’s method, molecular markers method, HSGCA, and HGCAMT methods, for their ability to classify the tested inbreds into heterotic groups; to identify inbred testers for heterotic groups and heterotic patterns among elite Chinese breeding lines.

**Conclusions:** Among the five different methods of classifying heterotic groups in Chinese *B. napus*, SCA method was the most promising one, followed by SCA-Yang’s and molecular marker methods. Chinese southern and northern rapeseed lines formed a different heterotic group.

**References:**


QTL analysis for pod shatter resistance in Chinese breeding populations of rapeseed

**Background:** Rapeseed shatter seeds upon maturity, making combine harvesting difficult. This becomes more serious under hot dry conditions at harvest time in the regions such as the Yangtze river valley in China. Development of varieties with pod shatter resistance suitable for mechanized harvesting has become the main breeding objective. Studies have shown that genetic variation exits in Chinese breeding populations and lines with both high and moderate resistance to pod shattering have high general combine ability (GCA) (Liu et al., 2013). SNPs on A9 were identified to be associated with pod shatter resistance in *Brassica napus* (Hu et al., 2012).

**Objectives:** One DH population and one natural population comprised mainly Chinese breeding lines with various resistance levels to pod shatter were used to analysis the genetic basis of pod shatter in *Brassica napus* combined with genome-wide SNP assay. By linkage and association analysis, QTL mapping and development of markers associated with pod shatter resistance are of great potential for accelerating variety development for pod shatter resistant and understanding the genetic mechanism of pod shatter.

**Methods:** One DH population comprising 96 lines was generated using a high resistant line × a susceptible line. Pod shatter resistance index (PSRI) were measured by random impact method in three environments. High density genetic linkage map was constructed using genome-wide single nucleotide polymorphism (SNP) markers assayed by the *Brassica* 60K Infinium BeadChip Array. Linkage analysis and SNP map construction were performed using QTL IciMapping V4.0 and JoinMap 4.0. WinQTLCart2.5 was used to detect QTLs. TASSEL was used for association analysis.

**Results:** Association analysis resulted in six QTLs located on A1, A6, A7, A9, C2 and C5, respectively, with the one on A9 explaining the largest phenotypic variation. Five, two and two QTLs were detected from DH population in three environments respectively. Two of the QTLs could be repeated detected across environments indicating that there are at least two additive genes involved in resistance to pod shattering and that the A9 and A6 loci were the main contributor to this resistance. The locus on A6 (BnSRL.A6) is a new one identified for pod shattering and fine mapping with near isogenic lines (NILs) is ongoing.

**Conclusions:** QTLs for pod shatter resistance were detected from Chinese breeding populations. Two major QTLs for pod shatter resistance are located on A9 and A6, with the one on A6 is newly identified and will be further investigated for pyrimiding pod shatter resistant loci in rapeseed varieties.

**Reference:**


Single-point mutation in the rapeseed acetohydroxy acid synthase (AHAS) gene confers resistance to imidazolinone herbicides and its application in hybrid rapeseed production

**Background:** Imidazolinone herbicides (IMIs)-resistant varieties, induced by mutations to acetohydroxyacid synthase genes (AHAS), are planted worldwide with many crops (Thompson and Tar'an B, 2014). However, in the case of rapeseed, which is a common source of edible oil for nearly one-half of China’s population, no IMI resistance has been reported for any of the varieties currently cultivated.

**Objectives:** We have developed an imazethapyr-resistant rapeseed (M9) derived from a naturally occurring mutant plant. The goals of this study were to determine the biochemical and molecular bases of herbicide resistance in M9, to develop molecular markers for the detection of herbicide-resistant genes, and to utilize herbicide-resistant traits to enhance seed purity in hybrid rapeseed production.

**Methods:** AHAS extraction and inhibition measurements of AHAS activity were performed. Three genes BnALS1-BnALS3 encoding ALS were isolated from the mutant and wild type by using PCR method. The RT-PCR analysis of AHAS transcripts was performed using TaqMan probe detection. An allele-specific PCR (AS-PCR) marker for BnAHAS1R was designed and detected in the F2, BC1, and BC2 populations. The resistant trait of M9 was introgressed into a susceptible restorer line of a CMS system using a marker-assisted backcrossing selection procedure.

**Results:** An in vitro AHAS activity assay indicated that the AHAS enzyme from M9 conferred a specific resistance to IMIs. Molecular analysis identified a single-point mutation leading to an amino acid substitution from serine 653 (AGT) to asparagine (AAT) at the herbicide-binding site of the rapeseed BnAHAS1 gene. This substitution mutation (Ser653Asp) did not change the transcription levels of BnAHAS1 in M9 compared with the wild type. An AS-PCR marker for the BnAHAS1R was cosegregated with IMI resistance in three populations. Finally, the CMS restorer line 10M169 was developed to show the resistance of M9 and the different purity of F1 seeds were generated from 10M169 and Ning A7 under different pollination conditions. The increases in seed purity under natural hybridization and hybridization in tents reached 13.41% and 16.41% after IMI treatment, suggesting that the herbicide-resistant trait can be utilized for the efficient elimination of false hybrids in hybrid rapeseed production, and leading to yield increases of up to 322 kg/ha and 394 kg/ha, respectively.

**Conclusions:** The molecular mechanism and molecular marker of herbicide resistance described herein provide the basis for the release of IMI-resistant rapeseed cultivars.

**References:**
Natural variation at *BnRSW* gene affects seed weight by regulating development of silique wall in polyploidy rapeseed

**Background:** Seed weight has been widely accepted as a complex trait controlled by polygenes in crops. Although some genes have been identified to regulate seed weight, mechanisms for seed weight regulation are still not well understood. Especially in polyploidy crops, no gene regulating seed weight has been cloned in polyploidy so far. *Brassica napus* L., as the world’s second leading crop source of vegetable oil, is a tetraploid (4x) species.

**Objectives:** We identified a major QTL on A9 chromosome that controls rapeseed seed weight (explaining approximately 30% of the phenotypic variation) and silique length. To gain a better understanding of how this QTL controls seed weight, we cloned and analyzed the targeted gene by fine mapping and association analysis.

**Methods and results:** Firstly, we used map based cloning and target-regional association to clone and characterize the genetic basis underlying the major QTL, *BnRSW* for seed weight. We uncovered a 165-bp deletion in *BnRSW* associated with the increased seed weight. *BnRSW* encodes an auxin response factor and shows inhibition activity on the auxin downstream genes. The deletion in *BnRSW* increases silique length and seed weight by decreasing the inhibition activity. Furthermore, reciprocal crossing result shows that this QTL affects seed weight by maternal effects. Based on the transcription analysis, we further proved that *BnRSW* regulates cell growth in silique wall by acting in the auxin response pathway.

**Conclusions:** Together, our results suggest that *BnRSW* regulates silique wall development and then finally determines the seed weight by controlling the amount of photosynthates as a new way of maternal regulation. Also, our study revealed one QTL gene in tetraploid and will provide the insights for QTL genes cloning in polyploidy crops.

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Resynthesizing *Brassica amphidiploids* for resistance to clubroot

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**Background:** Clubroot is an economically important disease affecting plants in the family *Brassicaceae* worldwide. In amphidiploid *Brassica* species, there are limited resistant sources available for the economically important canola crop *B. napus* and no resistant genotypes available for the mustard species *B. juncea* and *B. carinata*. Resistant germplasms resistant to a broad range of pathotypes of *P. brassicae* were identified in the progenitor diploid *Brassica* species *B. rapa*, *B. nigra* and *B. oleracea* (Peng et al. 2014), which could be used for developing canola and mustard crops for resistance to clubroot by re-synthesizing the *Brassica amphidiploids*.

**Objectives:** To introduce new sources of resistance to clubroot into the amphidiploid crops, *B. napus*, *B. juncea* and *B. carinata* lines were resynthesized through inter-specific hybridization using diploid progenitor species identified at Saskatoon Research Centre, Agriculture and Agri-Food Canada.

**Methods:** Four resistant *B. rapa* lines (Jazz, Milan, 96-6992 and T19), two *B. oleracea* (Tekila and Kilaherb), one *B. nigra* R line (CR2716), and two susceptible lines (ACDC, *B. rapa*; T010000DH3, *B. oleracea*) were used as the parents for interspecific crosses. Embryos at 15 days after pollination were cultured in the MS liquid medium with 2% sucrose. When cotyledons appeared, they were transferred into B5 solid medium supplemented with 2% sucrose. Plantlet roots were submerged in 0.2% colchicine solution for 2 hours, then washed for three times and transferred them to soil directly. Amphidiploids were identified by Ploidy Analyzer and then confirmed by the development of fully formed stamens.

**Results:** Three *B. napus* lines (Tekila × 96-6992, Kilaherb × Milan and T010000DH3 × Jazz) and two *B. juncea* lines (T19 × CR2716 and ACDC × CR2716) and one *B. carinata* (T010000DH3 × CR2716) were successfully re-synthesized. The presence of clubroot resistance genes derived from the *B. rapa* R lines in the amphidiploids were confirmed using SNP markers. Seeds were obtained by self-pollinating the re-synthesized *B. juncea* and *B. carinata* lines as well as the *B. napus* line derived from T010000DH3 × Jazz. Due to male-sterility in Tekila and Kilaherb, the re-synthesized *B. napus* from the cultivars were not able to produce pollen therefore they were crossed to a DH *B. napus* line DH16516.

**Conclusions:** Clubroot resistant germplasms in each of the three amphidiploid species were developed. Each will serve as new resistant sources for developing canola and mustard crops with resistance to clubroot thereby broadening genetic diversity for the crop improvement.

**References:**

Identification of QTL involved in resistance to clubroot in canola (Brassica napus)

**Background:** Clubroot, caused by Plasmodiophora brassicae Woronin, is an important disease of canola (Brassica napus L.). Over the past decade, clubroot has spread throughout central Alberta and also has been confirmed in a few fields in Saskatchewan and Manitoba. Breeding for resistance is the most effective management approach (Strelkov and Hwang 2014). European fodder turnips (B. rapa ssp. rapifera) were identified as the main source of clubroot resistance and have been extensively used in breeding (Diederichsen et al. 2009). Several resistance genes and QTL were identified in B. napus on chromosomes N02, N03, N08, N09, N13, N15, N16 and N19.

**Objective:** The objective of this study was to identify QTL conferring clubroot resistance in canola.

**Methods:** A doubled haploid (DH) population (N=133) of canola was developed from a cross between genotype 11-99 and genotype 12-1. The resistant parent 11-99 is a spring-type inbred line, and its resistance originated from ‘Tosca’ (B. napus). Three P. brassicae single-spore isolates (SSI) classified as pathotype 2, 3 or 5 on the differentials of Williams (1966) were used as inoculum. Seedlings were inoculated with a resting spore suspension (1×10⁷ spores/ml) and evaluated for clubroot reactions after six weeks with a 0-9 scale. A disease index (DI) was calculated according to the formula DI= [(0n0+1n1+…+9n9) ×100/(9×NT)] ×100%. One hundred and seventy five anchor simple sequence repeat (SSR) markers were used to generate a genetic map. Joinmap v. 4 was used to construct the map. The QTL for clubroot resistance were analyzed by composite interval mapping (CIM) with Map QTL v. 3.

**Results:** The resistant parent was resistant, while the susceptible parent was susceptible, to all SSI. The frequency distribution for resistance to the SSI in the DH population showed continuous segregation patterns. A genetic map was constructed with 175 SSR which included 18 linkage groups and covered 18 chromosomes (except N16). A total of six QTL were detected on two chromosomes that confer resistance to the three P. brassicae SSI, which explain 62.2% to 65.8% of the total phenotypic variance. One QTL located on N03 confers resistance to all isolates and explained 57.5% to 61.4% of the phenotypic variation. A QTL on N06 with a minor effect also was identified that contributed to resistance to all three isolates.

**Conclusion:** Two QTL were identified on a draft genetic map of canola consisting of 175 SSR markers. One QTL was located on N03 and confers resistance to all isolates, explaining 57.5% to 61.4% of the phenotypic variation. The other QTL was on N06 and had a minor effect on resistance.

**References:**
Transcriptome analysis of interspecific hybrid between *Brassica napus* and *B. rapa* reveals heterosis for oil rape improvement

**Background:** Heterosis is a prevalent phenomenon in evolution and breeding process of plants. Development of interspecific hybrids has been widely exploited for the heterosis breeding of *Brassica* crops. The hybrid between *Brassica napus* and *B. rapa* displays obvious heterosis in both growth performance and stress tolerance. The hybrid offspring obtains the advantage in many agricultural and developmental traits including biomass yield, plant height, vigor, stress tolerance from their parents, so as to improve the adaptation and enlarge the planting area in the world.

**Objectives:** In this study, the transcriptomes of *B. rapa* ssp. chinensis Makino and *B. napus* and their interspecific hybrid were sequenced to investigate the molecular basis of heterosis. Gene expression profiles of Ar and An genomes in two parents and interspecies hybrid were analyzed.

**Methods:** In the present study, *B. napus* (AnAnCC genome), *B. rapa* (ArAr genome), and its hybrid F1 (AnArC genome) were used to identify possible molecular mechanisms of heterosis at gene expression level. Total RNA of each sample was isolated using RNAprep pure Plant Kit. cDNA library was constructed following the manufacturer’s instructions of mRNA-Seq Sample Preparation Kit. Whole transcriptomes were sequenced using the platform of Illumina/Solexa. The uniquely mapped reads for a specific gene were counted by mapping reads to de novo assembled distinct sequences using soAP2 software, and the rPKm (reads Per Kb per million reads) values were computed as proposed by Mortazavi et al. RNAseq data were further validated using qRT-PCR for a selected number of genes using gene-specific primer sets.

**Results:** A total of 40,320 nonredundant unigenes were identified using *B. rapa* (AA genome) and *B. oleracea* (CC genome) as reference genomes. A total of 6,816 differentially expressed genes (DEGs) were mapped in A and C genomes, and 4,946 DEGs displayed nonadditively by comparing the gene expression patterns among the three samples. The coexistence of nonadditive DEGs including high-parent dominance, low-parent dominance, over-dominance, and under-dominance was observed in the gene action modes of F1 hybrid, which were potentially related to the heterosis. The nonadditive DEGs in hybrids from A genome mainly participated in metabolism and development, while those from C genome largely involved in stress resistances. The coexistence of multiple gene actions in the hybrid, and provided a list of candidate genes and pathways for heterosis. Furthermore, the expression bias of transposable element-associated genes from A and C genome was observed in the hybrid compared to their parents.

**Conclusions:** The present study could be helpful for the better understanding of the determination and regulation mechanisms of heterosis with purpose to *Brassica* improvement.
QTL mapping of traits associated with plant architecture and yield in *B. napus*

**Background:** Plant architecture directly affects the adaptability to cultivation, harvest index and potential yield of crops. Rapeseed plant architecture is a collection of many important agronomic traits including plant height, branch angle, number of branches, length of branches and silique density. In last decades, significant progress has been made in elucidating the molecular mechanism of plant architecture in rice, barley and maize. However, the molecular factors that shape plant architecture of *Brassica* crops are still largely unknown partly due to lack of mutants. Recently, we found a double haploid (DH) *B. napus* line (4942) exhibiting dwarf and compact phenotype. It has significantly fewer primary branches, shorter branch length and flowering period, higher silique density and no secondary branches.

**Objectives:** The aim of this study was to construct a linkage map using two *B. napus* genotypes with contrasting plant architecture for mapping of major quantitative trait loci (QTLs) for key agronomic traits.

**Methods:** A population of 181 DH lines derived from a cross between 4942 and 8008 (a *B. napus* inbred line with normal plant type) was used to construct a molecular linkage map using a 6K *Brassica* Infinium® SNP array and molecular maker techniques. Phenotypic data was collected from 16 traits associated with plant architecture and yield including two new indexes such as the length of silique layer and the width of silique layer under three environmental conditions.

**Results:** The map is comprised of three SCAR, 80IP, 160 AFLP, 253 SSR, and 1406 SNP markers distributed across 20 linkage groups and has a total length of 2,328.97 cm. A total of 337 quantitative trait loci (QTL) for 16 plant architecture and yield-associated traits were identified in three natural environments in the DH population. The proportion of phenotypic variance explained by the individual QTLs ranged from 2.6% to 60.0%. Maximum number of major QTLs were present on A01 followed by A09 linkage group. A trait-by-trait meta-analysis revealed 234 consensus QTLs, of which 70.5% were clustered and integrated into 59 pleiotropic unique QTLs by meta analysis. Two pleiotropic QTLs (mqA1.13 and mqA1.14) for length related traits such as plant height, the total and average length of primary branches, the length of silique layer and the width of silique layer were identified.

**Conclusions:** *B. napus* 4942 has promising plant architecture for mechanical harvesting. Our study is the first report on identification of QTLs for plant architecture traits in *Brassica*. The QTLs identified here provide a strong foundation for fine mapping and further cloning of the major QTLs shaping plant architecture, which would help in breeding new rapeseed varieties suitable for mechanization and unveiling the mechanisms that control the rapeseed plant architecture.
Single cell genomic sequencing in *Brassica napus* and wheat: Applications in monitoring recombination frequency

**Background:** Transfer of high value target loci from genetically diverse wild relatives to adapted elite varieties is seen as a key to the future of wheat breeding. Efforts to modulate homoeologous recombination between the chromosomes of alien donor and those of cultivated wheat via mutating the Ph1 (Pairing homoeologous 1) locus, chemical treatment or changes in environmental conditions are currently underway. However, the fast progress of such projects is hindered by the lack of a rapid screening method for monitoring the impact of modulation of homoeologous recombination in polyploid crops, such as *Brassica napus* and wheat. Currently used cytogenetic methods for assessing recombination frequency are cumbersome and time consuming.

**Objectives:** The main objective of this study is to establish an easy and efficient method for monitoring the impact of modulation of recombination in plants. We have devised a strategy to assess homoeologous recombination frequency in an F1 plant which leverages the combined advantages of single cell whole genome sequencing technology and the relative ease of enrichment of single microspores.

**Methods:** Single cell haploid microspores from an F1 plant is ideal material to quickly assess homoeologous recombination frequencies as it is relatively easy to isolate thousands of microspores carrying segregating genotypes. Our method involves DNA isolation from single microspores derived from F1 progenies using the Fluidigm C1 single cell auto prep system which offers a simplified single cell isolation and DNA extraction workflow. Subsequent sequencing of DNA and genotyping of multiple segregating microspores facilitates assessment of the frequency of homoeologous recombination.

**Results:** Based on our results, the Fluidigm’s C1 based single cell sequencing method works well for isolation of DNA from *B. napus* microspores. *B. napus* microspores were sorted successfully in individual Integrated Fluidic Circuit (IFC) wells. The cell capture frequency ranged from 40 to 55%. The capture frequency can be further improved by optimizing the concentration of cell suspension and elimination of clumps formed by dead cells. Bioanalyzer traces showed an enrichment of amplified DNA fragments at ~10 kb from all the IFC wells in which a microspore was captured. Empty wells did not have any DNA fragments which potentially ruled out the possibility of DNA amplification from contaminants. Successful PCR amplification of two well characterized *B. napus* genes further confirmed that the DNA isolated was derived from microspores. In the case of wheat, the bigger size of its microspores (40-60 µm) prohibits their flow and capture in the narrower IFC module/wells (maximum 25 µM). The use of smaller microspores (<25 µM) from very early stages of spike development is being tested to help resolve this issue. Alternatively, FACS based cell sorting system can be used for sorting larger wheat microspores.

**Conclusions:** Single microspores can be successfully captured, lysed and their DNA amplified using the Fluidigm C1 single cell module. Future work will consist of the optimization of microspore capture frequency and confirming uniform coverage of whole genome amplification.
Identification of genomic regions that control the diploidization rate of microspores in intervarietal substitution lines of rapeseed (Brassica napus L.)

**Background:** Isolated microspore culture (IMC) of Brassica napus treated with colchicine is an important technique that efficiently produces homozygous doubled haploid lines in one generation, thus accelerating crop improvement and breeding programs. A large variation in diploidization rates among genotypes can be detected even after identical treatments of microspores. Little is known about the genetic control of these differences. In segregating DH populations, genes that have an effect on the diploidization rate of microspores should lead to skewed segregation at markers linked to loci where the different alleles are segregating.

**Objectives:** Localize genomic regions that control the diploidization rate of microspores by evaluating the diploidization rates of intervarietal substitution lines (ISLs) of rapeseed (Brassica napus L.).

**Methods:** A mapping population of 197 diploid microspore-derived embryos (MDEs) was developed from isolated, colchicine treated microspores of one F1 plant of the cross ‘Express 617’ x ‘RS239’. Similar to the approach of Ecke et al. (2015) regions with skewed segregations that may carry genetic factors controlling diploidization rates were identified by genetic mapping in the MDE population and ISLs from the same cross as the MDE population with donor segments covering a number of these regions were selected. Diploidization rates of microspores from the selected ISLs were evaluated by measuring ploidy levels of MDEs with flow cytometry. The significance of deviations of the diploidization rates of the ISLs from the diploidization rate of ‘Express 617’, which is the recurrent parent of the ISLs, were tested by χ²-tests using the ratio of diploid and non-diploid MDEs of ‘Express 617’ as the expected ratio.

**Results:** A genetic map of 483 AFLP markers was constructed in the MDE population. A total of 75 markers in 13 regions showed significant deviations (P<0.05) from the expected 1:1 segregation ratio. Five regions and 10 ISLs were chosen for further studies. ‘Express 617’ showed a diploidization rate (DR%) of 51.8%. The DR% of the ISLs ranged from 23.9% - 58.7%. Two ISLs showed higher DR% and eight ISLs showed lower DR% compared to ‘Express 617’. The statistical analysis showed three ISLs, ER281, ER984 and ER964, to have significantly lower DR% of 23.9% (χ²=50.77), 25.6% (χ²=50.77) and 27.1% (χ²=44.13), respectively, than ‘Express 617’: No ISLs with significantly higher DR% were detected. The three ISLs were carrying two to four donor segments. After comparing the donor segments between significant and non-significant lines 6 genomic regions were identified distributed in 6 linkage groups that possibly carry genetic factors affecting the diploidization rates of microspores.

**Conclusions:** Three ISLs, ER281, ER984 and ER964, with significantly reduced diploidization rates compared to the recurrent parent ‘Express 617’ were identified. A total of 6 genomic regions on 6 linkage groups may carry genetic factors controlling diploidization rates of microspores.

**References:**
Introgression of resistance to blackleg from *Brassica juncea* into *B. napus* and analysis of blackleg resistance in synthetic hexaploid *Brassica* species

**Background:** Canola (*B. napus*), one of the valuable oilseed crops in the world, has been reported with significant yield losses due to blackleg disease caused by *Leptosphaeria maculans*. The primary method to control blackleg is to develop resistant cultivars. Blackleg resistance can be introduced from the B-genome containing *Brassicas* into canola. The synthetic hexaploid *Brassica* species (AABBCC) created with the genomes of *B. rapa* (AA) and *B. carinata* (BBCC) (Chen et al. 2011) have high levels of blackleg resistance that can be introduced into canola.

**Objectives:** To introduce blackleg resistance from *B. juncea* to *B. napus* and to analyze the high level of blackleg resistance in synthetic hexaploid *Brassica* species (AABBCC).

**Methods:** Fifty *B. juncea* accessions from the collection of University of Manitoba and eight lines of synthetic hexaploid *Brassica* developed by Dr. Meng were tested with cotyledon inoculation. All 'Meng' accessions were completely resistant to blackleg while most resistant and susceptible *B. juncea* accessions to blackleg were identified. Most resistant *B. juncea* were crossed with susceptible canola ‘Westar’ and all F1 plants were backcrossed to ‘Westar’ to produce BC1 generation. The BC2 generations are produced and being tested to find resistant progeny and eventually the blackleg resistance will be introduced into canola. To test blackleg resistance in synthetic hexaploid *Brassica* ‘Meng’, the interspecific hybridization between susceptible *B. juncea* and ‘Meng’ were made and embryo culture was used to obtain F1 plants. These F1 were backcrossed to the susceptible *B. juncea* to produce BC1 progenies.

**Results:** Hybrid seeds between *B. juncea* and canola were produced and backcrossed to canola. The seed setting rates of the F1 hybrids were low and a few to a dozen of seeds were obtained from different crosses. The lower survival rate was observed in BC1 than F1 where there was about 10 times of difference. In the crosses of *B. juncea* and ‘Meng’, the seed setting rates of the backcrosses to the susceptible *B. juncea* were very high and hundreds of BC1 seeds were harvested. These BC1 seeds are being tested and the resistance will be analyzed through genetic analysis of the BC1 and following generations.

**Conclusions:** Although the high level of blackleg resistance exists in the B-genome containing brassica species, it is difficult to introduce the blackleg resistance to canola. Understanding the mechanism of blackleg resistance in hexaploid ‘Meng’ will lay a foundation to introduce the blackleg resistance in the hexaploids to canola. The good seed setting indicated that genetic analysis of blackleg resistance by crossing synthetic hexaploid *Brassica* species to susceptible *B. juncea* is feasible since all hexaploid ‘Meng’ accessions are completely resistant to blackleg.

**References:**
Investigation of genetic resistance operating in oilseed rape against the light leaf spot pathogen, *Pyrenopeziza brassicae*

**Background:** Light leaf spot disease, caused by *Pyrenopeziza brassicae*, is currently the most damaging foliar disease on winter oilseed rape in the UK. *P. brassicae* is also able to infect and cause the disease on vegetable brassicas, including Brussels sprouts. Previously, severe disease epidemics have been recorded in Scotland and northern England, where the climatic conditions are favourable for disease development. However, according to recent disease survey data, the severity of epidemics has increased progressively across the UK since 2006 (http://www.cropmonitor.co.uk/). This frequent, widespread occurrence of light leaf spot has made it a high priority for many oilseed rape growing areas in the UK. Severe epidemics have also been reported in some parts of the continental Europe, including France and Germany. Breeding for host resistance can be used as an effective control strategy against this economically damaging pathogen. According to HGCA recommended list trials, there are commercial oilseed rape cultivars with good to moderate resistance against the pathogen. There is little information available about the genetic resistance operating in these cultivars.

**Objective:** To investigate genetic resistance in oilseed rape against *P. brassicae* to identify new sources of resistance, which can be exploited in oilseed rape breeding programmes.

**Methods:** Recent identification of a major gene locus for resistance against *P. brassicae* that has been mapped to the bottom end of chromosome A1 (Boys et al. 2012) and the genome sequencing data for *B. rapa*, *B. oleracea* and *B. napus* will be used for fine mapping and sequencing of this major gene. A DNA fragment of c. 260 bp was amplified from parental lines of a doubled haploid (DH) population segregating for resistance, using primers of a flanking marker closest to the resistance gene on chromosome A1. The PCR products will be sequenced and aligned to the *B. napus* genome sequence to locate the closest marker on the chromosome A1. SNP markers between this region and the telomere will be tested using DNA samples of the DH population to fine map disease resistance. In addition, QTL analysis will also be done on a population segregating for quantitative resistance against *P. brassicae* to map QTL and to identify candidate resistance genes.

**Results:** Physical locations of markers flanking the corresponding R gene on the chromosome A1 have been identified.

**Conclusion:** Fine mapping of the R gene at the bottom of chromosome A1 will become available in the future and will be used to sequence a resistance gene operating against *P. brassicae*.

**References:**
Synthesis and characterization of a wide hybrid between *Brassica napus* and *Raphanus raphanistrum*

**Background:** The *Brassicaceae* family includes an array of wild and weedy species which represent a massive reservoir of genetic and agronomic variation. The bringing of this variation into cultivated germplasm through interspecific/intergeneric hybridization is expected to further enhance the scope and pace of *Brassica* breeding efforts by broadening the genetic base of crop *Brassicas* (Garg et al. 2007, Nicolas et al. 2008). Wild radish (*Raphanus raphanistrum*) is an important source of resistance to blackleg, diamond back moth, pod shattering, acetolactate synthase herbicide, tolerance to salinity and downy mildew. Genetic relatedness between *Raphanus raphanistrum* and cultivated *Brassicas* will provide possibility to exploit desirable genes.

**Objectives:** To develop the Intergeneric hybrid between *B. napus* and *R. raphanistrum* and to study the genetic relatedness between genomes of *B. napus* and *R. raphanistrum*.

**Methods:** Sequential ovary-ovule culture was used to develop the intergeneric hybrid between *B. napus* and *R. raphanistrum*. Morphological, molecular, cytological studies and fluorescent genomic in situ hybridization were conducted to establish the hybridity and genomic relatedness. For cytological analysis young flower buds were fixed in Carnoy’s solution II (Ethanol: Chloroform: Acetic acid in a ratio of 6:3:1) and squash preparations were made in 2% acetocarmine. GISH analysis was carried on air dried metaphase spreads as per Heselop-Harrison protocol.

**Results:** F1 hybrid plants were morphologically intermediate between the two parents. Cytological investigations of the intergeneric hybrid (F1) was carried out to establish the extent of genomic relatedness between AC and RR genomes. The F1 hybrid plant had 2n=28 chromosome number. The cytogenetic analysis of the pollen mother cells of the F1 hybrids (2n=28) revealed the occurrence of varied chromosome configuration during diakinesis/metaphase-I with 10II+8I as the predominant configuration. The number of bivalents ranged between 7-13II. Maximum of 13II were observed in 8.24 per cent of the PMC’s. The mean bivalent frequency was 8.83. The quadrivalent and trivalent were also observed in 3.09 and 23.7 per cent of PMC’s respectively. Chromosome separation during anaphase was irregular with frequent chromosome laggards. Occurrence of more number of bivalents (13II) than expected along with a quadrivalent and a trivalent in few cells was indicative of allosyndetic pairing between the genomes under consideration. GISH studies also confirmed the hybridity and allosyndetic pairing between AC and RR genomes.

**Conclusions:** Presence of trivalent/quadrivalent indicated the allosyndetic pairing between the AC and RR genomes. This suggested the possible recombination between A, C and RR genomes. Therefore intergeneric hybrid, *B. napus × R. raphanistrum* has the potential to be used as a bridging species for transfer of desirable genes from the wild to the cultivated *Brassica* species.

**References:**
Development of nutritionally improved Indian Mustard (*Brassica juncea*) varieties having low erucic acid and low glucosinolate content using marker assisted selection

**Background:** India is one of the largest rapeseed-mustard growing countries in the world, occupying the third position in production after China and Canada with 12% world total production. Due to high content of glucosinolate, the Indian cultivars have limited preference in Southern and Central Zone of India and in International market as glucosinolate is undesirable to animal feed. On one hand erucic acid is an anti-nutrient while on the other presence of glucosinolate in the oil cake makes it unfit for cattle feed. TERI Developed double low strains of *Brassica juncea* however these lines did not had good seed yield. In order to develop high yielding double low variety of Indian mustard, TERI and Nirmal Seeds joined hands under the aegis of BIPP scheme of Department of Biotechnology, Government of India.

**Objectives:** The main objective of this project was to transfer low erucic (<2%) and low glucosinolate (<30 micro mole/g defatted meal) content in high yielding line Nirmal 100 using marker assisted selection.

**Methods:** The present investigation work underlying this paper, conducted during Rabi & Kharif season (field and polyhouse conditions) of 2011-2015 at Research farm of Nirmal Seeds Pvt. Ltd. Pachora, Dist. Jalgaon. Based on phynotyping (i.e. HPLC and GC analysis) and genotyping (molecular marker analysis) results, TERI N-4 was selected as a donor parental line and agronomically superior high yielding NML-100 *Brassica juncea* line was selected as a recipient parent. Sowing of the recipient line NML-100, donor line Teri-4, backcross seeds (BC1F1, BC2F1, BC3F1 and BC4F1) and self seeds (BC4F2) were conducted in their respective period in polyhouse and field condition. Backcrossed progenies were was validated for presence of glucosinolate and Eucric acid loci and AFLP markers was used for selection of recurrent parent genome (NML-100) recovery and reconfirmed with GC and HPLC analysis.

**Results:** Individual plants of donor line T-4 is used for crossing with recipient line NML-100. These plants have all low alleles in homozygous conditions for glucosinolate and erucic trait. Once the donor and recipient lines are selected, the erucic acid controlling genes were cloned and sequenced from these lines to use them for marker-assisted breeding in later generations. Plants which were close to recurrent parent genome were used for backcrossing programme. A total of 1153 BC4F2 plants were screened for GSL and Emeric acid loci. Finally 42 DL plants were recognized at BC4F1 containing 96% of recurrent parent genome. These BC4F1 population was selfed to fix the homozygosity and harvest BC4F2.

**Conclusions:** The 42 homozygous BC4F2 lines will be developed as double low variety ready for cultivation in India.
Quantitative trait loci (QTL) mapping in recombinant inbred lines of *B. rapa* for agronomic and seed quality traits

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**Background:** *Brassica rapa* L. (AA, 2n = 20), an important vegetable and oilseed crop, is one of the parental species of *Brassica napus* L. (AACC, 2n = 38). Total land area coverage of *B. rapa* species reduced to <2% in Western Canada in the last decades due to the release of early-maturing *B. napus* cultivars with high seed yield. However, earliness in current early-maturing *B. napus* cultivars is still not adequate especially in the short-growing season areas. *B. rapa* L is attractive for traits, such as, early-maturity, yellow seed associated with lower fibre content in meal, and reduced silique shattering. QTL mapping of these traits in *B. rapa* can facilitate understanding of the genetic control of the traits in the amphidiploid species, such as *B. napus* (AACC, 2n = 38) and *B. juncea* (AABB, 2n = 36), which shares the A genome of *B. rapa*.

**Objectives:** To understand genetic controls in recombinant inbred lines (RILs) from *B. rapa* cross for agronomic and seed quality traits and to map QTL for seed color, glucosinolate (GLS) content and silique length.

**Methods:** The F1 plants (Sampad × 3-0026.027) were self-pollinated for F2 seed. A total of 96 F2 plants segregating for different agronomic traits were subjected to a single seed descent for the development of RILs. The RILs were grown in a greenhouse, growth chambers and field trial. Seed color was visually assessed in individual plants, GLS content was estimated in μmol g⁻¹ seed by near-infrared reflectance spectroscopy and silique length was measured for five pods per plant in mm. QTL mapping was conducted using composite interval mapping (CIM).

**Results:** One major and a minor QTL on linkage group (LG) A9 and two minor QTL on LG A3 and LG A5 were detected for seed color, explaining ~2% to 64% of the phenotypic variance. QTL mapping detected three loci on linkage groups A2, A7 and A9 for GLS content. These QTL individually explained 5 to 22% of total phenotypic variation. For silique length, three QTL were detected on the linkage groups A3, A5 and A7, explaining 36.0–42.3% of the total phenotypic variance.

**Conclusions:** The two QTL for seed color on LG A9 are apart by ~70 cm in *B. rapa* map (Kebede et al. 2012). A single major QTL was detected on LG N9 _A9_ in *B. napus* (Fu et al. 2007). The alignment of common SSRs indicated that the SSR markers were spread more widely across LG A9 as compared to LG N9 of *B. napus* developed by Piquemal et al. (2005), which can explain for the presence of another QTL region on LG A9 in *B. rapa*. LG A9 carries a major QTL while A7 carries a minor QTL for total seed GLS content (Rahman et al. 2014). For silique length, a QTL on LG A5 was detected in all environments and explained about 9% of the total phenotypic variance (Kebede et al. 2014). However, no QTL was detected for silique length on LG A5 in *B. napus* (Chen et al. 2007; Qi et al. 2014). QTL mapping in *B. napus* indicated that the chromosome C5, which is homologous to LG A5, carries a locus controlling silique length (Chen et al. 2007; Qi et al. 2014). Thus, understanding the genetic composition of diploid parental species can give a better insight of the genetic architecture of the amphidiploid species.

**References:**


Transcriptome atlas of the *Arabidopsis* funiculus – a study of maternal seed regions

**Background:** The funiculus is the structure that connects the developing seed to the maternal plant, and is the only direct conduit for the transport of nutrients from the plant to the seed. While the use of genetic and molecular screens has contributed greatly to our understanding of seed development in recent years, our understanding of the funiculus development remains limited.

**Objectives:** Understanding the molecular genetics of funiculus development could contribute greatly to oilseed improvement research, but the accessibility of this structure and the analysis of large-scale datasets remains a challenge in the growing field of transcriptomics. We therefore studied the funiculus of the model plant, *Arabidopsis thaliana*, in order to provide insight into the biological processes and regulatory molecules that control these processes in space and time.

**Methods:** Using laser micro-dissection coupled with global mRNA profiling experiments of the seed transcriptome throughout development, we compared the funiculus with all subregions of the maternal (funiculus, seed coat) and zygotic (embryo, suspensor, endosperm) regions of the *Arabidopsis* seed. Using fuzzy-K means clustering, we generated dominant patterns of gene activity and performed Gene Ontology term enrichment analysis to uncover biological processes in the funiculus and other seed regions. These data are supported by our histological and anatomical analyses of the funiculus. Enrichment of sequence motifs, transcriptional regulators, and GO terms in funiculus mRNA populations was used to predict putative regulatory networks underlying funiculus development and function throughout seed development.

**Results:** The funiculus is a transcriptomically distinct region of the seed. Our data indicate that the funiculus is an energetically active region that is enriched for fatty acid metabolism, auxin response, and vascular development. The funiculus was also found to be enriched for glucosinolate biosynthesis, and many transcripts involved in glucosinolate biosynthesis and regulation were found to accumulate almost exclusively in the funiculus. Our analysis predicts MYC4 and AT4G00870 as regulators of glucosinolate biosynthesis and auxin response in the funiculus via interaction with the MYC2 binding motif. We also identify BEE2 and AT1G22490 as potential regulators of auxin response in the funiculus. We also identified several other transcripts that are specific to the funiculus in seed development, and unique to the funiculus in plant development overall.

**Conclusions:** Our study provides the first comprehensive analysis of funiculus development, setting a new foundation in the field of seed improvement research in *Arabidopsis* that can be extended to other members of the *Brassicaceae*. Understanding regulators of funiculus development and function in *Arabidopsis* may have great implications for our ability to enhance seed development via the manipulation of maternal tissues.
Gene-expression networks controlling seedling development and vigour in
Brassica napus

**Background:** Different Brassica napus genotypes show considerable variation for germination, seedling vigour and emergence when seeds are produced or sown in different environments. The mechanisms underlying this variation are unclear. Systems biological approaches, involving different levels of information from the genome, transcriptome and metabolome, can give deep insight into the regulation of poorly understood, environmentally sensitive traits like emergence and vigour in crops with complex genomes such as B. napus.

**Objectives:** The main goals of this study were to correlate seedling gene expression networks to emergence-related traits in diverse winter-type B. napus accessions, identify genes with high connectivity within trait-associated expression clusters and investigate interconnection of potential regulatory genes to quantitative trait loci (QTL) for germination and vigour traits.

**Methods:** Shoot and root transcriptomes from 4 week old seedlings of 42 B. napus genotypes with high, low and intermediate germination rate were assayed by Illumina sequencing of 100bp 3'-EST sequences. Sequence reads were mapped to Brassica unigenes and quantified. Weighted gene co-expression network analysis (WGCNA) was performed to cluster co-expressed genes into shoot and root gene co-expression networks and correlate gene expression modules (summarizing seedling expression clusters) to in vitro germination (early seedling development) and vigor traits (2 and 4 week old seedlings from multi-environment field trials). Trait-associated gene expression networks were investigated by functional annotation and co-localization of hub genes to QTL for germination and seedling development traits.

**Results:** Gene expression modules showing high eigengene correlations with field emergence and vigour traits were identified for both shoot and root networks, and some modules showed correlations to both germination and field emergence traits. Modules of interest exhibited hub genes related to growth and development. Of particular interest were hub genes, or genes with high interconnectivity to hub genes, that were located within QTL for early seedling development traits.

**Conclusions:** Co-localization of potential regulatory genes for vigour-associated expression networks to QTL regions identified promising candidates in seedling growth and vigor.
Investigating the influence of genome structure on QTL for disease resistance in *Brassica napus*

**Background:** Genome rearrangements in the allopolyploid *Brassica napus* genome have been shown to generate selectable genetic variation that can contribute to advantageous phenotypes, potentially including disease resistance.

**Objectives:** Within the French-German consortium GeWiDis ("Exploiting genome wide diversity for disease resistance improvement in oilseed rape") we are performing comparative analysis of structural organization and allelic diversity associated with resistance factors to important oilseed rape diseases. In particular we aim to determine how structural chromosome rearrangements affect quantitative trait loci (QTL) contributing to pathogen resistances and how these can be used to improve resistance in breeding.

**Methods:** Parental lines from doubled-haploid (DH) mapping populations, including donors of quantitative disease resistance factors, were resequenced to determine subgenomic structural variants including homoeologous and non-homoeologous chromosome exchanges. Sequencing and genome-wide SNP-based genotype data from DH lines were then utilised to trace structural variants in the segregating population and compare their positions to those of QTL for disease resistance.

**Results:** Preliminary results suggest that resistance loci can be influenced by homoeologous exchanges. These can cause gene dosage changes that may confer a selective advantage.

**Conclusions:** Analyses of genes in exchanged segments associated with resistance QTL is a promising new approach to deciphering the genetic basis of quantitative resistances in oilseed rape.
A review of the rise in volume and complexity of winter oilseed rape variety testing and the challenges ahead

Background: The size and complexity of variety trials has increased steadily over the last 30 years, requiring constant review of methodology to accurately demonstrate a yield improvement. However, the crop remains relatively poorly domesticated, with establishment problems associated with the small seed, as well as indeterminate maturity, pod shatter, lodging and inter-plot competition continuing to present difficulties.

Objectives: This paper reviews evolving trials methodology and identifies emerging challenges to provide a forum for exchange of new ideas.

Methods: NIAB has been conducting oilseed rape (OSR) trials since 1966 and set up the UK National and Recommended List (NL and RL) systems and has had involvement with all subsequent phases of trial development. The author draws on 25 years of direct experience of OSR variety trials and has accessed official published protocols and variety reports to produce this review.

Review findings: UK variety testing is similar in structure to that described by Kightley, (1993) with a two-year statutory NL programme which feeds the most promising varieties into a levy-funded (RL) system. Numbers peaked in 2014 with 107 NL1 entries and 71 varieties continuing into NL2 from the previous year. From 1992 onwards trials have become increasingly complex because of the entry of hybrids into trials, further increasing the already considerable inter-plot competition effects associated with different varieties (Talbot, 1993; NIAB, 1999).

Incomplete Block Design (Patterson, 1978) randomisations are used routinely to address field effects in large trials. These layouts are further adapted by blocking variety types to minimise inter-plot competition and, where necessary, using restricted neighbour design for height. At the plot level, drills modified to sow border plots between harvested areas have also contributed to reducing plot interference. Fitted Constant and REML Analyses (Kempton, 1997) have been used for linkage of data from different variety sets, sites and years. The large size of individual trials makes sowing and harvest operations increasingly lengthy and prone to interruption by rain but increasingly sophisticated machinery and access to Global Positioning Satellite (GPS) technology have introduced great efficiencies and improved operational accuracy. Modern plot combines have greatly reduced seed carry-over, allowing a move to shorter plots. On-board weighing, sampling and analysis systems have increased overall efficiency of harvesting and quality analysis but the precision of analytical methodology (NIrs) is not yet universally accepted.

On-going challenges include management of seed shedding risks in trials with diverse maturity types, and consideration of appropriate fungicide and nitrogen regimes. There are difficulties of finding long-rotation trial sites without volunteer problems and, increasingly, a need to adapt trial drills to min- or zero-till field conditions. New challenges include emergence of Verticillium brassicae as a widespread disease threat and the loss of control of cabbage stem flea beetles (Psylliodes chryscephela) as a result of the EU ban on the use of neonicotinoid seed dressings.

Conclusions: Constantly evolving, robust trials programs are required to that ensure that the yield potential and other characteristics of new varieties are correctly assessed.

References
Association mapping pilot study for the investigation of complex traits in canola

Association mapping has the potential to map complex traits at high resolution, allows for the identification of the best allele from a diverse panel of lines, and can make recommendations regarding the use of new germplasm containing these alleles in the breeding program. For this purpose a diverse panel of spring canola lines was selected consisting of lines from the Dow AgroSciences breeding program and exotic lines from around the world. All lines were genotyped through GBS generating over 70,000 markers. Field trials were conducted at two locations for two years and phenotypic data for a range of traits have been collected. Substantial phenotypic variation was observed for all traits, together with the observed medium to high heritability, should allow for genome wide association of these traits.

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Performance of MAXIMUS® semi-dwarf hybrids

Background: On-farm, high grain yield in winter canola is only realized in a crop not damaged by winter and plants not lodging until harvest. Pioneer Hi-Bred is using a dwarf gene (bzh) identified by INRA exhibiting additive inheritance (Barret et al. 1998). Using the OGU INRA hybridization system, female inbreds homozygous for bzh are crossed to tall restorers resulting in semi-dwarf MAXIMUS® hybrids.

Objectives: To demonstrate high performance of MAXIMUS® hybrids based on evaluations in large and small plot trials. In regions where rapeseed develops fast before winter and tall plant height is expected growers apply growth regulators in the fall and during elongation in the spring to reduce winter losses and to avoid lodging. Pioneer’s MAXIMUS® hybrids offer genetic improvements in both traits, namely winterhardiness and standability as well as high oil yields and additional agronomic benefits.

Results: In the period from 2006/7 to 2013/14 Pioneer Hi-Bred conducted more than 800 large plot trials in the PAct (Pioneer Accurate Crop Testing) system across Germany and Eastern Europe. The on-farm evaluation demonstrates the high yield performance of MAXIMUS® hybrids. In small plot trials the performance of the semi-dwarf hybrids is often underestimated. Moreover other important agronomic advantages of MAXIMUS® hybrids are only appreciated in large plots on farm (Feiffer and Koch. 2007).

Conclusion: Over the past 7 years hybrids like PR44D06, PX104, PX109 and PX113 have delivered step-wise improvements in yield and oil content. Additionally, MAXIMUS® hybrids provide significant agronomic benefits to European rapeseed growers. Despite a longer history and more intensive breeding efforts in tall hybrids, PACT results show that both growth types are at the same level in grain yield. Growers confirm that besides strong winter hardiness and standability, MAXIMUS® hybrids show less damage during spraying (Sauermann 2010), more synchronous maturation, very easy and economically beneficial harvest in comparison to Tall hybrids. Most farmers who have grown MAXIMUS® hybrids repurchase them underlining the high value of this innovative variety concept.

Reference:
Global gene expression responses to waterlogging in roots and leaves of rapeseed seedlings (*Brassica napus* L.)

**Background:** Rapeseed (*Brassica napus* L.) has the potential to follow rice as a rotation crop in paddy fields in Korea. Waterlogging is often a problem in paddy fields during the winter season (Ku et al. 2009). The effects of waterlogging stress include reduction in stomatal conductance, photosynthetic rate, and plant height, as well as premature senescence and disturbances to yield components (Ku et al. 2009; Zhou et al. 1997).

**Objectives:** To investigate the molecular responses of rape seedlings to waterlogging, we assayed global gene transcription in the aerial leaves and roots of waterlogged rape seedlings, as well as the physiological responses of seedlings to waterlogging.

**Methods:** Seedlings of ‘Tammi’ and ‘Youngsan’ cultivars were subjected to waterlogging for 3 and 6 days and recovery for 5 days. Physiological responses of seedling leaves to waterlogging were analyzed. We also examined NO production in roots of ‘Tammi’ cultivar during a 72-h waterlogging period. To examine changes in gene transcription in aerial leaves and roots of waterlogged seedlings and to investigate whether the observed physiological responses were correlated with the regulation of waterlogging-responsive genes, we analyzed the global transcriptional profile of leaves and roots of ‘Tammi’ cultivar seedlings exposed to waterlogging stress for a short period (36 and 72 h).

**Results:** Waterlogging stress caused a significant decrease in leaf chlorophyll content and premature senescence of the leaves. In addition, waterlogging stress repressed many genes that encode photosynthetic reactions, including the light reactions and carbon-fixing reactions. On the other hand, a majority of the genes that function in ROS scavenging, degradation (proteins, starch, and lipids), premature senescence, and abiotic stress tolerance were upregulated. In roots waterlogging for up to 72 h enhanced NO production rapidly in the roots. Maximum NO generation (sixfold higher than the control) occurred at hour 18, after which it decreased gradually until hour 72. Of 53,107 root genes assayed, 9,692 showed a twofold change in expression within 36 h of waterlogging. Two nitrate reductase (NaR) genes (TC201891, TC161540) and four nitrite reductase (NiR) genes (TC168889, TC164215, TC163914, TC185634) were potentially involved in NO production in response to waterlogging stress. Strong hypoxic induction of nonsymbiotic hemoglobin (Hb) gene (TC165566), which increased 656- and 645-fold at 36 and 72 h of waterlogging, respectively, could oxidize the NO overproduced in the roots. The up-regulation of many additional waterlogging-responsive genes with potential roles in the anaerobic respiration, sucrose and starch degradation, glycolysis, and pyruvate metabolism, may acclimate the plant to waterlogging-induced hypoxic condition.

**Conclusions:** Waterlogging stress caused a decrease of leaf chlorophyll content and premature leaf senescence. Waterlogging for up to 72 h enhanced NO production rapidly in the roots. Thesis physiological responses were associated with dramatic changes in gene expression profiles in the aerial leaves and roots of waterlogged rape seedlings.

**References:**

Genetic diversity of germplasm resources on winter rape (Brassica compestris L.)

**Background:** Winter turnip rape (Brassica campestris L.) began to be planted in spring rape production area of northwest China, with global climate warming and ultra cold-tolerance winter rape cultivar breeding. High resistance winter rape cultivar breeding provided with cultivar guarantee for development of new winter rape area. Winter rape production played important roles in Agricultural Production in China. But there was little research on genetic diversity of winter rape.

**Objective:** Genetic diversity of 51 winter rape cultivars in Gansu province were studied to provide the theory basis for collection, conservation exploitation, and utilization of germplasm resources and cold-tolerance breeding of winter rape.

**Method:** Genetic diversity of analysis was studied by SSR molecular marker and UPGMA clustering analysis.

**Results:** 15 SSR markers were screened for polymorphism among 51 winter rape cultivars. 96 polymorphic bands were detected. 11.45 the polymorphic rate of SSR markers was 100%, and the average allelic variants per SSR primer pair being 6.4. The range of PIC was 0.5834 - 0.8740. The mean value of PIC was 0.7794. The genetic similarity coefficient of 51 winter rape cultivars varied from 0.5000 to 0.969. The results of clustering analysis showed that 51 winter rape cultivars were divided two groups. The first group and the second group had 18 cultivars, which could be divided many sub-clusters.

**Conclusion:** Winter rape cultivars in Gansu province showed abundant genetic diversity, which also showed the genetic diversity of winter rape mainly caused by geographical and ecological environment.

**References:**

Genetic variation of new-type rapeseed derived from interspecific hybridization between *B. napus* and its parental species

**Background:** *Brassica napus* is originated from natural hybridization between *B. rapa* and *B. oleracea*. The narrow genetic basis of *B. napus* limited its improvement due to its intensive modern breeding and short history of origination and domestication. However, its parental species possess more diverse genetic basis than it. It suggests that utilization of *B. rapa* and *B. oleracea* offers an approach to improving *B. napus*.

**Objectives:** Two strategies introgressing genomic components of parental species into *B. napus* were reported, one was using hexaploid (AACCCC) crossing with *B. rapa*, the other was crossing *B. napus* with *B. oleracea* (Li et al. 2013, 2014). New-type *B. napus* from each of the crosses could have the potential to widen genetic basis of current *B. napus*.

**Methods:** 76 new-type *B. napus* (Na) derived from hexaploid (AACCCC) crossing with *B. rapa*, 51 new-type *B. napus* (Nc) derived from interspecific hybrid between *B. napus* and *B. oleracea*, are compared with 3 *B. oleracea*, 6 *B. rapa*, 52 current *B. napus* (16 spring, 16 winter an 20 semi-winter) via 155 SSR markers to clarify genetic variation.

**Results:** Diverse of *B. rapa* and *B. oleracea* are used to broaden genetic basis of *B. napus* via interspecific hybridization. Here, 486 polymorphic loci are amplified by 155 SSR markers in two kinds of new-type *B. napus*, comparing with *B. oleracea*, *B. rapa* and current *B. napus*. All the genotypes are separated into 3 groups: *B. rapa*, *B. oleracea* and *B. napus*, by principal components analysis. In which, the total variation explained by the first, second and third principal components are 20.33, 14.02 and 8.93%, respectively. Whereas all the *B. napus* are clustered into three groups: new-type *B. napus* (Na) introgressing A genome of *B. rapa*, new-type *B. napus* (Nc) having genomic components of *B. oleracea* and current *B. napus*. Genetic distance among populations of *B. napus* is further than within populations, except that between Nc and current *B. napus*. The genetic distance between Na and current *B. napus* (0.53 ± 0.11) is biggest than others, followed by the genetic distance between Na and Nc (0.34 ± 0.09). It indicates that the genetic variation of new-type *B. napus* introgressing A genomic components of *B. rapa* is different from the new-type *B. napus* having C genomic components of *B. oleracea*.

**Conclusions:** The genetic variation between two kinds of new-type *B. napus* are different. Both of them are different from parental species, and have the potential to broaden genetic basis of current *B. napus*.

**References:**
Qinfei Li, et al. (2014) Improvement of *Brassica napus* via interspecific hybridization between *B. napus* and *B. oleracea*. Molecular Breeding. 34:1955-1963
Qinfei Li, et al. (2013) A large-scale introgression of genomic components of *Brassica napus* into *B. rapa* by the bridge of hexaploid derived from hybridization between *B. napus* and *B. oleracea*. Thero Appl Genet. 126(8): 2073-2080
The efficient breeding of rapeseed male sterility hybrid induced by chemical hybridization agent and its production technology

**Background:** Hybrid rapeseed was focused because of its heterosis in yield and quality by breeders and producers generally, but how to raise the breeding efficiency continually, and to get high quality and enough quantity parent seeds for hybrids, it will be an eternal task of breeders. Though breeders have made new progresses in efficient breeding and parents reproduction, but these can not still meet the progress of rapeseed hybrid breeding.

**Objective:** Adopting simple and practical breeding method and mechanized reproduction technique make rapeseed breeding and parent seeds reproduction efficient, saving labours, high quality and yield.

**Method:** 1. The chemical hybridization agent (CHA) SX-1 was used to induce male sterility of hybrids for improving rapeseed varieties; and for artificial hybridization through smearing stem with CHA to induce sterility of rapeseed in the bolting stage; for creating more combinations for test through one male parent and more female parents (induce sterility of female parents) in net house; At the same time, DH was cultured to good F1 hybrids for creating new germplasms. 2. Reproduction more parents seeds of hybrids through mechanized pollination in big isolation net house; spraying CHA SX-1 and pesticides and fertilizers as well as harvesting through machineries for realizing entire mechanization of hybrid seeds production using CHA SX-1 to induce sterility in fields.

**Result:** CHA SX-1 inducing male sterility and microspore culture DH were used successfully in hybrid breeding, these improved efficiency of breeding greatly. In addition, pollination using machinery may get parent seeds with high yield and good quality in net house. In hybrids production field of rapeseed, mechanized spraying CHA SX-1 and pesticides and fertilizers can save a lot of labors and expenses, also assure quality of hybrids.

**Conclusions:** the results showed that the above-mentioned four methods were utilized in cross breeding of rapeseed successfully, breeding efficiency has been improved greatly. Meanwhile, entire mechanization of parents reproduction of hybrids and hybrids production will promote cross breeding of rapeseed to step into a new stage with high production, good quality and efficient.

**Reference:**
Male sterility induction mechanism study of the SX-1 in cytology and comparative proteomics

**Background:** Rapeseed (*Brassica napus L.*) is one of the main oilseed crops in the world. The major task for breeders is to increase the seed production at present. Hybrid cultivars have been used to increase the production of rapeseed worldwide successfully. Male sterility of rapeseed induced by chemical hybridization agents (CHA) is one of the main ways to produce hybrid rapeseed. SX-1 as a new-typed CHA had been widely applied on hybrid seed production with high efficiency in China. A series of sterile lines induced by SX-1, such as Y133, YD61A, had been selected out, and Qinyou33, Qinzayou4 and Qinzayou19 new hybrids cultivars were also cultivated successfully.

**Objectives:** Observing the anthers changes after SX-1 treatment and ultrapure water treatment in *B. napus.*

**Methods:** Firstly, the development of microspore and tapetum were observed using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Proteomic analysis was conducted in different development stages of anthers after *B. napus* treated with 6mg/L SX-1. These changes were analyzed using two-dimensional electrophoresis (2-DE).

**Results:** The results showed that, few obvious morphological changes were observed between microspore of control group and SX-1 treatment group in the beginning, all of them seemed to be full and round. However, nearly 100% of treated pollen grains were crimpled and these pollen coats lacked in the late stage of pollen grains in maturation process. Plasmolysis occurred in the crumpy pollen grains, the organelles were not evident and the microspores were almost empty of contents at last. Meanwhile, in the early stage of anthers development, developing tapetosomes and elaioplasts could be observed both in SX-1 treatment group and control group. However, tapetosomes and elaioplasts in tapetum treated with SX-1 became disordered and broken in advance in the late stage of pollen grains maturation process, resulting in tapetal extrusions and no oil body formation in treated pollen grains. These extruded regions appeared to have an extremely high density of organelles and electron-dense materials. These results suggested that SX-1 ruined the proper development of oil body in anthers. We also found that the filaments became shorter and thinner, the petal became smaller in the treated group than in the control group.

About 1000 protein spots were detected on each gel, a total of 130,220, 329, 366 protein spots were down-regulated and 87, 25,74, 60 protein spots were up-regulated, at four different developmental stages, in response to SX-1 treatment. Protein identity is in progress through liquid-chromatography–tandem mass spectrometry. By the available databases for rapeseed and other species, a comprehensive analysis of the *B. napus* anthers proteome could be performed.

**References:**


**ocsESTdb: a database of oil crop seed EST sequences for comparative analysis and investigation of a global metabolic network and oil accumulation metabolism**

**Background:** Oil crop seeds are important sources of fatty acids (FAs) for human and animal nutrition. Despite their importance, there is a lack of an essential bioinformatics resource on gene transcription in oil crops for a comparative perspective.

**Objectives:** In this study, we developed ocsESTdb, the first database of expressed sequence tag (EST) information from seeds of four oil crops with an emphasis on global metabolic networks and oil accumulation metabolism that target the involved unigenes.

**Methods:** Developed seeds were sequenced from cDNA libraries or directly from RNA and sequences were analyzed using multiple tools.

**Results:** A total of 248,522 ESTs and 106,835 unigenes were collected from the cDNA libraries of rapeseed (*Brassica napus*), soybean (*Glycine max*), sesame (*Sesamum indicum*) and peanut (*Arachis hypogaea*). These unigenes were annotated by a sequence similarity search against databases including TAIR, NR protein database, Gene Ontology, COG, Swiss-Prot, TrEMBL and Kyoto Encyclopedia of Genes and Genomes (KEGG). Five genome-scale metabolic networks that contain different numbers of metabolites and gene–enzyme reaction–association entries were analyzed and constructed using Cytoscape and yEd programs. Details of unigene entries, deduced amino acid sequences and putative annotation are available from our database to browse, search and download. Intuitive and graphical representations of EST/unigene sequences, functional annotations, metabolic pathways and metabolic networks are also available. ocsESTdb will be updated regularly and can be freely accessed at [http://ocri-genomics.org/ocsESTdb/](http://ocri-genomics.org/ocsESTdb/).

**Conclusions:** ocsESTdb may serve as a valuable and unique resource for comparative analysis of acyl lipid synthesis and metabolism in oilseed plants. It also may provide vital insights into improving oil content in seeds of oil crop species by transcriptional reconstruction of the metabolic network.
Phenotypic and molecular characterization of winter oilseed rape germplasms collected at the IHAR-NRI, Poznan, Poland

Background: Different methods are available to investigate the genetic diversity in winter oilseed rape breeding materials. Biochemical, phenological, agronomical traits and in recent years also DNA polymorphism analyses have been used to characterize and identify germplasms for use in winter oilseed rape cultivars breeding.

Objectives: The aim of this work was molecular characteristics and assessment of genetic diversity among oilseed rape cultivars and breeding lines of agronomic value, collected at the Plant Breeding and Acclimatization Institute-NRI, Research Division in Poznan, Poland.

Methods: The plant material comprised winter oilseed rape genotypes including Polish and foreign double-low and traditional (++ and +) cultivars, F1 ogura CMS hybrid and its parental components, in addition to domestic doubled haploid (DH) and recombinant lines developed from mutants with changed composition of fatty acids in seed oil, as well as selected high oleic genotypes, yellow-seeded and resynthesized lines.

Genomic DNA was isolated and degree of similarity among the studied genotypes was assessed using fluorescently labeled AFLP and STR markers separated by capillary electrophoresis. The UPGMA dendrogram was constructed based on the estimated Nei and Li genetic similarity (GS) coefficients. The presence of the ogura male-sterile cytoplasm (CMS) and the Rfo restorer gene was monitored with the multiplex PCR assay (Mikolajczyk et al., 2011), allelic forms of the FAD2 and FAD3 desaturase genes were identified by specific CAPS markers (Falentin et al., 2007) and SNaPshot analysis (Mikolajczyk et al., 2010), respectively.

First round of field trials has been conducted in the 2014-2015 growing season in two environments, in completely randomized block design and in four repetitions.

Results: The degree of similarity among studied genotypes was assessed with molecular markers using 10 AFLP primer combinations, and by PCR amplification with primer pairs specific for 48 STR loci. The constructed UPGMA dendrogram revealed pedigree chart of the genotypes. The ogura CMS was detected in 11 genotypes, the Rfo restorer gene – in 7, the fad2 mutant homozygous and heterozygous alleles – in 3 and 2 genotypes, respectively, whereas the fad3 mutant homozygous alleles were identified in 2 of the analyzed genotypes.

The evaluation of agronomical traits before winter dormancy revealed statistically significant differences among the genotypes.

Conclusions: This work will be continued on extended population of oilseed rape genotypes and the field trials will be continued in the 2015-2016 growing season, to establish association between the phenotype traits and molecular characteristics of the analyzed plant material of agronomical value.

References:


Organelle genome sequencing of Nsa cytoplasmic male sterility for identification of CMS gene in *Brassica napus*

**Background:** A novel cytoplasmic male sterility (Nsa CMS) was established by somatic hybridization between *Brassica napus* and its wild relative *Sinapis arvensis*. The sterility of Nsa CMS is more stable than the widely used Polima CMS and is of potential for safe hybrid production of oilseed rape. As a maternally inherited trait, CMS was often observed when an alien cytoplasm is transferred into a cultivated species (Igarashi et al. 2013). Genes responsible for CMS are usually located in new chimeric ORFs which may be caused by mutations, rearrangement or recombination in mitochondrial genome.

**Objectives:** In order to understand the mitochondrial genomic composition of the novel CMS and to identify genes responsible for the male sterility in Nsa CMS, comparative analysis of mitochondrial genomes of Nsa CMS line and its two original parental lines, *S. arvensis* var. Yeyou 18 and *B. napus* var. Zhongshuang 4 were carried out and structural differences among mitochondrial genomes were uncovered.

**Methods:** The etiolated one-week-old *S. arvensis* seedlings were used for mtDNA extraction. mtDNA sequencing was performed using the Roche 454 FLX system (Roche Applied Science, Indianapolis, USA) and the clean read sequences were assembled by Newbler Assembler Software Version 2.8. The contigs were joined by PCR sanger sequencing. ORF Finder, BLASTX, BLASTN, and tRNA-SE were used to identify mitochondrial ORFs, genes, rRNA, and tRNA. Circularized rRNA (CR)-RT-PCR (Kuhn and Binder 2002), was performed to determine orf347 and nad3 co-transcripts.

**Results:** We obtained the complete mitochondrial genome sequences of the *S. arvensis* (240,024 bp), Nsa CMS line (269,973 bp) and Zhongshuang 4 (221,862 bp). Nsa CMS mitochondrial genome showed a fusion type with 64.7% identity to *B. napus* and 92.5% identity to *S. arvensis*, indicating mitochondrial genome recombination mediated by protoplast fusion. Different mitotypes are co-exist substoichiometrically in Nsa CMS lines. Comparative analysis the mitochondrial genomes of Nsa CMS line and its maintainer line Zhongshuang 4 resulted in three candidate ORFs which might be CMS-associated genes based on their chimeric nature or they encode peptides with transmembrane domains. These genes are usually located on the edges of highly-rearranged CMS-specific DNA regions and from *S. arvensis*. One of the candidates, orf347, was co-transcribed with i encoding NADH dehydrogenase subunit 3, and thus was likely to be the CMS gene. Functional analysis of the candidate gene is in progress.

**Conclusions:** To our knowledge, this is the first time that organelle genome derived from a hybrid was sequenced and analyzed. The Nsa CMS mitochondrial genome was highly rearranged compared with its parental lines. Although large portion of sequence context was shared by mitochondrial genomes of CMS and *B. napus*, extensive genomic rearrangements were detected. orf347, which is from *S. arvensis*, was selected as a candidate CMS gene for further investigation.

**Reference:**
Effects of low nocturnal temperature on photosynthetic characteristics and chloroplast ultrastructure of winter rapeseed (*Brassica napus* L.)

**Objective:** To investigate the effects of low nocturnal temperature on photosynthetic apparatus of winter rapeseed (*Brassica napus* L.).

**Method:** An artificial climate chamber was used to simulate the effects of low nocturnal temperature on seedling and stomatal morphologies, chloroplast ultrastructure, photosynthetic parameters, and dry matter distribution and accumulation in two winter rapeseed cultivars, Longyou-7 with ultra cold resistance and Tianyou-2 with weak cold resistance.

**Results:** Compared with those at diurnal/nocturnal temperatures of 20°/10°C (control), rapeseed seedlings at 20°/5°C had increased leaf chlorophyll content, deepened green leaf color, decreased stomatal conductance (Gs), intercellular CO2 concentration (Ci), and photosynthetic rate (Pn), and improved root/shoot ratio; the majority of stomata remained open in Longyou-7 while those in Tianyou-2 were mostly closed or semi-closed. At diurnal/nocturnal temperatures of 20°/-5°C, rapeseed seedlings had decreased leaf chlorophyll content with increased Ci but decreased Gs and Pn; Tianyou-2 exhibited ruptured chloroplast membrane, dissolved grana, broken stroma lamella, and decreased root/shoot ratio, whereas Longyou-7 had chloroplasts retaining partial structure of grana with a small amount of starch granules in guard cells.

**Conclusions:** Low nocturnal temperature caused damage to the structure of photosynthetic membrane of chloroplasts and reduction of Pn in leaves of winter rapeseed, thus influencing photosynthetic processes in this crop. The reduction of Pn was mainly related to stomatal limitation at diurnal/nocturnal temperatures of 20°/5°C and non-stomatal limitation at diurnal/nocturnal temperatures of 20°/-5°C.
Disruption of a CAROTENOID CLEAVAGE DIOXYGENASE 4 gene converts flower color from white to yellow in Brassica species

**Background:** The distinctive yellow flower color of Brassica makes a striking contribution to the visual landscape of many arable rotations worldwide. The common flower color of Brassica species in U’s triangle (Nagaharu, 1935) is yellow, although some white-flowered varieties exist in subspecies of the B. oleracea cytode. White flower has been reported in rapeseed lines re-synthesized through interspecific hybridization between B. rapa and white-flowered B. oleracea (Chen et al., 1988; Heneen et al., 1995; Zhang et al., 2002). Genetic analysis of flower color in Brassica has been conducted since 1929 (Pearson, 1929), and has been shown to be controlled by a single nuclear gene in Brassica species containing the C genome or sub-genomes, with white color dominant over yellow (Pearson, 1929; Zhang et al., 2002; Liu et al., 2004; Huang et al., 2014). Genetic mapping has identified a number of molecular markers linked to the flower color locus on chromosomes C3 (Ramsay et al., 1996; Liu et al., 2004; Parkin et al., 2005; Geleta et al., 2012; Hu et al., 2014). However, to date the gene controlling flower color has not been identified, and the underlying molecular mechanisms and evolutionary processes in Brassica remain elusive.

**Objectives:** In the present study, we will reveal its molecular mechanism that controls carotenoid accumulation and discuss the evolutionary history of flower-colored trait in Brassica.

**Methods:** We measured the content of total carotenoids in petals from fully opened flowers of a white-flowered rapeseed line ‘2127’ and a yellow-flowered cultivar ZY821. Using HPLC, we also compared the carotenoid profiles in petals of ‘2127’ and ZY821. To reveal the molecular mechanism of flower color formation in Brassica species, we attempted to identify the flower color BnaFC gene using a positional cloning strategy. The likely enzyme activity of BnaFC was tested through headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS). The alleles of BnaFC were isolated and aligned in B. napus, B. oleracea and B. carinata.

**Results:** In Brassica napus yellow petals had a much higher content of carotenoids than white petals present in a small number of lines, with violaxanthin identified as the major carotenoid compound in yellow petals of rapeseed lines. Using positional cloning we identified a carotenoid cleavage dioxygenase 4 gene, BnaC3.CCD4, responsible for the formation of flower color, with preferential expression in petals of white-flowered B. napus and B. oleracea lines. Insertion of a CACTA-like transposable element 1 (TE1) into the coding region of BnaC3.CCD4 has disrupted its expression in yellow-flowered rapeseed lines. α-linone was identified as the major volatile apocarotenoid released from white petals but not from yellow petals. We speculate that BnaC3.CCD4 may use δ- and/or α-carotene as substrates. Four variations, including two CACTA-like TEs (alleles M1 and M4) and two insertion/deletions (INDELS, alleles M2 and M3), were identified in yellow-flowered B. oleracea lines. The two CACTA-like TEs were also identified in the coding region of BcaC3.CCD4 in B. carinata. However, the two INDELS were not detected in B. napus and B. carinata.

**Conclusions:** The results suggested that the insertions of TEs in BocC3.CCD4 predated the formation of B. napus and B. carinata, and that the two INDELS might occur outside of the origination centers of the two allotetraploids and not participate in their speciations.

**References:**

Identification of a new cytoplasmic male sterility type NRO4270A in *Brassica napus*

**Background:** The production of the hybrid seeds through cytoplasmic male sterility (CMS) is the most important way of heterosis utilization in rapeseed (*Brassica napus*). CMS is often associated with novel open reading frames produced through the rearrangements of mitochondrial genome, and they interfere with the pollen development (Iwabuchi et al., 1999; L’Homme et al., 1997). Several CMS systems have been reported in rapeseed, such as ogu, pol, tour and nap. *B. napus* NRO4270A CMS was obtained in the progenies of distant hybridization of *Raphanobrassica* (RRCC) (Chen et al., 2006) and *B. napus* (AACC) by us.

**Objectives:** In the present study, the phenotypes of NRO4270A CMS would be investigated, and its restorer and maintainer relationship would be identified. In addition, its stage and characters of pollen abortion would be revealed, and its cytoplasm type would be tested.

**Methods:** The sterility degree and sterility rate of NRO4270A CMS in field were investigated in Wuhan (winter rapeseed production area) and changyang or hezheng (spring rapeseed production area) in 2009-2013. Its restorer and maintainer relationship was compared with those of other CMS systems. The buds of NRO4270A CMS and its maintainer line were collected, and their paraffin sections were made and observed at different pollen development stages. The restriction fragment length polymorphism (RFLP) of NRO4270A mitochondrial DNA were analyzed and compared with those of other CMS systems.

**Results:** NRO4270A could not produce pollens and its male sterility was extremely stable, which could not be affected by environmental conditions, such as temperature and photo-period. Through restorer and maintainer relationship identification and RFLP analysis of mitochondrial DNA, NRO4270A is different from previous CMS systems, such as pol, ogu and kos. Microscopic structure analysis of NRO4270A anther development indicated that its pollen abortion occurred between the tetrad stage and mononucleate stage. At mononucleate stage, the microspore ektexine could not form, and tapetum vacuolated and expanded. Finally, tapetum and microspore completely degraded, and the pollen sacs became empty and could not produce pollen. Conclusions: It was suggested that NRO4270A was a new CMS system. Its discovery and utilization will overcome the problems of environmental sensitivity of some CMS systems and singleness of sterile cytoplasm type in rapeseed hybrid seed production.

**References:**
Anther-specific cysteine protease CP51 is essential for pollen exine formation in *Arabidopsis* and *Brassica napus*

**Background:** Cysteine proteases play important roles in intracellular protein degradation, programmed cell death and in responding to environmental stimuli.

**Objectives:** This study was to characterize a new cysteine protease.

**Methods:** Candidate genes were selected based on a microarray profiling study in which isogenic lines (the genic sterile and its backcrossing with normal fertility) were used. A putative cysteine protease named CP51 was transformed into *Arabidopsis* to study its function.

**Results:** We identified a new cysteine protease CP51 from *Brassica napus* and *Arabidopsis thaliana* which participates in exine formation and anther development. The gene encodes a papain-like subfamily (C1A) cysteine proteinase and is specifically expressed in anthers at stages 9-12 of *A. thaliana*, which was assessed by qRT-PCR and promoter-GUS fusion detection. RNAi transgenic *Arabidopsis* plants with reduced CP51 transcriptional levels exhibited male sterile phenotype with aborted microspores, shortened siliques and fewer seeds. Cytological analysis indicated that the tapetum degraded earlier and pollen abortion occurred due to defective pollen exine during the transition from the uninucleated stage to the binucleated stage. Scanning electron microscopy demonstrated that aborted microspores lacked complete or normal reticulate exine, and the intine membrane was extruded in pollens of CP51-RNAi plants. Transmission electron microscopy further revealed that the tapetum degeneration was initiated early and that normal tectum connections to the bacula were missing in anthers of CP51-RNAi plants. Taken together, these results suggested that CP51 participates in tapetum stability regulation and pollen exine formation.

**Conclusions:** CP51 is a member involved in the male gamete development.

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Evaluation of the Rfo introgression following recombination and mutation

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Background: The ogu-INRA CMS system is a cytological variant of the radish- (Raphanus sativus L.) derived ogu CMS (ogura; Ogura, 1968) system introduced through interspecific introgression into B. napus (Heyn, 1976). In this system, there are three different lines: A-lines are male sterile, B-lines are fertile maintainer lines for the respective A-lines and R-lines (restorer) are male fertile restorer lines.

The Rfo introgression is associated with poor agronomic performance due to a large unwanted piece of the radish chromosome that was introgressed with the Rfo gene. It contains 17 pentatricopeptide (PPR) motif repeats that confer fertility restoration (Hu et al., 2008). Previous reports have stated the Rfo locus and the PPR genes are likely to have evolved as a result of intergenic and intragenic recombination. Mutation is another strategy to induce change in the restorer genome. Ethyl methane sulphonate (EMS) has been extensively used to induce mutations in plants because it causes a high frequency of nucleotide substitutions (Talebi, et al., 2012). This approach to introduce variation has been used successfully in several Brassica species such as B. napus, B. juncea and B. rapa to induce fatty acid modifications in double haploids.

Objectives: This project will focus on evaluating the length and composition of the Rfo introgression present in restorer lines after undergoing recombination and mutation.

Methods: Four restorer (R-line) by non-restorer (B-line) crosses and four R-line by R-line crosses will be used for treatment with EMS. Seeds will be soaked in water for 12 hours, followed by soaking in EMS at concentrations varying from 0.5 to 1.2% for an additional 12 hours. These seeds will then be rinsed in water prior to planting. DNA will be extracted from vigorous, fertile M1 lines to sequence the restorer gene fragment and compare sequence variation between lines and to the control R-lines.

Results: Sequence differences in the PPR-B region will be observed when comparing the original R-lines and the mutagenized R-lines.

Conclusions: Recombination and mutation will generate changes that could potentially shorten the restorer fragment sequence and thus improve the agronomic performance of the restorer lines. Future work will focus on evaluating subsequent populations.

References:
Progress on self-incompatibility hybrid breeding in *B. napus* L

**Background:** Self-incompatibility (SI) has been used widely for hybrid breeding in vegetables *B. rapa* and *B. oleracea*, but rarely utilized in *B. napus*. As *B. napus* is an oil crop, its hybrids should be fertile for harvesting seeds and a SI line must be propagated on a large scale to produce many hybrid seeds. The SI of line S-1300 is recessive in most accessions but dominant in some genetic backgrounds (Ma et al, 2003), and so it has been utilized for three-component hybrid breeding via SI F1 hybrids (Fu, 1981). With development of a method of propagating SI lines on a large scale by spraying salt solution, we have claimed a two-line hybrid breeding method by self-incompatibility. How the method works in practice is not reported.

**Objectives:** Field data were collected and summarized to show the high vigor of the two-line hybrids. To confirm the efficiency of breeding a SI line, Microspore culture and Gene-based PCR markers have been adopted. F1 fertility is predicted and its purity is checked by the Gene-based PCR markers. A protocol of seed production system has been claimed in order to extend the hybrids.

**Methods:** Field trials were conducted during rapeseed growing season of 2008-2014 in farm fields at three locations under normal condition for crop production in Hubei province, China. Performance of some hybrids was from provincial and National official trials from 2008 to 2014 in China. All trials were designed as randomized complete blocks with three replications in each environment. Each plot was 20 m2. DNA isolation and PCR were carried out as described by Gao et al (2013) and PCR primers were from the literatures by Gao et al (2013) and Tang et al (2009).

**Results:** Four hybrids have been delivered, three have elite performance on provincial and National official trials, and three are selected for further trials, showing that the two-line hybrid breeding method has high efficiency. Many diverse genetically SI lines have been bred indicating SI lines are easy and fast to be improved. Hybrid seeds with high hybridity have been produced by a protocol.

**Conclusions:** The two-line self-incompatibility hybrid breeding method is effective and fast with some distinguished advantages such that hybrids have high vigor, SI lines are easy to be bred by Microspore culture and SCAR markers and F1 fertility and purity can be told by SCAR markers.

**References:**


Haplotype diversity across quantitative resistance loci associated with sclerotinia resistance in *Brassica napus*

**Background:** The omnivore fungal pathogen *Sclerotinia sclerotiorum* is a serious disease of many crop species including canola (*Brassica napus*). Resistance to sclerotinia is a desirable but rare trait. The Chinese *B. napus* cultivar Zhongyou 821 (ZY821) has a moderate level of quantitative resistance (He et al. 1987) and has been accessible to canola breeding programs world-wide since its release in the late 1980's. More recently, higher levels of resistance have been identified in *B. napus* germplasm lines from Pakistan, South Korea and Japan held at AAFC in Saskatoon. Bi-parental mapping populations of doubled haploid lines were developed from crosses with ZY821 and the new resistant sources with susceptible lines. Subsequently, the populations were phenotyped for stem rot resistance and genotyped with a combination of single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers. Numerous major and minor quantitative resistance loci (QRL) were identified through quantitative trait loci analysis, some of which mapped to the same chromosome and genomic block. In addition, QRL were identified by association mapping using a set of resistant and susceptible accessions in a world collection of *B. napus*. The resistant germplasm are mainly landraces and past breeding lines which are not adapted to the Canadian prairies and do not possess canola-quality seed traits.

**Objective:** The newly identified QRL are currently being introgressed into an elite breeding line to develop sclerotinia resistant germplasm. To target a specific QRL for introgression, it is imperative to elucidate whether genomic regions associated with sclerotinia resistance in different resistance sources are identical by decent or different allelic forms.

**Methods:** Existing SNP data at the previously detected sclerotinia resistance QRL of all lines used as parents of bi-parental mapping populations (PAK54, PAK93, DC21 and ZY821) and in association mapping is analysed to dissect relationships among the QRL by comparison with the larger association panel of lines. High density SNP array data is analysed using custom scripts and R packages to identify allele frequencies and haplotypes associated with the mapped QRL. The genetic relationships among the haplotypes underlying QRL-regions is further characterised through analysis of molecular variance and compared to genome-wide variation and population structure. Haplotypes of SNP markers across QRL is identified to infer allele frequency differences between the resistant lines. Additional haplotype characterization is conducted to look for signatures of selective sweeps.

**Conclusion:** Results will be presented identifying unique and shared alleles at previously mapped QRL conferring sclerotinia resistance in PAK54, PAK93, DC21 and ZY821. The analysis will reveal the presence/absence of conservation of haplotypes at the respective QRL among these resistant sources. This information is needed to prioritize resistance introgression targets in our canola breeding program.

**References:**
Yield stability and adaptability of NS spring rapeseed genotypes based on GGE biplot analysis

**Background:** Field experiments are usually being performed in different environments with an aim to evaluate yield stability of different crops under varying environmental conditions. Therefore, to reduce the possibilities of significant yield loss and to select specific cultivars for growing in target regions, the information of the effect of environmental factors on crop growth and development is essential (Marjanovic-Jeromela et al. 2011). The main environmental effects (E) and genotype environment interaction (GE) were recognized as the most important sources of crop yield variation (Yan et al. 2007). The GGE biplot technique (Yan 2001) is one of the tools used for GE interaction analysis. It can help to recommend genotypes for specific growing region taking into account the specificities of genotypes and growing conditions (Boshev et al. 2014).

**Objectives:** The objectives of the study were to evaluate grain yield stability, to graphically summarize the effects of genotype (G) and genotype by environment (GE) interaction, to identify “which won where”, and to recommend rapeseed genotypes for a specific growing region using GGE biplot.

**Methods:** The study was carried out in 2009 and 2010 at two locations in Serbia (Sombor and Novi Sad) and two locations in Macedonia (Skopje and Bitola), with 9 spring rapeseed genotypes. In order to evaluate and to quantify the magnitude of genotype x environment interaction effects on spring rapeseed yield, GGE biplot analysis was used to depict the stability and adaptability of genotypes at different locations and discrimination ability of the testing locations.

**Results:** The first two principal components explained 86.5% of the G+GE variation for seed yield (PC1 64.46%, PC2 22.048%). Both locations in Serbia were clearly separated from both locations in Macedonia. Sombor and Bitola were closest to the ideal location for rapeseed growing. Rapeseed genotype JR-NS-36 was the most stable and was the closest to the ideal genotype. However, the most suitable genotypes for growing in Serbia are JR-NS-6 and JR-NS-11, while JR-NS-9 had the highest and the most stable yield in Macedonia.

**Conclusions:** This technique can serve as a useful tool for recommendation of rapeseed genotypes for specific growing region, taking into account the specificities of the genotypes and environmental conditions.

**Acknowledgment:** This work is a part of the project TR31025 supported by Ministry of Education, Science and Technological Development, Republic of Serbia.

**References:**
Development of DuPont Pioneer proprietary Optimum® GLY Canola trait

Background: Adoption of herbicide tolerant (HT) canola in North America has been rapid with almost 99% of Canada's 20 million acres of canola now planted to HT types which were first introduced 20 years ago. Glyphosate tolerance represents approximately 50% of the total acreage and is projected to increase to 60-65% in the next 10 years. Canola Optimum® GLY is a DuPont Pioneer proprietary HT trait that has advanced to the pre-commercial stage with expected commercial launch later this decade, subject to appropriate regulatory approvals.

Objectives: To develop, obtain necessary approvals, and commercialize high-performing canola hybrids containing the Optimum® GLY herbicide tolerance event (DP-Ø73496-4) in North America and Australia. Secondly, to integrate current and future Protector™ traits into Optimum® GLY canola and tolerance to higher levels of glyphosate within an expanded application window (up to first flower).

Methods: The Optimum® GLY canola trait is based on the Glyphosate Acetyltransferase (GAT) gene that was optimized through the DuPont Pioneer proprietary DNA shuffling technology. The gene detoxifies glyphosate herbicide by acetylation to provide glyphosate tolerance. DuPont Pioneer was responsible for the development of the Optimum® GLY trait including a full range of trait development activities, including molecular characterization, indoor phenotypic and genetic characterization of events, trait introgression, field evaluation and seed production.

Results: The Optimum® GLY trait attributes include improved agronomics, better efficacy in the current label application window compared to current glyphosate herbicide tolerance, and commercial level glyphosate tolerance at late-stage plant development up to early flowering. Trials are currently being conducted to support a new label with higher levels of active glyphosate applied and an expanded application window to optimize weed control. Advanced pre-commercial hybrid testing and evaluations are ongoing. Optimum® GLY hybrids have been recommended for registration by the WCC/RRC in spring 2015 and will be candidates for commercial launch later this decade subject to appropriate regulatory approvals.

Conclusions: The commercialization of DP-Ø73496-4, Optimum® GLY Canola, will provide growers globally with a choice for glyphosate tolerant canola over a wide application window combined with high-yielding Pioneer genetics including key native traits, strong agronomics. In addition Optimum® GLY canola will provide additional integrated weed management options for growers.
Constructing a high density SNP genetic map and mapping QTL for yield-related traits using DH and IF2 populations in *Brassica napus*

**Background:** Yield is the most important and complex trait for rapeseed (*Brassica napus*). Quantitative trait locus (QTL) analysis has proved to be an effective approach to dissect complicated quantitative traits. The newly developed *Brassica* 60 K Infinium BeadChip Array is a very effective tool for SNP genotyping, and it has been successfully exploited in rapeseed’s seed fibre QTL analysis (Liu et al. 2013).

**Objectives:** To construct a high density SNP genetic map for mapping QTL for five yield-related traits in rapeseed using a double haploid (DH) population and an immortalized F2 (IF2) population derived from the DH lines.

**Methods:** Silique length (SL), thousand seed weight (TSW), seeds per silique (SPS), siliques per plant (SPP) and silique density (SD) of DH and IF2 populations were assessed in four different environments. Genome-wide single nucleotide polymorphism (SNP) of parental and DH lines were assayed by the *Brassica* 60 K Infinium BeadChip Array. Linkage analysis and SNP map construction were performed using QTL IciMapping V4.0 and JoinMap 4.0. WinQTLCart2.5 was used to detect QTLs.

**Results:** A 2217.2 cM SNP bin linkage map was constructed which contains 8876 SNP makers, and 7728 SNP markers were distributed among 900 bins. For QTL mapping, only one SNP maker of every bin was selected. The final high density linkage map for the DH population contains a total of 2046 non-redundant SNP makers, with an average distance of 1.08 cm between adjacent markers. Thirty-five and 23 significant QTLs were detected across environments for the five traits in the DH and IF2 populations, respectively. Amongst, 16 QTL were repeatedly detected in both populations. Eighteen QTL were repeatedly detected in the DH population and nine in the IF2 population across different environments. Two, two, two, one and one major QTLs were detected for SL, TSW, SPS, SPP and SD, respectively. Three of the major QTLs were repeatedly detected for two or three traits.

**Conclusions:** Major QTLs for five yield-related traits were successfully detected using a high density SNP bin map in DH and IF2 populations. The high density SNP bin map is very useful for QTL mapping, and SNP marker showing a great potential for marker-assisted selection. Some major QTLs for different traits were mapped in the same chromosome region, which gave a good explanation for significant phenotypic correlations of the traits at molecular level.

**References:**

Cell and tissue-specific RNA sequencing and laser microdissection of the *Brassica napus* (canola) maternal seed subregions

**Background:** *Brassica napus* (canola) contributes $19.3 billion to the Canadian economy each year due to its highly nutritive oil and protein reserves within the embryo. Embryo development and nutrient accumulation require the coordinated development and communication between all seed regions and subregions. The maternal tissues of the seed can be divided into a number of subregions including the outer and inner integuments surrounding the seed, the chalazal proliferating tissue (CPT) that subtends the chalazal endosperm, and the chalazal seed coat (CZSC) that serves as the first connection between the funiculus and the seed. While much research has been carried out on the filial embryo and endosperm, we have yet to fully understand the genes required to program the development of the maternal subregions at the cellular level in canola.

**Objectives:** Surprisingly, subregion-specific development and transcriptional circuitry of the maternal tissues of the canola seed has yet to be investigated. Our goal was to provide the first detailed anatomical description of the uncharacterized maternal seed subregions coupled with next-generation RNA sequencing to profile the genes and gene regulatory networks responsible for canola seed development.

**Methods:** The anatomy of the maternal subregions was studied using light and transmission electron microscopy across the mature ovule, globular, heart, and mature green stages of seed development. We then profiled each of the maternal subregions using laser microdissection coupled with next-generation RNA sequencing technology. These combined methods provide a high-resolution dataset of the transcriptional networks operative within the maternal seed. Hierarchical and fuzzy-K means clustering analyses combined with GO term enrichment were then used to predict and compare biological function and cellular processes within the CZSC, CPT, and inner and outer integuments.

**Results:** Vigorous bioinformatics analyses of RNA sequencing data revealed an impressive array of dominant expression patterns thought to control development of the maternal subregions in both space and time. Fuzzy-K means clustering analysis identified dominant patterns of gene activity between the different maternal subregions of the canola seed. Large numbers of transcripts were considered shared between all maternal subregions, while small numbers of genes were shown to be specific to each. Subsequent GO term enrichment showed that the CZSC has mRNAs associated with transport processes and also identified putative regulators of these processes operative in the CZSC and other maternal seed subregions. Light and electron microscopy identified vascular tissue of the funiculus, which supplies the seed with nutrients, terminating in the CZSC suggesting the CZSC is an unloading zone. In addition, plasmodesmata were identified between the cells of the CZSC, suggesting symplastic transport is likely present within this subregion.

**Conclusions:** Our combined anatomical and global transcriptomic datasets provides strong evidence that the maternal seed subregions in canola possess not only a structural and protective function but also serves a putative role in the transport of materials to the filial embryo and endosperm. Our data further provide a substantial informatics resource for those interested in oilseed genomics for improved seed development.

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How duplications in oilseed rape (Brassica napus L.) genome impact the organization of genomic regions involved in quantitative resistance to Leptosphaeria maculans

Background: All crop species are recent or ancient polyploids and have more or less duplicated genomes. Following polyploidy events, structural and functional modifications result in differential gene content or regulation in the duplicated regions, which can play a fundamental role in the diversification of the genes underlying complex traits.

Objectives: To better understand the functioning of the genetic factors controlling complex agronomic traits, it is necessary to analyze them in the light of the duplications in the genome. We have addressed this issue in oilseed rape, a species with a highly duplicated genome, with the aim of studying the consequences of genome duplications on the structural and functional organization of the regions involved in quantitative resistance to blackleg. This resistance has been shown to be controlled by many genetic factors (1, 2).

Methods: The genetic architecture of quantitative resistance to blackleg was evaluated using several methods: (i) linkage analyses on bi-parental populations with Darmor-bzh as common resistant parent, (ii) a QTL meta-analysis on a set of 7-years phenotyping data on the ‘Darmor-bzh x Yudal’ population and (iii) genome-wide association analyses in two panels of oilseed rape varieties. High density genotyping SNP were used for these analyses allowing to synthesize all the results on a dense integrated genetic map. These data were used to assess the proportion of resistance QTL located at duplicated positions (3). We then took advantage of the recently released genome sequence of Brassica napus (4) for the structural analysis of the duplicated regions. B. napus – Arabidopsis thaliana relatedness was used to explore gene ontology categories and the function of genes located in the duplicated regions.

Results: Numerous genomic regions were identified, which confirmed the high polygenic nature of this resistance. Their distribution was quite equivalent between A and C genomes of oilseed rape but a bias was observed in relation with the subgenomes deriving from the ancestral triplication event of Brassica clade. At least 44% of the genomic regions corresponded to homoeologous duplicated regions of five A. thaliana syntenic blocks (3). Comparative genomic analysis with A. thaliana showed that few genes were conserved in all the duplications of a given ancestral bock and that many of these genes were involved in stress response. NBS-LRR genes or resistance gene analogs were also present in some of these regions.

Conclusions: Most of the identified resistance associated genes corresponded to genes retained in duplicated regions and were involved in response to stress. It has actually been demonstrated in various other species that genes over-retained after whole genome duplication were involved in stress response, indicating a common evolution pattern across species. Comparative genomics also allowed us to draw hypotheses on the function of genes underlying the QTL located in these genomic regions.

References:

(2) Jestin et al., 2012. Open J Genet 2: 190-201.
Improving early generation selection in canola breeding

**Background:** Classical plant breeding has always relied on phenotypic selection as a keystone for any breeding program. Greatest advancement in genetic gains from plant breeding will be achieved by increasing heritability of traits under selection. The advancement of high throughput molecular markers have significant potential to assist plant breeders by providing a powerful tool for marker assisted selection (MAS). Similarly genome wide association studies (GWAS) will provide an additional tool to connect genotypic and phenotypic performance and for studying quantitative traits of interest such as yield and oil content. However, MAS and GWAS both require high quality and reliable phenotypic performance data.

**Objective:** Increase breeding methodology and selection efficiency in canola cultivar development programs.

**Methods:** Two studies were conducted. (1) Two hundred and ninety two spring canola and rapeseed (*B. napus*) accessions from a broad geographic distribution were grown in a greenhouse and leaf tissue analyzed with Sequenced-based Genotyping, producing more than 300,000 SNPs at genome-wide coverage. These accessions were grown in replicated field trials at one location in 2012 and two locations in 2013 and 2014. A wide range of morphological, agronomic, and quality traits were evaluated. (2) Nine commercial spring canola varieties were chosen from the germplasm collection (1) and crossed together in a 9x9 full diallel mating design. F1 plants were then self-pollinated to produce F2 seed, and these progeny were planted in a replicated covariance (reciprocal crosses) design in a greenhouse. Phenotypic data was collected (i.e. leaf characteristics, flower date, yield, pod density, etc.) for analyses.

**Results:** The plant traits days to flowering, leaf serration and lobing, and pod length showed high heritability between glasshouse plants and field evaluations, and between different sites and years under field testing. However, yield and oil content under glasshouse and field conditions was very poor. Heritability values for seed yield between field sites and years ranged between 0 and 0.4. Similarly, other important breeding traits had low heritability over locations and years. Greater variability of phenotypic performance over years and sites is perhaps the most limiting factor of success in plant breeding. In order for MAS, GWAS and quantitative trail loci to improve selection it is important that the genotype:phenotype relationship can be made reliably and have relationship over environments. Analyses from the diallel have yet to be completed at the time of submission. However, progeny performance of the diallel progeny will be predicted using available phenotypic and genotypic data available on the parent lines used and the results of these predictions discussed.

**Conclusion:** World-wide need for increased food production will necessitate larger genetic gains in our breeding efforts, which will require greater predictability in selecting superior cultivars. MAS and GWAS will have greater impact on cultivar development if these techniques can be applied at the parent level to select parents with greatest breeding value and thereafter identify specific individuals within progeny to be better cultivars.

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Transcriptomics analysis of *Brassica napus* using de novo assembly methods

**Background:** Canola (*Brassica napus*) is of significant global economic importance, this along with its unique evolutionary path that offers insights into polyploid evolution have ensured that it has been the focus of much genetic and latterly genomics research. All research in *B. napus* is complicated by the significant homoeology that exists between the subgenomes which resulted from the merger of the genomes of *B. rapa* (AA, n=10) and *B. oleracea* (CC, n=9), 7,500 to 12,000 years ago. This homoeology creates difficulties in determining the origin of gene transcripts and introduces ambiguity in assigning transcripts to a genomic location (Chalhoub et al, 2014, Harper et al. 2012). This is exacerbated when using the widely adopted short read sequencing technologies that allow unbiased assessment of the plant’s transcriptome, which is proving essential for the study of many agronomic traits. The current problem is how to adapt software tools designed for simpler genomes to provide efficient and effective analyses of transcriptome data of the complex *B. napus* genome.

**Objective:** To define robust protocols for analyzing short-read transcriptome data from *B. napus*, which are optimized for differentiating the homoeologous transcript pairs.

**Methods:** A transcriptome of *B. napus* morphotype ‘DH12075’ (leaf tissue) was created using experimental Illumina RNAseq data and the de novo transcriptome assembler Trinity (Grabherr et al. 2013). The resulting transcripts were compared by multiple sequence alignment to coding DNA sequence (CDS) data from ab initio gene prediction using MAKer (Cantarel et al. 2008). Simulated Illumina short-reads were generated from the same CDS data using ART software tool (Huang et al. 2012) and also assembled in Trinity. Accuracy of Trinity software was evaluated by multiple sequence alignment of the assembled CDS sequences to the original data.

**Results:** Initial assembly of simulated Illumina RNAseq reads resulted in 121,254 distinct transcripts (N50=423) and 125,586 total transcripts (including isoforms; N50= 432 bp) from the original 103,679 CDS, suggesting relatively good recovery. Assembly of experimental Illumina RNAseq data resulted in 56,025 distinct transcripts (N50 = 939 bp) and 87,545 total transcript isoforms (N50 = 1210 bp), this reduced number might be expected from a single tissue dataset. The assembled transcripts (both simulated and experimental) will be assessed for their integrity relative to the expected annotations. Multiple assemblies are currently being created with different k-mer lengths and additional software tools to study potential improvements.

**Conclusion:** The results of the different tools and assessment parameters will identify an optimal pipeline for transcriptome assembly in *B. napus*.

**References**


Development of molecular markers for Identification of *Fusarium* species

**Background:** *Fusarium* wilt disease in canola primarily caused by *Fusarium oxysporum* and *Fusarium avenaceum* has caused substantial losses. Unlike other plant pathogens *Fusarium* species are making an association where involvement of more than one pathogen makes disease more complex.

**Objectives:** To control fungal hazards of plants, animals and humans, there is a need for a rapid, easy and accurate identification system of *Fusarium* isolates with molecular methods. Identification of *Fusarium* species has always been difficult due to confusing phenotypic classification systems.

**Methods:** We have developed a fluorescent-based polymerase chain reaction assay that allows for rapid and reliable identification of seven toxigenic and pathogenic *Fusarium* species.

**Results:** The species includes *Fusarium avenaceum*, *F. oxysporum*, *F. sambucinum*, *F. culmorum*, *F. equiseti*, *F. solani* and *F. graminearum*. The method is based on the PCR amplification of species-specific DNA fragments, which were designed based on sequences.

**Conclusion:** Besides providing an accurate, reliable, and quick diagnosis of these *fusarium* spp for canola this can also be leveraged for other crops.

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High throughput phenotyping of OSR crops by Remote-, close range-, and in situ- sensing techniques: State of the art & first results

Context: The lack of suitable tools for rapid and non-destructive phenotyping methods may influence and hamper the physiological and genomic researches. Among the large number of high throughput phenotyping facilities at different scales (in situ, close range, and remote sensing), choosing the most appropriate data source, including the ‘vector’ and the ‘sensor’, remains a challenge.

At CETIOM, as part of the PHENOME (French phenotyping network) and RAPSODYN (optimization of the RAPseeds Oil content anD Yield under low Nitrogen input) projects, we establish, observe and follow the experimental platforms since 2012. These platforms have been designed for testing out the new phenotyping tools and methods. The final goal is to provide the high frequency measures of phenotypic traits of the plants at different growth stages using the data obtained from multiple sources. The expected results are mainly agronomical variables such as biomass, LAI, Nitrogen content, etc. The latters are basically estimated by combining statistical, physical and physiological models.

Methodology: We present, first of all, a summary of sensors and vectors tested at CETIOM experimental station in Dijon. Then some data, analysis methods and results of two different data sources will be shown: 1- the reflectance spectra obtained by a close range sensing device, the field VNIR spectrometer ASD (Advanced Spectrometry Device), 2- the RGB and multispectral images taken by a UAV (Unmanned Aerial Vehicle) system as a remote sensing tool. The pre-processed data has been used as input parameters of prediction models.

Results and discussion: The reliability of the estimated outputs, Green area index (GAI) and biomass, has been evaluated through the validation phase (lab traditional destructive methods). The first results show a good accordance between the data obtained from different sources and a reliable estimation of biomass and GAI ($R^2 \sim 0.7$).
Large scale SNP genotyping with optimized molecular marker sets for cost-efficient plant breeding in the Brassica species (B. napus, B. oleracea and B. rapa)

**Background:** Through the development of large genotyping arrays, it has now become routine to generate a wealth of genotyping data for individual plant lines. However, many of the genotype data generated in this way (e.g. with the Brassica 60K Illumina Infinium array) constitute of redundant information since the genotype data of many markers are in perfect linkage disequilibrium in breeding material and varieties.

**Objectives and methods:** With genotyping data generated from a large set of lines derived from various sources and countries in combination with mapping information for many markers, we have investigated in detail the extent of LD and marker haplotype groups in elite Brassica napus material as well as in B. rapa and B. oleracea to identify haplotype-specific markers of high quality. Taking all this information together, we have subsequently generated an optimized genotyping array for routine use in genetic analyses and breeding including genomic selection of oilseed rape and Brassica vegetables.

**Results and conclusions:** The array can now be used at much reduced costs compared to other arrays and without much loss of information compared to larger and more expensive arrays. In parallel and based on the same data set, we have generated an optimized marker collection based on individual SNP markers (KASP) for variety identification, variety purity analysis, marker-assisted backcrossing and other purposes.

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Achieving significant increases in productivity by exploiting Genotype - Environment - Management Interactions (GEMI)

**Background:** Climate change and input reduction in agriculture lead to a diversification of cropping environments with a higher expression of biotic and abiotic stresses. In this context, adapting the choice of cultivars according to their cropping environment is of special importance to increase winter rapeseed productivity. Crop cultivar assessment programs aim to evaluate relative performance of new cultivars, by subjecting them to multi-environment trials (MET), which are a series of field trials conducted across a range of geographic locations and sometimes over several years. Choosing a cultivar according to its global performance can be risky because of GEMI, which induce significant variations in the relative performance of cultivars when they are submitted to various climate conditions and crop management practices.

**Objectives:** The aim is to bring further statistical analysis of the data collected on MET, with a view to enrich the current information on commercial cultivars, and therefore improve recommendations on cultivar use. More precisely, one issue is to quantify GEMI for winter rapeseed. Another is to understand these complex interactions, and define adapted mega-environments for each cultivar. This is done by characterizing environments through the identification of production constraints like weather, soil and management variables.

**Methods:** We applied methodologies which have been developed for GEMI analysis on a rapeseed MET. First, rapeseed GEMI are described through an Analysis of Variance (ANOVA) model, partitioning yield variability into components linked to different sources of variation: genotype, environment, and GEMI. Then, a cluster analysis on GEMIs matrix allows us to define mega-environments (characterized by weather, soil and management practices conditions), where cultivars perform similarly against each other. Lastly, an existing approach like DiagVar (Lecomte 2005), combining agronomic diagnosis and dissection of GEMI, was applied to rapeseed MET, in order to characterize cultivar resistance. The used model expresses GEMI as the sum of the cultivar’s specific responses to each of those stresses and resources (Denis 1988).

**Results:** Winter rapeseed is an interactive crop and requires more understanding about GEMI. Their contribution to total yield is far less than the environment contribution but more than the genotype contribution. Choosing a cultivar solely on the global performance criterion is risky: in about 30% of cases it is a mistake. Cultivar characterization by GEMI is a good way to get information about cultivars.

**Conclusions:** Today rapeseed GEMI analysis is decisive for yield improvement, but it is not usual because of several rate-limiting steps in the approach. One of them is the characterization of environments which is difficult and not precise. In order to improve this step, diagnostic methods will be developed and shared. The use of existing crop models would be an additional option, which could make it possible to acquire non-measurable variables.

**References:**


Characterization of senescence-associated proteases activities related to with N leaf remobilization of winter oilseed rape at the vegetative stage

**Background:** Oilseed rape (*Brassica napus* L.) is a crop plant characterized by a weak N use efficiency (NUE) mainly due to a low N Remobilization Efficiency (NRE) during the sequential leaf senescence at vegetative stage.

**Objectives:** To characterize the key mechanisms able to improve the NUE of oilseed rape, the objective was to identify senescence-associated proteases activities implied in N remobilization during leaf senescence.

**Methods:** Plants were cultivated at the vegetative stage in greenhouse under limiting or ample nitrate supply (0.375 mM, LN; 3.75 mM, HN). We investigated leaf senescence processes (chlorophyll and anthocyanin contents; soluble proteins quantity) and proteases activities of a mature leaf becoming senescent during the experiment. The characterization of proteases activities was performed firstly by using in gelo analysis of the rubisco (rbcl) degradation with or without inhibitors of specific proteases classes. Then, to determine which proteases are responsible of the rbcl degradation, we performed standard protease activity profiling using activity-dependent probes specific of proteases classes.

**Results:** As expected, the mature leaf became senescent regardless of the nitrate treatment but LN conditions amplified senescence processes associated with a high degradation of soluble proteins. Then, characterization of proteases activities showed that: (1) aspartic proteases were active during senescence regardless of nitrate supply and (2) serine proteases, proteasome and particularly cysteine proteases (Papain-like cys proteases PLCPs and vacuolar processing enzymes VPEs) activities were increased when senescence processes were amplified by a LN supply. Moreover, three PLCPs were identified as playing a key role during leaf senescence. Furthermore, it was suggested that VPEs proteases might be responsible of the activation of some PLCPs.

**Conclusions:** As soluble proteins degradation in senescing leaves of oilseed rape is crucial for the improvement of N remobilization, characterization of proteases activities is a key for comprehension of N remobilization. Moreover, the NRE genotypic differences in LN conditions observed by Girondé et al. (2015) might be associated to different or contrasted proteases activities during senescence. Investigations of genotype variability were in progress in order to better characterize the proteases activities associated with a high N leaf remobilization efficiency.

**References:**

Characterization of the *Sclerotinia sclerotiorum* population from canola in western Canada needed for selection of partially resistant *Brassica napus* germplasm

**Background:** *Sclerotinia sclerotiorum* is a fungal pathogen with a wide host range which includes canola (*Brassica napus*). The disease can be controlled by fungicide application, but its sporadic occurrence reduces the likelihood of economic returns. As an alternative, quantitative resistance in *B. napus* germplasm is being utilized to develop Canadian varieties with improved level of stem resistance. Since canola is planted on 800,000 hectares in western Canada and sclerotinia is widespread, we sought to characterize the *S. sclerotiorum* population to ensure resistance in future canola varieties is effective against the prevailing pathogen population.

**Objectives:** To characterize genetic and pathogenic variability of *S. sclerotiorum* in western Canada, and examine whether quantitative resistance identified in *B. napus* germplasm is effective against the pathogen population.

**Methods:** In 2010, sclerotinia isolates were collected from canola fields in Alberta (AB), Saskatchewan (SK) and Manitoba (MB). A sub-set of 128 isolates and one isolate from 1992 were selected for genotyping. DNA were screened with 35 simple sequence repeat (SSR) markers designed using the *S. sclerotiorum* genome (Broad Institute) and 12 SSRs from Sirjusinh and Kohn (2001). Genetic variability was described by analysis of haplotype (Fabox), molecular variance and gene flow (GenAIEx), linkage disequilibrium (MultiLocus), population structure (Structure), and cluster analysis (nTSYS). Isolates representing 17 sub-populations were evaluated for pathogenicity by inoculating stems of six *B. napus* germplasm lines with mycelium followed by measurement of lesion length over time which was used in statistical analysis of phenotypic variation (SAS).

**Results and Discussion**

Screening of fungal DNA yielded 446 polymorphic alleles ranging from 2 to 35 alleles per marker. Each *S. sclerotinia* isolate was a unique haplotype, and 97% of the genetic variation was ascribed to isolates, while 3% was ascribed to geographical location. Gene flow (Nm) was highest between neighbouring Provinces MB and SK (54.0), less between AB and SK (13.4), and low between the two distant Provinces AB and MB (7.9). Analysis of linkage disequilibrium (LD) clearly showed a clonal population. Two distinct populations were identified by Structure analysis, while cluster analysis identified 17 sub-clusters. Pathogenicity tests showed the 17 representative isolates were statically different in pathogenicity, and the level of partial resistance differed among *B. napus* lines. Furthermore, there was a significant *S. sclerotinia* by *B. napus* interaction indicating the presence of pathotypes in the Canadian population similar to findings in Australia by Ge et al. (2012). Germplasm line, PAK54, from Pakistan had the highest level of partial resistance across all isolates and is therefore a good candidate for transfer of quantitative resistance to Canadian canola varieties. The other lines ranked PAK93 (Pakistan), K22 (Japan), DC21 (South Korea) and Tanto (France) since resistance was lower to one or more of the isolates.

**References**


Transferring *Sclerotinia* resistance from *Brassica incana* into oilseed rape

**Background:** Stem rot caused by fungal pathogen *Sclerotinia sclerotiorum* is a great threat for oilseed production in the world. In the previous studies, two resistance QTL totally explaining more than 30% and 60% of variances for stem and leaf resistance, were identified from *B. incana*, a wild *B. oleracea* with high level of resistance against *sclerotinia* (Mei et al, 2011 and 2013).

**Objectives:** the sclerotinia resistance should be transferred from *B. incana* into oilseed rape due to high colinearity between their genomes.

**Methods:** This line of *B. incana* as resistance donor was crossed with *B. rapa*, and the backcross progeny with *B. rapa* were selected with molecular markers linked with resistant loci togeter resistance evaluations.

**Results:** F1 was higher than *B. rapa* and partial to *B. incana* for the resistance, indicating that the behavior of resistance was partial dominant manner. Although low fertility was found in F1 and BC1F1 derived from parental *B. rapa*, the individuals of BC2F1 having the same chromosome number with *B. rapa* exhibited normal fertility. The lines with resistance loci from *B. incana* were significantly stronger than those without resistance QTL for resistance in BC2F1 and BC2F2. Further, 13 BC2F2 lines with 1.4- to 2-fold higher resistance in stem than a partial resistance line of *B. napus*, ‘Zhongshuang 9’, were chosen to develop new type *B. napus* via crossing with a hexaploid (AACC) derived from ‘Zhongshuang 9’ and resistant ‘B. incana’ (Mei et al. 2015).

**Conclusion:** Our data suggest the resistance from *B. incana* is transferred into *B. rapa*. Now new type *B. napus* lines are being selected in order to pyramid resistance in both A and C subgenomes.

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Genome-wide analysis of population structure and linkage disequilibrium in Chinese semi-winter rapeseed

**Background:** High-density SNP genotyping arrays are a powerful tool for genome-wide association studies and can give valuable insight into patterns population structure and linkage disequilibrium (LD).

**Objectives:** In this study we used the *Brassica* 60kSNP Illumina consortium array to assess the genetic diversity, population structure and the extent of LD and haplotype blocks in a diverse panel of 203 Chinese semi-winter rapeseed inbred lines.

**Methods:** Genome wide SNP data were used to calculate population structure. LD was calculated on the genome, subgenome and chromosome scale. Conserved homoeologous haplotype blocks were investigated for associations to known QTL for important traits.

**Results:** Population structure and principal coordinate analysis, using a subset of the SNPs, revealed diversification into three subpopulations and one mixed population, reflecting targeted introgressions from external gene pools during breeding. Pairwise LD analysis within the A- and C-subgenomes of allopolyploid *B. napus* revealed that mean LD, at a threshold of \( r^2 = 0.1 \), decayed on average around ten times more rapidly in the A-subgenome (0.25-0.30 Mb) than in the C-subgenome (2.00-2.50 Mb). A total of 3,097 conserved haplotype blocks were detected over a total length of 182.49 Mb (15.17% of the genome). The mean size of haplotype blocks was considerably longer in the C-subgenome (102.85 Kb) than in the A-subgenome (33.51 Kb), and extremely large conserved haplotype blocks were found on a number of C-genome chromosomes. Comparative sequence analysis revealed conserved blocks containing homoloegous quantitative trait loci (QTL) for seed erucic acid and glucosinolate content, two key seed quality traits under strong agronomic selection. Interestingly, C-subgenome QTL were associated with considerably greater conservation of LD than their corresponding A-subgenome homoeologues.

**Conclusions:** The data we present in this paper provide evidence for strong selection of large chromosome regions associated with important rapeseed seed quality traits conferred by C-subgenome QTL. This implies that an increase in genetic diversity and recombination within the C-genome is particularly important for breeding. The resolution of genome-wide association studies is also expected to vary greatly across different genome regions.
Comparative quantitative trait loci for silique length and seed weight in *Brassica napus*

**Background:** Silique length (SL) and seed weight (SW) are important yield-associated traits in rapeseed (*Brassica napus*). Although many quantitative trait loci (QTL) for SL and SW have been identified in *B. napus*, comparative analysis for those QTL is seldom performed.

**Objectives:** With the release of reference genomes for *Brassica* crops, such as *B. napus*, *B. rapa* and *B. oleracea* (Chalhoub et al., 2014; Liu et al., 2014; Wang et al., 2011), it should be feasible to conduct genomic comparative analyses in *Brassica* crops.

**Methods:** A *B. napus* DH population, consisting of 261 lines, from a cross between the European winter cultivar ‘Express’ (female) and Chinese semi-winter line ‘SWu07’ (male), were evaluated together with an F2 population (RC-F2) derived from DH lines for silique length and weight at maturity in the experimental field of Southwest University, Chongqing, China, in 2010, 2011 and 2013. The QTL were identified with the composite interval mapping (CIM) procedure of the software WinQTL Cartographer 2.5 by integrating the data of field trial and SSR. The SL and SW QTL regions were aligned to the reference genomes of *Brassica* crops by aligning QTL confidence intervals with the reference genomes of *Brassica* crops.

**Results:** 20 and 21 QTL for SL and SW were identified, totally explaining 55.1–74.3% and 24.4–62.9% of the phenotypic variations across three years, respectively. Of which, 17 QTL with partially or completely overlapped confidence interval on chromosome A09, were homologous with 2 overlapped QTL on chromosome C08 by aligning QTL confidence intervals with the reference genomes of *Brassica* crops. By high density selective genotyping of DH lines with extreme phenotypes, using a *Brassica* single-nucleotide polymorphism (SNP) array, the region of major QTL on chromosome A09 was aligned to a ~1Mb region on the reference genome of *B. rapa* and *B. napus*, respectively.

**Conclusions:** The alignment of QTL in rapeseed with *Brassica* reference genomes revealed homologous QTL on A09 and C08 chromosomes for SL. The major QTL on chromosome A09 was aligned to a ~1Mb region on the reference genome of *B. rapa* and *B. napus*, respectively.

**References:**
Nine years canola breeding from scratch at North Dakota State University

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**Background:** North Dakota is the leader in canola acreage and production with over 83% of U.S. acreage and produces about 84% of all U.S. canola (1.02 million acres and 1.7 billion pounds with a value of $364 million – 5 yr. average from 2009-2013; USDA-NASS). Canola ranked 4th most important field crops in North Dakota. Recently, construction of two canola based processing plants requires a three-fold increase of canola production in North Dakota. To meet the anticipated demand, it is necessary to improve the genetic potentiality of breeding lines adapted to this region. Without better performance and better adaptation, the demand for US canola will not be met, and the processing plants will need to look to foreign sources of feedstock. Basically, the spring canola varieties grown in this region are developed elsewhere and are may not highly adapted to this climatic and agronomic region. Therefore, North Dakota State University (NDSU) initiated a modern spring canola breeding program in 2006.

**Objectives:** The objectives of this program are to develop high oil per acre canola germplasm with better agronomy adapted to North Dakota.

**Materials and Methods:** A wide diversified Brassica germplasm with both roundup ready and conventional types have obtained from both private and public sectors across the world. Crossing and backcrossing have made between genetically diverse winter type with spring type canola, and spring type with spring type canola. Interspecific crosses were also made among B. napus, B. rapa, B. juncea and B. carinata. A modified pedigree breeding with early generation testing followed by multi-location field trials have been using in this program. A total of 367 diversified B. napus lines have been partially sequenced using GBS pipeline.

**Results:** A high oil variety (NDSU-662c) was released for the 1st time from this program in 2011. Three hybrids with high seed yield and high oil content were identified and are in process to release. Several hundreds of advanced breeding lines have been developed from the program. Canola double haploid production and molecular marker technology are already in place to accelerate the breeding line development program. The breeding program obtained a licensing agreement with INRA, France to utilize Ogura-CMS and restorer (R-2000) system for inbred line development for hybrid production. A collaborative breeding research has established with Monsanto and DL Seeds Inc. Both greenhouse and growth chamber are utilizing to grow canola in controlled environment. The seed quality lab is equipped with near infrared spectrophotometer for seed oil, seed protein and fatty acid profile analysis. Disease screening facilities are available both in greenhouse and in field under artificial inoculation system. The capacity of field plot testing and harvesting program is about 5,000 plots per year at seven trial locations across the state. Off-season (winter) nursery is located in Santiago, Chile that significantly reduces the breeding cycle. All necessary field and lab equipment have been purchased for this program.
Use of *Brassica rapa* L. to increase the genetic diversity in spring canola (*B. napus*)

**Background:** Genetic diversity in Canadian spring *Brassica napus* L. (AACC, 2n = 38) canola need to be broadened (Juska et al. 1997, Rahman 2013). Some efforts have been made to broaden genetic diversity and productivity of spring canola by use of winter type (Kebede et al. 2010, Rahman and Kebede 2012); however, very limited efforts made to utilize genetic diversity of its allied species as interspecific cross often introduce many unwanted alleles and cause meiotic anomalies in segregating population (Falk 2010, Rahman 2013).

**Objective:** To increase the genetic diversity in Canadian canola germplasm and to study the inheritance of glucosinolates content and response to selection for low glucosinolates content in the subsequent progenies. To estimate the genetic diversity in the newly developed *B. napus* lines.

**Method:** A breeding research was undertaken to broaden the genetic base of the Canadian spring *B. napus* canola through introgression of allelic diversity from *B. rapa* (AA, 2n = 20). For this, interspecific hybridization between *B. napus* and *B. rapa* were done and pedigree breeding was applied to develop canola quality euploid *B. napus* lines. Agronomic and seed quality traits, such as silique length, number of seeds per silique and seed glucosinolates content, and ploidy level of the interspecific crosses derived populations was assessed in different generations. Genetic diversity in the population was estimated by SSR molecular markers.

**Results:** Repeated selection for glucosinolates content resulted many low glucosinolates families in advanced generation. Ploidy level of many plants had reached close to the *B. napus* parent. SSR marker analysis revealed that the new interspecific derived families are genetically distinct from *B. napus* parent.

**Conclusion:** Intensive selection cycles for canola quality traits and ploidy level over generations in interspecific crosses progenies can produce desired result. Genetically distinct and fertile *B. napus* type canola quality germplasm was developed from in this experiment from *B. napus* × *B. rapa* F2 derived population.

**References:**


Genotyping-by-Sequencing, DArTseq platform for genome analysis in *Brassica napus*

**Background:** Genotyping-by-sequencing based on genome complexity-reduction methods provides a cheaper alternative for genome analysis. In this study, we report the utilisation of DArTseq™ markers for assessment of genetic diversity, construction of ten linkage maps and a consensus map comprising ~100,000 markers. We will also report marker-trait associations identified using classical QTL and genome-wide association (GWA) approaches.

**Objectives:** To test the usefulness of DArTseq markers for molecular breeding applications in canola.

**Methods:** DArTseq analysis was carried-out as described previously (Raman et al. 2014). Phylogenetic analysis among 219 accessions of *B. napus* and related species was performed using ~25,000 SNPs. A consensus map from ten DH populations originating from Australia, Canada, China and Europe was constructed as described previously (Raman et al. 2013). Mapping populations and a diversity panel comprising 180 lines of *B. napus* were phenotyped for agronomic traits such as resistance to blackleg, NDVI, CID, WSC, and flowering time for QTL and GWA analysis.

**Results:** DArTseq markers discriminated all accessions of *Brassica* into distinct groups representing different species/origin. We constructed the first consensus map consisting of 95,663 markers, comprising mainly DArTseq, and SNP markers present in 60K illumina infinium array. Generally the consensus map was in agreement with component maps of individual populations and the physical map positions of DArTseq markers on the published reference genomes of *B. napus, B. rapa* and *B. oleracea*. Data on trait-marker associations using classical QTL and GWA analyses will be presented. Some of the loci detected with GWA were confirmed by QTL mapping. Putative candidate genes implicated in target traits were identified within up to 40kb genomic regions.

**Conclusions:** DArTseq markers provided a suitable platform for molecular breeding applications such as assessment of genetic diversity, construction of genetic linkage maps, identification of molecular markers associated with traits of interest and delineation of genome structure based on chromosomal ancestral blocks of *Brassicaceae*.

**References:**

Candidate gene based association mapping for introgressed resistance against *Sclerotinia sclerotiorum* in *Brassica juncea*

**Background:** *Sclerotinia* stem rot caused by *Sclerotinia sclerotiorum* is a major threat to Oilseed *Brassica* production systems across the world. Complete resistance against this pathogen has never been reported. We had previously reported success in introgression of the high level of resistance to *Sclerotinia* stem rot in *B. juncea* from wild crucifers (Garg et al. 2010). For present communication we used *B. juncea-fruticulosa* introgression set to carry out candidate gene based association mapping to genetically characterize resistance by focusing on the genes of known function. The introgression set is known to carry varying proportions of genomic segments from *B. fruticulosa*.

**Objectives:** To associate the allelic variation at selected functional candidate loci, with introgressed resistance to sclerotinia stem rot.

**Methods:** A set of 208 BC1S5 genotypes were assessed for stem rot resistance. The resistance responses were evaluated by stem inoculation method described by Buchwaldt et al. (2005). These lines were sorted into five different classes based on stem lesion length. We downloaded the genomic sequences of 14 genes previously reported to be associated sclerotinia resistance in *Arabidopsis*. Sixty primers were designed for 14 candidate genes. These primers were used to amplify genomic region of the candidate gene in an association mapping set (91 genotypes). Proc glimmix in SAS 9.4 (SAS Institute Inc., Cary North Carolina, USA) is used for statistical analysis of phenotypic data. Marker-trait associations were investigated by using P3dem, P3demma models performed in TASSEL V 2.1 (Bradbury et al. 2007).

**Results:** Seven significant marker-trait associations could be recognized at a Bonferroni-corrected threshold (−log 10 (P) > 3.388). Of these two markers, NPR-4_1 (4.413) and CYP450-2_2 (20.532) (common in two models, P3dem, P3demma) were found to be significantly associated with candidate genes NPR and CYP450 that are known for defensive response against sclerotinia stem rot. Other markers MYB-1_3, IGMT5-1_2, CYP450-4_1, PAD3-1_1 and PAD3-2_2 also showed significant associations with sclerotinia resistant MYB domain, IGMT and PAD3 genes.

**Conclusions:** The candidate gene markers associated with sclerotinia stem rot resistance can potentially be exploited by breeders for screening of resistant genotypes, which further will improve the efficiency of MAS programmes and cloning of genes help in trait transfer to commercial mustard genotypes.

**References:**


Development of ultra-high erucic acid 
*Brassica carinata* using interspecific hybridization

**Background:** The competitiveness of Canadian agriculture and related industries depend on Canada’s ability to exploit new opportunities, particularly as they relate to the emerging bio-economy. Crop development and species diversity are an important aspect of the bio-economy, as is maximizing crop value through total crop utilization in the value chain. Ethiopian mustard has long been identified as a viable industrial oil crop for the hotter and drier regions of western Canada. Also, owing to its inherent mid to high erucic acid content it is an ideal candidate to be a superior feed stock for high value bio-products such as bio-fuels and lubricants.

**Objectives:** To develop an ultra-high erucic *B. carinata* strain

**Methods:** During the development of canola quality *B. juncea* a FAD2B mutant was identified which had high levels of oleic acid. This mutation was subsequently introgressed into *Brassica carinata*. The breeding line VR10-183 was developed as a stable FAD2B mutant after several backcrosses. Work to further increase erucic acid levels was undertaken via a second round of interspecific hybridization using *B. rapa* spp. yellow sarson cv. R500 (C22:1 content >50%) and the FAD2B mutant. The breeding line VR13-156 was identified after several backcross generations. It was field tested in 2014. Molecular markers were developed to aid in the selection process.

**Results:** The FAD2B *B. carinata* line VR10-183 contains 53% erucic acid. The FAE gene was isolated from R500 and compared to the FAE alleles in VR10-183. A CAPS marker was developed for screening purposes to ensure that all three copies of FAE were maintained during the crossing scheme. Subsequently VR13-156 was identified as being homozygous for both the FAD2B and R500 FAE alleles. The C22:1 content of greenhouse produced seed was 57.5%. Progeny from this material was tested in the field in 2014 with an average erucic acid content of 58.25%. A KASP marker was developed to aid in the introgression of the two genes into more genetically diverse backgrounds.

**Conclusions:** The successful development of an ultra-high erucic acid *B. carinata* was accomplished by interspecific hybridization and the successful transfer of the FAD2B and FAE alleles. A *B. carinata* line with high seed oil, early maturity and high seed yield will ensure that this species becomes the crop of choice for industrial oil applications.
Comparative transcript profiling of the bilocular and triolocular shoot apical meristems and ovarys in *Brassica juncea* L.

**Backgrounds:** Compared with bilocular plants, multilocar ones shown a higher yield per plant attributed by the increase in the number of seeds per siliqua. In recent years, some genetic analysis works in *Brassica juncea* have significantly widen our knowledge about the trait. However, the genes related to multilocular trait have not been cloned and the molecular basis of the locule number determination of *B. juncea* still remained unknown. The plant materials used in this study were BC5 and BC6 populations constructed by two *Brassica juncea* lines, J163-4 with triloculus and J248 with bilocular.

**Objectives:** The aim of this work was to identify the differences between the bilocular and triolocular shoot apical meristem (SAM) and ovary of *Brassica juncea* L. at the transcriptional level, and find out the different genes involved and their related functions. These results might be helpful to understand the molecular basis of the locule number determination.

**Methods:** Bilocular and trilocular SAM of BC5 populations and ovary of BC6 were used in this study. After the work of RNA isolation and purification, cDNA library construction, the libraries were paired-end sequenced with 100 bp on the Illumina HiSeq 2000. Clean reads were de novo assembled into transcripts by Velvet/Oases. Functional annotation of transcripts was performed using BLASTX searches against the *Arabidopsis*, KEGG, eggNOG databases and the non-redundant unigenes were obtained (E-value < 1e-5). The GO annotations for the unigenes were determined and the differentially expressed unigenes were obtained. The Real-time quantitative PCR verification was employed to verify these unigenes.

**Results:** A total of 139,339,803 sequences were successfully obtained and assembled into 168,783 transcripts, which composed the transcriptomes of the SAM and ovary. After functional annotation, 23,412 unigenes were got. A total of 825 and 709 unigenes were obtained with significant expression difference for SAM and ovary, separately. In SAM, these expression different unigenes were mainly enriched in metabolism, membrane transport, signal transduction pathways, while these unigenes in ovary were mainly enriched in energy metabolism, genetic information processing, cell growth death and some organismal pathways. Some unigenes involved in SAM homeostasis, phytohormone responses and signal transduction were significantly differently expressed.

**Conclusions:** These results showed that phytohormone responses and signal transduction may play a part in trilocular siliqua formation. In SAM, IAA19 and SAUR42 were significantly down expressed in triloclar sample and ARR16 up regulated. In our results, CLV3 and ROP9 were up regulated significantly in SAM.

**References:**

14TH INTERNATIONAL RAPESEED CONGRESS | ABSTRACTS | 273
Tribenuron-methyl induced male sterility resulted from anther-specific acetolactate synthase inhibition in *Brassica napus*

**Background:** Male sterility induced by chemical hybridizing agents (CHA) is a potent tool for utilizing crop heterosis. Sulfonylurea herbicide tribenuron-methyl (TM) inhibits branched-chain amino acid (BCAA) biosynthesis in plants by targeting acetolactate synthase (ALS) (Binder, et al. 2007), and low dose TM has been widely used as an effective CHA in rapeseed (*Brassica napus*) (Yu et al., 2006).

**Objectives:** Although TM-induced male sterility is wildly used for utilization of heterosis, the molecular basis of this trait remains unknown.

**Methods:** Targeted expression of csr1-1 (McCourt et al., 2006) and CYP81A6 (Liu et al., 2012), TM daubing in special branches, and silencing of genes in BCAA biosynthesis pathway ALS and KARI (Binder, et al. 2007) were executed. Several previously defined promoters were used, including Elongation factor-1α, 3SS, APETAL3, SUCCO TRANSPORTER 2, LIGHT-HARVESTING COMPLEX B2.1, and ABORTED MICROSPOR. At least 10 independent transformed lines were generated for each construct, and the pollen viability of rapeseed treated or control were analyzed by aceto-carmine staining.

**Results:** Both over expression and anther-specific expression of target-site resistance gene csr1-1 reversed the TM-induced male sterile phenotype in rapeseed. Targeted expression of csr1-1 in vegetative tissues or the early stages of stamen and petal development remained show male sterile phenotype after TM treatment. Sterile anthers only occurred when the side branches daubed with TM or in all higher branches above main stem daubed with TM, while the rest of the branches were fertile. The male fertile phenotype of lines expressing metabolism-based resistance gene CYP81A6 in mesophyll, phloem or vegetable tissues, and TM daub-stem experiment uncovered evidence that TM was mainly polar-transported from leaves to anthers through mesophyll and phloem. The percentages of BCAAs were significantly decreased after CHA TM spraying. Silencing of genes in BCAA biosynthetic pathway also led to low male fertility.

**Conclusions:** We reported a polar transportation of TM and anther-specific inhibition of ALS based mechanism to understand the selective male sterility in TM-induced rapeseed. As sterile anthers could be induced in special branches by daubing TM to selected branches, the costly and time-consuming manual emasculation in near-isogenic lines and construction of hybrid combinations would be replaced by daubing stem with TM.

**References:**

Fine mapping of the trilocular gene \textit{Bjmc1} and identifying the candidate gene in \textit{B. juncea}

\textbf{Background:} In rapeseed breeding, the most important goal is to develop cultivars with high yields. Previous studies have proved that multilocular rapeseed plants generally have higher yield than bilocular plants (Bechyné, 1995; Katiyar et al. 1998; Lv et al. 2012).

\textbf{Objectives:} In this study, we aim to investigate the inheritance of a landrace with trilocular siliques in \textit{Brassica juncea} in China, and to clone the trilocular gene \textit{Bjmc1}.

\textbf{Methods:} Based on several populations described by Xu et al (2014), BSA method were used to developed SSR and AFLP markers, and BAC clones of the purple-leaf mustard were used to develop SSR and SCAR markers. Finally, a large population (9300 individuals) of \textit{Bjmc1} constructed in BC4, BC5 and BC6 population was used to construct the map of \textit{Bjmc1} gene.

\textbf{Results:} The result showed that the trilocular trait was controlled by two independent recessive nuclear genes, \textit{Bjmc1} and \textit{Bjmc2}. \textit{Bjmc1} was preliminarily mapped by 24 AFLP markers and 7 SSR markers. Five AFLP markers linked to the target gene were successfully converted into SCAR markers (Xu et al, 2014). In order to fine mapping the \textit{Bjmc1} gene, the BAC library of purple leaf mustard (provided by Prof. Zhongsong Liu) was screened, and the positive clones were sequenced. According to the sequence, a SCAR marker (SC40) and two SSR markers (SR52 and SR151) were developed further. All the molecular markers that we have identified were used to screen the large population (9300 individuals). We found that EC14MC14 and SR151 were the closest markers in the map, and the genetic distance of these two genes were 1.1 and 0.04 cm respectively. In addition, the result indicated that SC40 was co-segregated with \textit{Bjmc1} gene. We sequenced the open reading frames (ORFs) around the SC40 ranging of 75kb in bilocular and trilocular plants respectively. Interestingly, no sequence difference was found in all the ORFs except the ORF40.

\textbf{Conclusions:} Since only the ORF40 showed sequence difference between the bilocular plants and trilocular plants and the homologous gene mutant of ORF40 in \textit{Arabidopsis} also exhibited multilocular trait, we predicted that the ORF40 was the candidate gene of \textit{Bjmc1}. And now the genetic complementation experiment is performing to test the candidate gene.

\textbf{References:}
Differential expression of small RNAs in the shoot apical meristem regulate the plant architecture in *Brassica napus*

**Background:** Increasing crop yield and mechanized harvesting are major challenges for modern agriculture. Plant architecture is an important agronomic trait, strongly influencing the suitability of a plant for cultivation, its yield and the efficiency with which it can be harvested (Reinhardt and Kuhlemeier, 2002). MicroRNAs (miRNAs) are endogenous small RNAs that play crucial regulatory roles in various developmental processes (Bartel, 2004). The molecular genetic bases of plant architecture focused on the activity of its shoot apical meristem (SAM), and initiates outgrowth of axillary meristem (AM), when AMs start to develop lateral organ branches, and correct timing for reproduction and senescence (Li, 2008). Although several genes have been found to regulate these traits, there is a lack of information on the expression profile of miRNAs regulate plant architecture in the oil crop *Brassica napus*.

**Objectives:** Desirable plant architecture greatly improve yield. The purpose of this research is to find miRNAs that regulate the development of SAM, and discovery some new miRNAs that have not been reported in *Brassica napus*.

**Methods:** A near-isogenic line of plant architecture was constructed. Squaring shoots from the normal plant architecture (Normal) and the mutant rod-like plant architecture (Rodlike) were sampled, respectively. Then two small RNA libraries and their corresponding degradome libraries were constructed and sequencing in Beijing Genomics Institute (BGI, Shenzhen, China). The bioinformatics analysis of sequencing data were analyzed as previously described. The stem-loop RT-PCR method was used in the quantitative RT-PCR experiments of miRNAs. To examine the miRNA-directed cleavage of their predicted targets in vivo, the RLM-5' RACE was used to find the cleavage site.

**Results:** Small RNA sequencing identified 108 known *Brassica* miRNAs and 261 novel miRNAs, including 53 novel miRNAs that were highly homologous to other plant species. To our surprise, only six known miRNA families were found to be differentially expressed, but more than 130 novel miRNAs were identified differentially expressed and most of them just expressed in one of the samples. In addition, a total of 258 transcripts in Normal and 239 transcripts in Rodlike were found to be targets identified through degradome sequencing. Analysis of correlated expression between differentially expressed miRNAs and their targets demonstrated that plant hormone signal transduction and many transcription factors cooperate to balance the stem cell maintenance and differentiation.

**Conclusions:** Our approach identified potential key regulators of miRNAs in the SAM of *Brassica napus* during shoot development. The results provide novel insight into the regulatory roles of miRNAs related to *Brassica napus* plant architecture. The discovery of novel miRNAs will sever as a foundation for further research in *Brassica napus* miRNAs.

**References:**
Characterizing growth physiology, flowering phenology, yield components and seed quality attributes in founder lines of a spring *Brassica napus* Nested Association Mapping population

**Background:** The Nested Association Mapping (NAM) strategy facilitates the dissection of complex traits important for crop improvement (McMullen et al, 2009). Recent field and greenhouse studies have focused on describing physiological and seed quality characteristics of the founder and reference lines from an annual *Brassica napus* L. NAM population. Both above-and below-ground growth characteristics were measured to characterize growth physiology, flowering and maturation phenology, yield attributes, non-target metabolite profiles of leaves and seeds and seed quality variation. Results of this study will also establish the links between phenotypes and molecular variants to accelerate genetic improvement of spring canola through association mapping.

**Objectives:** The objectives of the study were to: i) examine if differences between lines exist for photosynthetic assimilation rate (A), stomatal conductance (gs) and water-use efficiency (WUE) ii) to study the extent of plasticity in flowering phenology iii) to profile non-target metabolites of foliar and seed components, iv) to investigate the differences in root architecture that may exist among spring *B. napus* founder lines and v) to compare the results obtained from the greenhouse study with field evaluation of founder lines in 2014.

**Methods:** A total of 61 lines that included 50 NAM founder lines and the reference line, four additional diverse lines, historic commercial cultivars Tower, Westar and Midas and three representative commercial lines were used in this study. Four replications of the plants, grown under standard greenhouse conditions, were characterized throughout development for several growth physiology, flowering and yield-related traits. Chlorophyll content was measured using SPAD-502 chlorophyll meter, stomatal conductance using a leaf porometer, leaf area using a Licor LI-3100. Root systems were examined for root architectural traits. In the 2014 field season, four replications of the lines were grown at two locations and characterized for a range of plant, canopy and seed related characteristics.

**Results:** The results obtained from the 2014 field experiment demonstrated significant phenotypic variation for growth and developmental and seed quality traits among the lines. The present study further quantified this variation and explained the extent of genetic variability existing among the founder and reference lines in growth physiology, flowering phenology and yield related attributes.

**Conclusions:** The results from this study will be used in canola breeding programs to develop cultivars with improved agronomic and nutritional characteristics with the ultimate goal being identification of yield-stability related traits under variable environmental conditions. These results will be used to target phenotypic traits for study within the NAM population where the underlying components of the traits will be dissected and associated with genomic regions, identifying markers linked with economically important traits.

**References:**

An *Arabidopsis* gene (*AtGRP1*) increases seed yield when overexpressed in *Brassica napus*

A gene encoding a protein (GRP1) of unknown function was isolated from *Arabidopsis* and characterized. Overexpression of *AtGRP1* (35S::*AtGRP1*) in Arabidopsis resulted in plants with increased number of and taller racemes than the wild type and resulted in increased seed yield. An *AtGRP1*-YFP fusion introduced into tobacco protoplasts suggested that *AtGRP1* was located in the nucleus. Expression of the 1.9 kb *AtGRP1* promoter fused to GUS in *Arabidopsis* indicated that the gene was expressed strongly in vascular elements of leaves, stems, floral tissues and siliques but not in seeds. Generation of homozygous lines knocked out for *AtGRP1* was unsuccessful suggesting that *AtGRP1* may be essential for plant development. DNA sequences orthologous to *AtGRP1* were not detected in *B. rapa* or *B. napus* genome sequences so 35S::*AtGRP1* was introduced into *B. napus* cv Westar in order to assess if an observable phenotype similar to *GRP1* overexpression in Arabidopsis could be generated and to provide insights into the role of *AtGRP1* in plant related processes. Homozygous lines of transgenic *B. napus* overexpressing *AtGRP1* indicated an increased proliferation of racemes than the wild type and under strictly controlled growth cabinet conditions yielded 15-20% more seed than the wild type. Elucidation of the role of *AtGRP1* in plant development is in progress.

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Development of genic microsatellite markers and analysis of genetic variability in *Brassica juncea* L.

**Background:** *Brassica juncea* L. is the second major oilseed crop in India. Since the turn of the last century more than 150 high yielding varieties have been released. They possess a great deal of similarity with respect to morphological descriptors available for their identification and differentiation. Therefore, unequivocal identification of increasing number of varieties enforces to look for alternatives. We report the development of a comprehensive set of genic microsatellite (SSR) markers through deep transcriptome sequencing, and evaluation of its efficacy in revealing genetic variability in *B. juncea* varieties.

**Objective:** Development of a comprehensive set of genic SSR markers in *B. juncea*.

**Methods:** Total RNA isolated from *B. juncea* cv. CS-52 was used for preparation of pair end cDNA sequencing library. Expressed sequence reads were generated by whole transcriptome sequencing on Illumina MiSeq platform. High quality filtered sequence reads were assembled using CLC Genomics Workbench 7.5. The unigenes were used for mining genic SSR markers, and their primers were designed using BatchPrimer3 v1.0 software. Genic SSR loci with SSR lengths ≥ 18 bp were tested for amplification using genomic DNA from *B. juncea* cv. CS-52. The optimized SSR primers were then used for PCR amplification in nine *B. juncea* genotypes of diverse nature. The genotype profiles produced by SSR markers were used for calculating PIC values (Botstein et al. 1980). Thirty highly polymorphic SSR markers were used for analysis of genetic variability in 70 *B. juncea* varieties. The genetic similarity was estimated based on Jaccard’s similarity coefficient. All the 70 *B. juncea* varieties were clustered with the UPGMA analysis and SAHN procedure of the NTSYS-PC v2.10t (Rolf 2000).

**Results:** The Illumina MiSeq sequencing generated 47962057 expressed sequence reads. These reads were assembled into 45,280 unigene contigs from which a total of 4,100 SSR loci were identified. Trinucleotide was the most common repeat unit with a frequency of 59.88% followed by di- (38.73%), tetra - (0.71%), hexa - (0.44%) and pentanucleotide repeats (0.2%). PCR primers were designed from the unique sequences flanking 3,141 SSR loci out of which 460 were selected for primer synthesis. A total of 340 genic SSR loci amplified successfully of which 42.6% exhibited polymorphism among nine *B. juncea* genotypes with PIC values ranging from 0.18 to 0.81. Thirty highly polymorphic markers, selected on the basis of PIC values, were used for analysis of genetic variability in 70 commercial varieties of *B. juncea*. The dendrogram obtained by the UPGMA analysis clearly distinguished them in definitive groups corresponding well with their published pedigree.

**Conclusions:** We developed 4,100 genic SSR markers in *B. juncea* by whole transcriptome sequencing. Out of this 340 were validated using a set of nine diverse *B. juncea* genotypes. Genetic variability study among 70 commercial *B. juncea* varieties using 30 highly polymorphic markers selected from the 340 validated markers effectively distinguished even the closely related cultivars.

**References:**
Evaluation, characterization and classification of toria germplasm

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Background: Toria (Brassica rapa (L.) var. toria) holds promise among all the Brassicas due to its significance as a valuable donor source for high temperature stress at seedling stage. This crop is gaining importance globally due to its advantages over other oilseeds, viz., higher yield potential, low moisture requirement, higher return at low cost of production, wider adaptability for various farming conditions, etc., which is important towards having the next yellow revolution. Narrow genetic base of the crop for component traits is the main constraint for the cultivation of this crop.

Objectives: Evaluation of phenotypic diversity usually reveals key traits of interest to the plant breeder. Broadening of genetic base through utilization of diverse genetic resources and relevance of broad genetic base in evolving new cultivars by incorporating new genes in the existing ones is quite vital.

Methods: Agronomic characterization continues to be a useful tool for the classification of germplasm, as it allows plant breeders to select valuable genetic resources to be utilized later in different breeding programmes. Ninety two germplasm accessions of toria collected from different sources were grown evaluated for eight quantitative variables and 14 qualitative traits by giving scores in accordance with the standard DUS descriptor.

Results: Among all the 92 accessions studied, 76 lines were observed to have medium number of lobes (3-5) in the leaf except 16 lines which had many lobes (>5). Majority of the germplasm (79 lines) had narrow leaves (<7.0 cm). A total of 52 lines were early in flowering (31-40 days) whereas, all the germplasm was medium in maturity (81-100 days). On the basis of branches, most of the lines (89) were classified under intermediate category (8-14 branches). Four accessions were classified as short (<51 cm) on the basis of height, whereas, 11 lines were characterized as tall (76-100 cm). Half of the accessions were having medium main shoot length (41-60 cm) and siliqua density was found high in 48 lines. Moreover, most of the genotypes possessed short siliqua length (<4.5 cm). A large number of rapeseed mustard germplasm was also evaluated and characterized for various agro-morphological traits and biotic stresses by Misra et al. (2004) and Misra and Kumar (2009). In the present study, 12 lines were having few no. of seeds/ siliqua (<11), 62 lines recorded intermediate no. of seeds/ siliqua (11-20) and 18 lines exhibited many seeds/siliqua (>20). Medium seed size (3.0-4.0 g) was most common and observed in 52 lines. Seed yield/ plant grouped all the germplasm into 2 categories viz. Low (<10.0 g) and medium (10.0-20.0 g) of which 32 lines were grouped into low and remaining 60 in medium. On the basis of oil content, majority of the lines (65 lines) were characterized into medium (38-42 %) and the remaining 27 lines were having high oil content (43-46 %). Zada et al. (2013) also reported sufficient genetic variation in 134 germplasm collections of Ethiopian mustard on the basis of characterization for 33 agro-morphological characters ranging from seedling emergence to crop maturity.

Qualitative traits like leaf hairiness, leaf color and dentation of leaf margin were examined 45 days after sowing. The difference for hairiness grouped all the accessions into 3 categories (dense, sparse and absent). Leaf colour of more than 40% genotypes was dark and purple green, whereas 17 lines were having purple leaves. Lyrate dentation of leaf margin was the most common followed by auriculate type. Yellow and light yellow colour was most dominant for petal, whereas 5 lines had white petal colour. On the basis of siliqua surface texture all the genotypes were classified into three categories viz. intermediate (53 lines), constricted (28 lines) whereas, 11 lines had smooth siliqua surface texture. Semi-appressed siliqua angle with main shoot was most common (44 lines) followed by open (30) and appressed type (18 lines). Seed coat colour divided all the germplasm into five categories (yellow in 2 lines, dull grey in 16, reddish-brown in 37 lines, brown in 28 lines and black in 9 lines).

Conclusions: The characterization of germplasm will provide valuable information for strengthening of future breeding programme on toria. It will be helpful in formation of data base and reference lines/core collection for further use.

References:
Genetic enhancement of *Brassica carinata* through interspecific hybridization and population improvement

**Background:** *Brassica carinata* originated in highlands of Ethiopia is a natural allopolyploid of *B. oleracea* and *B. nigra*. It has been found to be a potential oilseed crop in different agroecological conditions due to its inherent properties (Malik 1990, Rakow and Getinet 1998). However, its cultivation has not been commercially feasible in India due to long maturity duration, tall plant stature and low oil content in comparison to widely grown *Brassica juncea*. It would be desirable to reshape the existing genotypes through introgression of earliness, short plant height and high oil content from *Brassica juncea*.

**Objectives:** to develop *Brassica carinata* genotypes which may be acclimatized in Indian agroecological situations through introgression of earliness, short plant height, bold seed size and high oil content from *B. juncea* with desirable traits of *Brassica carinata* (profused branching, more siliquae and tolerance/resistance against abiotic and biotic stresses)

**Methods:** Two approaches; interspecific hybridization and population improvement were followed to enrich the *B. carinata* gene pool. In interspecific hybridization; Pusa Swarnim (a variety of *B. carinata*) was crossed with Indian mustard variety ‘NRCHB 101’. Resulting F1 (ABBC) were grown and diploidization of genome was realized through colchicines treatment. Subsequent generations (F2-F5) were advanced through pedigree selection. In population improvement; one *B. carinata* population “MCB 1” was grown in vicinity to *Brassica juncea* gene pool and open pollination was allowed. Seed set on *B. carinata* genotype was harvested and random bulk was drawn to raise next population in vicinity of *Brassica juncea* gene pool. Three cycles were repeated and subsequent generations were advanced through pedigree selection. Developed genotypes of *Brassica carinata* and *Brassica juncea* were evaluated alongwith Pusa Swarnim and NRCHB 101 of *B. carinata* and *B. juncea*, respectively, as check cultivars. Observations were recorded on days to flower initiation, days to maturity, plant height, siliquae per plant, 1000-seed weight, shoot tip siliqua bearing, seed yield and oil content.

**Results:** Developed *Brassica carinata* genotypes exhibited genetic gain of -15, -30, 26, 2.5, and 50 percent for maturity duration, plant height, seed weight, oil content and seed yield, respectively, over check variety (Pusa Swarnim).

**Conclusions:** Developed *B. carinata* genotypes possess desirable traits of both species; *B. carinata* (tolerance against abiotic and biotic stresses, profused branching, shoot tip siliqua bearing and more siliquae) and *B. juncea* (earliness, high oil content, bold seed size, short plant height). High genetic gain for seed yield could be realized due to significant reduction in maturity duration making it acclimatized in prevalent conditions of high temperature at maturity.

**References:**

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The use of a new gene pool for obtaining forms of winter oilseed rape (*Brassica napus L.*) with changed quality characteristics

**Background:** Due to the non-food use of rapeseed oil, high oleic and low linolenic (HOLL) genotypes are under development and investigation worldwide in addition to obtaining breeding forms without antinutritive alkenyl glucosinolates. Allele-specific functional markers are very useful for selection of new forms with changed seed oil fatty acid composition by making the process more time- and cost-effective.

**Objectives:** The aim of this work was to develop new mutant oilseed rape HOLL genotypes with changed composition of the mono- and polyunsaturated C18 fatty acid in seed oil and characterized by high seed oil content and extremely low glucosinolate content in seed protein accompanied by MAS for detecting the wild-type and mutated alleles of FAD2 and FAD3 desaturase genes.

**Methods:** The plant material used in this study comprised the ho and ll oilseed rape mutant lines developed at the PBAI-NRI, Poznan, Poland (Spasibionek, 2006) as well as HO x LL recombinants and the selected lines with high seed oil and low alkenyl glucosinolate content. Total oil content was measured in whole dried seeds by NMR, whereas seed oil composition and glucosinolate content were determined by gas chromatography. Genomic DNA was extracted from young leaves using the Doyle method; allelic variants of FAD2 and FAD3 desaturase genes were determined by specific functional CAPS markers (Falentin et al., 2007) and by SNaPshot analysis (Mikolajczyk et al., 2010), respectively.

**Results:** New genotypes of oilseed rape were developed: HO-type, characterized by up to 82% of oleic acid in seed oil as well as HOLL recombinants with 81% of oleic acid and 1.9% of linolenic acid. Molecular analyses confirmed the presence of homozygous mutated alleles of fad2 and fad3 genes responsible for the oleic and linolenic acid content in seed oil of the obtained rapeseed lines. New lines of high, up to 53%, oil content as well as low alkenyl glucosinolate content – up to 0.2 μM g⁻¹ of seeds and low total glucosinolate – up to 2.0 μM g⁻¹ were selected.

**Conclusions:** The obtained lines make a valuable plant material for further study and field experiments. The new HO mutants and HOLL recombinants have been introduced into hybrid breeding to improve their agronomical value, whereas the selected forms with high oil content have been crossed with other valuable sources of genetic variability among winter oilseed rape cultivars.

**References:**


Association of domestication related genes with phenological periodicities in the available germplasm constellations of Brassica juncea and its diploid progenitors

**Background:** B. juncea (2n=36; AAbb) is an amphidiploid that arose from multiple natural hybridization events between B. rapa (2n = 20, AA) and B. nigra (2n = 16, BB). The crop was first domesticated as a vegetable crop and only later as an oilseed crop. Domestication related directional selection for specific alleles is known to occur at genes which control the major adaptation traits like initiation and duration flowering. These results in genetic bottlenecks in the genes and genomic regions subject to natural selection compared to the unselected ones. We have previously discovered evidence for selective sweep in flowering and shattering related loci (FLC, FT and SHP) in multiple populations of B. juncea and its diploid donors using the FST-based method (Kaur et al. unpublished).

**Objectives:** To study the association of the genes/genomic regions responsible for adaptation to domestication with the phenological periodicities in B. juncea and diploid progenitor species.

**Methods:** A worldwide germplasm collection comprising land races, historical and modern cultivars of B. juncea (88), B. rapa (83) and B. nigra (19) formed the basic germplasm. Data were recorded for initiation of flowering and completion of flowering. DNA markers were developed from the gene sequence and the genomic region around flowering associated genes (Flc and Ft). These markers were used to genotype entire germplasm. Association analysis was then carried out using software Tassel to document association between the phenotype and the molecular markers. Unique amplified products were also sequenced to understand allelic variation at a given genomic region.

**Results:** In general B. rapa germplasm was earliest to bloom and complete flowering. B. juncea behaved more like B. rapa. B. nigra was not only late to flower and also continued to flower for a longer duration than both the B. rapa and B. juncea. Polymorphism generated by gene-based microsatellite markers helped to identify selective sweeps for the target genes in the three test species. Selective sweeps were primarily recognizable in a very small genomic region around the gene under selection. Analysis of these showed differential responses to the directional selection for flowering habit. The associations between the genomic region and flowering phenotypes differed across B. juncea and parental diploids. To some extent, same was true for comparisons in multiple populations with in a same species.

**Conclusions:** B. juncea and its progenitor species showed differential responses to domestication related selection pressure for the same target trait.
Copy number variation generated by homeologous rearrangement creates phenotypic diversity

**Background:** Polyploidisation and subsequent diploidisation as a means of genome stabilization are acknowledged mechanisms in plant speciation. Rapeseed as an example for an amphidiploid crop has undergone several rounds of genome duplication, and genome stabilization is an ongoing process ever since. In the EraCAPS project "Evo-Genapus: Evolution of genomes: structure-function relationships in the polyploid crop species *Brassica napus*" we are investigating the influence of genome rearrangements on selection for adaptive and agronomic traits.

**Objectives:** We hypothesize that the genome restructuring observed in resynthesised *B. napus* represents an accelerated form of genome evolution that is ongoing in naturally derived, cultivated *B. napus*. We are testing this hypothesis on a genome-wide scale by investigating mapping populations derived from crosses between a winter oilseed rape inbred line variety and three different resynthesized rapeseed accessions. Observed structural genome variation related to trait variation of relevance for rapeseed as a crop will improve our general understanding of functional genome evolution in polyploid crops.

**Methods:** In this subproject we are quantifying different mechanisms of genomic changes, such as homeologous chromosome exchanges, to identify specific examples demonstrating the influence of genomic rearrangements on phenotypic variation. Comparative mapping of IlluminaTM *Brassica* 60k SNP markers and a large set of phenotypic data will enable us to associate phenotypic variation with the genomic constitution of the mapping parents. Whole-genome resequencing of the mapping parents allows us to calculate copy number variation along the genome and differentiate segments of altered copy number in comparison to the assembled genome of the European winter oilseed rape cultivar ‘Darmor-bzh’. These segments represent candidates for insertions/deletions and will be investigated in detail.

**Results:** As an example, homeologous rearrangements are common between chromosomes A9 and C8. We will present preliminary data suggesting that important seed quality QTL on chromosome A9 result from homeologous rearrangements that generated interesting variation for selection and breeding. We expect that knowledge about copy number variation and rearrangements will help to improve and focus selection processes in breeding for yield and quality traits in modern rapeseed lines.
Asexual propagation of the sterile plant from the recessive genic male sterility and utilization for production of the fully sterile line

**Background:** The three-line system of the recessive genic male sterility (RGMS) is one of very important ways for utilization of heterosis in oilseed rape (Brassica napus) in China. Compared with CMS, it is more difficult to produce the fully sterile line (the 100% sterile line), because 50% fertile plants from the homozygous two-type line need be thoroughly removed, and 50% sterile plants need be reserved to produce the fully sterile line. This step would result in reduction of output and lower physical purity. In order to overcome the questions, the study hoped that a large number of the sterile plants were obtained to produce the fully sterile line by tissue culture instead of by the homozygous two-type line of RGMS.

**Objectives:** The homozygous two-type line “20118Ab” of RGMS and the temporary maintainer line were used in this study. The homozygous two-type line “20118AB” is including 50% fertile plants (Genotype: AabbRfrf) and 50% sterile plants (Genotype: aabbRfrf). The genotype of the temporary maintainer line is aabbRfrf.

**Methods:** The fertility segregation of the homozygous two-type line “20118AB” and the offspring derived from sib-crossing and test-crossing with the temporary maintainer line were investigated in order to identify the genotype of the sterile plants “20118A”. Young branches were used as the materials for tissue culture from the sterile plants with the genotype aabbRfrf.

**Results:** The young branches were thoroughly sterilized and placed on the B5 medium with 3.0 mg/l 6-BA and 0.5 mg/l NAA. After about 30 days, the shoots would come out from the petiole or callus tissue. The new shoots could be inoculated into the new B5 medium with 1.0 mg/l 6-BA and 0.5 mg/l NAA for asexually propagation. The reproductive cycle is 2 weeks, and the coefficient is 6-8 times.

**Conclusions:** The oilseed rape has high propagation coefficient, so it is feasible to use asexual propagation for production of the fully sterile line. This way could simplify the steps and significantly increase production. The purity of the fully sterile line is up to 99%, so the article plants need no longer remove in production of hybrid seed. In conclusion, this study was very practical for production of GMS hybrid seed.

**References:**

Seed quality development in *Brassica napus*

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**Background:** *Brassica napus* L. is an intensively bred and highly valuable crop in Canada's agricultural industry. A significant problem with *B. napus* breeding programs is the limited genetic diversity available. Therefore, great effort is required to continue the growth of the Canadian canola industry by utilizing diverse germplasm within a breeding program. A set of 314 *B. napus* lines derived from re-synthesis between *B. rapa* and *B. oleracea*, in conjunction with ethyl methanesulfonate (EMS) mutagenesis and doubled haploid methodologies, has developed genotypes with increased diversity for seed quality. The discovery of modified seed quality provides new genetic sources for introgression into future breeding lines and cultivars.

**Objectives:** The objectives of this research are to phenotypically and genotypically analyze a diverse set of *B. napus* breeding lines for oil content, seed protein content, fatty acid content, and amino acid composition.

**Methods:** Phenotypic analysis was conducted on agronomic traits in 2014 field trials. Additionally, seed quality was analyzed using near-infrared spectroscopy and gas chromatography. Amino acid analysis will also be performed to reveal amino acid composition profiles. Genotyping will be performed using multiple markers on an ABI genetic analyzer along with genotype-by-sequencing (GBS).

**Results:** Phenotypic data has shown diversity for seed quality, including oil content (34 to 52%), seed protein content (23 to greater than 30%), and diverse fatty acid profiles. Populations will be analyzed using over 200 molecular markers and amino acid profiles will also be discussed.

**Conclusions:** Comparison of the phenotypic data with the genotypic data has helped to identify the genomic regions associated with these diverse seed quality traits. This data will be useful in QTL analysis and aid in further efforts to diversify *B. napus*.

**References:**

Introgression of resynthesized *Brassica napus* L. into parental lines of winter oilseed rape F1 hybrids

**Background:** Incorporation of resynthesized, (RS), *Brassica napus* L. genotypes in the breeding programs can be used to increase the variation in double low oilseed rape gene pool. But the RS lines of oilseed rape reveal poor performance for many agronomic traits and shows inferior seed yield as compared to current breeding material. However, after the crossing of resynthesized *Brassica napus* with 00-quality oilseed rape and then androgenesis in vitro of obtained F1 hybrid, it should be possible to select doubled haploids (DH) with improved seed yield and seed quality as well as significant genetic distance to present cultivars.

**Objectives and methods:** Until now resynthesized oilseed rape was obtained as a result of reciprocal crosses between three subspecies of Brassica rapa and two subspecies of *Brassica oleracea* using embryo rescue technique (Sosnowska, Cegielska-Taras 2014).

**Results:** As expected, the RS lines obtained in the study have been characterized by a high content of erucic acid in oil and glucosinolates in seeds. Several resynthesized oilseed rape lines were crossed with lines of double low quality winter oilseed rape. Populations of large number of androgenic plants semi-resynthesized (semi-RS) were developed from F1 hybrids using isolated microspore in vitro culture method. The seeds of all obtained semi-RS DH lines were analyzed biochemically with regard to 00-quality of seeds. Among the population of the semi-RS DH lines of winter oilseed rape with zero erucic acid content and low amount of glucosinolates were selected. Moreover, in this study the genetic similarity among some resynthesized lines, *B. oleracea* and *B. rapa* as parents and collection of winter oilseed rape genotypes was evaluated using AFLP markers.

**Conclusions:** Four selected semi-RS DH lines were characterized by either the zero erucic acid content in oil and glucosinolate content lower than 15 μmol g⁻¹ seeds. The dendrogram based on AFLP markers indicated that as well RS lines as semi-RS lines created distant group among studied 101 parental lines of winter oilseed rape F1 hybrids.

**References:**


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Agronomic and seed quality traits of DH populations of winter oilseed rape obtained from reciprocal crosses between black- and yellow-seeded DH line

**Background:** Oilseed rape is very important source of high nutritional quality oil with a balanced fatty acid composition, a valuable material for many industrial branches and a protein-rich meal for livestock feed. The yellow-seeded oilseed rape has thinner seed coat and thus higher oil and protein content as well as lower fibre content than black-seeded types. However, the introduction of this trait to oilseed rape involves reduction in yield and lowering of agronomic performance. Genetic information about the gene expression of quantitative traits can help to create an effective breeding strategy to develop new varieties.

**Objectives:** In the present study, the influence of maternal effects were evaluated on the basis of differences between the two DH line populations HZ and ZH obtained from reciprocal crosses between black-seeded DH H2-26 and yellow-seeded DH Z-114, derived from natural mutant with bright seeds and spring line of B. napus with segregating seed colour (Bartkowiak-Broda et al. 2009). The population, marked as HZ, consisted of 27 DH lines, derived from F1 hybrid DH H2-26 × DH Z-114 and the population marked as ZH consisted of 30 DH lines, derived from F1 hybrid DH Z-114 × DH H2-26.

**Methods:** Field experiments in a randomized complete block with four replications design were conducted in two seasons. Seed colour was determined with spectrophotometer Color Flex on the scale from 0 (black) to 5 (yellow). The experimental data were analyzed with uni- and multivariate statistical methods.

**Results:** Parental forms differed significantly in terms of seed yield, number of seeds per pod, thousand seed weight, protein, neutral detergent fibre (NDF), acid detergent fibre (ADF) and glucosinolate content, and seed colour. However, the maternal effects were revealed in DH line populations only for the thousand seed weight. In contrast, the influences of the paternal form were found on content of neutral and acid fibre and seed colour. The direction of crossing played a role in the frequency of the occurrence of transgression effects, and this was particularly in protein, NDF and ADF content. Positive transgression effects for protein content and negative transgression effects for NDF and ADF content were noted only among DH lines of ZH population, so in this population, which the paternal parent contained more protein, NDF and ADF. The use of multivariate statistical analysis allowed the simultaneous characterization and grouping of tested lines of HZ and ZH populations in terms of several traits.

**Conclusion:** Application of the statistical methods revealed the influences of the maternal parent on the thousand seed weight and paternal parent on content of fibre and seed colour.

**References:**
Genetic diversity for morphological and physiological traits in diverse rapeseed germplasm

Genetic diversity is the backbone of any plant breeding program. Seed companies and plant breeders involved in the development of crop varieties for specific consumer and industrial uses are often limited by either the lack of desired trait in existing genetic stocks or the narrow variability in the available germplasm for the trait of interest. It is always desirable to obtain or develop germplasm which possess the genes for economically important plant and seed traits. The Plant Genetic Resources Institute (PGRI) located at National Agriculture Research Center, Islamabad, Pakistan is holding a large number of Brassica accessions (land races) collected from diverse regions of Pakistan. Dow AgroSciences in collaboration with PGRI has characterized the germplasm for various morphological and physiological traits in triple environments of Pakistan and Canada. This research information will focus on explaining the genetic diversity for some traits such as physiological maturity, plant type, plant height, seed characteristics. The distribution patterns and conservation of traits will also be discussed. The data will provide insight into the geographical diversity of the Brassica germplasm.

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Introgression of imidazolinone tolerance into spring turnip rape (*Brassica rapa L*) and the launch of Aurea CL

**Background:** Imidazolinones (IMIs) are a family of herbicides that control weeds effectively. IMIs inhibit the function of acetolactate synthase (ALS), enzyme required for the synthesis of branched chain amino acids. Tolerance to IMI was developed in microspore mutagenized oilseed rape (*Brassica napus* L.) (Swanson et al. 1989). Mutants, carrying PM1 and PM2 ALS alleles, were found to have a superior tolerance to IMIs. Previously the imazamox, an imidazolinone herbicide, was shown to be very good in controlling the most troublesome weeds in oilseed fields in Finland (Haukkapää et al. 2005).

**Objectives:** The aim of this study was to incorporate the IMI tolerance into turnip rape (*Brassica rapa L*) and oilseed rape lines well suited to northern climates and to measure the actual level of tolerance of IMI tolerant lines to imazamox.

**Methods:** IMI tolerant lines were developed by crossing an oilseed rape line (originated from BASF Corporation, USA), containing both PM1 and PM2, to several turnip rape and oilseed rape breeding lines. The breeding work was done at the University of Helsinki in 2003-2007. Hybrids were backcrossed until the BC6 generation in turnip rape and BC4 generation in oilseed rape. After each backcross, the progeny was tested for the presence of the tolerance genes using a 30 g ai ha⁻¹ application rate of imazamox. PCR analyses were used for monitoring the presence of PM1 and PM2 alleles. IMI tolerance of the newly formed breeding lines was tested in field and greenhouse experiments. The level of IMI tolerance was tested with increasing doses of imazamox, and the activity of ALS enzyme and dry matter accumulation were measured as well.

**Results:** Introgression of PM1 and PM2 into several oilseed rape lines was successful whereas only PM2 was stable in turnip rape lines. Both field and greenhouse experiments showed that turnip rape carrying only the PM2 mutation withstood imazamox well. Furthermore the data suggest that turnip rape was more tolerant to imazamox than oilseed rape. IMI tolerance did not show any negative impact on the agronomic parameters of both *Brassica* species.

**Conclusions:** Based on these trials IMI tolerant turnip rape (*B. rapa*) line no: 4003 went through the official variety tests and was accepted on the official variety list in Finland as Aurea CL in 2011. Later it was approved on the EU list of cultivars as well. Since that it has been grown successfully on the farmers’ fields.

**References:**

QTL controlling pod shattering resistance in yellow-seeded Brassica napus line YN01-429

Background: The majority of the Canadian canola crop is swathed prior to combining, in part, due to susceptibility to mature pods shattering. A key element in optimizing straight cutting or direct combining of canola is a better understanding of the genotypic and physiological contributing factors to pod dehiscence. An initial investigation into variation in Canadian canola cultivars, hybrids and breeding lines showed that yellow-seeded lines possessed less shatter potential than many commercial black-seeded lines (Wang et al. 2007).

Objectives: 1) mapping of the genomic regions controlling reduction of pod shatter observed in YN01-429; 2) Determine if regions controlling shatter reduction were interspecifically-derived during the introgression of yellow seeded genes from B. carinata and B. rapa through linkage; and 3) Assess if pleiotrophy exists between reduction in shattering potential and other agronomic or seed fiber-related traits.

Methods: The elite yellow seeded breeding line was crossed to two different shatter susceptible, black-seeded lines and doubled-haploid populations were developed. Both populations were evaluated in full for one season at one or two sites and lines with extreme phenotypes were evaluated in a second field season. Days to flowering and maturity were recorded in each trial. Pod shattering phenotypes were assessed in non-harvested trials that were visually rated for pod shattering throughout the fall season. Linkage maps of the populations were developed with either SSR or SNP markers and QTLs for each site-year were determined. The trial was arranged in a split-plot where a harvested main-plot treatment produced seed that was analyzed for standard canola seed quality parameters and fiber fractions using NIR.

Results: In the first population evaluated, several QTL on various linkage groups were consistently detected across two sites and explained 6.4 to 16.1% of the variation. A QTL on N13 was confirmed the second season on population extremes. QTL analysis on the second population showed a unique single locus on N2 which explained 22.5% of the variation which was not detected in the first population examined. The shatter reduction QTL on N13 was shown to be derived from black-seeded pedigree parents used in the development of YN01-429 when surrounding SSR markers were assessed on all pedigree parents. In both populations, a major QTL for seed coat colour and fiber fractions was found on N9 and mapped to different linkage groups than shatter reduction QTL. Similarly, flowering and maturity QTLs mapped to different genomic regions. There were very little to no associations between shattering and these seed quality related traits, flowering or maturity ratings.

Conclusions: Uncharacterized variation for pod shatter exists within the B. napus genepool; however, several QTL control the trait and detection of these QTL is highly dependent on background genotype and environment.

References:
Characterization of genomic regions responsible for microspore embryogenic potential and direct embryo to plant conversion of a doubled haploid oilseed rape population

**Background:** Microspore culture is a very powerful technique in breeding of oilseed rape. Despite the progress achieved in optimizing in vitro culture protocols, tremendous differences remain in the microspore embryogenic potential among genotypes (Ferrie and Möllers 2010). Furthermore, breeding progress is hampered by a highly variable direct embryo to plant conversion of breeding lines. The Swedish spring cultivar Topas has been extensively studied for its excellent microspore embryogenic potential and the derived line DH4079 showed an outstanding embryo production of many thousand embryos per experiment, which has made DH4079 a standard in many investigations. In contrast, a very low embryo yield is obtained from inbred line 617 of winter oilseed rape cultivar Express, in the range of nil up to 50 embryos per experiment, under comparable conditions. Moreover, a moderate and a good direct embryo to plant conversion were found for DH4079 and Express 617, respectively. The genetic basis of such differences in microspore culture response and plant regeneration is still largely unknown.

**Objectives:** To identify genomic regions and candidate genes related to microspore embryogenic potential and direct embryo to plant conversion through the analysis and QTL mapping of both traits in a DH population derived from the cross DH4079 x Express 617.

**Methods:** In vitro propagated F1-plants of the cross DH4079 x Express 617 were used as microspore donors to generate a DH population of 200 lines. DH lines were grown and used as source of microspores, which were cultured following a standard protocol (Möllers et al. 1994). The number of regenerated embryos (MDE) per experiment was recorded. MDE were transferred to Gamborg B5 medium and following a cold treatment at 2 °C, direct embryo to plant conversion was scored. Experiments are repeated four times and mean values will be used for QTL mapping based on an Illumina Infinium Brassica 60K SNP molecular linkage map.

**Results:** So far 100 lines of the DH population were characterized for their embryogenic potential and direct embryo to plant conversion. Large and significant differences in microspore embryogenic potential were observed among DH lines, ranging from a few embryos to more than forty thousand as a mean of four experiments. Significant differences were also found for direct embryo to plant conversion, which ranged from 5 to 85%. Heritabilities for both traits were high (85% and 93%). A linkage map based on 3,142 SNP markers was developed and will be used for QTL mapping and candidate gene identification.

**Conclusions:** The DH population showed a continuous and significant variation for microspore embryogenic potential and direct embryo to plant conversion between genotypes, indicating that both traits are quantitatively controlled by several genes.

**References:**
Broadening of genetic diversity in spring canola (Brassica napus L.) by the use of the C-genome of B. oleracea var. italic and B. oleracea var. capitata

**Background:** Canola (Brassica napus, AACC, 2n = 38) is one of the important edible oil in the world after soybean and palm. In Canada, spring B. napus canola is one of the most important crops, contributes about $19 billion per year to the Canadian economy (Canola Council of Canada, 2013). Presence of genetic variability in breeding population is pre-requisite to develop new cultivars with improved yield to meet the demand of ever growing population in the world. Genetic diversity in spring B. napus canola is low; therefore, breeding efforts must be taken towards broadening of genetic diversity in this crop (Cowling 2007, Rahman 2013). This can be accomplished through introgression of genetic diversity from diploid progenitor species B. rapa L. (AA, 2n = 20) and B. oleracea L. (CC, 2n = 18), and other allied species of the family Brassicaceae.

**Objectives:** The research project was undertaken to develop canola-quality spring B. napus inbred lines with allelic diversity introgressed from the C-genome of B. oleracea var. italic (broccoli) and B. oleracea var. capitata (cabbage).

**Methods:** B. napus × B. oleracea interspecific crosses were made and the F1 plants were self-pollinated for F2 seeds. The F2 populations were self-pollinated for several generations with selection for different agronomic and seed quality traits. F4 plants were genotyped with SSR markers.

**Results:** All F5 generation families were free from erucic acid (0.18 ± 0.02 SE percent of total fatty acids) and mean of glucosinolate content in F6 was less than 20 µ mol g⁻¹ seed. About 69% of the F6 plants had partec value similar to the B. napus parent A04-73NA.

**Conclusions:** Results suggest that spring B. napus canola quality lines can be developed from B. napus × B. oleracea interspecific crosses for introgression of allelic diversity from broccoli and cabbage into B. napus.

**References:**

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Pod trait comparisons between a pod-shatter resistant accession and a pod-shatter susceptible accession in *Brassica napus L.*

**Background:** During rapeseed harvest, pod shatter loss proportion usually reaches up to 10% to 30%. In order to decrease the yield loss, it is necessary to breed varieties with pod-shatter resistance. Except for molecular assisted selection, it is more practical for breeders to differ the pod-shatter resistant accessions from pod-shatter susceptible accessions via pod traits.

**Objectives:** This article tried to find pod trait differences between pod-shatter resistant accession H155 and pod-shatter susceptible accession Qva (Wen et al. 2008). The pod traits included pod agronomic traits, valve anatomic structures and fiber content in pod valves.

**Methods:** Random impact test (RIT) for pod-shatter resistance of oilseed rape was developed by Morgan et al. (2000) and improved by Wen et al. (2008). Pod traits (pod length, pod width, beak length, valve thickness and seed number per pod) were obtained by a trial including three replications. Additionally valve anatomic structures were investigated by laser microscope and by transparent electron microscope. Total fiber contents in valves were tested according to the methods of Van Soest.

**Results:** Pod shatter resistance index difference between H155 and Qva reached 1% significant level. Pod length, beak length, valve thickness and seed number per pod of H155 were 8.71 cm, 0.50 cm, 1.84 cm, 0.22 mm and 32.8 seeds, respectively. Compared with H155, Qva had much shorter pod (3.74 cm), shorter beak (0.72 cm), thinner valve (0.15 mm) and less seeds per pod (10.2 seeds).

Results of anatomic studies of valve revealed that there were valve anatomic structural differences between H155 and Qva. In H155, cells in endocarp were long, perpendicular to endocarp and arranged tightly. The cell walls in endocarp, mesocarp and vascular bundle were seriously lignified. On the contrary, cells in endocarp of Qva were round and arranged loosely. Cell walls in endocarp of Qva were slightly lignified, and cell walls in mesocarp and vascular bundles were hardly lignified. Fiber contents in valves between H155 and Qva reached significant difference. Fiber content in H155 valves was higher by 5.37 times than that in Qva. Consequently, H155 had much stronger valve than that of Qva in mechanical strength.

**Conclusions:** Pod traits affects pod-shatter resistance in *Brassica napus L.* and accessions with longer and thicker valve have better pod-shatter resistance. Pod-shatter resistant susceptible accession have shorter, thinner and weaker valves than pod-shatter resistant accessions. Anatomic structure is another affecting factor of pod-shatter resistance. Those accessions with tightly arranged cells in endocarp and seriously lignified cell walls in endocarps, mesocarps and vascular bundles have strong mechanical strength and are pod-shatter resistant. These conclusions can help breeders to select pod-shatter resistant accessions without molecular assisted selection.

**References:**
Genome-scale diversity for prediction of hybrid performance in winter oilseed rape

Background: High-density single-nucleotide polymorphism (SNP) genotyping provides a powerful platform to investigate heterozygosity on a genome-wide scale and investigate the genomic basis of heterosis. Analysis of genome diversity patterns within and between divergent gene pools can potentially provide valuable input to statistical models for genomic prediction of hybrid performance. Ultimately, genome-based prediction could help breeders to introgress novel variation into hybrid gene pools and select the most promising parental combinations to maximize trait performance.

Objectives: In this work we are evaluating different approaches for predicting hybrid performance in winter oilseed rape. The data generated also provides insight into genome-wide diversity patterns caused by intense selection for important traits in breeding populations. Genomic selection strategies can potentially help breeders to enrich genetic diversity in depleted chromosome segments, with potential gains in hybrid performance facilitated by genome-based prediction models.

Methods: We are investigating genome-scale diversity by genotyping large breeding populations, representing divergent winter-type oilseed rape gene pools, with the Brassica 60kSNP Illumina consortium genotyping array. Patterns of diversity are analysed at the population and chromosome level using population genetic parameters and assessment of linkage disequilibrium. Phenotype data from F1 test hybrids, generated using these individuals as pollinators of four different male-sterile (MSL) mother lines and tested in multiple field environments, are being used to develop statistical models for prediction of hybrid performance based on parental genome profiles.

Results: Genome-wide patterns of linkage disequilibrium (LD) and diversity (FST) reveal chromosome regions with strongly eroded diversity in different winter oilseed rape breeding pools. These regions represent key targets for improvement of hybrid performance by introgressions of novel diversity into heterotic pools. Preliminary results of hybrid prediction in a large population of winter oilseed rape test hybrids demonstrate the power of genome-based modeling approaches for selection of high-performing hybrid combinations.

Conclusions: Genome-wide diversity data enable targeted improvement of parental pools for winter oilseed rape hybrid breeding and facilitate genomic prediction of hybrid performance.
Global DNA methylation dynamics during the microspore reprogramming to heat stress induced embryogenesis in *Brassica napus L.*

**Background:** Microspore embryogenesis is a unique and facilitating phenomenon in plants, and consists of the inducible reprogramming of microspores deviating their original pathway toward embryogenesis (Seguí-Simarro and Nuez, 2008). Although fruitful achievements have been made in the past, few studies have examined the relationship between DNA methylation and heat stress, which is a crucial triggering factor for microspore embryogenesis induction (Shariatpanahi et al., 2006), on a genomic scale.

**Objectives:** To reveal whether DNA methylation is as an crucial epigenetic regulatory way to switch the microspore cell fate to microspore embryogenesis after heat induction. If so, How it is working? Simultaneously, we hope to seek the important DMRs (Differential Methylation Regions) and the related genes that were corresponding to the heat treatments.

**Methods:** The high/low embryogenic potential cultivar Topas and Westar were chosen for uninucleate microspore isolation and in vitro treatments with 18°C and 32°C for 6 h, respectively. Then collected all these heat treated and untreated samples for total DNA isolation. The genomic DNA of each sample was sent to BGI (BGI Tech Solutions Co., Ltd, Shenzhen, China) for bisulfite sequencing. The qualified sequencing data was analyzed by Bismark, methylKit, and some other bioinformatic tools.

**Results:** DNA methylation status of the microspores originated form the cultivar Topas that with high embryogenic potential was more sensitive to heat stress compared with the low embryogenic potential cultivar Westar. And heat treatment definitely triggered DNA hypomethylation occurring on each Topas chromosome, especially on the CpG and CHG contexts. Additionally, in Topas microspores, the C genome DNA methylation status in CpG and CHG sites was more sensitive to 32°C heat treatment compared with that of A genome. Actually, our prediction also showed that the *Brassica napus* C genome possess more CpG islands frequency throughout the chromosomes, suggesting the evolution of C and A genome were asymmetric in allotetraploid plant *Brassica napus*. Furthermore, we identified the DMRs and the related genes, which were corresponding to the heat treatments.

**Conclusions:** The present study uncovered the evolutionary disequilibrium of the subgenome on DNA methylation pattern as well as their asymmetric response to heat during the microspore reprogramming to embryogenesis, meanwhile the heat stress induced DNA hypomethylation may be essential for microspore cell fate changing in *Brassica napus L*.

**References:**

Fine mapping of a yellow seeded gene in *Brassica juncea* L.

**Background:** Yellow seed of rapeseed is a very important trait; sometimes the yellow seed oil content is 1-3% higher than that of brown seed with the same genetic background (Liu et al. 1991). So yellow seed breeding is considered as one of the effective ways to improve the oil content. A yellow mustard is the main rapeseed variety in the northwest of China. The yellow seed of this yellow mustard was controlled by a major gene, and the gene was mapped to A09 Chromosome in *Brassica* (Xu et al. 2010; Huang et al.2012).

**Objectives:** In order to fine map the yellow seeded gene, we made use of the genome sequences of *B. rapa* to develop the SCAR and IP markers, fine map the yellow seeded gene. This study will provide a useful clue for cloning the yellow seeded gene.

**Methods:** A BC8S1 population consisting of 1256 individuals derived from a yellow seeded landrace “Wuqi mustard” and a brown seeded landrace “Wugong mustard” was developed for gene mapping. Through the analysis of previous markers’ sequences, we found that the gene was located between 23.304 and 28.224M in A09 chromosome in *B. rapa*. We randomly selected some genes in this region, and designed primers according to the sequences of these genes; at the same time, we also used the markers’ sequences of this region published in website (http://brassicadb.org/brad) to develop new SCAR markers. MAPMAKER/EXP 3.0 program was used for linkage analysis, a minimum LOD score of 3.0 was used for map construction.

**Results:** Five SCAR (sequence characterized amplified region) and five IP (intron polymorphism) markers were successfully developed, and all of IP markers were co-dominant. The markers IP4 and Y1 were located in the either side of the yellow seeded gene at a distance of 0.1 and 0.3 cM, respectively. The gene was mapped in a region of 0.54M in A09 chromosome. In this region, three IP markers IP1, IP2 and IP3 co-segregated with the targeted gene.

**Conclusions:** Thanks to the power of next generation sequencing technology, *B. rapa* genome has been completely sequenced and published in public domain. The sequence information can be unlimited employed in developing markers linked to genes of interest, fine mapping or cloning targeted gene in *Brassica*. Developing IP markers is a very effective way for gene mapping. The fine mapping of the yellow seeded gene will lay a solid foundation for the yellow seeded gene cloning and gene function research.

**References:**


**Title:** Genome wide identification and expression profiling of *B. rapa* annexins

**Background:** Annexins are multifunctional proteins first discovered in plants and are present in both eukaryotes as well as in prokaryotes. In plants, annexins have been shown to play vital roles in growth and development under normal and stress conditions (Jami et al. 2012). Whole genome sequencing of *Brassica rapa*, an important species of the genus *Brassica* with many subspecies grown all over the world as vegetable and oilseed crops, has made identification of gene families easier.

**Objective:** In the present study, we planned to identify the annexin family members from *Brassica rapa* genome database; characterize them through bioinformatics tools and study their expression patterns in response to different abiotic stress condition and signaling molecules.

**Methods:** Genome-wide identification of annexin gene family was achieved by performing a search with keyword “annexin” in *B. rapa* genome database (http://www.plantgdb.org/BrGDB). For the prediction of motifs and domains, SMART program (http://smart.embl-heidelberg.de) was used and the GSOS server (http://gsds.cbi.pku.edu.cn) was used for gene structure prediction. For expression profiling of the annexin gene family, 3d old seedlings were treated with salicylic acid (sA) 100 µm, sodium chloride (NaCl) 200 mM, methyl jasmonate (MJ) 100 µM, hydrogen peroxide (H₂O₂) 10 mM, methyl viologen (MV) 10 µM and abscisic acid (ABA) 100 µM. Total RNA was isolated from whole seedling and different tissues by trizol (Invitrogen, USA), which was followed by cDNA synthesis using total RNA (2 µg). Real time PCR was performed and data was analyzed by ∆∆Ct method.

**Results:** In a search with keyword “annexin” in BrGDB, we found 13 annexin like sequences (Bra034402, Bra036764, Bra039578, Bra031890, Bra024346, Bra017102, Bra000091, Bra017103, Bra000090, Bra033961, Bra009049, Bra009048 and Bra008892) in *B. rapa* genome. However, *A. thaliana* has only eight annexin in its genome. Further analysis of *B. rapa* genome by PLAZA (http://bioinformatics.psb.ugent.be/plaza/versions/plaza_v3_dicots/) WGMapping tool showed that this expansion of annexin family in *B. rapa* genome happened due to block duplication and tandem gene duplication. In a protein sequence analysis by SMART program we found two annexin domains in Bra000090 and Bra017103, while Bra039578 showed three annexin domains and one N terminal transmembrane region in its sequence. Other annexin members showed the presence of 4 annexin domains. Gene expression analysis of eight members of the *B. rapa* annexin family showed a spatial and temporal regulation under different stress treatments like NaCl, MV, H₂O₂, SA, MJ and ABA. In every treatment, Bra034402 expression level was very high in comparison to other annexins. In tissue specific expression Bra017102, Bra000090, Bra009049 and Bra009048 expressed at higher level in leaf than in other tissue. Bra034402 and Bra008892 showed more expression in stem followed by leaf and then in root. Bra024346 showed higher expression in root than other tissue. Bra033961 showed equal expression in stem and leaf which was higher than root.

**Conclusion:** Genome-wide identification and expression analysis will help in further functional characterization of annexin family members of *B. rapa*.

**References:**
Characterization of FAE1 in the zero erucic acid germplasm of *Brassica rapa L.*

**Background:** The modification of erucic acid content in seeds is one of the major goals for quality breeding in oil-yielding *Brassica* species. However, few low-erucic-acid (LEA, <2%) resources are available, and novel LEA genetic resources are being sought. Fatty acid elongase 1 (FAE1) is the key gene that controls erucic acid synthesis. However, the mechanism for erucic acid synthesis in *B. rapa* lacks systematic study.

**Objectives:** Most LEA cultivars of *B. rapa* were developed by the introduction of recessive alleles from the donor varieties SPAN, or their derivative lines. To expand the genetic resource of LEA genes, we attempt to isolate the zero erucic acid lines from Chinese landraces and the mechanism of LEA formation in *B. rapa* was explored.

**Methods:** The erucic acid contents of 1981 Chinese landraces were analysed and the accessions with erucic acid content lower than 20% were selected to isolate zero erucic acid lines by half-seed fatty acid analysis (Gupta et al. 2004). The sequences and expression profiles of FAE1 at different development stages were analysed among the *B. rapa* accessions with different erucic acid contents. The molecular marker based on the deletions in the promoter sequences of LEA accessions was designed and the association with erucic acid content was detected in the segregating population.

**Results:** We isolated lines with zero erucic acid from 1981 Chinese landraces of *B. rapa*. The variations FAE1 coding sequences were not correlated with the erucic acid content of *B. rapa* as reported for *B. napus*. The FAE1 gene transcript was more abundant in the high-erucic-acid (HEA) than in the LEA accession during the seed development. Moreover, the FAE1 promoter sequences of LEA and HEA materials shared 95% similarity. 28 bases deletions (containing a 24 bases AT-rich region) were identified at approximately 1300 bp upstream from the FAE1 start codon in zero erucic acid cultivars and landrace, which may cause the decrease of the expression. The molecular marker based on the deletions was designed, and the genotype with the deletions was co-segregated with the LEA trait in the segregating population.

**Conclusions:** The formation of LEA is not attributable to variations in FAE1 coding sequences, but may be attributable to the decrease of FAE1 expression. The promoter variations might modify the expression level of FAE1 and the results shed light on novel regulation mechanisms for erucic acid synthesis.

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Preliminary study on difference of main carotenoid contents in the petals with different colors in *Brassica napus L.*

**Background:** The color of rapeseed (*Brassica napus L.*) flower petals is commonly yellow, but it can also be in other colors, including white, milky white, pale-yellow, golden-yellow or orange, etc. Different colors of flower petals in oilseed rapeseed can be a useful indication character and also an agronomic character, which is of important values for breeding, genetic studies, and modern touristic agriculture. Some genetic studies have been made to understand the inheritance of flower petal colors in *Brassica napus L.* (Tian et al. 2007; Wen et al. 2010) However, no researches have been found to investigate the differences in pigment contents in the flower petals with different colors in the species. Carotenoid contents and compositions were observed to be associated with the yellow-to-orange colors of inforescence in *Calendula officinalis* L. (Pintea et al., 2003). The yellow color of flower petals in Japanese morning glory (*Ipomoea nil*) was also found to be related to the carotenoid compositions and the patterns of carotenogenic gene expression during the petal development (Yamamizo et al., 2010) [2]. In citrus, the difference in fruit skin colors was also observed to be correlated to the compositions of carotenoid contents in the peel. (Tao et al., 2003) In the present study, we investigated the difference in the contents of three main carotenoids (xanthophyll, lycopene and β-carotene) in the petals with different colors in *Brassica napus L.*

**Objectives:** The aim of the study was to investigate the difference in main carotenoid contents in the flower petals with different color in *Brassica napus L.*, and to observe the relationship between carotenoid contents and flower colors in *Brassica napus L.*

**Methods:** Fifteen *Brassica napus* lines in 4 types of petal colors, white (5 lines), pale-yellow (2 lines), yellow (4 lines) and golden-yellow (or orange) (4 lines), were selected to investigate the contents of carotenoids in the flower petals. The fresh petals were taken from each line at the full blossom stage and stored in plastic tubes under liquid nitrogen temperature for use. Fresh petals were ground into powder with liquid nitrogen, and near 0.5mg of powder was taken to extract the total carotenoids by referring to the earlier studies (Parlog et al. 2009, Pintea et al. 2003, Yamamizo et al. 2010) with minor modifications. Contents of the 3 main carotenoids (xanthophyll, lycopene and β-carotene) were determined with the HPLC method (Agilent 1200, USA), referring to the protocols adopted in previous studies (Parlog et al. 2009, Pintea et al. 2003, Yamamizo et al. 2010) with necessary adjustments, and were quantified based on the standard curves established with their standard samples.

**Results:** The average contents of xanthophyll were 5.27, 6.84, 8.29 and 58.30 μg/g-1 of fresh petal in the 4 flower color types of rapeseed lines, i.e., white, pale-yellow, yellow and golden yellow petal lines, respectively; The average contents of lycopene were 2.94, 33.15, 16.77 and 1267.0 μg/g-1, and the average contents of β-carotene were 2.68, 7.06, 27.59 and 9.87 μg/g-1 in the 4 different types of lines, respectively. The total contents of the 3 main carotenoids were 10.89, 47.05, 69.90 and 1363.99 μg/g-1 of fresh petal in the 4 types of rapeseed lines, respectively. It was evident that the golden-yellow petal lines contained a high content of xanthophyll and also a high content of xanthophyll in the flower petals; the yellow petal lines contained a high β-carotene content in the petals; and the white petal lines were low in all the 3 main carotenoid contents. The total contents of the 3 main carotenoids in the petals were shown in the order of white petal < pale-yellow petal < yellow petal < golden-yellow petal lines. The difference in contents of the 3 main carotenoids was small among different rapeseed lines with the same type of petal color.

**Conclusion:** The total contents of the 3 main carotenoids in petals were positively related to the shade of flower color in *B. napus L.* Flower petals with the same type of color contained close contents of carotenoid compositions. The yellow petal color and the golden-yellow petal color of flowers were highly correlated to the high contents of β-carotene and lycopene in the petals, respectively. White flower petals were in low contents of nearly all the kinds of carotenoids.

**References:**


Inheritance and molecular marker identification of the yellow seed character in Ya’an Yellow Rape (Brassica rapa L.)

Background: Ya’an Yellow Rape is a landrace cultivar of Brassica rapa L. long and widely grown in the Ya’an district, Sichuan Province, Southwest of P.R. China. This landrace has a high rate of yellow-seeded plants, containing high oil content, maturing early and adapting especially to the wet and sunshine-scarce mountainous regions. It is an elite landrace cultivar of B. rapa with potential uses in yellow-seeded cultivars breeding in both B. rapa L. and B. napus L. However, the genetic rules and gene markers of the yellow seed character are not clear. Studies have demonstrated that, compared to black and brown seeds, yellow seed in Brassica species has a higher oil content, a thinner seed coat, and a higher protein content in the meal (Jonsson et al. 1977; Liu et al. 1992). A research on the yellow seed trait of Ya’an Yellow Rape will facilitate the utilization of this special landrace resource in B. rapa L.

Objectives: We carried out the studies to: (1) investigate the inheritance pattern of the yellow-seeded trait in Ya’an Yellow Rape, and (2) identify SSR molecular markers tightly linked to the seed color gene in the line, and furtherly anchor the seed color gene to specific linkage group in B. rapa L.

Methods: One yellow-seeded inbred line (P1) and one brown-seeded inbred line (P2) developed from Ya’an Yellow Rape were selected and the six genetic populations, including P1, P2, F1, F2, BC1 and BC2, were prepared for the genetic study on seed color of the land cultivar. The populations were planted on the experimental station for rapeseed breeding in Dayi, Chengdu in 2013. Seed colors were investigated visually with 55, 66, 54, 295 and 152 random plants for P1, P2, F1, F2, BC1 and BC2, respectively. Segregation ratios were calculated in F2, BC1 and BC2 populations, respectively, and X² tests were made to evaluate the goodness of fit to the expected ratios. Two hundred and forty-nine individual plants were taken from BC1 (P1/P2//P1) to identify the SSR markers associated with the yellow seed color trait. Fifteen pairs of SSR primers were selected from previous reports (Li et al. 2012; Liu et al. 2009; Padmaja et al. 2013) and one pair of SSR primers, TT8-SSR, was designed based on the sequence of BtT8 gene that was reported to be the yellow seed color gene in B. rapa L. (Li et al. 2012). PCR amplifications were performed with these SSR primers and the SSR markers associated with the seed color genes were analyzed.

Results: Seeds from all the F1 plants, including reciprocal cross F1 hybrid, were in brown color, showing that the brown color seed trait was dominant, without any cytoplasmic effects. The ratio of brown-seeded plants : yellow-seeded plants in F2 generation was 245 : 71, well fitted to the expected ratio 3:1 ($x^2= 0.949$, $p= 0.84$). The ratio of brown-seeded plants : yellow-seeded plants in BC1 was 161:134, fitted to the expected ratio 1:1 ($x^2= 2.292$, $p= 0.24$). The BC1 generation (152 plants) comprised only brown-seeded plants, fitted to the expected ratio 1:0 ($x^2= 0.0$, $p= 0.84$). These observations indicated that only one Mendelian gene locus was involved in control of the seed color trait in Ya’an Yellow Rape.

Four SSR markers (gss29, gss29, gss44 and TT8-SSR) showed specific band patterns for each seed-color trait and were found to co-segregate with the seed color genes. The other two SSR markers, OL12-F02 and Na10-A08, were identified to be 0.8 cm away from the seed color gene and located on the same side of the gene.

Conclusions: The present study showed that the yellow-seeded trait in Ya’an Yellow Rape was controlled by a single recessive gene. The 4 SSR markers (gss29, gss29, gss44 and TT8-SSR) were completely associated with the seed color trait and can be used as good molecular markers for selection of the trait. The two SSR markers OL12-F02 and Na10-A08 were 0.8 cM apart from the gene and located on the same side, indicating that the two SSR markers were dramatically close to each other and on the same locus.

References:
Microsporogenesis of reverse thermosensitive male sterility 
Huiyou50S in Brassica napus

**Background:** Thermo-sensitive genic male-sterility (TGMS) has great advantages in hybrid crop production. Reverse TGMS line Huiyou50S in B. napus was bred from a spontaneous semi-sterile plant found in our breeding nursery in April of 2000 in a Chinese cultivar Huiyou50. Different from common TGMS, the reverse TGMS Huiyou50S is sterile when cultured in low temperature but fertile in high temperature.

**Objectives:** A comprehensive understanding of the development mechanism is essential for the efficient utilization of a male sterility before it is used to produce of F1 hybrids. The objectives of this study were to make further explanations for the developmental aberrations leading to male sterility.

**Methods:** Floral buds representing a wide range of developmental stages were collected from plants of male fertile and sterile. Anther development of Huiyou50S was observed by light and electron microscopy.

**Results:** Microstructural evidences indicated that the anther abortion occurred at the tetrad to early uninucleate microspore stage. The protoplast of uninucleate microspore condensed and then degraded, eventually, only the distorted shells remained. The tapetal cells vacuolated at tetrad stage and degenerated rapidly before microspore disintegration. Observed under transmission electron microscope, tapetum abnormality exhibited at the tetrad stage and then the cytoplasm of microspores was apparently disintegrated at mid-microspore stage. The tapetum was disrupted rapidly at vacuolated microspore stage, without elaioplast formed. Subcellular alterations in Huiyou50S anthers included undeveloped primexine in tetrads and microspores, dysfunctional plastids and a loss of recognizable elaioplast in the tapetal cells. The evidences suggested that tapetum abnormality was associated with microspore abortion in Huiyou50S.

**Conclusions:** The cytological evidence found in this investigation were important for other theoretical research, for example, right time for sampling in the differential analysis on the transcriptome, proteome, and metabolome, and some biological pathway involved in male sterility. Thereafter, it will promote the research on the development of plant male gametophyte and broaden the range of heterosis utilization of rapeseed.
Introgression of the clubroot resistance gene Rcr1 from Brassica rapa into B. napus

**Background:** Clubroot disease, caused by Plasmodiophora brassicae, poses a serious threat to canola production in Canada. Clubroot resistance (CR) gene Rcr1, which confers resistance to pathotype 3, was identified in a pak choi cultivar “Flower Nabana” (FN) (Chu et al., 2014). Resistance to pathotypes 2, 5 and 6 in FN was also mapped into the Rcr1 region and SNP markers tightly linked to Rcr1 were developed (Yu et al. 2014).

**Objective:** We introgressed Rcr1 from B. rapa into B. napus, which could enable canola breeders to accelerate incorporation of Rcr1 into their canola varieties. Furthermore, a common B. napus line carrying Rcr1 will be part of a set of isogenic spring type B. napus lines for identification of pathotypes of P. brassicae and monitoring in situ changes in race structure of P. brassicae within canola fields.

**Methods:** An interspecific cross between FN and a B. napus doubled haploid line DH16516 originating from the cultivar Topas was conducted. The resulting F1 was crossed with an elite AAFc breeding line S17V11-17667 to produce a BC1 generation. The genetic composition of the A-genome and the number of C-genome chromosomes in the BC1 population was determined using a genome-wide SNP (INCanSeq_6K_Illumina) analysis applied to the BC1 resistant plants. One BC1 resistant plant was selected for further backcross with DH16516 to produce BC2. The resulting BC2 plants were analyzed with a gene-specific SNP marker SNP_A03_13 for Rcr1 and self-pollinated to produce BC2S1.

**Results:** One BC1 plant (N66) was obtained that carried Rcr1 with limited amount of FN genetic background out of the 77 BC1 plants and a full set of C-genome chromosomes most similar to natural B. napus. Resistance to pathotype 3 was evaluated in 45 BC2 plants, which were also analyzed with the SNP_A03_13. The SNP marker co-segregated with the disease reaction phenotypes. Eleven BC2 plants heterozygous at the Rcr1 locus were self-pollinated to produce BC2S1. A total of 140 plants in one BC2S1 family were tested for resistance to pathotype 3 and they showed a 3:1 ratio for resistance: susceptibility. Also, B. napus plants homozygous at the Rcr1 locus were identified by using SNP_A03_08. In addition, the reaction of 36 BC2 plants to a new pathotype of P. brassicae, 15 of 36 plants were highly resistant to this new pathotype.

**Conclusions:** Spring type B. napus lines carrying the clubroot resistance gene Rcr1 from B. rapa were developed by employing a combination of molecular genetic approaches and conventional breeding methods.

**References:**
Chu M, T Song, K C Falk, X Zhang, X Liu, A Chang, R Lahlali, L McGregor, B D Gossen, F Yu and G Peng (2014) Fine mapping of Rcr1 and analyses of its effect on transcriptome patterns during infection by Plasmodiophora brassicae BMC Genomics, 15:1166

Separation of antifreeze protein and expression analysis of Copper and Zinc superoxide dismutase (Cu/Zn-SOD) gene from winter turnip rape

Background: Winter turnip rape (Brassica campestris L.) has been a valuable ecology and oil crops in cold and arid region of northwest China. Cold and extremely low temperature cannot make winter turnip rape successfully overwinter and limit its production.

Objective: “Longyou 6” and “Longyou 7” are two ultra cold-tolerance winter rape cultivar, which can resist an extremely low temperature (-32°C). Our objective is to separated antifreeze protein and clone related gene from winter turnip rape.

Method: Antifreeze protein was separated from “Longyou 6” by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and mass spectrometry. The cDNA sequence of gene was isolated by RT-PCR, the obtained cDNA sequence was analysed. Real time RT-PCR was used to assess the expression of related gene in response to lower temperature stress. The enzyme activity was measured by NBT deoxidization method in leaves and roots.

Results: Results showed that copper and zinc superoxide dismutase (Cu/Zn-SOD) was an important antifreeze protein. The cDNA of Cu/Zn-SOD was cloned from Longyou 7 by RT-PCR, using the primers designed according to the published crucifer Cu/Zn-SOD cDNA sequences. The sequence of Cu/Zn-SOD from winter turnip rape was 459 bp, encoding a predicted protein of 152 amino acid residues. It contained specific sequence characteristics and conserved domain of Cu/Zn-SOD superfamily. The expression analysis showed that the Cu/Zn-SOD was a differential expressed gene induced by lower temperature.

Conclusion: Cu/Zn-SOD was was an important antifreeze protein. The gene had genetic characteristics similar with other known species and it could provide reference for functional research, and it might play a role in cold tolerance of the Winter Turnip Rape.

References:
Study on the heterosis of photosynthetic traits of *Brassica napus*

**Background:** High photosynthetic efficiency combined with heterosis may be an effective way to break through the yield bottleneck of rapeseed. Studying the photosynthetic physiology and breeding for high photosynthetic efficiency should be important contents for the breeding of rape at present and in the future.

**Objectives:** The heterosis of photosynthetic traits of *Brassica napus* in different growth stages and different cross combinations were compared. In addition, the correlations of photosynthetic traits between hybrids F1 and their parents were analyzed in order to reveal the true circumstance of photosynthetic traits and provide basis for high photosynthetic efficiency breeding.

**Methods:** Four cytoplasmic hybrids F1 of *B. napus* (Zayou 59, Qinyou No.7, Qinzayou No.1, and Qinzayou No.3) and their parents and 19 pairs of orthogonal and reciprocal combinations were used as research materials in this experiment. By using some measurement instruments photosynthetic traits such as photosynthetic area, main photosynthetic parameters, net assimilation of total plant, net population photosynthetic rate and the chlorophyll content were determinate at different stages, and the heterosis of F1, correlations between parents and offspring and genetic effect of photosynthetic traits were also be studied in this experiment. At last, all the data were statistical analysis with SPSS 13.0 software.

**Results:** The photosynthetic area and the net assimilation of total plant of hybrids F1 were obvious positive heterosis in the whole growth stage. The main photosynthetic parameters were obvious positive heterosis in specific stage. The chlorophyll content was little heterosis and the crop growth rate was obvious negative heterosis in the whole growth stage. The heterosis of photosynthetic traits were that: the photosynthetic area > the net assimilation of total plant > the main photosynthetic parameters > the chlorophyll content > the crop growth rate; the net photosynthetic rate and chlorophyll content of orthogonal combinations were higher than reciprocal combinations by 2.34% and 1.26% respectively. The over-stand heterosis, mid-parents heterosis and over-parent heterosis of net photosynthetic rate of 10 orthogonal combinations were higher than corresponding reciprocal combinations and the three heterosis of chlorophyll content of 11 orthogonal combinations were higher than corresponding reciprocal combinations; the photosynthetic traits of parents, especially female parent, value of mid-parent and high parent were high correlation with hybrid F1. It was showed that the photosynthetic traits were controlled by both environment and gene, and it was play an important role in photosynthetic traits genetic that genetic effect of add and dominant.

**Conclusions:** The photosynthetic traits of *B. napus* had heterosis and the hybrid F1 was influenced greatly by parents on photosynthetic traits. In the breeding for high photosynthetic efficiency of rape, the photosynthetic traits of parents should be improved so as to change the photosynthetic traits of hybrid F1 and the high value parent should be as female parent.
A beautiful story about the AP3 gene mutations and petals development

Background: The emergence of core eudicot petals makes the world more various and colorful. However, origin and evolution of the petals is still mysterious. Despite more results suggest that evolution of core eudicot petals is more relate to APETAL3 (AP3) lineage duplication, but until now it is still unclear what happened in the lineage bringing the brilliant evolution.

Objectives: Our previous research distinguish that two kinds of higher homologous AP3 genes, B.AP3.a and B.AP3.b, specified petals and stamens development of Brassica. B.AP3.a regulates petal and stamen normal development, whereas B.AP3.b losing 24-bp sequence (8 residues) only specifies stamen development and has little effect on petals development. Due to the 24-bp difference existing natively and exhibiting two statuses before and after AP3 mutation, we inferred that the 24-bp foreign insertion probably led to the petals evolution of core eudicots. Further explore what bring functional differentiation of the AP3 genes will help us to uncover the secret of the origin and evolution of core eudicot petals.

Plant Materials and Methods: A series of AP3 related mutants of Brassica rapa and Brassica napus were used. In addition, the ap3-3 mutants, with homeotic identity of petals to sepals and stamens to carpels, of Arabidopsis thaliana (landsberg erecta) were used as the transgenic recipient. A series of molecular biological techniques were used.

Conclusions: The two higher homologous AP3 genes of B.rapa had obvious functional differentiation in the petal development: B.AP3.a as a major gene generated wild-type normal petals, while B.AP3.b as a redundant gene generated small dosage petals; In B. rapa, the mutations of B.AP3.a and B.AP3.b genes generated the sepal carpeloid mutant HGMSa. Brassica napus contained two B.AP3.a and two B.AP3.b, loss of the two B.AP3.a functions was the key reason for the apetalous mutation, however, the loss-of-function in all four AP3 genes led to the sepal carpeloid mutant AMSa; The 24-bp special sequence was the key loci to causing functional differentiation of the B.AP3.a and B.AP3.b genes; The euAP3 motif and PI-derived motifs of AP3 are irrelevant to petals formation, and the 8 amion acids (the 24-bp sequence) of AP3 protein were the key loci to specify normal petal development; The 24-bp special sequence insertion probably gave rise to the functional differentiation and promoted great evolution of core eudicot petals.

References:
The comparison analysis of transcriptome in waterlogging *Brassica napus L.*

**Background:** Rapeseed is the second largest oil crop worldwide. In China, the largest planting country in the world, 80% of rapeseed is planted along the Yangtze River. In this area, rapeseed is always planted in the paddy field as rotation crop just after rice and rainfall occurs a lot during the season, leading to serious waterlogging (Zou et al., 2013). Most researches were focused on physiological and morphological traits of the response to waterlogging in rapeseed. However, there is little study on molecular mechanism. The only research was carried by Zou using a tolerant variety, ZS9 (Zou et al., 2013).

**Objectives:** Studies reveal there is natural variation of waterlogging tolerance in rapeseed (Zou et al., 2013; Zou et al., 2014). One way of exploiting waterlogging tolerance is to unravel genetic mechanisms beyond natural variation. It will be helpful to perform comparative transcriptome analysis between tolerant and sensitive varieties under waterlogging.

**Methods:** By the comparison between transcript profiles of ZS9 and GH01, 2977 genes with similar expression patterns and 17 genes with opposite expression patterns were identified. Besides, 1438 genes and 1861 genes were indicated to be specifically regulated in ZS9 and GH01, respectively. Analysis of overlapped genes between ZS9 and GH01 revealed that waterlogging tolerant ability is kindly decided by the regulation ability of genes with same expression patterns. Moreover, the opposite gene expression patterns revealed ABA signal might contribute to waterlogging tolerance.

**Results:** By the comparison between transcript profiles of ZS9 and GH01, 2977 genes with similar expression patterns and 17 genes with opposite expression patterns were identified. Besides, 1438 genes and 1861 genes were indicated to be specifically regulated in ZS9 and GH01, respectively. Analysis of overlapped genes between ZS9 and GH01 revealed that waterlogging tolerant ability is kindly decided by the regulation ability of genes with same expression patterns. Moreover, the opposite gene expression patterns revealed ABA signal might contribute to waterlogging tolerance.

**Conclusions:** Thus, this study reduces the number of candidate genes evidently, and helps to focus on a limited number of the genes to be investigated by more specific and detailed functional analysis, which it can be useful for further study.

**References:**


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Genetic analysis of seed yield in *Brassica napus* L. by mixed major gene and polygene inheritance model

**Background:** Seed yield is a very complicated quantitative trait. Many researches have been performed on its inheritance in *Brassica napus* L. However, the genetic basis still is one of research focuses. The early opinion showed that seed yield was dominated by many minor-effect genes which genetic effects were assumed equal to each other. Other studies argued that many quantitative traits including seed yield were simultaneously controlled by few major genes and many polygenes. This latter is called the mixed major gene plus polygene inheritance model (or mixed inheritance model/mixed genetic model).

**Objectives:** To dissect the genetic components involved in seed yield per plant and their heritability. It will improve the selection efficiency for good genotype of seed yield.

**Methods:** The joint segregation analysis method of mixed major gene plus poly-gene genetic model was used to study the inheritance of yield per plant. According to the theory that major gene effects in a segregating generation is an independent normal distribution modified by the polygene and the environment, four populations of the parents P1, P2, F1 and F2:3 (derived from F2) were investigated. The most suitable genetic model was selected using Akaike’s Information Criterion and the fitness of the selection was tested by a set of fitness tests.

**Results:** The frequency distributions of yield per plant in F2:3 family populations showed the characteristics of mixture normal distribution, which indicated that the inheritance of seed yield per plant followed the major gene plus poly-gene model. Results indicated that genetic model C-0 was the most fitted genetic model for the seed yield per plant, no major gene controlled seed yield. In another word, yield per plant in oilseed rape was controlled by additive-dominance-epistasis polygenes. The additive effect of major gene was -3.88, which indicated locus of the allele from female parent decreased the yield per plant, on the contrary, the locus of the allele from the male parent increased it. The dominant effect of polygene was 20.73. The polygene heritability in F2:3 was 30.29%.

**Conclusions:** Genetic model C-0 was the most fitted genetic model for seed yield per plant. Seed yield per plant in *Brassica napus* L. was controlled by additive-dominance-epistasis polygenes. This indicated that additive-dominance-epistasis polygenes played a crucial role in the control of seed yield per plant. Dominant effects were higher than additive effects. The results provided strong evidence that seed yield per plant was a complicated quantitative trait; additive, dominant, and epistasis effects were the genetic basis of heterosis in rapeseed.

**References:**
Genetic mapping of a single co-localizing QTL for clubroot resistance to five pathotypes of *Plasmodiophora brassicae* in *Brassica rapa*

**Background:** Clubroot disease, caused by *Plasmodiophora brassicae*, was identified in canola fields in central Alberta in 2003 and is continuing to spread on the Canadian prairies, posing a serious threat to canola production in western Canada. There are at least five pathotypes of *P. brassicae* (2, 3, 5, 6 and 8) identified (Strelkov & Hwang 2014). Most resistance sources in *Brassica* species are race- or pathotype-specific (Diederichsen et al. 2009).

**Objectives:** We aimed to evaluate the resistance to different Canadian pathotypes of *P. brassicae*, map a clubroot resistance (CR) gene using tunable genotyping-by-sequencing, and develop SNP markers tightly linked to this CR gene in *B. rapa*.

**Methods:** One *B. rapa* canola breeding line T19, highly resistant to five pathotypes of *P. brassicae*, was crossed with a susceptible *B. rapa* canola DH line ACDC, and the resulting F3 was backcrossed to ACDC to generate a BC1 population. BC1S1 lines were produced by self-pollinating individual BC1 plants. The parents and 92 BC1S1 lines with 12 plants each were inoculated with single-spore isolates of pathotypes 2, 3, 5, 6 and 8, respectively. Plant inoculation, disease rating and index of disease (ID) calculation followed the methods described by Strelkov et al. (2006). DNA was extracted from young leaves following the protocols of the DNeasy Plant Mini Handbook (Qiagen). DNA sequencing and genotyping were performed at Data2Bio (Ames, Iowa, US).

**Results:** All ACDC plants developed severe galling, with IDs of 100% in response to each pathotype, while T19 exhibited a resistant response with IDs of 0. The distribution of IDs in the BC1S1 lines was divided into two classes: 43 lines were susceptible (ID > 60) and 49 lines were resistant or partially resistant (ID < 60), which fitted a 1:1 ratio (x2 = 0.53, P > 0.5). A genetic map consisting of 10 linkage groups with 1,438 high quality SNPs was constructed with an overall genetic length of 1,165 cM. A single co-localizing QTL namely Rcr4 on chromosome A03 was detected for all 5 pathotypes, and 7 SNPs tightly linked to the QTL were identified.

**Conclusions:** CR in the *B. rapa* breeding line T19 to all five pathotypes of *P. brassicae* was completely associated. It is controlled by a single co-localizing QTL Rcr4 on chromosome A03 of *B. rapa*. SNPs in the vicinity of Rcr4 could be used for marker assisted selection in canola breeding.

**References:**
High-density single nucleotide polymorphism (SNP) array mapping for yield-related quantitative trait loci in *Brassica napus* L.

**Background:** Seed weight (SW) and silique length (SL) are two important yield-related traits in oilseed rape. In the last two decades, large efforts have been devoted to unravel these complex traits through quantitative trait locus (QTL) mapping. While most studies were based on low density linkage maps constructed by limited molecular markers (e.g. RFLP, AFLP and SSR), they were insufficient to provide accurate QTL information that controls the traits. Nevertheless, large number of markers can be created by single nucleotide polymorphisms (SNPs), which are commonly used for the detection of genetic variations and construction of high-density genetic maps in rapeseed.

**Objectives:** Our goals are to construct a high density SNP map of *B. napus* and mapping QTL for SW and SL, using SGZ-DH population across eight environments. This approach would provide adjacent markers for rapeseed breeding and obtain the precise QTL intervals for map-based cloning of target genes.

**Methods:** A newly segregated DH population was obtained from the cross between “SG-DH198” (one line from SG-DH population) (Zhao et al. 2005) and a modern Chinese variety “Zheshuang 72” (SGZ-DH population 161 lines). Field experiments were conducted in eight environments, in which SW was from open-pollinated bulked seeds and SL obtained from measuring 10 siliques of 5 plants (main inflorescences). The *Brassica* 60K SNP Bead Chip Array was used to genotype 161 DH and parental lines. Imaging of the arrays was performed using an Illumina HiSCAN scanner, whereas allele calling for each locus was conducted by the GenomeStudio genotyping software v2011. The SNP marker data was scored according to the definition of Joinmap 4.0. WinQTLCart 2.5 software was applied to carry out QTL analysis.

**Results:** We constructed the SGZ genetic map that comprised of 1333 bins corresponding to 3516 SNP markers. The map spanned 1883.5 cM across nineteen chromosomes with an average interval of 1.41 cM between bins. Using composite interval mapping, four (qSWA2, qSWA7, qSWC1 and qSWC9) and six (qSLA6-1, qSLA6-2, qSLA7, qSLC4-1, qSLC4-2 and qSLC9) significant QTLs for SW and SL were repeatedly detected across environments, which together explained 41.61% and 66.55% of the phenotypic variations in population, respectively. Two major loci that co-localized on A7 (qSWA7/ qSLA7) and C9 (qSWC9/ qSLC9) showed pleiotropic effects for both traits, with “Zheshuang 72” as the favorable alleles. These results explained the significant correlations between SW and SL (R=0.20-0.36) as observed in population. The peak position of these two co-localized QTLs spans the physical regions of 18.21-18.23 Mb and 26.23-26.31 Mb in the *B. napus* genome sequence.

**Conclusions:** The SGZ-SNP map developed in the current study serves as an important tool for future comparative map study. The identified physical positions of QTL could benefit the cloning of candidate genes underlying QTL and assist the high yield breeding.

**References:**

Physical mapping of the OilA7 region and identification of a candidate gene in *Brassica napus*

**Background:** Oil content in rapeseed is one of the most important economic traits. Despite a large body of QTL information, map-based gene cloning in *B. napus* is rare. In our initial QTL analysis, OilA7 was detected in all eleven environments, showing the largest additive effect for oil content in SG population (Zhao et al., 2005, 2012). Using substitution mapping, OilA7 was validated and fine mapped in a reduced genomic interval (Zhao et al., 2011). Here, we present our work on further fine mapping and candidate gene analysis.

**Objectives:** Based on the fine mapping of OilA7 by developing locus specific markers and creating higher generations of substitution populations, we aimed to isolate the candidate genes in delimited QTL region by combining bioinformatics analysis and gene expression profile. To further validate the candidate genes, we adopted real-time PCR, allelic comparison of gene structures and transgenic analysis.

**Methods:** According to the flanking marker sequences of OilA7, homologous genomic regions from *Brassica* database (BRAD) (http://brassicadb.org/brad) were selected for marker design. Fine mapping was performed based on the marker genotypes of BC4F2 / BC4F3 plants and trait phenotypes of BC4F2:3 / BC4F3:3 families. Oil content was determined by Soxhlet extraction with two replicates. We used RNAsesy mini kit (QIAGEN) to isolate the total RNA from developing seeds (5-7, 15-17 and 25-27 days after flowering) of two parents (Sollux and Gaoyou) and from BC4F4 NILs. RNA samples were sequenced on an Illumina HiSeq 2500 with 100 cycle pair-end run. RNA-seq reads were mapped to a reference genome using TopHat. The transcript abundances were measured in FPKM (fragments per kilobase of exon per million fragments mapped) by Cufflinks 2.1.1. The differential expressions (FDR ≤ 0.05) were analyzed with Cuffdiff. To further validate the allelic difference of candidate genes, real-time PCR was adopted with NILs. The recombinant plasmids that contain target alleles from NILs are being sequenced.

**Results and Conclusions:** OilA7 locus was delimited in a genomic region on A7 ca. 300kb, where 8 of 60 genes (according to the BRAD) showed significant difference of transcript abundances between parents in at least one of three developmental stages. Bioinformatics analysis suggests two genes as the candidates for OilA7, one dictates the thickness of seed coat and the other codes a key enzyme in the fatty acid biosynthesis passway. Further, real-time PCR analysis on BC4F4 NILs suggests one of them to be the more potential candidate, since Gaoyou allele (increasing oil) behaved 25 times higher in gene expression than Sollux in the seed stage of 17-20 days after flowering. Sequencing two candidate genes and transgenic analysis are underway.

**References:**
Utilization of a novel recessive glossy mutation in hybrid breeding

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Background: The dominant sterility, controlled by epistatic interaction between the dominant GMS gene (Ms) and the suppressor gene (Rf), was reported by Li et al. (1985). Base on this GMS, the three-line system has been developed (Li et al. 1995), and its two hybrid cultivars, such as Heza No.7 and Heza No.9 have been registered in China recently. It is time-consuming and laborious to remove false hybrids from the 100% sterile population during hybrid seed production. In order to overcome this problem, the novel recessive glossy genes were transferred into the homozygous two-type line.

Objectives: The homozygous two-type line of the dominant GMS was used in this study including 50% fertile plants (Genotype: MsMsRfRf) and 50% sterile plants (Genotype: MsMsRfRf). The novel recessive glossy mutation was as the donor controlled by two recessive genes (g1 and g2).

Methods: Traditional plant breeding methods, such as crossing, self-pollination and test crosses were used. Fertility survey in segregating population was made during flowering period.

Results: The cross was made between the fertile plants (Genotype: MsMsRfRfG1G1G2G2) among the homozygous two-type line and a novel recessive glossy mutation (Genotype: msmsRfRfG1g1g2g2), and the sterile line with glossy trait (Genotype: MsMsRfRfG1g1g2g2) was obtained from the generation of self-pollination. The fertile plants from a cross the sterile line with glossy trait (Genotype: MsMsRfRfG1g1g2g2) × the homozygous two-type line fertile plants (Genotype: MsMsRfRfG1G1G2G2) × the homozygous two-type line fertile plants (Genotype: MsMsRfRfG1G1G2G2) × the novel homozygous two-type line with glossy trait (Genotype: MsMsRfRfG1g1g2g2) × the novel homozygous two-type line with glossy trait (Genotype: MsMsRfRfG1g1g2g2) × the novel homozygous two-type line with glossy trait (Genotype: MsMsRfRfG1g1g2g2). The new homozygous two-type line with glossy trait was produced by continuous sib-crossing between the sterile line with glossy trait and the fertile plants with glossy trait. The 100% sterile population with glossy trait was obtained from the sterile line with glossy trait × the novel recessive glossy mutation.

Conclusions: There are great differences between glossy trait and the normal trait in Brassica napus, which could be visually distinguished. Base on the glossy trait, it is easy and convenient to identify the purity of the 100% sterile population. More importantly, this trait was used to get rid of the false seeds mixed in the 100% sterile population. Because the false plants were removed before being planted for hybrid seed production, it is unnecessary to get rid of the false plants in hybrid production fields. So, there was significant savings in labor.

References:
Li, S. L., Y. X. Qian, and Z. H. Wu, 1985: Genetic study on genic male sterility and its utilization in Brassica napus L. Acta Agriculturae Shanghai 1, 1—12.
Genetic dissection of seed yield per plant in *Brassica napus* L. by QTLs mapping

**Background:** In crop breeding, high yield is the major objective. Therefore, seed yield is central to the analysis of genetic rules and exploration of approaches for crop breeding improvement. Among the approaches, heterosis utilization is the most important method for seed yield improvement. Seed yield of rapeseed is a complicated quantitative trait. To explore the genetic basis, many theoretical researches have been performed on heterosis. However, the genetic basis of heterosis continues to be a contentious issue. Nevertheless, QTLs mapping and genetic effects analyses of seed yield can help us to understand the origins of heterosis and improve the selection efficiency of superior genotypes for seed yield.

**Objectives:** To elucidate the genetic basis of heterosis better and improve the selection efficiency and accuracy of the superior genotypes for seed yield, QTLs and the genetic components involved in seed yield per plant were detected and dissected.

**Methods:** A linkage map is constructed with SRAP, AFLP and SSR markers by F2 populations. QTLs and epistasis are likewise detected. The statistic software of Windows QTL Cartographer Version2.0 and CIM were applied to identify the QTLs of seed yield.

**Results:** Four QTLs, yp13, yp9, yp12 and yp3 were detected and located on linkage groups 13, 9, 12 and 3, respectively. These QTLs each accounted for 7.26%, 7.53%, 6.99% and 10.27% of the phenotypic variation, respectively, alleles from female parent increase the effects of QTLs yp13, yp12 and yp3. On the other hand, the alleles from the male parent decrease the effects of QTL yp9. Genetic effects of all of the four main QTLs showed positive over-dominance. The 10 significant two-locus combinations controlling seed yield per plant were detected by two-way ANOVAs among seventeen co-dominant markers, which have significant effects on seed yield per plant in rapeseed. The interaction effects analysis also showed that DD and DA were the most significant type of interaction in seed yield per plant.

**Conclusions:** The results indicate that the number for two-loci combinations involving the entire genome detected in seed yield per plant was greater than that of one-locus. Epistasis interaction included loci of both QTL and non-QTL, in which the later was in the majority. The results also provided strong evidence that epistasis plays a significant role as the genetic basis of heterosis. In general, additive, dominant, and epistasis effects are the genetic basis of heterosis in *Brassica napus* L.

**References:**

Restriction enzyme digestion of chloroplast DNAs, PCR amplification of specific genes and molecular cloning of rbcL genes on Polima CMS and Shan 2A CMS

Background: Polima CMS and Shan2A CMS are two of the classic and effective cytoplasmic male sterility (CMS) lines in *Brassica napus* L., and the two famous CMS lines have played very important roles in study and utilization of rapeseed heterosis in China even in the world. In recent years, in cytoplasm study of the two sterile lines, about comparative study of mitochondrial DNAs (mtDNAs) of the two has been much more reported (Yang et al. 1998; Wang et al. 2001; Lin et al. 2006). However, about comparative study of chloroplast DNAs (cpDNAs) has been seldom reported.

Objectives: This research compared Shan2A CMS with Polima CMS in their chloroplast genomes through restriction enzyme digestion of cpDNAs, PCR amplification of the specific genes and molecular cloning of rbcL genes about the two CMS systems.

Methods: In accordance with the method of Liang et al. (1995), cpDNAs were extracted by seedling leaves from four materials including Polima CMS and its maintainer line PolB, Shan2A CMS and its maintainer line Shan2B, and several special gene fragments of cpDNAs (Accession number: AF267640, AY752707, AY752722, AY752708, AY752724) were amplified by PCR. The Rubisco large subunit (rbcL) genes (Accession number: AF267640), which were related to photosynthesis in the 5 special gene fragments, were further cloned and sequenced in the 4 materials. Restriction enzyme digestion and gel electrophoresis detection were also performed respectively to cpDNAs of the 4 materials using an excess of 4 kinds of enzymes including BamH, EcoR, Hind and Pst.

Results: The results were as follows: The same one target band was found respectively in horizontal submarine agarose gels in the 4 materials when 5 pairs of the special gene primers of cpDNA in rapeseed were used, and amplified products were consistent with expected target fragments. The sequencing results showed that the DNA sequences of amplification products of the 4 materials were exactly the same, their gene sizes are respectively 1733 bp, and the sequences are in accordance with that of the forecast purpose fragment (rbcL gene). The results of comparative analysis of enzyme fragment polymorphism indicated that the band types in the 4 materials are same and the enzyme digestion products have no difference, respectively.

Conclusions: The above results reflect the conservatism of chloroplast genomes of the two sterile systems in a certain extent.

References:


Characterization of candidate genes within QTL associated with resistance to stem rot in a doubled haploid breeding population of canola

**Background:** Stem rot in canola (*Brassica napus*) is a fungal disease caused by *Sclerotinia sclerotiorum*. Resistance to this economically important disease in canola is considered a quantitative trait. Genotyping-by-sequencing of an F2 population derived from a cross between doubled haploid parents susceptible (NEP32) or resistant (NEP63) to stem rot identified >20,000 polymorphic SNP markers between the two parents. These markers were used to construct a genetic map with 1847 marker loci with an average marker density of 8 cM per marker that corresponds to 0.37 Mb of the genome. Composite interval mapping identified five significant QTL on chromosomes A01, A03, C01 and C08 associated with resistance to stem rot that account for 10 to 19% of the phenotypic variation (Chittem et al. 2015a). RNAseq data obtained from the two parents (NEP32 and NEP63) in response to inoculation with *S. sclerotiorum* identified several differentially-expressed genes within each of the five QTL associated with resistance to stem rot (Chittem et al. 2015b).

**Objectives:** Determine if differentially-expressed genes identified in the parental germplasm and that fall within the five QTL associated with resistance to stem rot correlate to resistance or susceptibility of the F2 progeny.

**Methods:** Sequence obtained from the parental lines (Chittem et al. 2015b) and the draft genome of canola (Chalhoub et al. 2014) were used to identify candidate genes within the five QTL associated with stem rot resistance. Primer pairs to at least one gene showing significant differential expression among the five QTL were designed using the Primer Select program of DNASTAR Lasergene B software. A subset of canola plants with varying levels of stem rot resistance within the F2 progeny were inoculated with or without *S. sclerotiorum* (NE 152). RNA extraction, cDNA synthesis, and qRT-PCR were conducted as previously described by Doğramaci et al. (2014).

**Results:** Candidate resistance genes within the five QTL associated with stem rot resistance are being characterized to determine if their expression levels correlate to differing degrees of resistance in the F2 breeding population. Candidate genes being tested for correlation to stem rot resistance in this study include: BnaA01g14790D (putative nucleotide hydrolase), BnaA03g36620D (putative alternative oxidase), BnaC01g24700D (putative Myb-like transcription factor), BnaC08g37640D (putative CCAAT-binding transcription factor), and a novel transcript of chromosome C01 (putative ethylene-responsive transcription factor).

**Conclusions:** will be based on outcome of results.

**References:**

- Chittem K, del Rio-Mendoza LE, Goswami RS. Identification of candidate resistance genes to *Sclerotinia sclerotiorum* in canola using next generation sequencing. USDA-ARS National Sclerotinia Initiative 2015a Annual meeting, January 21-23, 2015, Bloomington, MN.
Analysis of the interaction between the avirulence genes AvrLm4-7 and AvrLm3 in Leptosphaeria maculans

Background: Leptosphaeria maculans is the fungus responsible for phoma stem canker (blackleg), a damaging disease on canola (Brassica napus). The deployment of resistant B. napus cultivars is known to be the most effective way to control this disease. Specific resistance genes (Rlm) are efficient whenever the corresponding avirulence (AvrLm) allele is prevalent in fungal populations. A complex relationship has been identified between two avirulence genes of L. maculans: AvrLm3 and AvrLm4-7. When an isolate possesses both avirulence genes, only the avirulence towards Rlm7 is expressed. The AvrLm3 avirulence phenotype is only expressed when an isolate displays a deleted or a non-functional allele of AvrLm4-7.

Objectives: Following the cloning of AvrLm4-7 (1), we cloned AvrLm3 to decipher the functional relationships between AvrLm3 and AvrLm7 and to understand the role of each avirulence protein in pathogenicity.

Methods: An AvrLm3 candidate gene was identified combining several approaches: genetic mapping, BAC-clone sequencing and RNA-seq of plant-pathogen interaction. Complementation and silencing assays were performed to validate the AvrLm3 candidate and the functional antagonism between AvrLm3 and AvrLm7 phenotypes. The presence of AvrLm3 and its allelic diversity were analyzed in a large collection of world-wide isolates.

Results: The AvrLm3 locus is genetically close to AvrLm4-7 and located in a genomic region partially absent from the assemblies of the reference sequence genome of L. maculans as well as from a series of resequenced L. maculans genomes. AvrLm3 encodes for a small secreted protein, rich in cystein residues and the gene is highly expressed at early infection stages. The genotyping of field isolates showed that AvrLm3 is highly conserved in field populations, with no isolates displaying deletion of AvrLm3. Only one avirulent allele but several virulent alleles have been identified. Complementation of an avirulent isolate towards Rlm3 with a functional allele of AvrLm4-7 leads to avirulence towards Rlm7 and to the loss of the avirulence towards Rlm3.

Conclusions: We cloned a new avirulence gene of L. maculans which has classical AvrLm genes characteristics. AvrLm3 is involved in a complex interplay with AvrLm4-7. A similar interaction has only been identified in one other ascomycete, Fusarium oxysporum (2). In the context of the outbreak of isolates virulent towards Rlm7 due to the massive deployment of Rlm7, Rlm3 is becoming effective again. Therefore, this negative interaction between two avirulence genes offers opportunities for the development of strategies for sustainable management of resistance genes. A modelling approach is in progress to determine which strategy, among pyramiding or alternating Rlm3 and Rlm7, is the best to be deployed.

References:
(1) Parlange et al, 2009 Mol Microbiol 71 851-863
(2) Houterman et al, 2010 Plos Pathog 4 e1000061

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Identification of cotyledonary resistance to *Leptosphaeria maculans* (the causative agent of blackleg) in cultivated *Brassica oleracea* accessions

**Background:** The genetic resistance to blackleg in canola cultivars can be easily eroded by the changes in the virulence of field populations of the causative agent *Leptosphaeria maculans*. Breakdown of the host resistance has been reported in several countries in Europe and in Australia (Rouxel et al. 2003, Li et al. 2003).

**Objective:** In Australia, the major genes *Rlm2, Rlm9, LepR3* have broken down and there are no longer effective for breeding purposes. As a result, there is a constant challenge to discover novel sources of blackleg resistance in related plant species. To date, major resistance genes to blackleg have been discovered only in the A genomes (*Brassica rapa* and *B. napus*) and B genomes (*B. carinata* and *B. juncea*) with none reported in the C-genome.

**Method:** For this study, 37 cultivated *B. oleracea* accessions were screened in controlled environment conditions. The gene profile was identified using a differential set of 12 field-derived single-spore isolates of *L. maculans* (Marcroft et al. 2012). The experimental design was randomised block design with 30% partial replication. Cotyledons of 10 days old seedlings were wounded and inoculated with 10 µL of each of the individual isolates. Lesion scores were determined 14 to 21 days after inoculation.

**Results:** Three cultivated *B. oleracea* accessions have shown high levels of cotyledonary resistance to a number of the differential isolates.

**Conclusion:** This paper reports the identification of resistance to blackleg in the C-genome of diploid *Brassica oleracea* accessions, for the first time. These resistant lines will be utilised in a re-synthesis program and molecular markers for these genes will be sought.

**References:**


Standing up against a bully: Plant defense mechanisms underlying blackleg resistance in *Brassica napus*

**Background:** *Leptosphaeria maculans* (Blackleg) is a devastating fungal pathogen of *Brassica napus* (canola) and causes millions of dollars in crop damage and loss to growers around the globe each year. Current blackleg disease management strategies rely heavily on gene-for-gene resistance, however the genetic mechanisms mediating these interactions in canola remain poorly characterized.

**Objectives:** We are interested in understanding defense strategies against the devastating fungal pathogen, blackleg in canola at the molecular level using cutting edge transcriptomics. We studied the transcriptome in a commercially available resistant and susceptible line before, during and after blackleg inoculation of the cotyledons to identify the genes and gene regulatory processes associated with plant resistance.

**Methods:** We sequenced canola cotyledons in Westar (susceptible) and Dl15 (*LepR1*) before, during and after the infection processes at the level of the RNA. A total of 36 RNA sequencing libraries were analyzed using robust bioinformatics strategies to identify differentially expressed genes between control and infected tissues. Fuzzy K-means clustering and GO term enrichment analyses revealed dominant patterns of genes activity thought to control cellular defense mechanisms. We complemented our transcriptome series with a comprehensive histological analysis at the light and electron levels.

**Results:** Both susceptible and resistant cultivars had an increased abundance of transcripts associated with canonical defense responses like jasmonic acid, ethylene, and salicylic acid signaling, production of antifungal compounds and phytoalexins, and programmed cell death. Populations of transcripts were found to accumulate specifically in the *LepR1*-mediated resistant cultivar. This included a variety of highly active kinases and other transcripts that code for proteins that have demonstrated the ability to carry out the oxidative burst. In addition, transcripts coding for transcription factors, calmodulin binding proteins, and other upstream signaling proteins were found to be up-regulated only in the *LepR1* containing cultivar.

**Conclusions:** We provide the most comprehensive blackleg infection dataset produced to date in the cotyledon of *B. napus*. A subset of transcripts were found to accumulate specifically within the *LepR1* resistant cultivar during infection and may coordinate the defense response necessary for plant resistance at the seedling stage of development. These data provide an informatics resource for those interested in the genes responsible for mediating blackleg resistance in canola.
Optimal agronomic conditions for spring and winter canola production in northern Idaho

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Background: Lack of economically viable alternative crops to grow in rotation with small grain cereals has increased producers interest in growing spring and winter canola. Higher yielding canola cultivars combined with competitive prices has resulted in an increase of canola acreage in the Pacific Northwest region. Although adapted canola cultivars are now available to growers, few attempts have been made to optimize productivity through agronomic management of the crop.

Objectives: The aim of this two-year study is to optimize growers’ productivity and profitability with a range of adapted winter and spring canola cultivars in specific environments. Agronomic factors examined include planting date, seeding rate, and fertility management in two different tillage systems.

Methods: Two winter canola cultivars were planted at two locations in July and in August in the fall of 2013 and 2014. Similarly, four spring canola cultivars were planted at two locations as early as possible and two weeks later in the spring of 2013 and 2014. At each planting, cultivars were seeded at three rates and were grown with six nitrogen levels in replicated yield trials. A range of morphological characters were recorded throughout the growing season. At harvest seed yield and oil content was recorded.

Results: Amanda was the highest yielding winter cultivar (3,582 kg ha-1) over all factors examined and responded significantly better to increased nitrogen levels compared to Hyclass-125 (3,168 kg ha-1). Early planting winter canola produced higher seed yield (3,517 kg ha-1) compared to later plantings (3,235 kg ha-1), and yield from intermediate and high seeding rates were not different. Interactions between spring cultivars and most agronomic factors were often significant but small in magnitude compared to the main effects. All the spring canola cultivars produced higher yields when planted early, at intermediate seeding rates and with moderate to high nitrogen availability; although, as with the winter cultivars, the spring cultivars responded differently to nitrogen availability. Highest spring yield was 2,684 kg ha-1 for the cultivar DKL 30-42 with 249 kg N ha-1. Agronomic factors had little effect on seed oil content for either winter or spring cultivars tested.

Conclusions: In general early planting was advantageous for both winter and spring canola cultivars. Low seeding rates can reduce yield potential in most but not all situations. Cultivar choice has the largest impact on overall yield potential. However, cultivars did interact with regard to nitrogen application and it is critical to determine optimal nitrogen requirements on a cultivar by cultivar basis.
On-farm studies of canola insect pests: effects of insecticide application and seeding date on seedpod weevils, lygus and seed yield

**Background:** Cabbage seedpod weevil is a chronic exotic pest of Canola in southern Alberta and Saskatchewan. The main damage is caused by larvae feeding on seeds, therefore, growers spray insecticide at early flower to target adults. Lygus bugs, on the other hand, are native pests that attack many crops including canola and are controlled, also with insecticides but sprayed later at the pod stage. In the drier Short Grassland Eco-Regions of Alberta the dominant species are *L. elisus* and *L. borealis* and *L. keltoni* (Carcamo et al. 2002). Lygus bugs can increase rapidly under dry hot weather through reproduction and dispersal from other sites as plants senesce (Otani and Carcamo 2011).

**Objectives:** Determine impact of spraying insecticide for cabbage seedpod weevil at early flower on abundance of lygus bugs at early pod in commercial farms and the effect of seeding date on the management of this pest complex.

**Methods:** Insects were sampled using a sweep net in 75 farms from 2010 to 2013. Four to eight strips (about 25 m wide and 50-100 m long) along the borders of the fields were left unsprayed to assess the impact of the insecticide on insects and seed production. Yield was collected from all these sites using 64 quadrat samples (0.25 m²) per site. For 20 fields of these fields we also obtained the yield from the farmers combine monitors. Also, damage to pods by seedpod weevil was determined from sub-samples from the main racemes of selected fields. For all fields we obtained information on seeding dates and cultivars seeded and local rainfall for selected fields.

**Results:** Early seeding in April increased the risk of having high weevil pests but decreased the risk of lygus. Sites planted latest, during the last two weeks of May, had the most lygus and the fewest weevils and those planted at a normal period (first 2 weeks of May) had intermediate numbers of both pests. Spraying insecticide at early flower resulted in fewer lygus at the pod stage in most fields and a yield increase over the untreated strips of approximately 100 kg/ha (2 bu/ac) across all sites. However, there was very high variability from site to site and year to year. Correlation analysis suggested that lygus bugs at early flower were not related to yield or to lygus numbers at later crop stages.

**Conclusions:** Growers should not extrapolate economic thresholds for lygus below one per sweep even at high canola prices. Canola fields planted early with less than 2-3 weevils per sweep should not be sprayed prophylactically for lygus bugs.

**References:**

Mapping and identification of clubroot resistance genes in *Brassica nigra*

**Background:** Clubroot disease caused by *Plasmodiophora brassicae* is one of the most serious diseases that affect the plant family *Brassicaceae*. It is an emerging threat to canola and mustard production in western Canada. To manage the disease, it is very important to identify and use new sources of clubroot resistance (CR) for developing canola and mustard cultivars. *Brassica nigra* lines with a broad spectrum of resistance to clubroot were recently identified (Peng et al. 2014).

**Objectives:** The project aims to identify CR genes and fine map the genes in *B. nigra* for facilitating map-based gene cloning; and to develop robust genetic markers tightly linked to the CR genes for use in marker-assisted selection in canola and mustard breeding programs.

**Methods:** Plant materials were as follows: resistant (R) lines BA and PI; susceptible (S) line CR2748. The two CR *B. nigra* lines were crossed with CR2748, respectively to produce the F1. F1 plants were self-pollinated to produce F2. F1 plants from CR2748 X PI and CR2748 X BA were backcrossed with CR2748 plants to produce BC1 populations. Genetic mapping of CR genes was carried out using bulked segregant RNA-seq described by Liu et al. (2012). RNA was isolated from each bulk using RNeasy Plant Mini Kit (Qiagen). The cDNA libraries were prepared using TruSeq RNA Sample Preparation Kits v2 (Illumina). RNA-seq was carried out on MiSeq platform. Validation and genotyping of SNP markers were carried out using Kompetitive Allele Specific PCR method.

**Results:** Complete resistance to clubroot was found in all F1 plants derived from crosses of CR2748 with PI and BA, respectively. Evaluation for resistance to clubroot showed ratios of 1R:1S in BC1 and 3R:1S in F2 for both R genotypes, indicating that CR is controlled by a single dominant gene in either PI or BA. Short reads from R and S bulked RNA-seq samples in the BC1 population derived from PI were assembled into the *B. rapa* reference genome v1.5 respectively. A CR gene designated Rcr6 in PI was mapped in a region homologous to *B. rapa* chromosome A08. Three SNP markers (SNP_A08_14, SNP_A08_15 and SNP_A08_17) linked to Rcr6 were developed. Plants in the BC1 population with BA were also analyzed with the SNP markers linked to Rcr6 and results showed that resistance in BA was not associated with the Rcr6 linked SNP markers, indicating that the CR gene namely Rcr8 in BA is not in the Rcr6 region.

**Conclusions:** This is the first report on mapping of CR genes in *B. nigra*. Two CR genes Rcr6 and Rcr8 were identified in *B. nigra*. Rcr6 was genetically mapped and SNP markers linked to the gene were developed.

**References:**
Blackleg and clubroot – Disease resistance development in Cargill Specialty Seeds and Oils

**Background:** Blackleg (*Leptosphaeria maculans*) and clubroot (*Plasmodiophora brassicae*) are major diseases of canola across the western prairies of Canada. Current farming practices including tight rotations impose extreme selection pressure resulting in the shifting of pathogen populations. This has lead to more severe disease epidemics and the generation of new and increasingly more virulent pathotypes of these species, which puts the canola industry in western Canada at risk.

**Objectives:** Develop a plan to combine durable disease resistance breeding, strategic deployment of resistant canola hybrids along with integrated pest management to slow down the erosion of resistance against these two diseases and ensure the high yielding potential of Cargill canola hybrids.

**Methods:** New germplasm disease screening, evaluation of pathogen avirulence gene profiles, genetic marker-assisted breeding, indoor and outdoor evaluation of seedling and mature plant resistance under high disease pressure and long term resistance gene deployment were used to reach the goals of high yield and disease resistance.

**Results:** Blackleg disease resistance levels in both public disease trials at different geographic locations and through a series of post registration evaluations indicate that Cargill canola hybrids harbor high and durable resistance to blackleg. Meanwhile clubroot resistance in Cargill products is not only effective against the predominant pathotypes found in Alberta today, but also against new pathotypes recently identified.

**Conclusions:** Exploring different genetic pools and fully understanding the structure of pathogen populations greatly help in the deployment of effective resistance genes to control blackleg and clubroot. Combining major gene resistance with quantitative trait loci is the key for durable resistance.

**References:**
Effect of spore load on growth of clubroot-resistant canola and Napa cabbage

**Background:** Clubroot caused by *Plasmodiophora brassicae* Woronin reduces yield in canola (*Brassica napus* L.) and *Brassica* vegetables such as Napa cabbage (*B. rapa* L. ssp. *pekinesis*). Genetic resistance is essential for clubroot management. However, studies indicate that high spore loads may reduce growth and delay development in clubroot-resistant cultivars of canola. (Deora et al. 2012, Hwang et al. 2011). This indicates that resistance to clubroot involves a high level of metabolic activity in the plant.

**Objectives:** To compare the growth of clubroot-resistant canola and Napa cabbage cultivars at two adjacent field sites that differ only in spore loads of *Plasmodiophora brassicae*.

**Methods:** One susceptible and three clubroot-resistant cultivars of canola and of Napa cabbage were direct seeded in soil naturally infested with *P. brassicae* at the Muck Crops Research Station, Holland Marsh, Ontario. Each trial was conducted as a randomized complete block design with four replicates. Plant growth was assessed weekly in canola by measuring plant height from hypocotyl (at soil surface) to shoot apex. Plant growth in Napa cabbage was assessed weekly by measuring the leaf length of the third and fourth youngest leaves. Area Under the Growth Stairs was calculated using weekly measurements. At 9 weeks after planting, the proportion of canola plants at selected developmental stages (vegetative, bud, flowering, pod development) was assessed. Plants were harvested and weighed and roots were assessed for clubroot incidence and severity using a standard 0–3 rating scale. The data were combined across trials and analyzed using a mixed model analysis of variance to examine the interaction between cultivar and location (PROC MIXED, SAS software version 9.1).

**Results:** There were no symptoms of clubroot in the resistant cultivars, but severe clubroot developed (100 DS) in the susceptible canola control at both sites. At the location with a higher spore load (1 x 10^6 resting spores g^-1 dry soil), the height of the resistant canola cultivars was reduced by 39% (_6 SE) and leaf length of Napa cabbage was reduced by 19% (_3 SE) relative to the site with a lower spore load (1 x 10^5 spores g^-1 soil).

**Conclusions:** These field results support the observations from controlled environment trials (Deora et al. 2012) that high concentrations of resting spores of *P. brassicae* cause a reduction in the growth of clubroot-resistant cultivars of canola. A similar pattern of reduction was also observed for vegetative growth of Napa cabbage.

**References:**


Optimizing blackleg chemical control: Baseline sensitivity of QoI fungicides and effect of timing of application on disease control

**Background:** Blackleg, a disease caused by the fungus *Leptosphaeria maculans* is resurging as an important threat to canola (*Brassica napus*) production in North Dakota. Most commercial cultivars planted in the region are resistant to *L. maculans* strains containing *Avrlm2* and/or *Avrlm3* but new strains capable of defeating these resistance genes are increasing in prevalence (Nepal et al., 2014). Consequently, the use of fungicides to manage this disease will increase in the near future as blackleg outbreaks intensify. Four of the five fungicides registered in North Dakota to control blackleg are Quinone-outside inhibitors (Friskop et al., 2015). Since many plant pathogens have developed resistance against fungicides of this group (Ma and Michailides, 2005), it is expected that *L. maculans* isolates with resistance to these compounds may develop in the future. To delay development of resistance to these compounds it is necessary to monitor changes in sensitivity and optimize fungicide applications.

**Objectives:** Develop baseline sensitivity information of *L. maculans* to azoxystrobin and pyraclostrobin and determine the association between timing of fungicide application on disease control.

**Methods:** Replicated trials to accomplish these objectives were conducted twice. Sensitivity of *L. maculans* isolates to these fungicides was estimated using the methodology described by Wise et al. (2008) and was expressed as the concentration that reduced spore germination by 50% (EC50). Mean and median EC50 values were calculated for samples collected between 2004 and 2012. To determine the relation between timing of application and efficacy of control, trials were conducted in greenhouse conditions. Cultivar Westar was inoculated with a mixture of five virulent isolates 12 days after planting and sprayed with commercial doses of the fungicides -2, 0, 2, 4, 8 or 16 days after inoculation. Disease severity was estimated 12 days after inoculation and prior to harvest.

**Results:** Sensitivity to azoxystrobin was normally distributed in the population sampled and had a mean EC50 of 0.07 µg ml⁻¹ and a median of 0.08 µg ml⁻¹. Estimation of sensitivity to pyraclostrobin is under way. Greenhouse trials indicated that both compounds provide the best protection when applied no more than four days after inoculation. Delaying fungicide application by 16 days increased plant mortality significantly.

**Conclusions:** North Dakota *L. maculans* populations are very sensitive to azoxystrobin at present time. Growers should apply fungicides as early as possible after the crop has emerged; delaying the application could compromise the efficacy of control.

**References:**

Changes in pathogenic variability of *Leptosphaeria maculans* in North Dakota

**Background:** Blackleg, caused by *Leptosphaeria maculans*, is once again becoming a threat to the canola (*Brassica napus*) industry in North Dakota (del Río Mendoza et al., 2012). This resurgence is powered by a shift in the virulence profile of *L. maculans* populations in the state that was first noticed in 2003 (Bradley et al., 2005). A more recent paper that documented the change by comparing the prevalence of strains from pathogenicity groups 2, 3, T, and 4 among isolates collected between 2004 and 2009 concluded the previously dominant PG-2 had been replaced almost completely by strains from groups 3 and 4 (Nepal et al., 2014). Strains from these groups are in general more aggressive and capable of overcoming resistance carried by most commercial varieties grown in the region (Nepal et al. 2014). The sequencing of *L. maculans* avirulence genes has made possible to determine their prevalence in populations using PCR assays (Gout et al., 2006; Fudal et al., 2007; Parlange et al., 2009; van de Wow et al., 2014). Combining traditional screenings with PCR assays could provide a more complete image of the variability present in *L. maculans* populations in North Dakota.

**Objectives:** Characterize the prevalence of pathogenicity groups and known avirulence genes in *L. maculans* isolates from North Dakota.

**Methods:** Single-spore cultures from *L. maculans* isolates collected in 2014 were evaluated in greenhouse to determine pathogenicity groups by inoculating them on differential cultivars ‘Westar’, ‘Glacier’, and ‘Quinta’ as described by Nepal et al. (2014). Screening these isolates with additional differentials is under way. Presence of avirulence genes *AvrLm1*, *AvrLm6*, *AvrLm4-7*, and *AvrLm11* is being carried out using primers and PCR assays described in the original papers.

**Results:** Preliminary results suggest that PG-4 continues to be the most predominant pathogenicity group in North Dakota. Assays to determine the prevalence of avirulence genes is under way.

**Conclusions:** Will be based on outcome of results.

**References:**


Control of Sclerotinia stem rot in oilseed rape: Initial investigations and plans for future work at the Centre for Crop and Disease Management

**Background:** The fungus *Sclerotinia sclerotiorum* causes disease in over 400 species of plant including oilseed rape, in which it is commonly termed Sclerotinia stem rot (SSR). Disease only occurs when environmental conditions are suitable for fungal germination and growth, though *S. sclerotiorum* can remain soil borne for up to a decade in the form of tough resting bodies called sclerotia. In Australia, as oilseed production has increased in response to global demand, SSR incidence has been on the rise. There is now an urgent need to develop new ways of quelling yield losses attributed to this disease.

**Objectives:** With our fledgling research program at the Centre for Crop and Disease Management based at Curtin University, Perth, we plan to explore several lines of investigation regarding SSR management, with an aim of understanding key aspects of fungal pathogenesis. We will conduct an assessment of *S. sclerotiorum* global genetic diversity through a collaborative effort; this will include improvement of the *S. sclerotinia* reference genome with up-to-date sequencing technology. We will conduct a genome wide association study on *Brassica napus* varieties in an attempt to elucidate its genetic potential for SSR resistance. We will test fungicides that are not registered in Australia for their efficacy against locally collected fungal isolates. And we will use the model species *Arabidopsis thaliana* to identify the most promising genetic modifications for SSR resistance, with the overall aim of introducing these into *B. napus*.

**Methods:** Intergenic spacer (IGS) sequences of 70 *S. sclerotiorum* isolates collected from four regions in Western Australia were sequenced and compared. These isolates were then phenotypically screened using a mycelial compatibility group (MCG) test to determine whether those with similar IGS sequences were vegetatively compatible. Several fungicides currently unregistered in Australia for the control of SSR were tested in vitro on local *S. sclerotiorum* isolates and their EC50 concentrations were determined. The genome of the reference isolate was re-assembled using reads generated with Pacific Biosciences single molecule real time sequencing technology.

**Results:** The 70 *S. sclerotiorum* isolates fall into four distinct IGS groups, which have been identified in several other regions globally. These groups largely correspond to MCGs based on in vitro screening for vegetative compatibility. Unregistered fungicides of the strobilurin, azole and carboxamide classes have good activity against local *S. sclerotiorum* isolates in vitro. Re-sequencing of the reference genome allowed for closure of approximately 1 Mb of sequence gaps, leading to an improved and more reliable sequence.

**Conclusions:** IGS sequencing indicates that Australian *S. sclerotiorum* isolates share genetic similarities with the global population. Correlation of sequences with MCGs indicates no unusual propensity for outcrossing or other forms of genetic recombination in these isolates. There is a rationale for field-trialling and registering several antifungal chemistries currently out of reach of Australian oilseed growers. The improved genome of *S. sclerotiorum* is close to the size predicted by optical mapping in a previous study. This new sequence will be an important resource for global isolate comparison and the *S. sclerotiorum* research community.

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Genome sequencing provides insight into pathogenicity of *Plasmodiophora brassicae*

**Background:** Clubroot disease of *Brassica* crops is caused by the biotrophic parasite *Plasmodiophora brassicae* (Pb). Infection of canola (*Brassica napus*) by Pb stimulates gall formation on the roots, which alters source/sink relationships, impedes water transport and results in significant yield loss in severely infected crops (Dixon, 2014). The most virulent pathotype of Pb on canola in Canada is pathotype 3 (Pb3), as defined on the differentials of Williams (1966). In contrast, pathotype 6 (Pb6) is virulent mainly on vegetable *Brassicas* (Strelkov and Hwang, 2014). As an obligate biotroph, Pb is highly adapted to suppress host defence through secretion of effector proteins. Effectors are defined as small, secreted proteins produced by the pathogen to help with pathogen colonisation through the suppression of host immunity (Win 2012).

**Objectives:** Identification and functional characterization of Pb effectors

**Methods:** The genomes of Pb3 and Pb6 were sequenced from isolated resting spores using the 454-titanium platform. RNA from root tissues of *B. napus* cv. DH12075 infected with Pb3 was extracted at 1-week intervals (up to 6 weeks after inoculation), and the transcriptome sequenced by Illumina technology. A subset of predicted effectors was cloned and characterised by transient expression in planta.

**Results:** The Pb3 and Pb6 genomes were assembled into 109 and 356 scaffolds, respectively. The total number of predicted genes for Pb3 was 10,851 and for Pb6 10,070. A total of 590 effector genes were predicted for Pb3 with 317 of them ranging in size between 1000 bp to 300 bp. We have identified effectors that are able to suppress PAMP-triggered immunity (PTI) in a transient expression assay in tobacco leaves.

**Conclusion:** This study provides the first insights into the genome of *P. brassicae*, an obligate soil-borne parasite that has been difficult to study as it resides within the root tissue of its host. The Pb genome is small (24.2 Mbp) and highly compact with less than 2% repeats. We have identified a subset of genes as potential virulence factors and provided evidence for their function in the suppression of plant defence.

**References:**


Williams, P.H., 1966. A system for the determination of races of *Plasmodiophora brassicae* that infect cabbage and rutabaga. Phytopathology, 56, 624-626

Diversity of symptoms in *Arabidopsis* plants infected by a Canadian strain of AY-WB

**Background:** Phytoplasmas are non-culturable bacteria that are able to infect many plant species including ornamentals, crops, and perennial plants. Plants that are infected by a phytoplasma species often develop symptoms ranging from yellowing, witches’ broom, virescence, phyllody, stunting, little leaf, big bud or other symptoms. Insects (primarily leafhoppers) act as vectors to transmit the pathogen. Some of the symptoms caused by phytoplasma are strain specific while other symptoms are common features of phytoplasma infection among various host plants. The Aster Yellows phytoplasma strain Witches’ Broom (AY-WB) infects many species of crucifer family including *Brassica napus* (canola/rapeseed) and *Arabidopsis thaliana*. AY infection can be devastating, leading to severe yield loss (Olivier et al., 2010), and it is of great concern that the frequency of AY infection in Canada is on the increase.

**Objectives:** Using *Arabidopsis* as a model to study AY-WB disease of canola.

**Methods:** The SK AY-WB strain of phytoplasma was transmitted to *Arabidopsis* Col-0 using the leafhopper species *Macrostella quadrilineatus* (Mq). Plants were subsequently observed for the development of the symptoms. The presence of the phytoplasma was detected in the infected plants as described by Dumonceaux et al., (2014). Moreover, the possibility of the transmission of phytoplasma through the seeds of the infected *Arabidopsis* plants was examined.

**Results:** *Arabidopsis* Col-0 plants showed a range of symptoms after infestation by Mq leafhoppers carrying SK AY-WB. The symptoms varied from stunting, anthocyanin accumulation, yellowing, witches’ broom, to phyllody, virescence, little leaf, big bud and apical dominance. The multiplicity of symptoms was surprising as it encompassed the symptoms of a wide diversity of phytoplasma strains on different hosts. Infected plants showed a drastic drop in the seed production, and the seeds were shrivelled in the heavily infected plants. Progeny of the plants with severe symptoms showed overall reduced growth, anthocyanin accumulation in younger leaves, and yellowing of the leaves compared to the healthy control plants. Traces of phytoplasma were monitored and detected by PCR in the progenies of infected plants.

**Conclusion:** This study provides the first evidence that SK AY-WB of Canola could infect *Arabidopsis* using infected *M. quadrilineatus* leafhoppers. Plants that were infected by the phytoplasma showed various symptoms typical of different phytoplasma species. The *Arabidopsis* – AY-WB pathosystem described here provides an interesting model to study the interaction of AY-WB with its host plant.

**References:**


Potential establishment of *Leptosphaeria maculans* (phoma stem canker) on Chinese oilseed rape

**Background:** Phoma stem canker (blackleg), caused by *Leptosphaeria* species, is a serious disease of oilseed rape (*Brassica napus*) that produces considerable worldwide losses. In China, phoma stem canker has not generally been a serious problem and only the less damaging *L. biglobosa* has been isolated from diseased crops. However, it is possible that the more damaging *L. maculans* may spread to China since it has spread into areas where only *L. biglobosa* had been present, such as Canada or Poland (Fitt et al. 2008).

**Results:** Both *L. biglobosa* and *L. maculans* were detected on crop debris and seed in shipments of oilseed rape seed imported into China through Shanghai or Wuhan ports in 2009-2011 (Zhang et al. 2014). Incidence of phoma stem canker in pre-harvest surveys from 2005 to 2012 was greater on winter oilseed rape along the Yangtze River in central China (in May) than on spring oilseed rape in north China (in August). When the causal pathogen was isolated from stem cankers, it was always identified as *Leptosphaeria biglobosa* by morphology in culture and/or by species-specific polymerase chain reaction and no *L. maculans* was isolated. Descriptions of the observed spread of *L. maculans* into areas previously colonised by *L. biglobosa* across a spring oilseed rape growing region (Alberta, Canada, westwards, 1984-1998) were used to estimate the potential westward spread of *L. maculans* in China across spring oilseed rape growing regions (north China). Descriptions of the spread across a winter oilseed rape growing region (Poland, eastwards, 1984-2004) were used to estimate spread across a winter oilseed rape growing regions (central China). The rates of spread were estimated as 47 km per year across spring oilseed rape in north China and 70 km per year across winter oilseed rape in central China. Dispersal modelling suggested that the rate of spread of *L. maculans* across Alberta, Canada (c. 17 km per year) could be explained by wind-borne dispersal of ascospores.

**Conclusion:** It is important to develop strategies to decrease the risk of spread of *L. maculans* into China.

**References:**
Clubroot disease in rapeseed - A persistent challenge thanks to varying pathotypes

**Background:** Clubroot, caused by *Plasmodiophora brassicae*, is a soil-borne disease of high importance for *Brassica* crops. Infestation with the pathogen causes thickening of roots and subsequent gall formation. On heavy infested fields a seed yield reduction of 50% or more can be observed. By today, the disease is wide-spread and prevalent on every continent. Clubroot infection can be monitored on ca. 8% of the European cultivated rapeseed area. Clubroot became a major disease in North America, Australia, Asia and especially in Europe. High incidence of the disease is noted across Europe including regions in Poland, Germany, France, Czech Republic, UK, Denmark and The Netherlands.

Infestation with *Plasmodiophora brassicae* can be reduced by different means including wide crop rotation, high grade agronomical practice, weed control and amelioration liming. However, the method of choice to reduce damage on rapeseed caused by *Plasmodiophora brassicae* is breeding for resistant varieties (DIEDERICHSEN et al. 2009).

Pathotype-specific resistances in Europe: For success of resistant varieties, monitoring of pathotypes of *Plasmodiophora brassicae* in rapeseed cultivation areas is of prime importance. Pathotypes are quite similar phenotypically but differ towards their virulence against different host plants. Due to specific host-pathogene interaction it is possible to classify pathotypes. In Germany pathotype P1, P3 and P5 (according to SOMÉ et al. 1996) are the most common ones. European breeders used this knowledge for the development of new rapeseed varieties with resistance to the prevailing pathotypes. This work led to the development of two clubroot resistant rapeseed varieties, Mendel and Tosca registered in Europe by 2000. Due to the fact, that the resistance takes effect only on the prevailing pathotypes in Europe, it is called pathotype-specific resistance.

Challenges for resistance breeding in Europe: The introduction of Mendel to the European market helped farmers to cultivate rapeseed even on infested fields. Today’s new clubroot-resistant varieties possess better seed yield and agronomic characteristics reflecting the investment of plant breeding into better control of the disease. Pathotypes of *Plasmodiophora brassicae* have the ability to widely adapt to environmental changes and to overcome the resistance of Mendel. Especially in Germany resistance breaks have been reported in different regions over the last years (ZAMANI-NOOR 2014). Today, it is the challenge for European rapeseed breeders to detect new sources of resistance against clubroot and to integrate them in new competitive rapeseed varieties.

**References:**


Clubroot control in oilseed rape using host resistance – potential and challenges

Clubroot has gained significant relevance in European oilseed rape production and is expected to further increase its impact due to close crop rotations and climate change. To control this disease an integrated approach needs to be applied, which is primarily based on the use of resistant cultivars. A number of resistant cultivars has been released in the past decade, all of these are basically sharing the same resistance source. The performance of resistant cultivars in respect to agronomy and efficacy of their resistance towards local Plasmodiophora brassicae isolates will be presented. Compatible races are present but so far these did not cause wide-spread break-down of resistance efficacy. Some future perspectives of clubroot resistance breeding will be addressed including new resistance sources from Brassica and Raphanus and clubroot reactions of interspecific/ intergeneric hybrids will be presented. Basic considerations on resistance management for this soil-borne disease and its application for clubroot resistance will be discussed.

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Leptosphaeria maculans effectors involved in the oilseed rape systemic colonization

**Background:** The blackleg, caused by the fungus *Leptosphaeria maculans*, is one of the most devastating diseases of oilseed rape. This disease is essentially managed through the breeding and use of resistant varieties. Specific resistance genes, interacting with the corresponding avirulence genes according to the gene-for-gene model, are used for genetic control in the fields. However, the fungus may overcome these resistance genes. In that case, *L. maculans* colonizes the plant tissues, from the leaves to the crown, during a systemic and asymptomatic phase. It eventually switches to a necrotrophic stage leading to the damaging crown canker. The fungus may either successfully escape plant defenses leading to the stem canker development, or the plant can develop an “adult-stage” resistance (1) leading to a reduced stem necrosis incidence. To date, we have no information on the fungal and plant genes involved in the molecular dialogue during systemic infection and stem canker development.

**Objectives:** Similarly to other models in which concerted waves of expression of effectors genes occur (2), we hypothesized that a set of fungal proteins, called “late effectors”, are expressed only during systemic growth and/or stem necrosis development, and are necessary for the endophytic colonization of the plant. The objective of this project is to identify and characterize late effectors specifically expressed during the systemic colonization, and to understand whether “matching” resistance genes exist in the plant and may be involved in adult-stage resistance.

**Methods:** A RNAseq analysis evaluated fungal and plant genes expressed at three stages of the infection under controlled conditions (early infection, endophytic colonization and stem necrosis).

**Results:** *L. maculans* specifically expresses 165 genes encoding Small Secreted Proteins (SSPs) during stem infection. Except for their expression kinetics and genome location, these SSP display all characteristic features of early infection effectors like avirulence genes. Thus these SSPs are promising candidates as effectors involved in the systemic colonization. Among those 165 genes, 10 candidate effectors were chosen for further characterization based on their high level of expression, and their level of conservation in *L. maculans* populations.

**Conclusions:** These results suggest that successive waves of effector genes could be expressed by *L. maculans*, enabling it to carry out its complex life cycle. To elucidate whether late effectors could be recognized by plant resistance genes, it is envisaged to characterize and to genetically manipulate these late effector genes in order to express them during the early stages of infection. This could give us access to new tools for the identification of efficient resistance sources.

**References:**

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Utilizing propidium monoazide (PMA) to differentiate viable and non-viable *Plasmodiophora brassicae* resting spores

**Background:** Management of clubroot (*Plasmodiophora brassicae* Woronin) of canola (*Brassica napus* L.) on the Canadian prairies has proven to be difficult, partly due to the longevity of *P. brassicae* resting spores in soil. One way to quantify the number of soil-borne spores is by using quantitative polymerase chain reaction (qPCR) assays. However, qPCR does not distinguish between DNA from viable and non-viable spores. Propidium monoazide (PMA) has been used in conjunction with qPCR (PMA-qPCR) to discriminate between viable and non-viable microorganisms (Moyné et al., 2013). PMA does not appear to readily cross through the external membrane of living cells, but seems to cross into non-viable cells of many microbes. Once inside the cell, photo-activation causes it to bind to double-stranded DNA. This alteration of the DNA inhibits subsequent amplification by qPCR.

**Objectives:** To assess the potential for using PMA-qPCR to discriminate between viable and non-viable resting spores of *P. brassicae* for accurate quantification of viable spore loads.

**Methods:** Resting spores were isolated from clubbed roots of cabbage. Spores were subjected to heat treatments (80 °C for 0, 5, and 10 min) to produce a mixture of viable and non-viable spores. The spores were then treated with 0, 40, 80, and 120 µM PMA (Biotium Incorporated, Hayward, CA, USA) (Moyné et al., 2013). This was followed by qPCR analysis including a competitive internal positive control (Deora et al., 2015). The data were log transformed and tested for significance using ANOVA and Tukey’s Honestly Significant Difference test at P < 0.05.

**Results:** Heat treatment did not affect estimates of spore concentration based on regular qPCR. However, estimates of resting spore concentration in samples heat-treated for 5 and 10 min were substantially reduced after PMA treatment. PMA-qPCR with 40, 80, and 120 µM PMA estimated the spore number in samples that were not heat-treated at 2–5 x 10^6, whereas samples that were heat-treated for 5 min were estimated to contain 0.9–4 x 10^5 spores (62–96 % reduction), and samples that were heat-treated for 10 min were estimated to contain 0.2–1 x 10^5 spores (95–99 % reduction).

**Conclusions:** This study indicates that PMA-qPCR may be useful in discriminating between viable and non-viable resting spores of *P. brassicae*. Further testing is required to determine if the results from PMA-qPCR are consistent with bioassays of resting spore viability, and if this approach can be applied successfully to a wide range of soil samples.

**Acknowledgement:** Funding was provided by the Canola Science Cluster of the Canola Council of Canada and Agriculture and Agri-Food Canada.

**References:**

Vertical distribution of resting spores complicates clubroot management using fumigants

Background: Clubroot (Plasmodiophora brassicae Woronin) has become an important disease in canola (Brassica napus L.) crops in western Canada. It is spreading quickly in central Alberta, but occurs in isolated patches in other regions.

Objectives: To examine the potential of fumigation to reduce / eliminate resting spores of P. brassicae in infested patches of fields and to assess the vertical distribution of resting spores in various soils.

Methods: Two replicated field trials were conducted in Ontario in 2014 to assess the efficacy of the fumigants metam sodium and chloropicrin in soils naturally infested with P. brassicae. Treatments of metam sodium and chloropicrin were applied at 15–20 cm depth. After application, the area treated with chloropicrin was covered with a totally impermeable film (TIF) for 14 days. The trials were then seeded with a susceptible host and rated using a 0-3 scale at 6 wk after planting.

In 2013, soil cores were collected to a depth of 53 cm from two sampling locations at each of three sites naturally infested with P. brassicae: two sites with mineral soil and one with high organic matter content. Three replicate cores were collected within 1 m² at each sampling location. Resting spore concentration at selected depths in each core was initially assessed using standard qPCR methods. However, there were several instances of negative results from samples where spores were known to be present based on plant symptoms. As a result, a multiplex qPCR assay including a competitive internal positive control (CIPC) was developed to estimate the level of amplification inhibition in the qPCR reaction (Deora et al. 2015). This modification was used to assess each sample.

Results: Moderate rates of metam sodium provided excellent control of clubroot under controlled conditions (data not shown). Chloropicrin was only assessed in field trials because of applicator safety considerations. At a site where disease pressure was high, metam sodium had no measurable effect on clubroot severity, but chloropicrin substantially reduced severity and increased plant growth. At a second site where disease pressure was low, moderate rates of chloropicrin eliminated clubroot and increased plant growth. The variability among assessments of resting spore concentration in soil cores was high, both vertically and horizontally at all four sites.

Conclusions: Neither fumigant eliminated the pathogen at heavily infested sites. Reductions in clubroot severity will likely be more consistent where spore concentrations are lower and the treatment includes a TIF cover. Across all sites, spore numbers declined with increasing depth down to 20 cm depth, but were present to the bottom of each core (53 cm). Localized high spores concentrations, and spores deep in the soil profile, will make it difficult to use fumigants to disinfect sites, but also complicate activities for industries such as utilities and road construction that move large quantities of soil.

References:
Transcriptome and proteome analysis of *Leptosphaeria-Brassica* interaction

**Background:** The hemibiotroph fungus *Leptosphaeria maculans* (blackleg) causes severe yield reduction on canola (*Brassica napus*). *L. maculans* penetrates the leaf tissue through wounds and stomata. Hyphae grow in the intercellular space and move toward the stem as infection progresses and eventually cause lesions at the base of the stem.

**Objectives:** Proteome and transcriptome of *L. maculans* and canola were carried out to understand the molecular aspects underlying the *L. maculans*- *B. napus* interaction.

**Methods:** Cotyledons of cultivar ‘DH-Topas’ that is susceptible to *L. maculans* isolate Lm00100 were sampled at 0, 2, 4, 6 and 8 days after inoculation (dai). RNA was extracted and sequenced using the Illumina HiSeq 2500 platform. RNA-sequence reads were mapped to the host and pathogen genome using CLC Genomics Workbench (1). The statistical software, DESeq2 (2) was used to determine the differentially expressed genes. Protein extracts of apoplastic fluid of cotyledons were subjected to label-free quantitative proteomics analysis to determine the protein profile of *L. maculans* during the same time course.

**Results:** We catalogued the expression profiles of 101040 *B. napus* and 12469 *L. maculans* genes. Among the differentially expressed genes were hydrolytic enzymes, nutrient transporters and a large number of *L. maculans* small-secreted protein-encoding genes.

**Conclusions:** We applied RNA-sequencing in combination with label-free quantitative proteomics and were able to gain information to better understand the pathogenicity of *L. maculans*. Patterns of gene expression during infection provided insight into the colonization and acquisition of nutrient by the pathogen and regulation of pathogen effector genes.

**References:**

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Pyrethroid resistance of pest insects in oilseed rape in Germany

Pyrethroid resistant pollen beetles (*Meligethes aeneus*) are widely distributed in Germany after the first resistant beetles were detected about 14 years before. 53% of populations sensitive in biotests in 2005 declined to 0% in 2011 already. Very clear resistance increased quite fast from 33% in 2005 to 99% in 2014. One reason of the fast development and spread of resistance are limitations in the use of other modes of action in oilseed rape, high infestation pressure in some years and regions, and too many sprays ignoring damage thresholds. In addition only pyrethroids are able to provide sufficient control for some of the pests occurring during the growing season and some products cannot be applied during the flowering period because of bee issues. Results of a monitoring program on pollen beetles show a still increasing resistance of the beetles. Monitoring of the other pest insects of oilseed rape in Germany showed no resistance yet in pest species including *Ceutorhynchus napi* and *C. pallidactylus*, *Dasineura spp.* and *Phyllotreta spp.*, but a clear resistance to pyrethroids has developed in *C. obstrictus* and *Psylliodes chrysocephala* since some years. Resistance of both species has not spread over the whole of Germany yet and resistance factors in laboratory biotests are far below resistance values known for *Meligethes aeneus.*

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**Arabidopsis** species provide insights for anticipatory breeding of durable white rust resistance in **Brassica** crops

**Background:** Major effect genes that encode receptor-like proteins can potentially provide long-lasting resistance to a particular pathogen, if they can detect specific molecules that are vital (highly conserved or slowly evolving) to pathogen fitness. **WRR4** in **Arabidopsis thaliana** provides a **Brassicacea** example. An allele of this TIR-NBS-LRR gene from accession Columbia confers White Rust Resistance to four specialised races of **Albugo candida** from different **Brassicacea** hosts (Borhan 2008). An **Albugo** protein was predicted which is encoded by a conserved allele in all four races and is detected by the **WRR4-Col** protein. **WRR4-Col** is functional in transgenic oilseed **brassicas** (Borhan 2010).

**Objectives:** Every pathogen is capable of evolving new virulence, so combined effect from complementary R-genes is essential for truly durable disease resistance. Early identification of virulent pathogens that can overcome individual R-genes is therefore essential to anticipate which alleles could be assembled for the most effective R-gene combination. Our objectives have been to identify WRR4-virulent isolates of **Al. candida** from wild host species, and to use these to identify the next complementary R-gene.

**Methods:** White rust in **A. thaliana** is usually caused by **Al. laibachii** under field conditions. However, we attempted to collect **Al. candida** by springtime sampling of white rust from floral tissue in natural populations of **A. thaliana** that were growing in close proximity to **Capsella bursa-pastoris** (a prolific source of **Al. candida**). We also used perennial **Arabidopsis** species as a pot-grown bait to trap white rust throughout the year. PCR-based markers were used to identify **Al. candida** from diseased tissue. When identified, an isolate was propagated in **Arabidopsis** accessions lacking **WRR4-resistance**, and then tested for virulence in **Columbia**. If found, then **Col-0** virulent isolates could be used in the next round of R-gene identification by screening a global collection and recombinant inbreds of **A. thaliana** for association and recombination mapping of resistance.

**Results:** Floral tissue proved to be a useful source for **Al. candida** from natural infections of **Arabidopsis**. Although isolates were difficult to propagate and Col-virulence was rare, three have been identified including one from a natural population of **A. thaliana** and one each from bait plants of **A. lyrata** and **A. halleri**. Resistance to all three isolates has been mapped to the **WRR4** locus which contains two additional TIR-NBS-LRR genes.

**Conclusions:** An alternative allele of **WRR4** or an allele from a neighboring gene may provide broader spectrum resistance to **Al. candida**. Combining this allele with the Col-allele could provide a durable combination for white rust resistance in transgenic brassica crops. The new isolates are avirulent in brassicas and are therefore not a direct threat to resistance that already occurs crops. However, identifying the causal mutation for Col-0 virulence would be useful for diagnostics to detect recombinants in pathogen populations that could potentially threaten crop production. Genome analysis of Col-virulent isolates will be used to determine whether causal mutations occur in a conserved effector protein of **Al. candida**.

**References:**
Effects of quantitative resistance on R gene-mediated resistance against *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape)

**Background:** Phoma stem canker, caused by *Leptosphaeria maculans*, is a damaging disease on oilseed rape in the UK and can cause up to 50% yield losses (Fitt et al., 2011). Use of durable host resistance to control this disease is becoming more important. Resistance against *L. maculans* may be major resistance (R) gene-mediated resistance or quantitative resistance (QR). R gene-mediated resistance is race-specific and often rendered ineffective due to pathogen population changes from avirulent to virulent (Sprague et al. 2006). QR is race non-specific and is considered more durable but it is a partial resistance. Recent work showed that QR decreased the speed of increase in frequency of virulent alleles overcoming Rlm6 resistance against *L. maculans* (Delourme et al. 2014). This suggests that QR affects the effectiveness of R gene resistance.

**Objectives:** To investigate effects of QR on resistance against *L. maculans* mediated by different R genes.

**Methods:** Eight winter oilseed rape cultivars with different types of resistance were used in field experiments at 11 different sites in the 2010/2011, 2011/2012 and 2012/2013 growing seasons. Six cultivars had an R gene in a background with or without QR: DK Cabernet (*Rlm1* + QR), Capitol (*Rlm1*), Adriana (*Rlm4* + QR), Bilbao (*Rlm4*), Excel (*Rlm7* + QR) and Roxet (*Rlm7*). Two cultivars had QR without known R genes: Es-Astrid and NK Grandia. The field experiments were arranged in randomised block designs with three replicates. The severities of phoma leaf spots in autumn and stem canker in summer were assessed.

**Results:** For cultivars carrying *Rlm1* or *Rlm4*, which were only partially effective because the frequencies of the corresponding avirulent alleles of *AvrLm1* or *AvrLm4* in *L. maculans* populations were less than 50%, there was no effect of quantitative resistance on severity of phoma leaf spots but there was an effect on severity of stem canker. The severity of stem canker on DK Cabernet (*Rlm1* + QR) was less than that on Capitol (*Rlm1*), suggesting that *Rlm1* is more effective when it is introduced into a host background with QR than without QR. Similarly, less severe stem canker on Adriana (*Rlm4* + QR) than on Bilbao (*Rlm4*) suggested that *Rlm4* is more effective when it is introduced into a host background with QR than without QR. Cultivars Roxet and Excel both carried an effective resistance gene *Rlm7*, because the frequency of the corresponding avirulent allele of *AvrLm7* in *L. maculans* populations was greater than 97%, there were no significant differences between them in severities of both phoma leaf spots and stem canker. Of the two cultivars with only QR, Es-Astrid developed less stem canker at most experimental sites than NK Grandia.

**Conclusions:** There were effects of background quantitative resistance on the effectiveness of R gene-mediated resistance in *Brassica napus*. Quantitative resistance increased effectiveness of R gene-mediated resistance against *Leptosphaeria maculans* in oilseed rape.

**References:**


Effects of temperature on R gene-mediated resistance against *Leptosphaeria maculans* (phoma stem canker) in *Brassica napus* (oilseed rape)

**Background:** Phoma stem canker (*Leptosphaeria maculans*) is an economically important disease on oilseed rape (*Brassica napus*) in Europe, Australia and North America. Effective control of this disease relies on use of two types of resistance; major resistance (R) gene-mediated qualitative resistance and quantitative resistance (QR) (Delourme et al. 2006). However, effectiveness of host resistance is known to be affected by environmental factors, including temperature. *Rlm6*-mediated resistance to *L. maculans* is effective at 20°C but not at 25°C (Huang et al. 2006). At least 16 R genes (*Rlm1-Rlm11, Rlms, LepR1-LepR4*) have been identified in *Brassica* species but only two (*LepR3 and Rlm2*) have been cloned (Larkan et al. 2013, 2015). With predicted global warming, there is a need to investigate effects of temperature on effectiveness of R gene-mediated resistance against *L. maculans*.

**Objectives:** To investigate effects of temperature on effectiveness of resistance against *L. maculans* mediated by different R genes.

**Methods:** Oilseed rape cultivars or breeding lines with different R genes in backgrounds with or without QR were inoculated at 20°C and 25°C. Cotyledons of 12-day old plants were wounded and a 10 µl drop of 10^7 spores/ml conidial suspension placed over the wound. Symptoms were assessed at 16-18 days-post-inoculation on a 0-9 scale (0: no symptoms; 9: large grey lesions with pycnidia). To avoid the effects of background QR and investigate plant defence responses, near-isogenic lines of Topas (susceptible) with R genes *Rlm4* (Topas-*Rlm4*) or *LepR3* (Topas-*LepR3*) were used. Cotyledons of 12-day-old plants were each infiltrated with 10 µl of 10^6 spore/ml conidial suspension at 20°C and at 25°C.

**Results:** There were differences in temperature-sensitivity between the ten different R genes (*Rlm1, Rlm2, Rlm3, Rlm4, Rlm5, Rlm6, Rlm7, LepR1, LepR2 and LepR3*) tested and there were differences in response to temperature for the same R gene in cultivars with or without quantitative resistance. There were differences in defence responses between Topas-*Rlm4* and Topas-*LepR3*, with *Rlm4* responding more quickly and more strongly than *LepR3* at the higher temperature.

**Conclusions:** Background QR affected the temperature-sensitivity of R gene-mediated resistance. Understanding effects of temperature on interactions between hosts and pathogens will help breeding of cultivars with durable, temperature-resilient resistance.

**References:**


Fine mapping of a clubroot resistance gene using SNP markers based on RNA-seq

**Background:** Clubroot disease, caused by *Plasmodiophora brassicae*, is one of the most devastating diseases of canola (*Brassica napus, B. rapa*) in western Canada. It is difficult to control using chemical treatments because the pathogen can persist in the soil as resting spores for many years (Voorrips 1995). Therefore the development of resistant cultivars is considered to be the most effective way to control the disease. However, sources of clubroot resistance (CR) in canola are very limited. Interestingly, materials effectively resistant to a broad range of pathotypes of *P. brassicae* have been identified in one of the *B. napus* progenitor species, *B. rapa* (Peng et al. 2014). Objectives: A Chinese cabbage cultivar “Jazz” showed strong resistance to five pathotypes of *P. brassicae* prevalent in Canada (Peng et al, 2014). In this study, we identified a CR gene namely *Rcr2* in “Jazz” and genetically mapped the gene using bulked segregant RNA-Seq (BSR-Seq) analysis. SNP markers tightly linked to the CR gene were developed for use in marker assisted selection (MAS).

**Methods:** A single resistant (R) plant was crossed with a doubled haploid *B. rapa* canola susceptible (S) line AcDc and the resulting F1 was back-crossed with AcDc to produce a BC1 population. Pathotype 3 of *P. brassicae* was used for inoculation. Leaf tissue from 30 R and 30 S plants were collected and bulked respectively as a biological replicate, with three replicates. RNA was isolated from each bulk using RNeasy Plant Mini Kit (Qiagen). BSR-Seq was performed following the methods described by Liu et al (2012). Validation and genotyping of SNPs were carried out using Kompetitive Allele specific PCR (KASp) method (http://www.lgcgroup.com/). Genetic distance was determined with Joinmap 4.1.

**Results:** A total of 9.76 Gb and 10.76Gb raw reads from R bulks and S bulks were obtained respectively. Approximately 173 K SNPs were identified between R and S bulks. One significant peak was observed on 23-26 Mb of chromosome A03, which was predicted to contain the causal gene *Rcr2* in the region via BSR-Seq. A total of 302 informative genes with 1269 SNPs were detected in the region. Subsequently the BC1 population consisting of 1000 plants was genotyped with 17 SNPs using KASP method and *Rcr2* was fine mapped between two SNP markers SNP_A03_19 and SNP_A03_67, 0.1 and 0.3 cM from *Rcr2*, respectively. The physical distance between the two SNPs is 0.21Mb.

**Conclusions:** A large number of SNPs tightly linked to *Rcr2* were identified through RNA-seq. Fine mapping of *Rcr2* in the study will enable cloning of the gene.

**References:**

**Plasmodiophora brassicae** in soil, water and OSR plants from Poland – bioassay, LAMP and qPCR detection

**Background:** Clubroot has become one of the most serious threats to OSR cultivation in Poland. The disease is caused by a damaging protist, *Plasmodiophora brassicae* Woronin.

**Objectives:** The aim of this work was to survey agricultural soils and water from natural reservoirs close to agricultural fields as well as plants in arable fields to determine the occurrence, quantify the biomass and identify the races of *P. brassicae*.

**Methods:** Plant samples were collected in 2010-2014 from 256 fields of oilseed rape. Agricultural soils were collected randomly from 1168 fields. Water samples were collected from 9 reservoirs, including 6 ponds and 3 drainage ditches, as well as from 6 small puddles in OSR fields. Infestation of all soil samples was studied using a biotest. Out of these 59 samples of soils collected in Poland and one soil sample from Sweden, of known high level ofinfestation by *P. brassicae*, were also assessed using approaches based on Loop-mediated Isothermal DNA Amplification (LAMP) (Kaczmarek et al. 2014) and a quantitative real-time PCR method with Taqman probes (Wallenhammar et al. 2012). Races of the pathogen were evaluated according to Somé et al. (1996).

**Results:** Bioassays, LAMP and qPCR techniques differed with sensitivity, but all detected successfully the pathogen. Additionally bioassays facilitated studies on its races. Plants of OSR with advanced symptoms of clubroot were numerous on farmers’ fields and were also detected on roots of varieties regarded as resistant or tolerant to *P. brassicae*. The concentrations of the pathogen DNA in some soils reached 459 pg g⁻¹ of dry soil, which exceeded 280 million of spores in soil volume. High amounts of *P. brassicae* DNA were also detected in water from puddles collected on fields with infested soil. In water reservoirs *Aspergillus fumigatus*, *A. viridans*, *A. niger* and *Rhizopus nigricans* were also present. Clubroot disease occurred in all the major OSR cultivation areas of Poland, including regions previously regarded as safe or free from the disease. Fields with numerous plants with clubroot symptoms were found in Pomerania, Opole region, Lower and Upper Silesia, Varmia and Mazury. While pathogen incidence was low in other regions, but no region of Poland was exempt from the disease. Apart from OSR, clubroot was also observed on field-planted Chinese cabbage and broccoli. On the whole, the pathogen was found in 86 out of 295 counties (29.2%) that were monitored using a biotest. Extensive screening for races done in 2014 found the prevalence of P1 and P3 (88%) in comparable amounts in most regions, even though races P2, P4 and P5 were also detected in some locations. Regardless of pathogen race, all the methods used detected successfully *P. brassicae* in tested materials.

**Conclusions:** *Plasmodiophora brassicae* is widespread in Poland at very high levels of host and environment infestation. There is a high demand for OSR cultivars with multiple resistance to several races of the pathogen.

**References:**
Exploiting mycoviruses to control Stem rot of oilseed rape caused by *Sclerotinia sclerotiorum*

**Background:** Stem rot caused by *Sclerotinia sclerotiorum* is the most important disease on oilseed rape in China. Due to no resistant-cultivar is available, the loss caused by stem rot every year in China is very huge, about 2 billion $ RMB (or 0.3 billion US$) per year. It is necessary to seek for efficient way to control stem rot. Mycoviruses are viruses that infect fungi and are common in nature. Hypovirulence-associated mycoviruses when released in field may cause debilitation of fungal pathogen, thus, they have a potential to control fungal crop diseases (Xie and Jiang, 2014). However, mycoviruses are transmitted vertically via host reproduction or horizontally via host hyphal anastomosis which only occurs between vegetative compatible individuals. Thus, the efficacy of mycoviruses in field is limited by host vegetative incompatibility reaction. Since the vegetative compatibility of *S. sclerotiorum* in field is very complicated, it may not very easy to exploit hypovirulence-associated mycoviruses to control stem rot of oilseed rape.

**Objectives:** The objectives are screen hypovirulence-associated mycoviruses which could transmit and spread in field efficiently and exploit them to control stem rot of oilseed rape.

**Methods:** Strains of *S. sclerotiorum* were originally isolated from sclerotia produced on the lesion of diseased plant. Strains showed abnormal phenotype were screened to detect if they were infected by mycoviruses either with RNA_seq analysis, or extraction of viral genome RNA and DNA from hyphae. Mycoviruses horizontal transmission test were conducted with dual culture on PDA plate. Mycoviruses that could be efficiently transmitted among host’s different vegetative compatibility groups were further tested their potential to control stem rot of oilseed rape by spraying hyphal fragments suspension on plants of oilseed rape, and then inoculating virulent strain on the same plants.

**Results:** More than 160 mycoviruses were detected in *S. sclerotiorum*, these mycoviruses either have dsRNA genome, or (+)ssRNA genome, or (-)ssRNA genome or ssDNA genome. Some mycoviruses could be grouped into existing Families, while some mycoviruses were novel, and their classification status were uncertain. Among them, two mycoviruses showed strong infectivity. One is unclassified geminivirus-like DNA virus, *Sclerotinia sclerotiorum* hypovirulence associated DNA virus 1 (SsHADV-1) (Yu et al, 2010); another is a typical partitivirus, *Sclerotiorum sclerotiorum* partivirus 1 (SsPV1) (Xiao et al, 2014). Field tests showed that SsHADV-1 could control stem rot efficiently (Yu et al, 2013); the field test for SsPV1 will be conducted in March this year.

**Conclusions:** Various mycoviruses could infect *S. sclerotiorum*, and some of them could be used to control stem rot of oilseed rape. Our study also suggested that mycoviruses may be an important force to suppress the virulence and population of *S. sclerotiorum* in nature.

**References:**


RNAi-induced resistance against insect pests in rapeseed

**Background:** As one of the major crops worldwide, rapeseed/oilseed rape/canola is exposed to various biotic and abiotic stress factors, causing heavy yield losses. Damage by insects represents a serious threat for farmers. The pollen beetle (Meligethes aeneus), one of the most harmful insect pests in Europe, can cause yield losses of up to 50%. The most frequently applied plant protection strategy constitutes the use of various classes of insecticides, particularly pyrethroids. Heavy pesticide usage causes not only negative impacts on the environment, but may also facilitate fast development of insect resistance against specific chemical classes. New strategies are therefore needed to control insect pests in rapeseed.

**Objectives:** Different technologies based on genetic engineering can potentially help to improve plant resistances to insect pests in order to support classical breeding methods. RNA interference (RNAi) using double-stranded (ds) RNA has been demonstrated as a promising method for pest control in crops.

In this study we aim for proof of concept that RNAi can induce highly-specific resistance against the pollen beetle whilst protecting beneficial organisms.

**Methods:** Candidate genes essential for beetle fitness were identified from gut-expressed pollen beetle expressed sequence tags, based on homology to genes showing lethal knockouts in the model beetle Tribolium (T. castaneum). Potential candidates were tested and analyzed by in vivo feeding trials with adult pollen beetles collected from flowering fields. Daily mortality rate was measured for 14 days after oral application of various concentrations and different dsRNA molecules derived from candidate target sequences.

After validation of the best candidate sequences, an RNAi-construct was created containing the target sequence coupled with inverted pollen-specific promoter sequences. The plasmid was used to generate insect-resistant prototype plants via Agrobacterium-mediated transformation.

**Results:** Candidate genes and pollen-specific promoter sequences were successfully identified. Potential lethal-acting target genes of the pollen beetle were identified, isolated and tested for lethal effects, specificity and mode of action.

Feeding applications confirmed the proof of concept by demonstrating a significant reduction of beetle vitality after oral uptake of dsRNA from target genes.

The best potential target gene sequences have been transferred and integrated into the genome of *B. napus*, opening the way for first tests with prototype plants expressing promising RNAi targets.

**Conclusion:** Alternative pest control strategies are increasingly necessary to reduce pesticide applications, for protection of the environment and non-target organisms, to avoid insecticide resistances and therefore, to decrease yield losses caused by major insect pests. A suitable approach to breeding for pollen beetle resistance could be to breed plants expressing beetle-specific dsRNA sequences in the pollen. After feeding on pollen, activation of the RNAi mechanism can lead not only to immediate lethal effects, but also to long-term reduction in the fitness of beetle populations.

**References:**


Identification of genome-wide pathways for enhancing clubroot 
(*Plasmodiophora brassicae* Woronin) resistance in *Brassica napus*

**Background and Objectives:** *Plasmodiophora brassicae* Woronin is an obligate pathogen that causes clubroot disease in *Brassica napus* L. and other *Brassica* crops. This disease is a threat for canola production in Western Canada. Building durable genetic resistance to this disease is an objective of many research groups. We present our multidisciplinary studies on the genetic and molecular aspects of the disease: root transcriptomics; microRNA analysis and biochemical analyses of two parental lines of *B. napus* differing in susceptibility to *P. brassicae* pathotype 3 and in a biparental population of the two lines.

**Methods:** Three disease developmental stages were investigated: primary (10 Days after Inoculation, DAI), secondary (22 DAI) and advanced disease development stage (42 DAI). Transcriptomics identified differences between the parents and pools of susceptible and resistant doubled-haploid segregants of a mapping population.

**Results:** Resistance response at the molecular level was evident even at the primary stage, the secondary and advanced disease development stages showed greater level of expression of PR proteins, jasomonic acid (JA), salicylic acid (SA) and signaling. In conjunction with phytochemical analyses, cell wall lignification was identified as a significant response in clubroot resistance. Disease susceptibility in contrast, was associated with higher expression of sugar breakdown and hexose transport, auxin metabolism and transport indicating pathogen modulated transcriptome level changes for subverting host nutrition. The susceptible parent and the DH pools of susceptible lines showed substantially greater number of differentially expressed genes at the secondary infection stage.

**Conclusions:** The lesser perturbation to gene expression in the resistant lines suggest stronger influence of the resistance gene derived from cv. Mendel in the spring *B. napus* canola. MicroRNA analysis at secondary infection stage indicated genome-wide differences for their targets in resistant and susceptible reaction. A model that incorporates the inferences is presented.
Recurrent selection for resistance against mustard aphid, *Lipaphis erysimi* (Kaltenbach) in a set of *Brassica juncea - B. fruticulosa* introgression lines

**Background:** Mustard aphid, *Lipaphis erysimi* (Kaltenbach) is a key pest of oilseed *Brassica* in India. At present there is no genetic solution to the management of this pest. The development of aphid resistant cultivars is considered critical for effective and environment friendly method of pest management. A wild crucifer, *Brassica fruticulosa* was previously found to be resistant to mustard aphid (Kumar et al. 2011). A complete set of introgression lines was developed using single pod descent method following first cycle of backcrossing. The objective of this breeding scheme was to obtain optimal coverage of *B. fruticulosa* genome in the background of *B. juncea* with high level of aphid resistance.

**Objectives:** To enhance the level and stability of introgressed resistance against mustard aphid.

**Methods:** A set of 533 introgression lines developed from selfing of the BC₁ progenies of *B. juncea / (B. fruticulosa/ B. rapa)* combination were screened under field conditions during 2009-10 crop season. The lines showing resistant reaction were further screened in the successive crop seasons from 2010-11 to 2013-14 to identify introgression lines with consistent reaction. Aided epiphytotic conditions were created by artificial release of aphids @ 20 aphids/ plant. Five plants per replication were selected at random to study aphid population, injury symptoms on 0-5 scale and per cent plant infestation following the procedure of Bakhetia and Sandhu (1973).

**Results:** Proportion of plants harbouring lower aphid population was higher during 2009-10 but there was a general decline in the proportion of such plants in each successive year. While in the first three years, the maximum aphid population varied from 175.5 to 330.0 aphids/ plant, in the 2013-14 crop season, only 55.1 aphids/ plant were observed on selected resistant lines. Almost similar trend was observed w.r.t. Aphid Infestation Index (AII). However, the results from the parameter of per cent plants infested were not categorical. Out of the initial 533 introgression lines, 8 lines showed consistent resistant reaction over five cropping seasons. These included: Ad3L61, AD3L370, Ad3L401, Ad3L460, AD3L462, AD3L503, AD3L506, Ad3K190. These inbred lines had the lowest score for aphid population/ plant, per cent plant infestation following the procedure of Bakhetia and Sandhu (1973).

**Conclusions:** Recurrent selection of introgression lines with lower aphid infestation over five years resulted in significant improvement in the level of introgressed resistance against mustard aphid.

**References:**


Kumar, S., C. Atri, M.K. Sangha, S.S. Banga, 2011. Screening of wild crucifers for resistance to mustard aphid, *Lipaphis erysimi* (Kaltenbach) and attempt at introgression of resistance gene(s) from *B. fruticulosa* to *B. juncea*. Euphytica 179: 461-470.
Potential of *Brassica carinata* as a trap crop for managing large white butterfly, *Pieris brassicae* (L.) on *Brassica juncea*

**Background:** The large white butterfly, *Pieris brassicae* (L.) is an important pest of oilseed *Brassica* after mustard aphid in this part of the country (Kumar 2011). “Alternative” environment friendly pest management methods to manage insect pest populations and to improve habitat diversification have received considerable attention in recent years. Several of these strategies exploit the differential host preference by many insects. In this strategy, trap crop is planted near to main crop which results in the reduction in the number of insect-pests reaching main crop by concentrating them on the trap crop. This approach enables a reduction in the amount of insecticide required for pest management or may negate the need for insecticides altogether. Previous observations at this institution have shown that *P. brassicae* exhibits differential oviposition preference on *B. carinata* over *B. juncea*.

**Objectives:** To test whether *B. carinata* can be used as an effective trap crop against *P. brassicae*.

**Methods:** *Brassica juncea* cv. PBR 210 was grown at Punjab Agricultural University, Ludhiana, India during 2012-13 and 2013-14 crop seasons. There were two treatments: *B. juncea* bordered by four rows of *B. carinata* cv. PC 5 and the other left unbordered. There were three replications for each treatment in a plot size of 6 x 5 m planted in a randomized complete block design. At the pest appearance, data on the number of *P. brassicae* larvae per plant were recorded at weekly intervals from ten plants per replication selected at random. Yield data were recorded at harvest.

**Results:** *B. juncea* plots bordered with *B. carinata* harboured significantly less number of larvae than unbordered *B. juncea* plots. In 2012-13 crop seasons, the mean larval density on *B. juncea* bordered with *B. carinata* was 1.5 larvae/plant as against significantly high larval density of 10.7 larvae/plant on unbordered *B. juncea* plots. Interestingly, the larval density on *B. carinata* border rows was exceptionally high (50.4 larvae/plants) indicating the increased preference of butterflies to oviposit on *B. carinata*. The grain yield from bordered plots (1782.5 kg/ha) was also significantly higher than that from unbordered plots (1322.8 kg/ha). Almost similar trend was observed in 2013-14 crop season both for larval density and grain yield. Laboratory experiments have also indicated increased preference of butterflies for oviposition on *B. carinata*.

**Conclusion:** Since *P. brassicae* shows increased oviposition preference for *B. carinata*, this *B. carinata* has potential to be used as a trap crop to attract this pest. However, there is a need to establish the spatial and temporal pattern of deployment which will ensure the most effective trap crop system.

**References:**

Design and implementation of web-based open access rapeseed-mustard bibliographic information system

**Background:** The advancement of research in the field of rapeseed-mustard diseases is clear to everyone. The numbers of articles are being published each year, resulting in increase of knowledge at every moment. Problem identification, future planning, implementation, and interpretation of individual research studies all depend on ready access to all of the relevant existing rapeseed-mustard diseases research knowledge. Need, felt to collate vast and scattered information published in several publications on different aspect of research on rapeseed-mustard disease in the form of bibliography database. An online bibliographic database (an organized digital collection of references to published literature in specific research domain) may be general in scope or cover a specific research discipline and usually focus on a particular domain of knowledge, and contain various types of publications including journal, conference proceedings, reports, newspaper articles, patents, books, government and legal publications, etc. [Ng et al., 2010].

**Objective:** The broad objective to develop this online bibliography database is to enabling researchers in the field of rapeseed-mustard disease to gain access to research articles data in this field, the long-term mission is to acquire existing and new research result and convey for new studies to the scientific community, students, and research centers in particular and our whole society in general.

**Methods:** The design of a bibliographic information system has to take into account two possible requirements: the completeness of the data held in the database, and ease of use for the intended users. System design and development usually proceeds through several phases of a software development life cycle (SDLC) that includes: feasibility study (problem identification); requirements analysis (users’ requirements); choosing the system design and architecture; testing; implementation and evaluation. The system design is based on 3-tier architecture, separating data, logic and presentation tiers. This makes the application easier to maintain in the future, as well as to further upgrade with new features. HTML, CSS, MySQL and PHP was used in implementing this bibliographic information system. (Oguntoyinbo, et al, 2013)

**Results:** The system developed RMBiblio, offer researchers easy and fast access of most comprehensive resource for research articles in rapeseed-mustard disease research. System supports a wide range of publication types, and has features like advanced search option, extraction of publications statistics based on a variety of visual form based queries, etc. Metadata formats suitable for describing scientific publications have been used in creating the database. Searching facility has been implemented using MySQL full text search options, the rows returned are automatically sorted with the highest relevance first. HTML, CSS, was used for development of user interface; PHP is the middleware and MySQL for the backend.

**Conclusions:** RMBiblio provides much needed exposure to rapeseed-mustard disease research published and acts an important resource to rapeseed-mustard information seekers. The system is receiving response from the rapeseed-mustard community both at national as well as international level.

**References:**
Effect of blackleg resistance on dispersal of *Leptosphaeria maculans* at the landscape scale

**Background:** Previous studies have indicated that introduction of *Leptosphaeria maculans* to China may result in widespread dispersal there. Data collected from over 700 rape crops in Alberta, Canada from 1985 to 1998 have been used to simulate the potential for dispersal through China (Fitt et al. 2008), but these models have not accounted for effects of host resistance.

**Objectives:** Measures taken in Alberta in the early 1980s to slow spread of blackleg through the province may be of value to China. One of the core strategies of the Alberta plan was to encourage the development and deployment of blackleg resistant cultivars. Here, we focus on the potential benefits to China of genetic resistance and crop rotation on dispersal of blackleg disease.

**Methods:** We modelled *L. maculans* spread westwards from a single entry point across a 500km × 250km grid. A potential of 200-400 spores per unit percentage increase were spread throughout the simulated landscape with random angle. The distance travelled by each ascospore was determined by the inverse of the half Cauchy distribution (Savage et al. 2012, Lo-Pelzer et al. 2010, Xu and Rideout 1998), of which the parameter mu was used to fit the model to the empirical rate of spread, as determined from the Alberta data. Spores landing on a plant in a particular block were assumed to cause infection if a generated random number was less than Infrate, the parameter we used to adjust host resistance. Final disease prevalence and average distance of spread were calculated over each series of 100 simulations.

**Results:** At an Infrate of 0.25, 82% of the simulations showed virtually no spread over 15 years, i.e. spread of blackleg through a landscape dominated by highly resistant cultivars is expected to be essentially zero. The percentage of “no-spread” simulations decreased to 20, 8, and 5% when Infrate parameter values were increased (i.e. cultivar resistance decreased) to 0.50, 0.75, and 0.99, respectively.

**Conclusions:** Employment of resistant cultivars appears likely to effectively reduce the rate of blackleg dispersal in the event of an introduction of *L. maculans* to China.

**References:**


Modulation of the resistance QTL effects under abiotic stresses: Case study of resistance to clubroot in rapeseed under nitrogen constraint.

**Background:** The use of resistant varieties is a major component of integrated strategies for the control of plant diseases. In a perspective of low input agriculture development, taking into account abiotic challenges in the evaluation of resistant factors is essential for constructing and managing resistant varieties in different cropping systems.

**Objectives:** The aim of this work was to estimate the impact of nitrogen constraint on the genetic architecture of clubroot resistance in *Brassica napus*.

**Methods:** 108 doubled-haploid (DH)-lines derived from the cross between Darmor-bzh (harbouring partial quantitative resistance to clubroot caused by *Plasmodiophora brassicaceae*) and Yudal (susceptible) were tested for clubroot resistance under two N conditions (N+= 8 mM of nitrate vs N=1 mM) and using two single spore isolates (K92-16 and Eh). The overall experiment was carried out twice each with three replicates. Disease scoring was done 49 days post inoculation. For each isolate and each year a mixed linear model was fitted. Genotype (G), GxN, and NxReplicates effects were considered as random and N and Replicates effects as fixed. The estimates of G and GxN effects were used for QTL mapping, as well as delta defined as the difference between GxN+ and GxN- estimates. The genetic map consisted of 3592 unique SNP locus obtained from the infinium 60K array and covered 2128.2 cM. QTL mapping was carried out using Multiple QTL Mapping (MQM) implemented in R/qtl package.

**Results:** For each trait, the genetic architecture consisted in a major QTL and few moderated QTL. The main QTL for DI-eH (Disease Index using Eh) and DI-K92-16 were located on C9 and on the bottom of A3, respectively, these QTL showing epistatic interactions with the others QTL. N supply impacted DI QTL differently according to the isolates. The DI-eH QTL showed N modulation for most of QTL: The effect of C9-QTL decreased whereas the effect of C2 and C3 moderated-QTL increased under N limited conditions. For DI-K92-16 only one QTL for the GxN variable was identified on the bottom of A3. Moreover, a QTL can be N-responder for Eh and non-responder for K92-16 as shown for the QTL on the top of A3.

**Conclusions:** The genetic control of clubroot resistance is modulated by N constraint, the effect of the main DI-QTL varying between the two N conditions. This modulation also depends on the *P. brassicaceae* isolates.
Epidemiology of sclerotinia stem rot of canola in New South Wales, Australia

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**Background:** Sclerotinia stem rot is a yield-limiting disease of canola caused by the necrotrophic fungal pathogen, *Sclerotinia sclerotiorum*. In Australia, the disease is prevalent in high-rainfall districts of southern New South Wales (NSW) where canola is frequently grown. Surveys conducted in Australia from 1998 – 2000 showed that the disease poses a threat to the Australian canola industry (Hind et al. 2003) and yield losses as high as 24% have been recorded under the Australian conditions (Hind-Lanoiselet and Lewington, 2004). The development of stem rot is highly sporadic due to its dependency on specific environmental conditions and inoculum levels. The only post sowing management tool currently available to producers is foliar fungicides. Therefore, a reliable forecasting system is needed to ensure that any in-crop foliar fungicide applications will be effective and economical.

**Objectives:** Understanding the epidemiology of sclerotinia stem rot in NSW is therefore paramount to assist in the strategic application of foliar fungicides. This can be done through identifying the ‘trigger points’ that lead to sclerotinia stem rot development by examining the interaction between environmental conditions, pathogen life cycle and the host.

**Methods:** Six commercial canola crops located in high disease risk districts of southern NSW were monitored for development of sclerotinia stem rot. Fifty petals collected from 50 different racemes separated one meter apart were collected from each crop and plated weekly to determine the level of petal infestation. Relative humidity and temperature at each crop were monitored using data loggers located in-crop, and rainfall data was taken from the nearest Bureau of Meteorology weather station. Measurement of the crop height, flowering stage and the presence of apothecia were recorded weekly. The types of infection (main stem, branch and leaf) were also recorded out of 100 plants within a crop to determine the level of stem rot incidence weekly.

**Results:** Four crops in NSW were found to develop significant levels of disease. Apothecia was scouted in all crops early before the commencement of flowering, therefore high levels of petal infestation (>90%) was detected upon flowering. Plants started to show symptoms of branch and stem infections commencing from the middle to late flowering period, which coincided with a prolonged period of high relative humidity (>90%) and rainfall events. Branch and stem infections increased up to approximately 40% and 30% respectively at some sites, even after the flowering period had finished and the relative humidity decreased.

**Conclusions:** Preliminary data showed that high initial inoculum levels, rainfall events and high relative humidity played a significant role in the development of stem rot during the flowering period. Once the flowering period had ended, branch and stem infections still progressed, mainly due to frequent rainfall events which enabled lodged infected senescent petals or leaves to cause further infection.

**References:**
Study of sclerotinia stem rot caused by *Sclerotinia sclerotiorum* on pre-flowering plants of *Brassica napus* in Sichuan province of China

**Background:** Sclerotinia stem rot (SSR) caused by *Sclerotinia sclerotiorum* is the most devastating disease on oilseed rape (*Brassica napus*) in China. Previous observations showed that in most cases, epidemics of oilseed rape SSR begin from ascospore-contaminated flower petals, which cause infection on leaves and stems when they fall on leaves and stems. In late December of every year since 2011, a kind of white mould disease on the stem base of oilseed rape plants at the bolting stage was observed in oilseed rape fields in Chengdu, Meishan and Guanghan County of Sichuan Province, China. The disease started by formation of water-soaked lesions, which spread rapidly on stems, leaf petioles and leaves. White cottony mycelia and black-colored sclerotia (6 mm in diameter on average) were produced on the lesion surface.

**Objectives:** The symptoms were very similar to sclerotinia stem rot caused by *Sclerotinia sclerotiorum*. This study is focus to disease type and disease incidence on pre-flowering plants of *Brassica napus* in Sichuan province of China.

**Methods and results:** The diseased plants usually showed wilting appearance. *Sclerotinia* collected from diseased plants were, surface-sterilized for 5 min in 5% NaOCl, rinsed in sterilized water, placed on potato dextrose agar (PDA) and incubated at 20°C for 5 days. A total of 6 single-sclerotium isolates were obtained. All these isolates grew rapidly at 20°C on PDA and formed white-cottony mycelia and black-colored sclerotia. The morpho-cultural characteristics of these isolates appeared similar to *S. sclerotiorum*. Strain CanSS-QM1 was used for further identification by analysis of the ITS sequence and by specific PCR detection. The ITS sequence was cloned by PCR using the genomic DNA from CanSS-QM1 as template and ITS1/ITS4 as primers. The sequence (GenBank Acc. No. KC748491) showed 100% identity to that of *S. sclerotiorum* strain Ep-1Pb (GenBank Acc. No. GQ404793). In the specific PCR detection, a 292-bp DNA fragment specific for *S. sclerotiorum* was obtained in nested PCR using two primers ITS4/ITS5 and XJJ21/XJJ22. This confirms the above-mentioned morpho-cultural identification. Pathogenicity was determined by placing mycelia of the strain CanSS-QM1 on detached leaves of *B. napus* cv. Chuanyou 58 at 20°C under humid conditions for 72 h. Necrotic lesions were produced from each inoculant and a fungus similar to strain CanSS-QM1 in morpho-cultural characteristics was re-isolated. The disease incidence varied in different fields and years: 5~85% in 2011 and 1~11% in 2012. Meanwhile, this disease was also observed in Sichuan on the stem base of a few cruciferous vegetables and weeds, including *B. juncea* var. *crassicaulis*, *B. campestris* var. *purpurea*, *Capsella bursa-pastoris* and *Cardamine hirsuta* L., with the incidence of 13% to 39%.

**Conclusions:** It shows that SSR caused by *S. sclerotiorum* on pre-flowering plants of oilseed rape in Sichuan province is increasingly widespread.

**References:**
Field effect of the seed-coating on clubroot of oilseed rape

Background: Sichuan Province is the major oilseed rape production base in China, perennial planting area of about one million hectares. In recent years, the clubroot breaks out and spreads out on a large scale and the stricken area comes to 10~20 thousand hectares. The lost of production in serious area reached more than 80%, which is a great threat to the local oilseed rape production. Consequently, an effective control technology is urgent need.

Objectives: With the purpose of effectively controlling for clubroot of oilseed rape, we tried various studies on reagent combination, and then screened out BY1 seed coating formula. After that, we performed field efficacy experiments by seed-coating for clubroot on oilseed rape during 2013 and 2014.

Methods: We made the coating operation according to the weight ratio of 1:50. Lately on October 5, 2013 we conducted the field contrast test in the serous stricken area of Guanghai City (located in the central of Sichuan Province). Before direct seeding, the soil was ploughed, and the seeding rate was 2.5 Kg/ha. The seeding machinery is supported by Jingyang agricultural machinery cooperation. We used the seeds without coating as the contrast and the experimental plot area is 300 square meters. The repeated trials were conducted 4 times. The mature oilseed rape was reaped by harvester. Seed moisture determination was measured by the moisture test apparatus, and then dry weight under the 10% moisture content condition was calculated.

Results and Conclusions: After seeding 15d, we found that the seedling rate of coated seeds was 50~65 plants per square meter, while the contrast had 30~35 plants per square meter. The production of oilseed rape which treated with seed-coating was 2.465 t/ha, while the contrast had 1.442 t/ha. The results showed that production increased 1.023 t/ha and the growth rate reached to 71.4% in some serious plots, which indicated that the BY1 had great control effect for clubroot on oilseed rape. In other words, seed coating BY1 could significantly increase the emergence rate, survival rate and could decrease the damage of the clubroot.
Building a new defense line against *Sclerotinia sclerotiorum* infection

**Background:** Stem rot caused by *Sclerotinia sclerotiorum* is a major yield-limiting factor in canola production. There is great variation between oilseed rape varieties and thus the resistance has become a major measure for control of this disease in China. There is however no complete immunity found in host plants, and the pathogen can fully infect plant (Liu et al. 2005) in varieties with the highest resistance, e.g. under high disease pressure in field. Thus there is an urgent need to develop new resistant varieties with significantly higher resistance against the pathogen.

**Objectives:** This study is to develop a new strategy that can significantly defend the pathogen infection on leaves and stem.

**Methods:** A comprehensive approach was developed to construct this strategy where high throughput genomics technologies were employed.

**Results:** The core idea is based on that the majority of disease lesions on leaves, stems and pods come from petal-mediated infection, and high and specific expression of foreign genes in petals will not have negative effect on plant development and avoids concern on effect of transgenic products on food chains, allowing us to use petals as a bioreactor to produce potent antifungal agents. The new strategy includes multiple components: highly efficient and short antimicrobial peptides (AMPs), highly specific and strong petal promoter with sustainable expression activity to sustain a large amount of AMPs in petals, and elements of anti-degradation of expressed proteins. We have screened all relevant genomic databases of animal, microbes and plants and predicted a large number of putative AMP genes. To experimentally test activity of candidate genes, we have established a set of high throughput methods, i.e. using the overlapping PCR to synthesize AMPs which are subsequently cloned into pET30a - EDDIE - GFP expression vector developed to express His-EDDIE-AMP fusion proteins in *Escherichia coli*, purification of the inclusion bodies and self-cleavage. We have obtained several recombinant AMPs with high antimicrobial activity. The above mentioned promoter was identified through in-depth RNA-seq of different tissues and subsequent function analysis. To increase more efficient inhibition on the fungus, multiple AMP genes were expressed together in a tandem array in which individual AMP release was allowed through enzymatic cut. After assembling these components, transgenic petals showed very high resistance in the model *Arabidopsis*. And transgenic *B. napus* is being tested.

**Conclusions:** The method based on bioinformatics tools and the described vector-screening is useful and high-throughput for discovery of AMPs. This new strategy is expected to efficiently complement naturally immune resistance bred through traditional or marker-aided selection to build two defense lines and thus efficiently prevent infection from the pathogen in oilseed rape.

**Reference:**
Defining the molecular recognition of *Leptosphaeria maculans* effector AvrLm1 by *Brassica napus* disease resistance protein LepR3

**Background:** The fungus *Leptosphaeria maculans* is the causal agent of blackleg posing a major threat to canola/oilseed rape (*Brassica napus*) worldwide. In *Brassica*-*L. maculans* pathogen system, 16 R genes that confer race-specific resistance to blackleg have been identified, and two of them, LepR3 and Rlm2, have been recently cloned (Larkan et al., 2013; Larkan et al., 2015). Genetically, LepR3 recognizes AvrLm1 and Rlm2 recognizes the recently cloned AvrLm2 gene (Ghanbarnia et al., 2014). Recognition of Avr protein by its cognate R protein often culminates into a hypersensitive response (HR) around the infection site. Nicotiana benthamiana have served as a model plant to study the function of R/Avr proteins by *Agrobacterium* mediated transient expression.

**Objectives:** We exploited the *N. benthamiana* model plant to investigate recognition mechanism of AvrLm1 by the recently discovered LepR3 gene.

**Methods:** *Agrobacterium*-mediated transient expression was used to ectopically express AVR or R genes in *N. benthamiana*. Validation of protein-protein interaction was performed by co-immunoprecipitation and bimolecular fluorescence complementation (BiFC). Recombinant tobacco rattle virus (TRV)-based virus-induced gene silencing (VIGS) was used to silence the Nbsobir1 in *N. benthamiana*.

**Results:** Co-expressing LepR3/AvrLM1 gene pair in *N. benthamiana* results in development of HR observed in the infiltrated region. But, the truncated AvrLM1 lacking indigenous signal peptide is unable to induce LepR3-mediated HR, indicating that AvrLM1 is perceived by LepR3 extracellularly. LepR3 physically interacts with the *B. napus* receptor like kinase, Bnsobir1. Silencing of Nbsorbir1 compromises LepR3-mediated HR developments, suggesting that LepR3-mediated resistance to *L. maculans* in *B. napus* requires Sobir1. Structure-function analysis of AvrLM1 protein reveals that C-terminal region is required for LepR3-mediated HR in tobacco and resistance to *L. maculans* in *B. napus*.

**Conclusions:** This is the first report on the use of tobacco as a model plant to study the function of R and Avr genes from *B.napus*-*L.maculans* pathosystem. Using this model system we were able to rapidly determine Bnsobir1 as an essential partner of LepR3 signalling complex and to define the AvrLM1 effector domain. We also showed that in Sobir1 silenced tobacco plants, LepR3 failed to induce HR in response to AvrLM1, providing additional support for the role of Sobir1 in LepR3 mediated defence response.

**References:**


Growth promoting ability of *Trichoderma* spp. isolates on rapeseed

**Background:** *Trichoderma* species have world-wide distribution in different types of soil. In addition to their antagonistic effect on a vast number of soil pathogens, *Trichoderma* species are known for their good growth promoting effects on numerous crops (Asaduzzaman et al. 2010).

**Objectives:** The aim of this study was to determine the ability of Serbian *Trichoderma* spp. isolates to promote the growth of rapeseed.

**Methods:** This study used ten *Trichoderma* spp. isolates originating from different soil types and localities in Vojvodina (Serbia). All isolates were refined to single-spore isolates for further research. The growth promoting activity was tested on 100 rapeseed seeds (cv. Zlatna) treated with *Trichoderma* spp. suspensions (2.5x10^6) for 30 minutes according to modified Mukhtar et al. (2012) method. Seeds with Trichoderma coating were dried at room temperature for 24 hours and germinated on double wet filter paper under optimal laboratory conditions. Seeds treated with sterile distilled water were used as control. Germination energy and germination was calculated on days 5 and 7 respectively, as a percentage of germinated seeds. Vigor index was calculated according to formula by Asaduzzaman et al. (2010). On day 7 root and shoot weight and length were also measured. All obtained data were analyzed in Statistica 10 by Duncan test (percentages were previously transformed in ArcSin√%).

**Results:** Seven out of ten tested *Trichoderma* isolates showed significant positive effect on at least one measured parameter of rapeseed seedlings. The isolate K176 can be singled out as the most effective, with the significant increase of five measured parameters – shoots length and weight, germination energy, germination and vigor index. Isolates K178 and K179 significantly increased root length and weight as well as vigor index of seedlings. Isolates K173, K174 and K175 significantly increased shoot lengths and weights, germination energy and germination, while isolate K150 significantly increased only seedling root weight.

**Conclusions:** Seven out of ten tested *Trichoderma* isolates expressed good growth promoting ability on rapeseed and those isolates should be further tested in more comprehensive research under the greenhouse and field conditions.

**References:**


Biological efficacy of *Trichoderma spp.* isolates against *Sclerotinia sclerotiorum* on rapeseed

**Background:** *Trichoderma* species occur in soils and plant organic matters worldwide, and are well known as effective antagonists to a variety of soil fungal pathogens. *Sclerotinia sclerotiorum* is a cosmopolitan pathogen that colonizes over 400 plant hosts including oilseed crops, and can be efficiently controlled by *Trichoderma* spp.

**Objectives:** Due to good preliminary results in dual culture tests which indicated antagonistic activity of Serbian *Trichoderma* spp. isolates (Tančić et al. 2014), the aim of this research was to test the ability of those *Trichoderma* spp. isolates to protect rapeseed seedlings from *S. sclerotiorum*.

**Methods:** The study used ten *Trichoderma* spp. isolates from different soil types and localities in Vojvodina province (Serbia), and one *Sclerotinia sclerotiorum* isolate from sunflower grown at Rimski Šančevi, near Novi Sad (Serbia). The biological efficacy was tested on 100 rapeseed seeds (cv. Zlatna) treated with *Trichoderma* spp. suspensions (1x10^6) for 30 minutes according to modified Mukhtar et al. (2012) method. *Trichoderma*-coated seeds were dried at room temperature for 24 hours and placed in four replicates on wet double filter paper in Petri dishes. Next to each seed the 3 mm plug of *S. sclerotiorum* mycelia was placed. Seeds treated with sterile distilled water with no pathogen were used as a negative control, and seeds treated with sterile distilled water with the pathogen plugs were used as a positive control. Seeds were incubated under the optimal laboratory conditions. On day 7 biological efficacy of *Trichoderma* spp. isolates was estimated and calculated according to Liu et al. (2009). Data were analyzed in Statistica 10 by Duncan test (percentages were previously transformed in Arcsin√%).

**Results:** Biological efficacy of all tested *Trichoderma* isolates was statistically significant as compared to the positive control. Good antagonism with over 50% biological efficacy was registered in 6 isolates (K150, K160, K173, K176, K178, and K179). Germination was significantly higher in all treatments compared to the positive control. In 7 treatments germination was at the same level of significance as germination in negative control. Two treatments even had higher germination than negative control but not significantly different. Lower germination rates were mostly connected with lower biological efficacy of the isolates. The exception was the isolate K114 with low biological efficacy and high germination, which indicated a good growth promoting effect of the isolate but low protection from the pathogen.

**Conclusions:** Six *Trichoderma* isolates which showed biological efficacy over 50% should be included in further more comprehensive research.

**References:**


Decline in the concentration of resting spores of *Plasmodiophora brassicae* following a susceptible crop

**Background:** The concentration of resting spores of *Plasmodiophora brassicae* Woronin in soil increases rapidly when susceptible crops are grown in short rotation. Some of these spores survive for many years (Wallenhammar 1996), but the rate of decline of resting spores in soil is unknown. However, a recent study indicates that there may be a practical advantage to a moderate (2- to 3-yr) break between a susceptible cultivar of canola (*Brassica napus L.*) and a clubroot-resistant cultivar (Peng et al. 2013).

**Objective:** To monitor the survival of resting spores for up to 6 years following a susceptible canola crop.

**Methods:** Small blocks (8 x 30 m) in a field infested with *P. brassicae* at the AAFC research site at Normandin, Québec, have been in cropping rotations over many years that includes clubroot susceptible canola. Resting spore populations in soil were quantified after continuous canola and break intervals of 1, 2, 3, 5, and 6 years between canola crops. Two blocks were selected for each length of break (0 to 6 year). Each block was divided into two parts, and five soil cores were collected and bulked within each part to form a representative sample. The concentration of resting spores in each sample was assessed using a multiplex qPCR protocol with an internal control (Deora et al. 2015). Three biological and three technical replicates were conducted for each sample. Log transformation, ANOVA, single df contrasts, and regression were used to assess the change in spore concentration over time.

**Results:** The concentration of resting spores in soil declined over time as the length of break from the susceptible canola crop increased. Regression analysis indicated a quadratic relationship of $y = 1E + 07e-0.759x$. $R^2=0.65$. Compared to continuous canola (1.3 x 108 spores g-1 soil), resting spore concentration declined by 96% after a 1-yr break, 99% after a 2-yr break from canola, but then declined very slowly.

**Conclusions:** These results support a previous report (Peng et al. 2013) that large numbers of resting spores die or disappear in the first 1 to 2 years after a susceptible crop, but that some resting spores are persistent and may survive for many years. This observation has important implications for the importance of cropping rotations in management of clubroot on canola on the Canadian prairies.

**References:**


Why is clubroot a problem on canola in Alberta but not in Ontario, Canada

**Background:** Clubroot, caused by *Plasmodiophora brassicae*, has been a major disease of canola in the province of Alberta since pathotype 3 (P3) was identified on that crop in 2003. The disease spread rapidly and has caused total crop losses in some fields. In contrast, clubroot has caused losses on vegetable crops in Ontario since the 1930’s (Reyes et al. 1974) but does not appear to be a problem on canola.

**Objective:** To investigate the susceptibility of canola to *P. brassicae* at a high organic matters soil in Ontario and examine other factors that may affect the spread of clubroot in Ontario relative to Alberta.

**Methods:** Field trials were conducted in 2011, 2012, and 2014 at the Holland Marsh, Ontario, Canada at a site naturally infested with P6. There were 33 canola lines / cultivars tested in 2011, 13 in 2012, and 22 in 2014. Each trial included the clubroot-susceptible line ACSN39 as a control. The trials were arranged in a RCBD with four replicates. Clubroot symptoms were assessed on up to 50 plants per replicate using a 0–3 scale, and a disease severity index (DSI) was calculated. Cultivars were designated as moderately resistant if they had DSI values less than 30 and resistant if values were ≤ 10.

**Results:** High levels of clubroot developed each year on the susceptible line ACSN39 (66, 100, and 71 DSI, respectively). In 2011, 22 of 33 lines had a DSI < 30, and 12 of these had a DSI < 10. In 2012, 3 of 13 lines were classified as resistant and 7 of 22 lines in 2014. All of the cultivars that were resistant to P3 were also resistant to P6, but lines resistant to P6 were generally susceptible to P3 (data not shown). Canola is often grown in a 2-year rotation in Alberta, but crop rotations are generally much longer in Ontario, because the acreage is lower. There were only 14,000 hectares of canola in Ontario in 2014, compared to almost 2.6 million hectares in Alberta. Soil pH is similar in both provinces, but soils in Ontario may have higher levels of calcium. Canola may be seeded earlier in the spring in Ontario and winter canola is sometimes grown. Plant growth when temperatures are cool (< 15 oC) supresses clubroot development (Sharma et al 2011b).

**Conclusions:** The low level of clubroot on canola in Ontario can be attributed, in part, to the resistance of many canola cultivars to P6. Even cv. Westar, which is routinely used as a susceptible control, was partially resistant. Long cropping rotations and early spring or fall seeding may also suppress the establishment of *P. brassicae* in Ontario soils.

**References**
Abundance of cabbage stem weevil, *Ceutorhynchus pallidactylus*, and rape stem weevil, *C. napi*, in northern Serbia

**Background:** Expected increase of the acreage under rapeseed puts more pressure on insect control issues. Two weevil species, *Ceutorhynchus pallidactylus* Marsh., cabbage stem weevil, and *C. napi* Gyll., rape stem weevil, are the largest potential pest threats in Serbia.

**Objectives:** Stem weevils damage rapeseed crop proportionally to their abundance. Weevils presence and activity can be monitored using different methods, in order to obtain data on flight activity, population growth and date of maximum flight. Such information can be a valuable tool in estimating the necessity of chemical control. Furthermore, by combining different monitoring methods an accurate estimate of the number of weevils in the field can be obtained.

**Methods:** The monitoring of these pests was conducted in the vicinity of Crvenka, northern Serbia (N 45° 39' 24.6", E 19° 30' 57.8"). Assessments were made every week during spring in 2011, 2012 and 2013, using three different methods, yellow water traps (Moeicke dishes), entomological net (sweep net) and visual method. For this purpose four yellow traps were used. Sweep net was used at four different points in one field with 25 sweeps for each place. Visual assessments were made on 25 plants near each water trap. Collected specimens were identified in entomological laboratories at the Faculty of Science in Kragujevac and Institute of Field and Vegetable Crops using these keys: Alonso-Zarazaga 2004; Angelov 1979; Freude et al. 1983.

**Results:** Flight dynamics showed that the highest abundance of both species was observed in late March. Afterwards, in early April, the number of collected specimens rapidly decreased which can partially be explained by chemical control of pollen beetle carried out during the first week of April. Later in the season only individual specimens were sampled. Rape stem weevil (316 specimens) was three times more numerous than cabbage stem weevil (114 specimens). Regarding rape stem weevil sampling methods efficacy, yellow water traps were the most effective (282 specimens), followed by the visual method (51 specimens) and sweep net (28). In case of cabbage stem weevil sampling methods efficacy, the sweep net method was the most efficient (59 specimens), followed by yellow dishes (39 specimens) and the visual method (16 specimens).

**Conclusions:** Both species overwinter as adults and the beginning of rapeseed growing season coincides with the beginning of stem weevils’ flight, which is usually early March in Serbia. It is advised to use a combination of methods to obtain better insight into the abundance of these harmful insects and their control, while the best results can be obtained with yellow water traps and visual method.

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**References:**
Pathogenicity of *Leptosphaeria maculans* isolates obtained from *Brassica napus* (oilseed rape) cultivars with the *Rlm7* resistance gene

**Background:** *Leptosphaeria maculans* causes phoma stem canker disease on oilseed rape (*Brassica napus*) (Fitt et al., 2006). The disease results in yield losses worldwide and causes losses to farmers of more than £100M p.a. at a price of £370 per tonne in the UK. A cost-effective and environmentally friendly method for control of the disease is the deployment of cultivars with resistance against *L. maculans*. Resistance against *L. maculans* involves both major (R) and minor resistance genes (Delourme et al., 2006). Major gene resistance operates on a "gene-for-gene" concept.

**Objectives:** To examine pathogenicity of *L. maculans* isolates obtained from cultivars with the resistance gene *Rlm7* on different *Rlm7* cultivars in controlled environment conditions in order to investigate differences between cultivars with the same R gene.

**Methods:** Isolates obtained from winter oilseed rape cultivars with the resistance gene *Rlm7* were examined for their pathogenicity by inoculation onto cotyledons or true leaves of the susceptible cultivar Drakkar (no R gene against *L. maculans*) and cultivars with the resistance gene *Rlm7* (Excel, Roxet, Hearty and line 01-23-2-1). After assessment of lesions, cotyledons and true leaves were detached 17 and 21 dpi, respectively, and incubated in darkness under high humidity to assess pycnidial development and conidial production.

**Results:** All the isolates tested on Drakkar produced typical large/grey lesions (susceptible phenotype) on both cotyledons and true leaves; large numbers of pycnidia with conidial masses were produced within and outside the lesions on cotyledons after 5 days of incubation and on true leaves after 3 days of incubation. All the isolates tested on the four *Rlm7* cultivars produced small lesions surrounded by dark margins (resistant phenotype) on cotyledons with no difference in lesion area between isolates. However, there were differences between isolates on true leaves of Roxet but not on true leaves of Excel, Hearty and 01-23-2-1. Most of the isolates produced small numbers of immature pycnidia (without conidial masses) on the lesions on cotyledons after 5 days of incubation and on true leaves after 3 days of incubation of the four cultivars with the *Rlm7* gene. Further incubation, when cotyledons and true leaves senesced, induced mature pycnidial development outside the lesions with conidial masses.

**Conclusions:** Different cultivars with the *Rlm7* gene may respond differently following inoculation with *L. maculans* isolates in controlled environment conditions. Asexual reproduction can take place on resistant cultivars when the tissue senesces.

**References:**


Phoma stem canker on oilseed rape cultivars with good resistance against *Leptosphaeria maculans*

**Background:** Phoma stem canker, caused by the related pathogens *Leptosphaeria maculans* and *L. biglobosa*, is an economically important disease of oilseed rape (*Brassica napus*) worldwide (Fitt et al., 2006). There is a ‘gene-for-gene’ interaction between *L. maculans* and *B. napus* at the leaf spot stage of the disease. It has been suggested that one of the resistance genes that has been widely deployed in new oilseed rape cultivars across Europe, Rlm7, is more durable than other commercially available R genes (Clarke et al., 2011). Monitoring phoma stem canker severity in crops and the frequency of the virulent isolates in pathogen populations can help to predict increases in pathogen virulence and to manage the risk of severe disease epidemics.

**Objectives:** To examine the *Leptosphaeria* populations on Rlm7 cultivars in the UK, to investigate emergence of isolates virulent against Rlm7 and to determine molecular mechanisms leading to virulence. To examine the importance of the co-existing pathogen *L. biglobosa* in determining severity of phoma stem canker on cultivars with the Rlm7 gene.

**Methods:** Leaves with phoma leaf spots were sampled from cultivars carrying the Rlm7 gene and a cultivar with no known R genes (Drakkar) in autumn/winter the UK (2011/2012, 2012/2013 2013/2014 cropping seasons). Severity of phoma leaf spotting caused by *L. maculans* or *L. biglobosa* was assessed. Single pycnidial isolates were obtained and pathogen identification was done by observations on PDA and species specific PCR. Frequencies of the avirulent AvrLm2, AvrLm3, AvrLm4 and AvrLm7 alleles in *L. maculans* populations were investigated at different sites in the UK by inoculation on cotyledons of cultivars with the corresponding R genes.

**Results:** There were differences in severity of phoma leaf spotting on Rlm7 cultivars between seasons. The number of *L. maculans* leaf spots on Rlm7 cultivars increased from 2011/2012 to 2012/2013. Phoma leaf spotting caused by *L. biglobosa* was more severe on the Rlm7 cultivars than on Drakkar. All *L. maculans* isolates obtained in 2011/2012 were virulent against Rlm2 and Rlm3 and avirulent against Rlm7. Isolates virulent against Rlm7 that were found in 2012/2013 were avirulent against Rlm3. Isolates virulent against Rlm4 were present in the *L. maculans* populations.

**Conclusions:** Breeding for resistance against *L. maculans* may affect susceptibility of cultivars to *L. biglobosa*. There may be opportunities for sequential deployment of different R genes in oilseed rape crops.

**References:**

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Molecular characterization and identification of fungi causing rapeseed stem canker in Serbia

**Background:** During the 20th century the pathogenic fungus *Leptosphaeria maculans* was on the quarantine list in Serbia. The pathogen was first isolated in Serbia in 1995 from seed and consume cabbage, and in 1987/88 from rapeseed. Cultivation of rapeseed intensified towards the end of the 20th century, and based on the examination of production fields during 2005 and 2006, parasitic fungus *Leptosphaeria maculans* was isolated in all production areas of the Province of Vojvodina, Serbia. More frequent symptoms on the aboveground plant organs lead to the conclusion that this parasite on rapeseed might become economically important in the future.

**Objectives:** The aim of this study was to identify pathogenic fungi causing stem cancer in rapeseed in Serbia by using molecular methods.

**Methods:** Stem canker of rapeseed is economically important disease in Europe, Australia and North America. This disease is caused by two species: *Leptosphaeria maculans* (Desm.) Ces. et de Not (anamorph *Phoma lingam* (Tode ex Fr.) Desm. and *Leptosphaeria biglobosa* Shoem. and Brun. *B. napus* plant material infected with *L. maculans* and *L. biglobosa* was collected from nine sites in Serbia (Karavukovo, Crvenka, Prigrevica, Subotica, Rimski šančevi, Srbobran, Beška, Banatsko Karađorđevo, Srpski Miletic). Infected tissue samples were taken in 2008-2010 from root, upper and basal stems, leaf, flower and pods. Total of 119 isolates from Serbia and two from Great Britain were analyzed using PCR and PCR-RFLP. Digestion of PCR products was performed with 5 selected endonucleases: BamHI, HaeIII, RsaI, EcoRIi and AluI. From a total of 119 isolates originating from Serbia and two representative isolates, 15 isolates were used for PCR-RFLP analysis.

**Results:** Based on DNA amplification with PN3 and PN10 primers, band length was 580 bp in isolates K-111 to K-118, and 560 bp in the remaining isolates (K-1 to K-25, St-1 to St-28, GS-1 to GS-27, C-1 to C-6, L-1 to L-10, S-1 to S-11, LJ-1 to LJ-6). PCR-RFLP analysis showed that K-111, K-112, K-113, K-115 and K-116 differed in one or two restriction locations from isolates K-2, St-16, GS-25, L-5, C-3, LJ-2, and S-1.

**Conclusion:** Based on the PCR analysis of all isolates originating from Serbia, it was determined that 111 belong to *Leptosphaeria maculans* and 8 to *Leptosphaeria biglobosa* NA1 (*Leptosphaeria biglobosa* brassicae).

**References:**


Influence of timing of volunteer oilseed rape control on clubroot disease development and inoculum density of Plasmodiophora brassicae

Background: Clubroot of oilseed rape (OSR; Brassica napus) caused by Plasmodiophora brassicae Woronin is a disease of increasing importance in OSR growing regions worldwide. The disease is difficult to control and disease management depends mostly on resistant cultivars, long crop rotation and the soil alkalization.

Objectives: Till date, any fungicide has been discovered to give complete control over clubroot and presently, the number of resistant cultivars is limited. Previous studies indicated that clubroot severity on OSR increases with increasing density of P. brassicae inocula (Hwang et al. 2011). Besides, it was shown that OSR volunteers and weeds play a critical role in development of clubroot epidemics by increasing the number of resting spore populations in the soil (Murakami et al. 2002; Ahmed et al. 2011). Therefore, it is necessary to study and develop management strategies for decreasing inoculum density in the soil.

Methods: A series of experiments were conducted under controlled glasshouse conditions with a susceptible OSR variety to clubroot to assay the effect of timing of foliar application of the herbicide glyphosate and mechanical destruction of OSR volunteers in reducing inoculum density and subsequent clubroot disease severity. Oilseed rape plants were inoculated artificially with injecting spore suspension (2*10^7 spores/ml) beside root hairs at BBCH 11-12. To determine the effect of timing of applications, plants were terminated early (7 dpi) or late (21 dpi). The amount of disease severity and the number of resting spores per gram of root was assessed for each treatment at 35 dpi. Later on, according to the Koch’s postulate test, the effect of early or late volunteers and weeds destruction on pathogenicity factor of new resting spores was evaluated on new set of OSR plants.

Results: Visual disease assessments after early and late applications showed well variation among the treatments in symptom development. Changing the time of application had significant effect (P ≤ 0.05) on control efficiency. Results from this study demonstrated that the early application of glyphosate as well as the early mechanical destruction significantly decreased, relative to untreated control, the development of clubroot symptoms and suppressed the establishment and survival of the resting spores. Afterwards, the disease incidence and severity were significantly decreased in new plants which inoculated with the spore extraction from early treated roots.

Conclusions: Our results described that early treatment of OSR volunteers and weeds reduced significantly symptom severity and spore production.

References:
Life history of the rape pollen beetle, *Brassicogethes viridescens*, in a canola crop on Prince Edward Island

**Background:** The rape pollen beetle, *Brassicogethes viridescens*, an introduced species to eastern North America from Europe, is causing damage to local *Brassicaceae* plants, including canola (*Brassica napus*). With a small canola industry, ~3000 acres in PEI annually, eastern Canada has a low potential for economic loss due to this species. However, this species is projected to migrate further west into the prairie ecozone of Canada where ~10,000,000 acres of canola are grown annually (Mason et al., 2003); creating a high potential for economic loss.

**Objectives:** The key objectives of this study include: (1) Determining time periods and relative degree-days for several life cycle stages of *B. viridescens* relative to *B. napus* phenology, and comparing *B. viridescens* life cycle time periods on PEI to those in central Europe. (2) Determining the damage caused by *B. viridescens* feeding and oviposition in canola buds on PEI.

**Methods:** The study was conducted at Agriculture and Agri-Food Canada’s Harrington Research Farm, PEI [46.343/-63.164] in 2014. Several trapping techniques were used to collect various stages of *B. viridescens* during its life cycle within canola plots and the hedgerow. These techniques included sticky traps (to intercept emerging adults and new generation adults entering overwintering sites), manual collection of buds (to record eggs and larvae, and damage), pupation traps, and adult emergence traps. Degree days were calculated using the equation DD = (T°-t(t)) and the relationship between buds with damage and buds with young was tested using linear regression.

**Results:** The active period of *B. viridescens* life cycle (overwintered adult emergence to new generation entering overwintering sites) occurred between late June and early September in 2014 and required 799.2 DD above 10°C. Life cycle time periods were later in PEI compared to those reported from central Europe. Mating of *B. viridescens* adults and development of the young was found to coincide with plant phenology. A significant positive relationship was found between the number of buds with damage and the number of buds containing *B. viridescens* young (p<0.001).

**Conclusion:** Emergence of overwintered adults occurred in late June (average temperature: 16.15°C), much later than emergence in central Europe (late-April; average temperature: 5-10°C;Nilsson, 1989). This indicates that other factors are involved in the emergence of adults besides temperature, such as photoperiod, or adaptation by *B. viridescens* to *B. napus* phenology in North America. The degree-day values calculated in this study can be used to predict *B. viridescens* life stages in canola fields, estimate potential damage by relating percent damaged buds to the percent young within buds and time control applications more precisely.

**References:**
Mining QTL for candidate genes involved in resistance of oilseed rape against *Verticillium longisporum* by an integrative omics approach

**Background:** *Verticillium longisporum* is an increasing threat to winter oilseed rape production in Europe. Resistance against *V. longisporum* is quantitatively inherited. Commercial European winter oilseed rape cultivars exhibit no or very low levels of quantitative tolerance or resistance. Quantitative trait loci have been identified in biparental crosses using resynthesized lines with the most effective partial resistance originating from the C genome of *Brassica oleracea*. Resistance against *V. longisporum* is expressed internally in the vascular tissue of the hypocotyl and phenolic compounds have been found to be associated with resistance expression (Eynck et al. 2009, Obermeier et al. 2013).

**Objectives:** The integration of data obtained from the genetic analysis of segregating populations and breeding material applying a combination of targeted and untargeted high-throughput omics-based technologies (genomics, metabolomics, transcriptomics) might allow more efficient identification of involved pathways and candidate genes than by any of these technologies alone. This integrative approach might speed up the development of broadly applicable diagnostic molecular markers for use in marker-assisted breeding for *V. longisporum* resistance.

**Methods:** A doubled haploid mapping population produced from an inbred line of a German rapeseed cultivar and a resynthesized rapeseed line segregating for *V. longisporum* resistance (Express617 x R53) was used for comparative genetic, genomic, transcriptomic and metabolomic analysis. The non-targeted analysis included the production of a high density genetic map using genotyping-by-sequencing and 60K SNP chip Illumina Infinium genotyping and quantitative trait locus (QTL) mapping for disease related traits and comparison with global RNA-Seq data. The targeted analysis included the identification and quantification of phenolic metabolites and lignin monomers in the hypocotyls of the mapping population by RP-HPLC and GC-MS to evaluate association with resistance expression and identify metabolite QTL and genes co-localizing with resistance QTL.

**Results and Conclusions:** Omics-based dissection and integration of metabolomic and genetic data from the mapping population with transcriptomic data obtained by RNA-Seq analysis from contrasting genotypes allowed efficient mining of QTL for candidate genes and pathways involved in resistance expression. This approach allowed to establish an improved understanding of the multiple mechanisms and pathways involved, rank them by relevance and prioritize candidate genes from the QTL interval. The contribution of different interconnected pathways and genes to resistance expression in the highly complex pathogen-host interaction will be discussed. Based on this approach competitive allele-specific PCR (KASP) markers have been derived and are being used by German oilseed rape breeders for marker-assisted selection.

**References:**


Evaluation of seed treatments for control of leafhoppers and suppression of aster yellows in hybrid canola

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Background: Aster yellows (AY) caused major yield losses to canola in western Canada in 2012 (Miller et al. 2013). The phytoplasma is transmitted by the aster leafhopper, *Macrosteles quadrilineatus* that is brought into western Canada on winds from the southern US in early spring. AY symptoms are more severe when canola seedlings are infected in wet soil than in dry soil (Olivier et al. 2014). AY is difficult to control. An application of dimethoate is the only method currently registered for leafhopper control in canola but no economic threshold has been established (Anonymous 2015). Neonicotinoid seed treatments provide effective leafhopper control at early seedling stages in other crops. The treatments are currently registered for flea beetle control on canola in Canada and have not been evaluated against leafhoppers.

Objectives: Assess the effect of soil moisture on the efficacy of neonicotinoid seed treatments for control of leafhoppers and suppression of aster yellows at different growth stages of hybrid canola.

Methods: Untreated seeds and seeds treated a fungicide (Tribune) or neonicotinoid seed treatment containing imidacloprid (Gaucho CS FL), clothianidin (Prosper Evergol) or thiamethoxam (Helix, Helix Xtra) were grown in dry soil (20-30% moisture content) and wet soil (70-100% moisture content). Plants at the cotyledon and 1st-4th true-leaf stages were placed in cages and exposed to AY-infected leafhoppers (n = 6 adults/plant) at 20°C for 72 h. Leafhopper mortality was assessed after 24 and 72 h. Plants were grown at 20°C under high light intensity (>400 μmol/m²/s). AY symptoms were assessed after 6, 8 and 10 weeks using a five-point rating scale (Olivier et al. 2014). Plants were harvested at maturity to determine seed yield.

Results and Conclusions: Seed treatments and soil moisture had a significant effect (P≤0.001) on leafhopper mortality at the cotyledon and early true-leaf stages. At each growth stage, mortality in the neonicotinoid seed treatments was higher in dry soil than in wet soil. In dry soil, Prosper, Helix and Helix Xtra provided excellent control of adult leafhoppers after 24 h at the cotyledon stage (93-96%), 1st & 2nd true-leaf stage (90-97%) and 3rd & 4th true-leaf stage (95-100%). Mortality in the treatments after 72 h exceeded 95% at each growth stage. Treatments were less effective in wet soil. Mortality with Prosper, Helix and Helix Xtra in wet soil averaged 68-81% at the cotyledon stage, 65-74% at the 1st & 2nd true-leaf stage and 81-88% at the 3rd & 4th true-leaf stage. Mortality in the treatments after 72 h averaged 86-91%, 71-89% and 95-99%, respectively. AY symptoms and yield are currently being assessed. Preliminary results suggest that greater than 80% control of leafhoppers within 24 h may be required to prevent AY infection and yield loss in dry and wet soil.

References:
Distribution and characterization of clubroot pathotypes in the main WOSR production areas in France

Background: Clubroot is an important soil-borne disease of Brassica crops caused by Plasmodiophora brassicae, an obligate biotrophic protist. The disease is characterized by the development of galls on the root system disturbing water and mineral nutrition of the plant, leading to premature death of the plant and causing significant yield losses. The pathogen can remain in the soil as viable spores during 20 years. Using resistant varieties is one of the best ways to control this disease in Brassica napus crops. To date, no official test exists for registration of clubroot resistant varieties to the French Official Catalogue of Plant Varieties.

Objectives: To determine the distribution and characterization of the P. brassicae pathotypes in the main Brassica production areas in France and to develop a protocol for evaluating the resistance to clubroot in WOSR varieties.

Methods: Location and characteristics of infested fields were monitored by CETIM through an online reporting system (http://www.cetiom.fr/hernie/). Field sampling was done in Centre, Bourgogne, Bretagne, Lorraine and Poitou-Charentes regions in France, targeting different environmental situations. Biological characterization of P. brassicae field populations isolated from infected plant clubs was done using the differential host set defined by INRA of Rennes, allowing the identification of eight pathotypes (Some et al., 1996; Manzanares-Dauleux et al., 2001).

Results: A total 70 samples was analyzed. Pathotypes P1 to P6 were identified in the territory, P1, P2 and P3 being the most frequent. More than half of the isolates were identified as P1. Several isolates belonging to pathotypes P1, P2 and P3 were able to overcome the resistance of Mendel (reference resistant variety).

Conclusions: Isolates from the most frequent pathotypes in France (P1, P2 and P3) have been chosen in order to test the resistance of the WOSR varieties. A complete protocol to evaluate resistance has been proposed to the French registration committee. This protocol is now currently used by GEVES to evaluate resistance of WOSR varieties for registration.

References:
Twenty years of canola variety performance in the Pacific Northwest

**Background:** The Pacific Northwest (PNW) has a long history of growing small grain cereal crops, such as wheat and barley. Only about 2% of land in the PNW that is suitable for cereal grain rotations is planted to canola. Canola is a good rotational crop when grown with wheat and barley and adds diversification to dry-land farmers of the PNW. Rotational benefits include improved control of pests, grass weeds, and diseases in cereal production. A major constraint on increasing canola acreage has been the availability of suitably adapted cultivars.

**Methods:** Researchers at the University of Idaho established the Pacific Northwest Spring and Winter Variety Trials in the early 1990s and have conducted winter and spring cultivar trials throughout the PNW region for the past 20 years, testing cultivars and breeding lines from the University of Idaho alongside entries from private and other public breeding programs. The trials were typically grown at four locations in Idaho, three in Washington, and two in Oregon.

Over the 20 years of testing, 266 spring cultivars from 25 different companies and 160 different winter varieties from 20 different companies or breeding programs have been tested. ‘Westar’ spring canola was included in all spring trials as a control, and ‘Bridger’ winter rapeseed was included in all winter trials as a control. Both of these controls were used as checks to determine the proportion of yield improvements that were attributable to advances in crop genetics.

**Results:** The yield of the three best winter canola cultivars increased from 3,400 kg ha⁻¹ to over 4,400 kg ha⁻¹, an increase of 52 kg ha⁻¹ each year. The cultivar ‘Bridger’ showed a yield increase from 2,601 kg ha⁻¹ to 3,070 kg ha⁻¹, 23 kg ha⁻¹ per year. This yield increase for Bridger can be attributed to improvements in agronomic practices, including new pesticides. Comparison of the genetic and non-genetic yield gains shows that winter canola genetic improvements contributed yield increases of 604 kg ha⁻¹ (55%), while agronomic improvements contributed 483 kg ha⁻¹ (45%).

Yield of the best cultivars entered into the spring variety trials have shown an improvement in yield from 1,950 kg ha⁻¹ to over 2,500 kg ha⁻¹. ‘Westar’ also showed a yield increase from 1,665 kg ha⁻¹ to 1,844 kg ha⁻¹. Comparing genetic with non-genetic yield gains, spring canola cultivars showed improvement due to genetics of 470 kg ha⁻¹ (70%), while agronomic improvements accounted for an increase in yield of 188 kg ha⁻¹ (30%).

**Conclusions:** Genetic improvements of cultivars and improved agronomic practices have increased yield potential for spring and winter canola significantly. In recent years, the acreage of canola in the PNW has risen and continues to increase. This is due in a large part to the availability of new and adapted cultivars in combination with improved agronomic practices.
Transcriptome sequencing and gene networks analysis reveals pathogen effectors manipulate the JA and SA pathways of *Brassica napus*

**Background:** The white mold fungus *Sclerotinia sclerotiorum* is a devastating necrotrophic plant pathogen with a remarkably broad host range (Guyon et al. 2014), however, very few studies have investigated host-pathogen interactions about the genetics and biochemistry.

**Objectives:** Identification of gene networks relate to disease-resistance in *Brassica napus* or pathogenicity in *S. sclerotiorum* will help us understanding the mechanism of rape-*S. sclerotiorum* interaction and guide the commercial rapeseed breeding.

**Methods:** A uninfected leaf of ‘NRS-1’ together with hypha of ‘S06S2’ were used as one sample. Three infected leaves and pathogenic mycelium collected at 6h, 24h and 48h, respectively, were used as the other three samples. All these four samples total RNA were used for transcriptome sequencing, respectively.

**Results:** A total of 80.46M high-quality reads were obtained by Illumina HiSeqTM 2500 sequencing of the rape-*S. sclerotiorum* interactome. ToPhat software was used to distinguish rape and *S. sclerotiorum* sequences. 52,159 rape unigenes and 13,313 *S. sclerotiorum* unigenes were predicted to be expressed specifically during the rape-*S. sclerotiorum* interaction. Among those unigenes expressed in ‘NRS-1’, 2,528 at 6h, 2,064 at 24h and 2,475 at 48h were detected as Differentially Expressed Genes (DEGs). The same method was also used to analyze *S. sclerotiorum* unigenes, and we found 698 DEGs, 475 DEGs and 540 DEGs, respectively. A method based on the BLAST program was modified to obtain DEGs annotation from Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. At 6h, ‘NRS-1’ expressed 127 genes were annotated to ribosome assemble. Then at 24h, 10 genes were annotated to alpha-Linolenic acid metabolism and 48h, 35 genes were annotated to plant-pathogen interaction and indolylmethyl-glucosinolate biosynthesis were expressed specifically. All these pathways were related to jasmonic biosynthesis and signaling transfer. Additionally, Shikimic acid pathway, which was reported as another way of salicylic acid synthesis in plants, was found in *S. sclerotiorum* at 24h. The expression patterns of the 22 genes belong to these pathways were analyzed by RT-qPCR to explore their putative functions.

**Conclusions:** Our data reveals the different stage when *S. sclerotiorum* infecting, *B. napus* will activate defence genes against fungal infection and most of defence genes depend on the inducing by JA pathway. The reason that necrotrophic fungal pathogen *S. sclerotiorum* suppress JA-dependent defenses may caused by effectors which targeting inhibiting JA signaling but activating SA pathway in *Brassica napus*.

**References:**
Using genome-wide SNPs to facilitate the selection of genetic background for efficient introgression of clubroot resistance from diploid *Brassica rapa* vegetable species into amphidiploid *B. napus canola*

**Background:** Diverse host resistance mechanisms help durable resistance for clubroot (*Plasmodiophora brassicae* Woronin) management on canola. Most clubroot resistance (CR) genes have been found in the diploid species *B. rapa* (*2n = 20, AA*), but >90% of canola cultivars in Canada are the amphidiploid *B. napus* (*2n = 38, AACC*). Interspecific hybridization can be used to move CR genes from *B. rapa* into *B. napus*, but most BC1 plants carry univalent C chromosomes resulted from unpaired C chromosomes from the triploid F1 (*2n = 29, AAC*). Additionally, varying amounts of undesirable background may be carried over from the CR donor. Normally repeated backcrossing is used to stabilize suitable chromosomal content and eliminate undesirable genetic background.

**Objective:** Use SNP markers to select plants with the most desired genetic composition at early stages of introgression to accelerate the development of CR canola germplasm.

**Methods:** Interspecific crosses were made between *B. rapa* ssp. *chinensis* ‘Flower Nabana’ (FN) carrying the CR gene Rcr1 (Chu et al. 2014) and *B. napus* line DH16516 originating from ‘Topas’. Resistant F1 progenies were crossed further with the *B. napus* canola DH line SV11-17667 to produce a BC1F1 population. SSR flanking markers and a bioassay were used to select plants carrying Rcr1. The genetic composition of the A and C genomes were examined using an Infinium 6K SNP array. Plant development and yield potential were assessed for selected plants with varying genetic content and composition under controlled conditions and resulting seed were analyzed for fatty-acid profiles.

**Results:** Rcr1 was present in about half of the BC1F1 population. The background SNP analysis found that each of the resistant plants carried nine C genome chromosomes from SV11-17667, but 1 to 9 from DH16516, resulting in varying numbers of univalent C chromosomes in the BC1F1 plants. Additionally, some of the polymorphic A-genome SNPs that showed the same segregation pattern as in the parental line FN were found also in these BC1F1 plants, ranging from 22% to 64%. This potentially denotes varying amounts of genetic background carried over from FN. It was noteworthy that plant # 66, which carried Rcr1, a full complement of C-genome chromosomes and 40% of SNPs shared with FN, exhibited morphological characteristics and fatty-acid profile similar to those of SV11-17667. These yield/quality traits often were better than those observed for other resistant BC1F1 plants carrying 1 to 8 univalent C chromosomes.

**Conclusions:** Background selection using the SNP array allowed rapid identification of a desirable line in a population derived from the crosses between *B. rapa* and *B. napus* species, showing the potential of using the technology to accelerate CR introgression into *B. napus canola*.

**References:**

The profile of avirulence alleles in the population of *Leptosphaeria maculans* (blackleg) in western Canada

**Background:** Blackleg disease of canola (Brassica napus), caused by the fungus *L. maculans* (Desmaz.) Ces. & de Not, has increased in western Canada in the past several years, with severe cases reported on R-rated canola cultivars. Resistance conferred by major-gene and quantitative (adult-plant resistance - APR) exists in Canadian canola cultivars, with >70% carrying Rlm3 and 56% with APR. Other specific resistance genes are uncommon. Recent analysis of *L. maculans* race structure in random field samples confirmed an earlier notion (Kutcher et al. 2007) that Rlm3 was no longer effective against blackleg in western Canada.

**Objectives:** To better understand the race structure and dynamics of *L. maculans* population in western Canada, and investigate potential adaption of the pathogen to resistant cultivars.

**Methods:** Approximately 50 trap plots/strips of cv. Westar (carrying no resistance genes) were seeded across canola-growing regions in western Canada in 2012 and 2013. Diseased stems were collected from 10-15 locations each year and up to 25 *L. maculans* isolates were tested from each location using host differentials carrying known resistance genes to profile Avr alleles in the pathogen population. Likewise, diseased samples from commercial fields with varying blackleg incidence/severity were also collected and *L. maculans* isolates tested. A total of 424 and 300 isolates from the trap plots and commercial fields, respectively, were tested using an established protocol (Kutcher et al. 2007).

**Results:** In trap plots, AvrLm1, AvrLm3, AvrLm9, AvrLep1 and AvrLep2 showed low frequencies (0-9%), whereas AvrLm2, AvrLm4, AvrLm6 and AvrLm7 were common (>70%), with AvrLm7 at 99% in each region by 2013. The low frequencies of AvrLep1 and AvrLep2 may be related to the test protocol used. The results indicate that the resistance genes Rlm2, Rlm4, Rlm6 and Rlm7 are generally effective against the current pathogen population. Some regional differences were observed: AvrLm4 was noticeably lower in Alberta (35%) than in Saskatchewan and Manitoba (75%), while AvrLm2 was most common in Saskatchewan (100%) relative to the other two provinces (60-70%). In commercial fields, AvrLm1, AvrLm3, AvrLm9 and AvrLep2 were absent or at very low frequencies. Varying severity of blackleg in these fields, however, can't be explained by the pathogen race structure alone; AvrLm3 was generally missing in the pathogen population and only the resistance gene Rlm3 had been used in Canada. Likely APR is common in current canola cultivars, otherwise more severe blackleg could have occurred in many regions. Often the Avr profile in a severely diseased field did not differ substantially from that in other commercial fields or trap plots in the same region.

**Conclusions:** Analysis of Avr allele frequencies in the *L. maculans* population indicates that Rlm2, Rlm4, Rlm6 and Rlm7 are effective against the current pathogen population in western Canada. Varying severity of blackleg on R-rated cultivars may be caused by multiple factors, in addition to variations in Avr allele frequencies observed.

**Reference**

Choice of cultivar – the key tool in oilseed rape disease control

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Background: Oilseed rape is an important crop in Lithuania in terms of the area grown and fungicide inputs. A high concentration of oilseed rape in a crop rotation in recent years and its frequent return to the same field have resulted in high pressure of the most important diseases such as sclerotinia, blackleg, black spot, verticillium wilt and others. Different disease management tools must be used giving priority to environmentally friendly measures and any fungicide use has to be balanced against the risks of losses to diseases.

Objectives: The aim of this study was to establish the resistance of different rapeseed cultivars (cvs.) to the main diseases in the natural infection conditions.

Methods: Field experiments were carried out during the 2013-2014 growing seasons with 21 cvs. of winter and 21 cvs. of spring oilseed rape, which represent a wide range of resistances and susceptibilities to diseases using predominantly cvs. available on the national market. Visby and Landmark, the currently most popular cultivars, were used as references for winter and spring oilseed rape, respectively. The experimental design included the 2 main plots: 1. natural infection, with no fungicide; 2. fungicide applied at GS 65. Disease pressure was established according to the assessment scales (Aubertot et al. 2004; Krüger, 1991). The seed yield of each cultivar was estimated.

Results: Sclerotinia, blackleg and verticillium dominated in the natural field conditions in winter and spring oilseed rape during the experimental year. Winter oilseed rape cv. Visby was the most resistant to blackleg; however, it was the most susceptible to sclerotinia. Cv. Cult OP was 2.4 times less damaged by sclerotinia compared to the reference Visby. Cv. Nelson H was the most susceptible to verticillium wilt. The best yield response to fungicide application at GS 65 was achieved in cv. Remy OP, lower response was in cv. Cult OP. Blackleg severity was 2.6-3.0 times higher in spring oilseed rape cvs. Lennon OP, Fenja OP and Majong H compared to the reference Landmark OP. Cv. Smilla H exhibited the highest resistance to sclerotinia. The highest verticillium wilt severity was recorded in cvs. Smilla H and Traper H, lower severity in cvs. Kaliber H and Fenja OP. The highest yield potential was shown by cvs. Lennon OP, Majong H, Fenja OP and Jegger OP.

Conclusion: Choice of cultivars with satisfactory disease resistance attributes can reduce the need for fungicide input.

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References:


Levers of tolerance to floral bud damage in *Brassica napus*

**Background:** Floral bud damage generated by pollen beetle is a source of dramatic nuisibility on the final crop yield (Lerin, 1987). However, nuisibility remains variable according to plant compensation that implies the number of fertile branches, the number of pods, and the pod weight (Tommey and Evans, 1992; Pinet, 2015).

**Objectives:** Objective of the study is to assess the respective implication of the key levers cited above in the compensation in seed yield. Therefore, damage of varying intensities and different modalities (floral bud or inflorescence clipping) has been applied to plant with varying branching potential generated by the interaction between genotypes and nitrogen fertilisation levels.

**Methods:** Three varieties with contrasting architectures were studied during two years (2007 and 2008) combined to two levels of nitrogen fertilization (high: 140 and 150kg ha⁻¹ for year 1 and year 2; and low: 70 and 40kg ha⁻¹ for year 1 and year 2). Three intensities of inflorescence clippings were applied in year 1 on five plants before flowering (5% to 50% of floral buds destroyed according to treatments). Three intensities of floral bud clipping were applied in year 2 on 13 plants (23% to 94% of floral bud destroyed according to treatments). At harvest the number of pods, axes, and pod per axis were counted, the dry mass of different compartments of the plant was weighted on plant sample of varying sizes according to years and treatments.

**Results:** Results are that the major lever of compensation of the damage in terms of seed yield was the number of pods, before the pod weight. Primary branches present prior to clipping mainly carried the compensating pods, and the proportion of yield beard by the secondary branches was increased. A correlation between the compensation in seed yield and the number of fertile axes was evidenced. Variations in branching and biomass allocation were observed between the clipping modalities that can be explained by the fact that floral bud clipping did not affected the vegetative mass of the plant while the clipping of the whole inflorescence did.

**Conclusions:** Reproductive morphogenesis is a key process of plant answer to floral bud damage. This study helps understanding plant tolerance to pollen beetle on *Brassica napus*. A dynamics and spatial study on pod setting on the plant would help refine the mechanisms involved and their genotypic variability (Pinet, 2010).

**References:**


Evolution of *Leptosphaeria maculans* populations in a small area of the region Centre (France) following the introduction of oilseed rape hybrids carrying the *Rlm7* specific resistance gene

**Background:** The specific resistance to blackleg conferred by *Rlm7* has been used in commercial oilseed rape (*Brassica napus*) cultivars since 2004. Varieties carrying *Rlm7* have since become widespread today with a very large market share at the national level. In order to evaluate consequences of the selection pressure exerted by the *Rlm7* resistance gene on populations of *Leptosphaeria maculans* (causal agent of blackleg), samples of isolates of *L. maculans* were collected for ten years on plants both with and without *Rlm7* in a small area in the central region of France.

**Objectives:** The main objectives are:

1. To improve tools to be able to follow qualitative evolution of fungus populations.
2. Follow *L. maculans* population evolution over a long period in the centre of France, which is a production area where the disease risk is important.

**Methodology:** Each year from 2004, sampling was done in 20-30 different fields in late November or early December. One leaf per plant and one isolate from a blackleg lesion per leaf were sampled to reach 200-250 isolates. Isolate characterization was done by classical cotyledon tests, by molecular PCR, or by HRM PCR technique which was improved in recent years.

**Results:** The HRM PCR distinguished different alleles of the *AvrLm4-AvrLm7* locus and is a very powerful and time saving method. We found avirulent isolates with *AvrLm7* present on *Rlm7* hybrids, which confirmed previous results. This interaction produced foliar lesions but no further development of the disease was observed. There was an increase of virulent *AvrLm7* sub-population on *Rlm7* hybrids and on non-*Rlm7* hybrids. It would appear that the specific resistance *Rlm7* seems to be broken down. All virulent *AvrLm7* isolates are also found to possess the avirulent allele *AvrLm3*. These interactions are under further investigations at INRA Bioger.

**Conclusion:** Break down may occur after seven years of intensive use of *Rlm7* genotypes. Nevertheless agronomically this disease dynamic is under control and final symptoms are still very low (G2 index). Several arguments may explain such situation. It may be due to the high frequency of dry autumns in the region, or due to a fitness deficit of *AvrLm7* isolates, or due to interactions with other resistance genes (QTLs or Rlm3).

**References:**


**Aknowledgements:**

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Transfer of sclerotinia resistance from wild relative of *Brassica oleracea* into *Brassica napus* using a hexaploidy step

**Background:** Stem rot caused by fungal pathogen *Sclerotinia sclerotiorum* is a great threat for rapeseed (*Brassica napus*) production around the world. A high level of resistance against *Sclerotinia sclerotiorum* was documented in wild *B. oleracea* (*B. incana*), but not in cultivated rapeseed (*B. napus*) (Mei et al. 2011). Inheritance investigation and QTL analysis revealed the additive genetics for *Sclerotinia* resistance in *B. incana* (Mei et al. 2013).

**Objectives:** This study was conducted to transfer *sclerotinia* resistance from *B. incana* into rapeseed.

**Methods:** A strategy was proposed using hexaploids (AAcccc) derived from crosses between the wild *B. oleracea*-related *B. incana* genotype ‘c01’ and the Chinese rapeseed variety ‘Zhongshuang 9’ as a bridge. Progenies (BC1F1 and BC1F2) that generated by backcrossing the hexaploid to ‘Zhongshuang 9’ were screened firstly by five molecular markers linked to the major resistance QTL in *B. incana* and secondly by resistance evaluation. Resistant individuals in BC1F2 were checked for chromosome numbers.

**Results:** Among 73 BC1F1 individuals, eleven that harbouring resistance loci were selected to develop vegetative clones for resistance evaluation. Of these, five exhibited significantly higher resistance than ‘Zhongshuang 9’ and the most resistant individual was chosen to develop the BC1F2 progeny. Finally, five individual genotypes with nearly two-fold higher resistance than ‘Zhongshuang 9’ were identified from 100 BC1F2 individuals. Hereof, one rapeseed-type individual with 38 chromosomes and good self-fertility (15.0 ± 3.56 seeds/pod) was identified.

**Conclusions:** Our results indicate that the proposed strategy is effective for transferring sclerotinia resistance from *B. oleracea* into rapeseed.

**References:**
- Mei JQ, Qian LW, Disi JO, Yang XR, Li QF, Li JN, Frauen M, Cai DG, Qian W (2011) Identification of resistant sources against *Sclerotinia sclerotiorum* in *Brassica* crops with emphasis on *B. oleracea*. Euphytica 177:393-400
Investigating the durability of blackleg resistance genes in *B. napus* and the emergence of virulent isolates in *L. maculans*

**Background:** *Brassica napus* (Canola/oilseed rape) is grown extensively in Australia, North America, Europe and Asia. This crop is susceptible to a few major diseases including blackleg or stem canker caused by the ascomycete fungus *Leptosphaeria maculans*. The disease has a great economic impact worldwide due to serious yield loss. A number of single dominant R-genes to *L. maculans* have been identified in different *Brassica* species; however a single R-gene may not offer durable resistance to the disease. Studies have shown the breakdown of single resistance gene, Lepr3 and Rlm1 in Australia and France, respectively. In recent years Rlm3 has been reported to have broken down in Canadian canola fields. This indicates the need for proper management strategies for the development of host resistance. Moreover, effective management of resistance depends on the understanding host-pathogen interactions as well as pathogen adaptation.

**Objectives:** The aim of this study is to generate useful data to implement a canola cultivar rotation strategy in the Canadian Prairies based on an understanding of R-gene durability and the nature of emergence of virulent blackleg isolates.

**Methods:** The experimental design of the project consisted of 10 separated plots with 3 replications; 5 plots for examining durability of a single R-gene and 5 plots for determining the virulence changes in isolates. Each plot was sprayed with either 90% avirulence isolates for durability or 100% avirulence isolates for emergence. Five single Rlm Topa lines developed by AAFC Saskatchewan were used in the study along with a Topa check as a control (no R-genes). Each plot was set up for canola-wheat-canola rotation over 2-years. In addition, the Topa lines carrying different single R-genes were rearranged randomly for both trials. Disease incidence, disease severity and representative yield were recorded from all plots. Moreover, the pathogen isolates collected from either infected stubble or a 7-day Burkard spore trap were subjected to differential testing as well as PCR identification for the presence of six avirulence genes to monitor changes in pathogen virulence.

**Results:** Disease incidence varied among the cultivars in both the durability and emergence trials; however, similar level of disease incidence was observed in each cultivar between the trials. The emergence trial revealed higher disease severity of 2.5 on a 0-5 scale in the cultivar harbouring the single R-gene, Rlm3. The rest of the cultivars showed ≤ 1.0 disease severity irrespective of trial. PCR analysis of *Leptosphaeria maculans* isolates cultured from the stubble indicated that the isolates were carrying AvrLm5, AvrLm6 and AvrLm11. PCR analysis of ascospores collected via the spore trap indicated the presence of *Leptosphaeria maculans* as well.

**Conclusions:** The first year of data collection indicates the possibility of Rlm3 break down and adaptation of the pathogen. The study will continue for four years to observe the likelihood and timeline for the breakdown of R-genes and the emergence of new races. The data will be informative in planning canola rotation on the Canadian prairies.
Role of non-conventional chemicals in induced resistance against *Sclerotinia sclerotiorum* the incitant of Sclerotinia stem rot of Indian Mustard

**Background:** Sclerotinia stem rot of Indian mustard incited by *Sclerotinia sclerotiorum* has become an important disease in recent times in India. Wide host range, soil borne nature, difficult to manage with fungicides and lack of complete genetic resistance in all economical crops prompted us to study on the induction of systemic resistance against this pathogen. However, it is well known that plants in many crops have evolved an array of defense mechanism to combat invasions by plant pathogens. But, in crops where this mechanism is lacking, the induced resistance can be activated by treatment of plants with either natural or systemic chemicals (Kessmann et al., 1994).

**Objectives:** This fungus produces oxalic acid, which creates an optimal pH for the activity of enzymes related to pathogenesis. The non-conventional chemicals when apply can induce some defense through their activities on biochemical changes in plants. Hence, the present study was undertaken to screen non-conventional chemicals and to examine the changes due to these in biochemical parameters.

**Methods:** Forty-five to fifty days old plants raised in the pots in screen house were sprayed by non-chemicals and after 24 h pure culture of pathogen was inoculated by stem inoculation method and observations on disease severity was calculated using standard scale. In control, the plants were sprayed with sterile water before challenge inoculation. Five plants/pots were maintained and the pots were arranged in CRD with four replications. Leaf tissues were taken at various intervals (0, 3 and 6 days) after pathogen inoculation from both control and treated plants and immediately homogenized and stored in liquid nitrogen. These samples were powdered and used for analysis of different biochemical changes that have taken place in the host tissues.

**Results:** As the concentration of different non-conventional chemicals increased from 10, 50 and 100 ppm there was reduction in Sclerotinia rot disease. Maximum induction of resistance was recorded by the application of salicylic acid at 100 ppm concentration after six weeks of challenge inoculation with *S. sclerotiorum* followed by acetylsalicylic acid, indole butyric acid and indole acetic acid. Total phenols, PPO, PAL, PO and chitinase activity was at peak after 3 days of challenge inoculation with *S. sclerotiorum* and thereafter the activity of all these parameters slightly declined after 6 days of inoculation in all non-conventional chemicals tested. However, SA has shown maximum rise of total phenol at 3 days after challenge inoculation followed by ASA and IBA, respectively. Similarly, PPO, PAL, PO and chitinase contents were increased to the maximum in treatment with SA followed by ASA and IBA.

**Conclusion:** The application of SA at 100 ppm concentration significantly enhances the activity of total phenol, PPO, PAL PO and chitinase after challenged inoculation of the pathogen and activates systemic resistance against stem rot disease in Indian mustard.

**References:**

**Pseudomonas chlororaphis PA23 biocontrol of Sclerotinia sclerotiorum on canola: Understanding populations and enhancing inoculation**

**Background:** Biological control is emerging as an attractive tool to manage plant diseases due to an increasing demand for sustainable approaches with minimal adverse environmental impact. Biocontrol by *Pseudomonas chlororaphis* strain PA23 has previously been shown to effectively control *Sclerotinia sclerotiorum*, one of the most important diseases of canola in Western Canada (Fernando et al., 2007). PA23 biocontrol is mediated through the production of secondary metabolites that inhibit fungal growth. These compounds include the antibiotics pyrrolnitrin and phenazine, along with HCN, protease and lipase (Poritsanos et al., 2006).

**Objectives:** The purpose of this research is two-fold. Firstly, to determine how time of application affects PA23 biocontrol. This will reveal at what point post inoculation PA23 begins to produce antifungal metabolites; how long PA23 populations survive on canola; and the period of time PA23 affords canola protection from sclerotinia infection. Secondly, to research the effects of supplements on PA23 population size and antifungal activity. These findings will be valuable for designing inoculation formulations that promote PA23 establishment and biocontrol in the environment.

**Methods:** Antifungal metabolite gene expression will be monitored in vitro using quantitative reverse transcription PCR (qRT-PCR) at different bacterial growth phases (log and stationary growth). In vivo, we will investigate how long PA23 provides control by inoculating PA23 at different times (1, 4, 7 days) prior to *Sclerotinia ascospore* application. Petal infestation and plant infection by the fungus will be assessed. PA23 populations on petals will also be monitored by washing the petals and plating for colony forming units (CFU). The impact of supplements on antifungal activity will be assessed in radial diffusion assays, after which qRT-PCR will be used to determine how best to control for sclerotinia in a greenhouse setting.

**Results:** Preliminary results suggest that PA23 provides long term control of Sclerotinia in a greenhouse setting. When PA23 was inoculated 7 days prior to ascospore inoculation, there was greater control than either 4-day or 1-day advance inoculation. qRT-PCR suggests that the antibiotic pyrrolnitrin is upregulated in stationary phase. Initial radial growth assays indicate that glucose and fructose amendment provides PA23 with better conditions to retard Sclerotinia mycelial growth.

**Conclusions:** This research will allow for a better understanding of how *P. chlororaphis* strain PA23 interacts with canola and imparts biocontrol over time. Understanding PA23 population dynamics, antifungal gene expression, as well as optimal application formulations is necessary before this bacterium is ready for implementation into a sustainable disease management strategy.

**References:**
Studies of clubroot (*Plasmodiophora brassicae* Wor.) on oilseed rape in the Czech Republic

**Background:** The clubroot, caused by *Plasmodiophora brassicae*, previously a problem in vegetable, is appearing on oilseed rape in the Czech Republic. The infested stands are reported across the whole country, mainly in north and north-east of the country. The pathogen is probably spread in the whole country; its occurrence depends on weather conditions during the sawing period (in mid-August). Research on *P. brassicae* in the Czech Republic is therefore important for the development of effective management strategies under Czech environmental conditions.

**Objectives:** The prevention is only way of protection against clubroot. When the field is already infested solution could be using resistant winter oilseed rape cultivars, but the right crop rotation is essential too. Therefore, one part of research is targeted on resistant cultivars. Pathotype monitoring is necessary for complex knowledge of clubroot. Insight on the amount of inoculum – the spore load, which can be done by quantitative PCR analysis (qPCR) is valuable for assessment of pathogen potential.

**Methods:** Experiments with clubroot resistant cultivars were made in the field and greenhouse. In the greenhouse (conducted in 2013, repeated in 2014), six clubroot resistant cultivars were grown in infested soil collected from 14 Czech fields, and assessed for disease severity. The presence and amount of clubroot inoculum in soil samples was also tested by conventional and qPCR analysis. In the field, seven resistant cultivars were grown on clubroot infested field, disease development monitored monthly. Yields were measured during cropping. The experiment will be repeated in season 2014/2015. Finally, a set of 16 *P. brassicae* field isolates from across the Czech Republic were assessed for pathotype designation on the differential hosts of Williams, Somé et al., and the European Clubroot Differential set.

**Results:** Greenhouse testing brought various results, depending on source of soil samples. The control cultivar was highly infested with one exception. All cultivars showed good resistance on all tested localities. Index of Disease (ID) was lower than 25% (except one). The results from both the field and the greenhouse are similar. All resistant cultivars showed good level of resistance on infested field (except one). The control cultivars were highly infested. The pathotype designation revealed 6 pathotypes according Williams classification (the most frequent pathotype 7). Somé et al. classification identified 3 pathotypes (the most frequent P3). ECD set determined 8 pathotypes (the most frequent ECD 16/14/15). The qPCR analysis revealed high spore load in infested soil samples, highest in locality Modlibohov (5 mil. spores/1 g soil) and the lowest in locality Zirovnice (70000 spores/1 g soil).

**Conclusions:** Tested clubroot resistant cultivars are suitable for growing in clubroot infested soil and can be recommended for agricultural production as one of the protection ways. The PCR and qPCR are reliable methods for clubroot detecting and quantification. The identified pathotypes can be useful in testing of new clubroot resistant cultivars.

**References:**


Somé et al. 1996: Plant Pathology, 45:432–439

Williams 1966: Phytopathology 56:624-626
COLEOTTOOL: Development of molecular tools to identify the main weevil species attacking oilseed rape, and their parasitoids

**Background:** In Europe, the oilseed rape crop is very dependent on insecticides. The first way of reducing pesticide use is to guarantee the quality of diagnosis in the fields to avoid unnecessary treatments. For instance, it is very difficult to identify weevil larvae. Another way is to develop new crop protection strategies, for example by enhancing biological control in agroecosystems. Today, references and knowledge about natural enemies are poor. In France, the last inventory of the parasitoids of oilseed rape pests date back to the 60’s. It is in this context that the French Ministry for Agriculture is supporting this 3 years project that has started in 2014.

**Objectives:**

1) To establish an inventory of parasitoid species specific to the key weevil pests of oilseed rape.

2) To provide validated molecular tools to identify weevils at any stages, and their parasitoids.

**Methods:** Weevils and parasitoids are caught by beating, Malaise traps or yellow traps in several fields all around France. Weevil larvae or eggs are reared in a laboratory to identify the hosts of each parasitoid species. The COI mitochondrial fragment and the ITS2 nuclear marker will be sequenced. These sequences will feed a database that will be searchable by BLAST. The molecular identification tool will be validated and tested in field experiments in the last year of the project.

**Results:**

1) To give a clear picture of weevils’ parasitoids in France. Revisions of the Zoological Nomenclature could be done if necessary.

2) To develop validated and operational methods to identify at least 40 insect species at any stage of development thanks to sequences.

**Conclusions:** The main goal of this project is to develop molecular tools that will be available online. These tools should make it possible to identify and hopefully quantify insects (pests and natural enemies) in different kind of environments and at different stages. This should lead the way to new researches to identify agricultural practices or landscape impacts on the pests and their natural enemies.

**References:**

Identification of races of *Leptosphaeria maculans* infecting high erucic acid rapeseed (HEAR) in western Canada

**Background:** Erucic acid is a very important raw material for the industry and can be used as an additive in lubricants and solvents, a softener for textiles and an amide derivative in polymer synthesis. HEAR contains 43% minimum erucic acid in the seed oil and is the most economical source of this long-chain fatty acid (Mastebroek et al., 1997). HEAR is grown by contract growers. Blackleg disease is caused by the pathogen *Leptosphaeria maculans* (Desmaz. Ces. & De Not., and is responsible for significant yield loss in oilseed rape and canola (*Brassica napus* L.) worldwide. Genetic resistance has been used effectively to control blackleg in western Canada. Specific resistance genes are an effective means of disease control when the pathogen population is mainly avirulent on the cultivated varieties carrying the corresponding resistance gene (Kutcher, H.R. et al., 2010). However, the resistance has been breaking down in recent years due to changes in the pathogen populations and new races.

**Objectives:** The objective of this study is to determine the race structure of *L. maculans* from stubble samples collected in HEAR fields in western Canada. The pathogen structure information could be used in the development of blackleg disease control strategies and breeding for resistant cultivars of canola and rapeseed varieties of HEAR in western Canada. This research will provide a current picture on the distribution of different races and potentially detect novel races.

**Methods:** Blackleg infected stubble was collected from the HEAR commercial fields in western Canada in 2014 and 2015 in Manitoba, Saskatchewan and Alberta. The pathogen was isolated from infected stubble onto V8 juice media. Single spore isolates were then cultured from pycnidia. Multiplex PCR was used to discriminate between *L. maculans* and *L. biglobosa*. PCR was performed on *L. maculans* isolates using primers obtained from *AvrLm1*, *AvrLm5*, *AvrLm6*, *AvrLm4-7* and *AvrLm11*. For *Avr* genes which have not been cloned were characterized using a differential set of *B. napus* genotypes. Inoculations were made using single-spore cultures, diluted to a 2 x 10⁷ CFU/ml and inoculated onto wounded cotyledons seven days after planting. Phenotypic evaluation was made 10 days after inoculation using a 0-9 scale (Sawatsky, W. M., 1989).

**Results:** Preliminary results indicate the frequency of avirulence alleles *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4*, *AvrLm5*, *AvrLm6*, *AvrLm7*, *AvrLm9*, *AvrLmLepR1*, *AvrLmLepR2* and *AvrLmLepR3* varied from 5 to 100% depending upon the geographic location. Four avirulence alleles were observed at a higher frequency, including *AvrLm2*, *AvrLm4*, *AvrLm6* and *AvrLm7* (77 to 100%). The corresponding R genes (*Rlm2*, *Rlm4*, *Rlm6* and *Rlm7*) could be candidates for use in canola/rapeseed cultivar development in western Canada.

**Conclusions:** This knowledge, coupled with the knowledge of the existing R genes present in current canola/rapeseed cultivars could be used to aid in cultivar development. This information could also help canola/rapeseed growers in the selection of the appropriate cultivars for different growing regions in western Canada. This updated race structure data for *L. maculans* in western Canada is helpful in the development of improved blackleg disease resistance strategies that might prevent or delay resistance breakdown. This research could improve the management of blackleg using cultural methods, by reinforcing the importance of crop rotation to producers and the industry.

**References:**

Variation for pod shatter resistance in an international germplasm collection of Brassica rapa

**Background:** Sclerotinia stem rot caused by Sclerotinia sclerotiorum is a major factor limiting the production potential of Brassica oilseeds. Synthetic chemical fungicides used for its management do not provide satisfactory control. At present, there is no reliable resistance source available in cultivated Brassica. Three sets of B. juncea introgression lines from already reported resistant wild, Erucastrum cardaminoide and E. abyssinicum (Garg et al. 2010) were used in these studies.

**Objectives:** Stabilizing and enhancing resistance responses of introgression lines to diverse S. sclerotiorum isolates.

**Methods:** About 7,000 plants from three introgression sets were screened against S. sclerotiorum isolate (Ludhiana), using stem inoculation technique described by Buchwaldt et al. (2005). These were narrowed down to 617 and then 94 progenies over three selection cycles. Introgression lines showing consistent reaction over three years formed the basic germplasm to initiate next phase of phenotypic selection to further improve average resistance responses. In the second phase, selected progenies were challenged by four diverse S. sclerotiorum isolates (Bharatpur1, Bharatpur2, Ludhiana and Bawal). These four isolates were separated in to different clades on the basis of morpho-cultural and molecular variation. Average lesion length and proportion of plants showing hypersensitive response were used as selection criteria.

**Results:** After three years of rigorous screening; 22, 42, 66, 71 and 36 progenies were selected based on their consistent resistance responses to all four isolates. Intensive selection led to a significant decline in mean lesion length. Against Bharatpur-1 isolate, the lesion length declined from 4.41 cm to 3.65 cm while, frequency of plants showing hypersensitive reaction (HR) remained almost constant. Similar trends were also observed against Bharatpur 2, Ludhiana and Bawal isolates. These sets showed decline in average lesion length from 5.38 to 2.69, 4.52 to 3.67 and 7.09 cm to 3.53 cm, respectively. Twenty two progenies were identified for resistance against all the four isolates. Of these, four progenies namely, ARL-42, A-825-1, JBR-31 and ADRL-7 exhibited consistent resistant reaction over the six years. These progenies showed lowest mean lesion length for all the four isolates. These progenies bred true for their resistant responses and also showed minimal within-progeny variation.

**Conclusion:** Six cycles of selection enhanced and stabilized resistance against S. sclerotiorum. Four progenies exhibited consistent resistant responses over six years of selection. All the selected progenies carry euploid chromosome number and bred true for resistance. Molecular characterization is underway.

**References:**


**Characterization of Sclerotinia sclerotiorum necrosis inducing proteins**

**Background:** Sclerotinia sclerotiorum causes stem rot in Brassica napus which leads to lodging and severe yield losses. The fungus releases acids, enzymes and toxins that destroy the host before it can mount a defense. As it consumes the necrotic tissue, the rot spreads leading to stem collapse. *S. sclerotiorum* secretes two necrosis and ethylene-inducing proteins (SSNEP1 and SSNEP2) that cause significant necrosis when infiltrated into plant tissues (Bashi et al. 2010). Necrosis is also caused by polygalacturonase enzymes (SSPG3 and SSPG6) which can be partially overcome by the expression of *B. napus* polygalacturonase inhibitor proteins (Bashi et al., 2013).

**Objectives:** The objectives of this study are: (i) to use transcriptomic and bioinformatic analyses to identify additional candidate necrosis-inducing effectors (NEs) from *S. sclerotiorum*, and (ii) to examine their contribution to disease using an in planta expression system and *S. sclerotiorum* gene knockouts. Ultimately, these NEs may be used to develop an effector-assisting breeding strategy to screen *B. napus* cultivars for stem rot resistance.

**Methods:** The genomic sequence of *S. sclerotiorum* isolate 1980 has been published. The proteins encoded by these genes were subjected to several bioinformatic filters to identify small, secreted proteins. Illumina RNA-seq analysis was conducted to obtain information about the expression of all *S. sclerotiorum* genes through the earliest (1 hours) to the latest (48 hours) stages of infection on *B. napus*. Proteins were considered to be candidate NEs if they passed the bioinformatic filters and if the expression of their corresponding genes was induced within 1-6 hours of inoculation as these proteins are most likely to impact host-pathogen interactions leading to pathogen establishment. The candidate NEs were tested for their ability to cause necrosis using an *Agrobacterium tumefaciens* transient infiltration system.

**Results:** Potential *S. sclerotiorum* NEs were identified using computational and RNA-seq analyses. The *S. sclerotiorum* genome encodes ca. 900 secreted proteins (i.e. possess a signal peptide), of which 100 were found to be small (less than 250 amino acids), cysteine-rich proteins that do not possess transmembrane domains, a GPI-anchoring site or a vacuolar sorting (NPIR) motif; these are signatures of secreted NE proteins. The expression of these candidate genes was determined from the RNA-seq information, yielding 26 that were induced to relatively high levels during the early phases of the infection. These were subjected to in planta expression and several new *S. sclerotiorum* NEs were identified.

**Conclusions:** *S. sclerotiorum* secretes a substantial repertoire of proteins that can induce necrosis in host tissue and contribute to disease progression and severity in *B. napus*. These may serve as targets for developing resistance to stem rot disease.

**References:**
Genetic diversity among isolates of *Albugo candida* in India using newly identified SSR markers

**Background:** White rust caused by an obligate oomycete *Albugo candida* affects both the vegetative and reproductive stages of the crop. Variability among the *A. candida* isolates has not been studied adequately in India. Knowledge of population structure of a plant pathogen at genetic level is expected to provide greater insights for understanding the relation between disease severity and prevalence of particular genetic group(s) of fungus. Simple Sequence Repeat (SSR) markers are widely accepted for studying genetic variability within a fungal species due to high reproducibility, multi-allelic nature, relative abundance, wide range of distribution and high mutational rate (Schlotterer 2004).

**Objectives:** Development and validation of new SSR markers for *A. candida* and their use for establishing genetic variability among *A. candida* isolates in India.

**Methods:** The availability of *A. candida* whole genome sequence (Links et al. 2011) provided a chance to identify SSRs in silico. *A. candida* genome is represented by 252 scaffolds covering 34.5Mb of the genome. This genome sequence was used for in silico identification of SSR motifs using MISA perl script. White rust infected leaf samples of *B. juncea* were collected from mustard growing areas of north-west India. A set of twenty new designed SSR markers were used to establish genetic diversity among eleven *A. candida* isolates.

**Results:** In total 145 SSRs were identified from the genome sequence of *Albugo candida*. Twenty primer pairs were synthesized. These markers proved to be pathogen specific as there was no amplification when DNA from uninfected mustard leaves was used as template. Eighteen polymorphic markers amplified a total of 54 alleles with an average of 3 alleles per locus. Primer pair for Acssr1 showed the maximum PIC value (0.769). Eleven isolates could be resolved into four clusters. The first cluster comprised of isolates Acms, AcFr and Acbt. The second cluster included four isolates (AcJs, AcMk, AcKg and AcDh), the fourth cluster consisted of three isolates (AcRp, AcLg and AcLd2b) while third cluster included only one isolate from foot hills of Himalaya (Achr).

**Conclusion:** Genome sequence is needed to design molecular markers such as ITS, COS2 and LSU, and could be applied to establish the interspecies differences rather intraspecies variations. Newly designed SSR markers were effective in revealing the genetic diversity among the different isolates of *A. candida* and could help unravel the high degree of genetic diversity among the *Albugo* species complexes.

**References:**
Identification of pathogenicity of *Plasmodiophora brassicae* from three different regions in Sichuan, China

**Background:** Clubroot disease in *Brassicaceae* is caused by a soil-borne pathogen, *Plasmodiophora brassicae*. Different physiological races or pathotypes in *P. brassicae* have been identified in China, among which the race No.4 was considered the prevailing race in the Sichuan plain area (Shen et al. 2009; Ding et al. 2013; Ji et al. 2013). Pathogenicity of different races may be largely different to host plants. Therefore, identification of physiological races or pathogenicity of pathogens from different rapeseed (*Brassica napus* L.) regions is an important fundamental task for control of clubroot in rapeseed production.

**Objectives:** To mainly observe the difference in pathogenicity of *P. brassicae* from three different severely infested areas in Sichuan Province, China.

**Materials and methods:** In present study 20 rapeseed materials were used, including 19 inbred lines and 1 hybrid variety. “Deyou 6”, the present control variety of regionalized tests of rapeseed in Sichuan Province. Clubroot tumors and infested soils were collected from three different regions in Sichuan Province, including Guanghan (plain area), Dayi (plain area) and Kangding (western high-mountain area), for the tests. Two methods were used to identify the difference in pathogenicities of the pathogens: 1) artificial inoculation with spores isolated from the root tumors, and 2) direct test with infested soils. Artificial inoculation was conducted by injection of 1ml spore suspension with a density of 1 x 10^7 spores / ml into soil near the seedlings at 2-leaf stage. Tests were made in 4 replications. The disease occurrence was observed with 20 plants for each line 50 days after inoculation or seeding. Disease incidence (DI) and severity index (SI) were calculated based on the disease observations.

**Results:** In the artificial inoculation test, average DI's were 63.36%, 32.83%, 4.29%, respectively; average SI's were 39.1, 1.71, 2.1, respectively, for the 3 different source of pathotypes. In the soil test, average DI's were 64.49%, 29.08%, 12.73%, respectively; average SI's were 46.96, 18.81, 8.97, respectively. It was obvious that the Guanghan pathotype showed the strongest pathogenicity and the Kangding pathotype showed the weakest pathogenicity. Results of the two methods were consistent.

**Conclusions:** The pathogenicities of *P. brassicae* from the three different regions in Sichuan Province were different. The orders of occurrence were Guanghan pathotype > Dayi pathotype > Kangding pathotype.

**References:**
Report on a new insect pest on oilseed rape in Tibet, China

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Background: Oilseed rape is a very important oil crop in China. It can be damaged by many insect pest species and many yields were lost in every year. More and more new insect pest species begin to damage oilseed rape because of the climate and crop cultivation system change now. Investigate and study new insect pest species that damage on oilseed rape are very important and significant to control them.

Objectives: A new insect pest on oilseed rape was found in Tibet, China, in 2014. Its bio-characteristics, occurrences rules, harmful characteristics and what species it is should be studied by systemic investigation and filed census. Then we can make a safe and high-efficiency standard procedure to control them.

Methods: The new insect pest bio-characteristics were observed by stereomicroscope, occurrences rules and harmful characteristics were investigated by filed systemic census. According to the bio-characteristics, occurrences rules and harmful characteristics compare them with literature to identify what species it is. Using agricultural, physical, biological and chemical methods to integrate control them.

Results: The insect pest adults appear in spring, first appear in April later to early May. They damage oilseed rape seedlings from cotyledon to three leaves stage. The adults eat leaves. Some adults also can be found under ground near to the roots of oilseed rape. There are more than 30 adults per square meter in filed when we investigated in June 17, 2014.

The size of adults are 6.1~7.9mm long, the pronotums are 1.7~2.2mm wide and the backs are 2.3~3.0mm wide. They have two geiculate antennae, each antenna has 9 segments. The scapes are 0.7~0.9mm long and the flagellums are 1.0~1.4mm long. The ends of antennae are a little more swollen. All adults are black color, prognathous mouthparts. They have some microgrooves on its two elytrons, but they can not fly.

Conclusions: The new insect pest belongs to *Leptomias* sp., *Culculionidae*, Coleoptera. It has not been identified and confirmed what species it is yet.

References:
Deciphering the role of cyclophilin A (Cyp4) in blackleg causing fungi \textit{Leptosphaeria maculans} and \textit{L. biglobosa} on oilseed rape

**Background:** Oilseed rape is challenged by a number of fungal pathogens. One of the most economical important diseases is phoma stem canker (blackleg) that cause serious loses globally. The disease is caused by the fungal species complex \textit{Leptosphaeria maculans} and \textit{L. biglobosa}. However, several studies have shown that \textit{L. maculans} is much more aggressive on oilseed rape (Shoemaker and Brun, 2001). This phenomenon can only in small parts be explained by its attribute to produce the host-unspecific toxins sirodesmins (Sock and Hoppe, 1999), which are not produced by \textit{L. biglobosa}. However, it is still unclear which main factors underlie these huge differences of aggressiveness. In spite of the fact that cyclophilins are highly conserved family throughout genera, however the gene is not well studied in \textit{L. maculans} and \textit{L. biglobosa} (Singh et al., 2014). Similarly, it has been shown that cyclophilins may contribute to the virulence of certain fungal pathogens (Viaud et al., 2002).

**Objectives:** To perform comparative analysis of cyclophilin gene family in \textit{L. maculans} and \textit{L. biglobosa}. Functional characterization and delineating the precise role of cyclophilin A (Cyp4) in virulence.

**Methods:** Genome-wide identification of cyclophilin gene family, Culturing of various fungal isolates, Pigmentation and sirodesmin analyses, Host pathogen interactions, RNA isolation and cDNA synthesis, qRT-PCR and cloning of Cyp4.

**Results:** Through comprehensive whole genome analyses of \textit{L. maculans} and \textit{L. biglobosa}, we identified seventeen and fifteen genes respectively encoding cyclophilin in these two fungi. Further to gain more insight, in silico analyses of sequences followed by cloning of cyclophilin A (Cyp4) in various isolates showed the compelling differences between two species at sequence level as well. In addition, expression levels of the Cyp4 in the mycelium found to be relatively high in \textit{L. maculans} as compared to \textit{L. biglobosa}. However, the expression analyses not only demonstrated a significant difference among species but also significant intraspecific variation. Furthermore, pigmentation and sirodesmin analyses distinguished \textit{L. maculans} and \textit{L. biglobosa} isolates. Disease assessment studies of \textit{L. maculans} and \textit{L. biglobosa} isolate on various oilseed cultivars also exhibited different level of pathogenicity.

**Conclusions:** Taken together our finding for the first time shed light onto the significant differences between the two species at sequence level. In addition ongoing ad planta studies may further support our hypothesis that cyclophilins and their expression may explain the difference in virulence on oilseed rape against \textit{L. maculans} and \textit{L. biglobosa}.

**References:**


Shotgun label-free proteomic analysis of clubroot resistance mediated by the resistance gene *Rcr1* from *Brassica rapa* ssp. *chinensis*

**Background:** Clubroot disease, caused by the soil-borne phytopathogen *Plasmodiophora brassicae* Woronin, is one of the most serious diseases on *Brassica* crop species worldwide. For decades, host resistance has been the major tool in management of clubroot. Although several resistance genes were characterized and employed in commercial cultivars, mechanisms of clubroot resistance, especially the molecular mode of action, remains sketchy.

**Objectives:** The major purpose of this work was to profile and compare the proteomes of canola plants with and without the clubroot resistance (CR) gene *Rcr1*. The functional annotation of differentially accumulated proteins (DAPs) identified by the proteome comparison was analyzed to reveal their biological context. The biological pathways associated with DAPs were highlighted and subjected to biochemical and genetic studies.

**Methods:** A segregating BC1 *B. rapa* canola population was inoculated with *P. brassicae* and genotyped using flanking markers for the presence/absence of *Rcr1* gene. The total proteins were extracted from root tissues at 2 weeks after pathogen inoculation and analyzed using UHPLC-MS/MS. The spectra collected from the MS were processed through the global proteome machine (GPM) software using the X!Tandem algorithm. The abundance of each identified protein was calculated as normalized spectral abundance factor (NSFA), which was then subjected to a t test to determine DAPs. The identified DAPs were annotated using Blast2GO based on gene ontology terms and their expression patterns were analyzed using the Mapman software.

**Results:** A total of 527 DAPs were identified in samples carrying *Rcr1*, relative to those without this CR gene. Functional analysis of DAPs identified a potential signaling pathway associated to the resistance by *Rcr1* that was distinct from other commonly reported modes of recognition receptors for fungal and bacterial pathogens. This novel signaling pathway appeared to act in a calcium-independent way through an unknown cascade of mitogen-activated protein kinases (MAPK) and would require the ubiquitin-26S proteosome, which was previously demonstrated to function in abiotic stresses, especially the cold stress. Furthermore, our study also identified a range of biological processes that were differentially regulated in resistant plants. The putative pathogen-induced defense responses included a potential ROS accumulation, sulfur-containing glucosinolate breakdown and lignin biosynthesis. A variety of host metabolites typically induced by *P. brassicae* in compatible interaction, including glycolysis and arginine catabolism, were significantly down-regulated in resistant plants carrying *Rcr1*.

**Conclusion:** The proteomic study showed that clubroot resistance mediated by *Rcr1* would consist of induced pathogen-recognition and signaling pathways in defense responses, as well as suppression of pathogen-induced re-programming of host metabolism favoring clubroot development. The results provided important insights into the mechanisms of clubroot resistance, and offered candidate targets for further biochemical and genetic studies to verify the action mode for *Rcr1* and other CR genes.

**Reference:**


Characterization of differentially regulated host metabolism conferred by the clubroot resistance gene \textit{Rcr1} using metabolome profiling and targeted metabolite analysis

\textbf{Background:} Clubroot disease, caused by the soil-borne phytopathogen \textit{Plasmodiophora brassicae} is one of the most devastating diseases on \textit{Brassica} crop species worldwide. Host resistance has proven to be the most effective and economical approach for clubroot disease control. Better understanding of clubroot resistance (CR) mechanisms, especially relating to specific CR genes, will facilitate selection and use of these genes in breeding for reliable and durable resistance. The CR gene \textit{Rcr1} was previously identified from \textit{Brassica rapa} ssp. chinensis, and initial transcriptomic and proteomic analyses indicated potential changes in metabolism in plants carrying \textit{Rcr1} gene.

\textbf{Objectives:} The major purpose of this work was to identify and characterize the metabolic changes in a segregating \textit{B. rapa} canola population carrying \textit{Rcr1} gene induced by \textit{P. brassicae}. A focus was placed on the metabolism highlighted by in transcriptomic and proteomic analyses.

\textbf{Methods:} Root samples were collected at 15 days post inoculation, when the secondary infection by \textit{P. brassicae} just started but the clubbing symptoms were not yet visible. The presence of \textit{Rcr1} was verified with flanking markers to separate plants into resistant and susceptible groups. After pulverization in liquid nitrogen, samples were extracted and analyzed by liquid chromatography mass spectrometry (LC/MS) using an LTQ-Orbitrap Classic mass spectrometer. The metabolites were characterized based on search against an in-house built library for canola and quantified in comparison to the control (without \textit{Rcr1}). In addition, synchrotron-based Fourier infrared spectroscopy was employed to profile the metabolites in the same set of sample.

\textbf{Results:} The global profiling of metabolites identified 83 differentially accumulated metabolites in resistant samples relative to susceptible samples. Caulilexin C, a phytoalexin derived from indole-containing compound metabolism, was significantly induced in resistant samples, along with several other indole-containing metabolites which appeared to be related to differentially regulated auxin metabolism. The defense-related phytohormone jasmonic acid, as well as several additional metabolites involved in jasmonic-acid signaling pathways, was also highly induced in pathogen-infected resistant samples. Furthermore, spectroscopic analysis revealed potential changes in plant cell-wall compound organization, particularly the lignin composition.

\textbf{Conclusion:} Metabolomic analysis identified putative contributions of several metabolic processes to clubroot resistance mediated by \textit{Rcr1}. The induction of jasmonic acid as well as the intermediate molecules involved in signaling pathways indicated its pivotal role in inducing further downstream defense responses, one of which is the high accumulation of phytoalexins derived from the metabolism of indole-containing compound. The altered metabolism in the plant cell wall indicates a potential role of cell reinforcement against the infection into cortical tissues.

\textbf{Reference:}


Swede midge distribution across prairie Canada

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Background: Swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae), was first identified in North America from southern Ontario cole crops in the year 2000 (Hallett and Heal 2000). The pest and its impact spread quickly from this region to adjoining jurisdictions. Economic injury to spring oilseed rape (canola) in northern Ontario has become so severe that the current recommendation is to cease canola production in the area for 3 years. “Jumping larvae” were collected from canola in Saskatchewan in 2003, with the first swede midge identification from the Canadian prairies in 2007 in northeastern Saskatchewan. However, swede midge injury to canola crops on the Prairies was not reported until 2012, with no reports of injury to cole crops in the region to date.

Objectives: Because the pest poses a major risk to the 7 million ha canola industry on the Canadian prairies, we wished to determine the current distribution and population growth of swede midge in the region to assess the potential for economic injury here.

Methods: To determine swede midge distribution, surveys of canola fields for midge injury were conducted in 2012-14 in Saskatchewan, and swede midge pheromone traps were placed in or near canola and cole crop fields across the Canadian Prairies in 2013-2014. Emergence cages were placed over canola stubble at sites in northeastern Saskatchewan in early spring 2014 and removed in August, and at new locations in the same fields at the end of July and removed in September. Presence and number of swede midge males were determined.

Results: Swede midge adults were found on pheromone traps across Saskatchewan and several locations in Manitoba in 2014, greatly extending the known range of the insect. However, at a maximum of eight males per pheromone trap per week, numbers were much lower than typically found in eastern Canada. Visual symptoms of damage to canola occurred later in 2014 than in 2013, perhaps a reflection of the very cold and wet spring in 2014. First emergence of adults from the soil in 2014 was in early July, about 4-6 weeks later than seen in eastern Canada. Two to three generations may occur on the Prairies as opposed to the more typical four in eastern Canada.

Conclusions: The rapid expansion of swede midge across Saskatchewan and Manitoba supports the premise that it could have a serious economic impact on canola production in the region. The low numbers found may be an indication that the midge is early in its establishment phase as an invasive species, or may reflect its lower reproductive potential here than in eastern Canada, or may result from a combination of both these factors.

References:

Understanding effector-triggered defence responses against the phoma stem canker pathogen *Leptosphaeria maculans*

**Background:** Effector-triggered defence responses of oilseed rape against the phoma stem canker pathogen *Leptosphaeria maculans* are not well understood although the importance of salicylic acid and ethylene signalling has recently been suggested. Many aspects remain to be clarified, including variation in host defence responses against *L. maculans* with different effectors or effector combinations. Furthermore, the expression of receptor-like proteins (RLPs) and their encoding genes has to be solved to better understand effector-triggered defence responses against this apoplastic fungal pathogen (Stotz et al., 2014).

**Objectives:** To better understand effector-triggered defence responses by challenging susceptible and resistant oilseed rape genotypes containing different resistance (R) genes with *L. maculans* isolates that differ in effector gene composition. To clarify the contribution of RLPs to race-specific resistance by studying expression of LepR3 at mRNA and protein levels.

**Methods:** *L. maculans* isolates used differ in expression of AvrLm1 and AvrLm4. Susceptible and resistant oilseed rape cultivars with corresponding R genes were tested. Effector-triggered defence responses were analysed using quantitative RT-PCR, diaminobenzidine and nitroblue tetrazolium staining to monitor hydrogen peroxide and superoxide production, respectively. The expression of LepR3 was studied using qRT-PCR and immunoblotting with an antibody generated against the encoded RLP.

**Results:** The strength of the host defence response was a function of the *L. maculans* effector combination. The expression of LepR3 mRNA was low and constitutive in a susceptible cultivar. Bands of ~80 and ~140 kDa were recognised by the antibody against the LepR3 protein. The expression of this RLP appeared to decrease in response to infection by an *L. maculans* isolate with the corresponding effector AvrLm1.

**Conclusions:** Effectors appear to differ in their ability to suppress host defence responses. The LepR3 antibody offers a new tool to better understand the fate of RLPs during host defence responses against *L. maculans*. Subcellular localization of the LepR3 protein during the defence responses will be needed to further characterise the process of effector-triggered defence.

**References:**

Control of cabbage stem weevil in winter and spring oilseed rape

**Background:** In Lithuania, like in many other countries, rape has become one of the most promising crops recently. In the intensive cropping systems, one of the most important factors limiting rape productivity is insect pest damage. In Lithuania, little is known about the harmfulness of stem pests in rape. The area of rape has rapidly increased recently, which is expected to result in the invasion from cabbage stem weevil (*Ceutorhynchus pallidactylus*) in rape fields.

**Objectives:** The study was aimed to estimate *C. pallidactylus* infestation level in rape and to determine the optimal application timing of insecticides with a different mode of action.

**Methods:** Experiments were conducted in the winter and spring rape crops in the Institute of Agriculture, LRCAF, in 2011–2013. Contact insecticide lambda–cyhalothrin 0.15 l ha⁻¹ and systemic insecticide thiacloprid 0.3 l ha⁻¹ were used in the experiments. *C. pallidactylus* control included the following treatments: unsprayed; cover spray against pollen beetles and pod pests with deltametrin 0.15 l ha⁻¹; contact and systemic insecticides were applied when plants were <10 cm, 10–20 cm, 20–30 cm and insecticides were applied twice (< 10 cm and 20–30 cm). Thirty samples of rape stems per plot were taken to be examined for pest damage.

**Results:** The main flight of *C. pallidactylus* started at the end of stem elongation in winter rape and at plant emergence in spring rape. *C. pallidactylus* infestation in spring rape was 2.2-4.3 times less compared to winter rape. At all application times, contact and systemic insecticides effectively reduced the number of pest-injured stems in winter rape. Compared with the control and cover spray plots, a significant yield increase and the highest benefit was obtained in winter rape treatment applied with systemic insecticide at the beginning of active migration of adults, when stems were 10–20 cm high (GS 39). In spring rape, both insecticides at all application times significantly reduced the number of *C. pallidactylus* larvae-injured stems compared with control plots, and in some cases compared with cover spray plots. However, seed yield increased when systemic insecticide had been applied when spring rape was 20–30 cm high (GS 53). It is likely that systemic insecticides applied against *C. pallidactylus* at the beginning of spring rape inflorescence emergence provided control of pollen beetle (*Meligethes aeneus*) too.

**Conclusions:** At all application times, contact and systemic insecticides effectively reduced the number of *C. pallidactylus*-injured stems in winter and spring rape. A significant yield increase of winter rape was obtained only when systemic insecticide had been applied at the beginning of active migration of adults at 10–20 cm plant height. Spring rape productivity increased when systemic insecticide had been applied at 20–30 cm plant height and both insecticides had been applied twice.

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Monitoring of plant and airborne inoculum of *Sclerotinia sclerotiorum* in spring oilseed rape using real-time PCR

**Background:** Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is a major disease of spring oilseed rape in Sweden. The pathogen survives in the soil for long periods as sclerotia. Ascospores are produced in moist conditions by carpogenic germination and constitute the major source of inoculum for infection by *S. sclerotiorum*. Infection is suggested to mainly occur when ascospore-infected petals fall and stick to the leaves, allowing the pathogen to penetrate the petiole and infect the stem. The impact of Sclerotinia stem rot is dependent on weather conditions and the timing of ascospore release, the disease causes severe damage which varies from year to year in spring oilseed rape in central Sweden, and thus oilseed rape fields are presently subject to large-scale chemical treatment. The disease forecasting service available to Swedish farmers is a regional risk assessment based on local climate and field information and is like other forecasting schemes not a satisfactory tool for individual fields.

**Objectives:** Our objectives were to develop and validate a *S. sclerotiorum* specific real-time PCR assay that has the potential to be used as a tool in a field specific disease risk assessment and to increase our knowledge about the infection process by determining the presence of naturally occurring inoculums (ascospores) of *S. sclerotiorum* (i) on oilseed rape petals, (ii) on leaves of the plant and (iii) in air using collection tapes from a Burkhard spore sampler.

**Methods:** A real-time PCR assay was developed and used to determine the incidence of *S. sclerotiorum* DNA on petals and leaves of spring oilseed rape as well as in air samples. Five field experiments were conducted from 2008 to 2010 to detect and study pathogen development. The presence of Sclerotinia DNA on petals and leaves at different leaf levels of the plant of two different cultivars was determined regularly during the flowering period. Air samples were collected using a Burkad 7-day continuously recording spore sampler starting in late May 2010.

**Results:** The real-time PCR assay proved fast and sensitive and the relationship between percentage of infected petals determined using a conventional agar test and the PCR assay was linear (R²>0.76). There were significant differences in *S. sclerotiorum* incidence on petals at different stages of the flowering period. The incidence of *S. sclerotiorum* DNA on the leaves varied (0-100%), with significantly higher incidence on leaves at lower leaf levels of the plant. In one field experiment, *S. sclerotiorum* DNA was not detected on petals during flowering, whereas the pathogen was detected on the leaves, with a corresponding stem rot incidence of 7%. The amount of *S. sclerotiorum* DNA in air sampled using the spore trap revealed that the spore release did not coincide with flowering on that experimental site.

**Conclusions:** Using a real-time PCR assay to assess the incidence of *S. sclerotiorum* on oilseed rape leaves could potentially improve disease risk assessment in a disease support system based on predictive tests, field data and local climate.

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Transferring blackleg resistance from *Brassica carinata* to canola using synthetic hexaploid species (AABBCC)

**Background:** Blackleg caused by *Leptosphaeria maculans* is one of the most serious diseases in canola production particularly in Australia, Canada, and Europe (Rimmer 2006). A high level of blackleg resistance shows in *B. carinata* (BBCC) and synthetic new *Brassica* species ‘Meng’ with three genomes (AABBCC) from the crosses of *B. rapa* and *B. carinata*.

**Objectives:** Genetic resistance to *L. maculans* is considered to be the preferred approach for controlling this disease. However, the resistance determined by a single race-specific gene is not durable and has been reported to be broken down. Transferring the resistance from the B genome-containing *B. carinata* and ‘Meng’ appears to become a feasible approach to achieve a complete blackleg resistance in canola.

**Methods:** The resistance to blackleg from *B. carinata* (BBCC) ‘T4001’ and ‘Meng’ (AABBCC) is transferred into the susceptible *B. napus* (AAcc) ‘Westar’ through interspecific hybridization followed by backcrossing to ‘Westar’ twice or more times and selfing to produce pure lines. Blackleg isolates were used to select the resistant individuals in each generation through cotyledon inoculation. The blackleg resistance introgressed from *B. carinata* and ‘Meng’ into canola will be tested with various blackleg isolates belonged to PG2, PG3, and PG4. Embryo rescue tissue culture was used to obtain F1 plants of the crosses of ‘Meng’ and ‘Westar’.

**Results:** When the F1 plants were backcrossed to ‘Westar’, the seed setting of backcrosses between ‘Meng’ and ‘Westar’ is much better than using *B. carinata* to cross with ‘Westar’ directly, which makes it much easier to transfer blackleg resistance from ‘Meng’ to canola. Currently, we observed at least two kinds of resistance interactions in the BC1S1 of ‘Meng’ and BC2S1 of *B. carinata* according to the inoculation results.

**Conclusions:** In order to further control blackleg disease, transferring new resistances from the B-genome *Brassica* species into canola has been attempted for a long time. It is not easy to obtain the complete blackleg resistance as that showed in *B. carinata* due to low fertility, poor seed set, and unstable introgression, even though the advanced breeding methods have been implemented. However, our results showed a strong possibility to transfer the high level of blackleg resistance from ‘Meng’ into canola.

**References:**

Analysis of the host hormonal signaling involved in *Brassica napus* - *Leptosphaeria maculans* Pathosystem

**Background:** Canola (*Brassica napus cv.*) is the number one oilseed crop in Canada. Blackleg, caused by *Leptosphaeria maculans*, is the most economically important disease of canola. Evidence from previous studies has shown that plants are able to adjust their hormonal signals to combat different types of plant pathogens. Because this response is highly dependent upon the disease causing pathogen and the nature of infection within the host, it is crucial to study the hormonal signaling profile exhibited by canola when encountering *L. maculans*. Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are well known hormones involved in plant defense. The expression of genes such as WRKY70, NPR1, PR4 and BON1, have been shown to be linked to hormone signaling.

**Objectives:** To understand the regulation of the downstream signaling under each hormone and to determine which hormone(s) may play an important role in canola when combating blackleg, the expression of genes (e.g. WRKY70, NPR1, PR4 and BON1) controlled by SA, JA and ET signaling were monitored using RT-qPCR.

**Methods:** The *L. maculans* isolate HCRT75 8-1 (AvrLM2, AvrLM4-7, AvrLM6 and AvrLM5) was used in this study, as it displayed different levels of disease severities (susceptible, intermediate and resistant reactions based on lesion size on cotyledons) when inoculated onto different canola cultivars. The *L. maculans* isolate was inoculated on the cotyledons of Westar (no Rlm gene), Surpass400 (RlmS and LepR3) and 01.23.2.1 (Rlm7), and the RNA was extracted from the cotyledons at three time points (3, 7 and 10 days post inoculation). To analyze the expression levels of the marker genes under SA, JA and ET signaling, RT-qPCR was carried out on cDNA synthesized from the extracted RNA.

**Results:** Among the analyzed genes, some of the transcription factors controlling the defense response (such as predicted BON1) seemed to be very important for rapid and effective resistance. The expression of BON1 on 01.23.2.1 was down-regulated at 7 dpi while in Westar it was up-regulated. The onset of the expression of some genes was more important than the expression levels. For instance, the early (3 dpi) and late (10 dpi) induction of PR4 distinguished between the resistant (01.23.2.1 and Surpass400) and susceptible (Westar) cultivar. In Surpass400, however, the BON1 was induced at 7 dpi and illustrated early induction of PR4. The data from WRKY70 and NPR1 suggested that early (3 dpi) induction of SA signaling is crucial for resistance because Surpass400 and 01.23.2.1 had higher expression of these two genes than Westar at 3 dpi.

**Conclusions:** Results confirm that the extent of plant defense in canola against blackleg is dependent on factors involving the hormone signaling in planta. It appears that the expression levels and/or onset of these factors determined the levels of resistance.
Effect of water flooding on survival of *Leptosphaeria biglobosa* ‘brassicae’ in stubble of oilseed rape (*Brassica napus*) in central China

**Background:** Blackleg (phoma stem canker) caused by *Leptosphaeria maculans*/*L. biglobosa* is an economically important disease on oilseed rape and many cruciferous vegetables (Fitt et al., 2008). Oilseed rape–rice rotation is a routine cultivation practice in central China.

**Objectives:** This study was conducted to assess the effect of flooding on survival of *Leptosphaeria biglobosa* ‘brassicae’ (Lbb) in the stubble of winter oilseed rape (*Brassica napus*).

**Methods:** Basal stems with typical blackleg symptoms were collected and cut into small (2 cm) pieces that were either submerged in water at 16/20, 20/28, 28/33 and 33/40°C (12 h/12 h), respectively, or kept dry at room temperature (control). Moreover, in a field experiment, the stem pieces were placed on the soil surface in a rice field or in a cotton field and either flooded in water or not flooded, respectively. After 1, 2, 4, 6 and 8 weeks, the stem pieces were sampled for retrieval of Lbb on V8-juice agar and for determination of dry weight (Peloula et al., 2013). Selected Lbb isolates from the stem pieces were identified by PCR (Cai et al., 2014).

**Results:** Results from the two experiments showed that compared to the controls, flooding for 1 to 2 weeks substantially reduced recovery of Lbb and flooding for 4 weeks resulted in negligible recovery of Lbb. All the 99 selected isolates produced a 444-bp DNA fragment in the PCR, confirming that they belong to Lbb. Results also indicated that flooding caused rapid decomposition of the stem pieces. After flooding for 8 weeks, the dry weight of the stem pieces was reduced by 28 to 42% in the laboratory experiment and by 26 to 36% in the field experiment.

**Conclusions:** Oilseed rape–rice rotation is probably an efficient way to reduce longevity of Lbb in stubble of winter oilseed rape in central China.

**References:**


Transcriptomic responses of *Brassica napus* L. to *Leptosphaeria maculans* infection as revealed by RNA sequencing

**Background:** *Brassica napus* L. (canola) is one of the most important oilseed crops grown worldwide. Blackleg, caused by the fungal pathogen *Leptosphaeria maculans*, is one of the major constrains for canola production in North America, Australia, Europe and many other regions around the world (Fitt et al., 2006). The disease is mainly controlled by utilization of resistant cultivars, crop rotation and fungicide applications. There is a typical gene for gene interaction between *B. napus* and *L. maculans* at seedling stage. However, *L. maculans* populations can rapidly adapt to selection pressures imposed by single resistance genes and cause resistance breakdown. It’s very important to understand host defense mechanisms, especially at the transcriptomic level in the *B. napus*-*L. maculans* pathosystem to achieve a better control of blackleg. RNA sequencing (RNA-Seq) is an ideal strategy to study transcriptomic responses of *B. napus* to *L. maculans* infection.

**Objectives:** This study aims to analyse the defense transcriptome profile of *B. napus* in response to *L. maculans* infection using RNA sequencing approach.

**Methods:** A susceptible canola variety Westar and a canola line DF78 displaying resistance to a *L. maculans* isolate D3 were used for the study. Infected and mock inoculated canola cotyledons were sampled at 3 days post inoculation (dpi), 7 dpi and 11 dpi. Total RNA were extracted from plant samples and RNA sequencing libraries were prepared as described by Kumar et al. (2012) with some modifications. Sequencing was performed on Illumina HiSeq2500 platform. RNA-seq reads were mapped to *B. napus* reference genome. Differentially expressed genes (DEGs) in response to *L. maculans* infection were identified. Functions of DEGs were characterized by homology to *A. thaliana*, with special focus on genes that are reported to be involved in *B. napus*-*L. maculans* pathosystem or plant defense mechanisms in general. Real-time quantitative PCR (qPCR) was performed to validate expression of a few plant defense genes.

**Results:** A total of 36 samples were sequenced, with an average of 13.3 M reads per sample. After *L. maculans* infection, number of genes that were upregulated in infected tissues increased over time both in resistant ( incompatible interaction) and susceptible ( compatible interaction) plants. Gene pattern analysis showed a set of transcripts that were highly expressed only in fungal infected plant samples. A few pathways and a list of candidate transcripts that might have played major roles in defense against *L. maculans* were identified. Compatible and incompatible interactions in this pathosystem showed both common and different defense mechanisms.

**Conclusions:** RNA-Seq is a promising strategy to discover host transcriptome responses in *B. napus* – *L. maculans* pathosystem. Defense pathways and transcripts that were involved in this pathosystem in both compatible and incompatible interactions were identified in this study. Results of this study expanded our current understanding of *B. napus* defense responses to *L. maculans* infection.

**References:**

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Development of clubroot resistant hybrids in omega-9 canola

Clubroot disease, caused by *Plasmodiophora brassicae*, is a widespread disease that causes serious problems in many *Brassica* growing areas. Symptoms of the disease include formation of galls on the roots of susceptible plants which results in stunting, wilting and even plant death. Clubroot was first discovered in western Canada in 2003 in Alberta, and since then the cases of clubroot infection in the field have been reported increasing and this has become a significant concern to the canola industry in Canada. Due to the fact that resting spores of *P. brassicae* can survive in soil for up to 20 years and no chemical control of the clubroot is currently available, the development of resistant cultivars and cultivation of resistant hybrids is the most efficient way to control this disease. However, resistant sources to clubroot have only been described in European winter canola (*Brassica napus*) and rutabaga as well as in other *Brassica* species. So far up to eight major genes and a few QTLs contributing to clubroot resistance have been reported, but none of these are directly applicable in marker assisted breeding due to various marker platforms used to discover the genes. Omega-9 canola is Dow AgroSciences’ proprietary Nexera varieties with high oleic and low linolenic fatty acid composition. Here we present the development of a molecular marker associated with clubroot resistance in *B. napus*, and the development of the first Omega-9 canola hybrids with clubroot resistance.
Effect of processing conditions on the chemical composition and nutritive value of canola meal for broiler chickens and pigs

**Background:** Studies have shown that the nutritive value of canola meal (CM) can be enhanced or diminished by the processing conditions used in the canola processing plants. Excessive heating during pre-press solvent extraction may result in reduced digestibility of amino acids (AA), particularly lysine.

**Objective:** To evaluate the effect of processing conditions on the chemical composition and standardized ileal digestible AA contents of CM for broiler chickens and growing pigs.

**Methods:** Three surveys involving 11 Canadian crushing plants were conducted to determine the effect of processing and meal pelleting on the chemical composition of CM. As expected, some variations were observed in the contents of lys (2.0-2.29 %DM), glucosinolates (2.0-10.1µmol/gDM), and dietary fiber (33.3-41.9 %DM), the components known to be affected by heat treatment. Based on their levels, 6 representative samples were selected and used in an AA digestibility study involving 240 1-d old broiler chicks and 18 ileal cannulated barrows (BW=23.3 kg). Chicks were housed in cages (7 per cage) and were randomly assigned to 8 semipurified diets (including 2 additional diets containing pelleted CM from 2 plants) formulated to contain 22% CP. Semipurified diets for the pigs were also formulated to contain 18 % CP using the same set of CM samples. A casein-corn starch diet was included in the pig study to determine ileal endogenous AA losses. Pigs were housed individually in pens and were fed the 9 diets in a completely randomized design for 3 periods. All data were subjected to ANOVA using the GLM procedure of SAS.

**Results:** Standardised ileal digestibility values for Arg, Lys, Met, and Thr averaged 88.0, 79.9, 89.7, and 75.2 %, respectively in the broiler study and 87.5, 78.8, 85.4 and 74.8 %, respectively in the pig study. There were variations (P<0.05) between plants in the standardized ileal digestible content of all AA in both chickens and pigs. In the broiler study, the standardized ileal digestible contents of Arg, Lys, Met, and Thr averaged 2.24, 1.80, 0.55, and 1.09 %, respectively, and ranged from 2.18 to 2.50 % for Arg, 1.74 to 2.00 % for Lys, 0.49 to 0.65 % for Met, and 1.00 to 1.38 % for Thr while in the pig study, they averaged 2.22, 1.78, 0.52 and 1.07 %, respectively and ranged from 2.00 to 2.44 % for Arg, 1.61 to 1.96 for Lys, 0.45 to 0.63 for Met, and 0.94 to 1.34 for Thr. Pelleting reduced (P<0.05) the standardized ileal digestible content of all AA in chickens and all AA except Pro in pigs with crushing plant x pelleting interaction for all AA.

**Conclusions:** The high dietary fiber and the corresponding low glucosinolates observed in some crushing plants could have been caused by CM overheating. There was relationship between the chemical composition of CM and their nutritive values for broiler chickens and pigs.
Dual purpose canola: Increasing the value of winter canola by harvesting forage and seed

**Background:** Dryland agriculture in the Pacific Northwest (PNW) of the USA is dominated by small grain cereals. Low precipitation falls mainly in winter and early spring, as a result summer fallow is common. Yield of winter canola crops in the PNW are higher than other USA regions. Despite strong rotational benefits canola acreage in the region has not risen to meet demand from local crushers and much of this is because of high profitability in growing winter wheat.

**Objectives:** To examine potential of dual-purpose winter canola in a biennial system for forage and seed production. Feasibility is determined by considering forage quality and yield, along with the associated seed yield. Canola and canola mixtures are examined.

**Methods:** Three different are presented. (1) Two winter canola cultivars were sown at three planting densities over four planting dates (May through September) in four years. Vegetative biomass during the first year was harvested and ensiled to determine silage quality. Canola was allowed to overwinter and seed harvested the following summer. (2) 'Amanda' winter canola was planted in early May and forage harvested at weekly intervals from 4 weeks after planting through mid-August. The following summer seed yield was determined on all forage harvest treatments. (3) Winter canola was planted in early June inter-seeded with either spring wheat or spring pea. Forage mixtures were harvested when the wheat seed was at the milk stage. Thereafter, canola was allowed to overwinter and seed harvested the following summer.

**Results:** (1) Cultivars produced similar forage yield and quality and canola silage (Canolage®) quality was exceedingly high. Total dry matter forage yield was greatest for May plantings (5.2 t DM ha⁻¹), while August seeded canola yielded 2.4 t DM ha⁻¹. Planting dates had significant, but inconsistent effects on seed yield. Early-planted winter canola withstood multiple forage harvests without an impact on seed yield most years and economics indicate it as a feasible management practice. (2) Average dry matter forage yield was 4.0 Mt ha⁻¹ with highest forage production of 6.7 Mt ha⁻¹. Under these dryland conditions, timing of forage harvest had no impact on subsequent seed yield, and average seed yield was 3,378 kg ha⁻¹. Maximum combined income from forage and seed occurred when forage was harvested after 1,011 growing degree-days. (3) Forage yield was maximized with canola seeded at 9 kg ha⁻¹ with spring wheat at 22.4 kg ha⁻¹, producing 9.0 Mt ha⁻¹ dry matter forage, with a relative feed value of 278 and a crude protein of 18.7%. Subsequent canola seed yield from this canola:wheat mixture was 3,483 kg ha⁻¹.

**Conclusions:** Dual purpose early planted winter canola in the PNW has potential to increase grower profitability and hence increase canola acreage in the region. The PNW imports large quantities of forage and feeds to supplement a growing need for livestock feed, therefore, a local market exists for forage and seed meal.

**References:**


Environmental effects on oil quality of high oleic-low linolenic (HOLL) and low linolenic (LLIN) spring canola

**Background:** Partially hydrogenated vegetable oils, which contain trans-fats, have adverse effects on human health. Traditional canola oil requires partial hydrogenation to avoid off-flavors when used for high temperature frying and to increase shelf life. Rancidity and off-flavors in oil are caused by high linolenic content, which has led to the development of high oleic - low linolenic acid (HOLL) and low linolenic acid (LLIN) canola cultivars. Availability of these cultivars in the Pacific Northwest would be of particular interest to the potato fry industry which requires large volumes of these oils. However, these new HOLL and LLIN cultivars must have stable quality over the environmental conditions throughout the Pacific Northwest.

**Methods:** Four LLIN lines and four HOLL lines were grown along with two standard canola cultivars (‘Westar’ and ‘Profit’) over 2 years in multiple location field trials throughout the Pacific Northwest. Two later plantings were included to simulate increased biotic and abiotic factors such as heat stress and insect infestation. Prior to flowering, racemes were covered with Delnet® pollination bags to avoid cross pollination. At harvest, seeds from the covered plant racemes were harvested by hand and used for fatty acid testing. Fatty acid profiles were determined using gas chromatography. The remainder of each plot was combine harvested, weighed to determine yield potential, and a seed sample analyzed for oil content.

**Results:** Interactions between cultivars and environments were often significant, though they were usually small compared to the main effects between cultivars. Cultivar x site and cultivar x year interactions were not significant for linoleic and linolenic acid, indicating genetic stability over a wide range of environments. Cultivar x site and cultivar x year interactions were observed for oleic acid indicating a potential environmental interaction. It is not known whether the LLIN and HOLL lines in this study have the documented FAD-2 or FAD-3 genes, which have shown environmental instability in some previous studies. However, it should be noted that the original LLIN and HOLL lines developed in breeding programs tended to show poor adaptability, either through gene drag from the mutagenesis techniques used in their development, or from the ‘novel’ fatty acids interfering with seedling growth. HOLL and LLIN breeding lines showed good adaptability for yield and oil quality. Therefore either negative genetic drag resulting from mutagenesis has been selected against or these lines have been selected such that they are no longer negatively impacted by the modification of fatty acids.

**Conclusions:** The results from this study show that recently developed LLIN and HOLL cultivars are adapted to a wide range of environments in the Pacific Northwest and maintain high quality oil suitable for non-hydrogenated fry oils while producing competitive seed yield and seed oil content.
Analysis and evaluation on quality characteristics of 500 rapeseed lines

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**Background:** Erucic acid, glucosinolates and oil content are important quality indexes to the evaluation of rapeseed. Breeders have made great efforts to improve rapeseed quality. So far, lots of work about quality characteristics of crops was done by the data processing software. Collecting representative rapeseeds widely and analyzing the quality of them, then multiple correlation coefficient and cluster analysis were done by data processing software. It’s significant in rapeseed quality improvement.

**Objective:** At present, germplasm resources of *Brassica napus* L. are very rich. In order to know whether branch height had any effect on rapeseed quality, seeds from different branch height of the plant were collected and analyzed separately. On the other hand, correlation and distribution among quality characteristics of 500 rapeseed lines were studied, which could provide guidance for cultivar breeding quickly.

**Methods:** When rapeseeds matured, seeds were collected respectively from different branches of 50 rapeseed cultivars. After that, quality characteristics were measured by NIRS. The software SPSS 19.0 was used to data statistic analysis. Non parametric tests were applied to significance analysis. Quality characteristics of 500 rapeseed lines were studied by using multiple correlation coefficient and cluster analysis.

**Results:** Branch height has significant impact on protein, glucosinolates and oil content, but indistinctively influence on erucic acid. The highest oil content and glucosinolates in the whole plant was discovered in the upper part of main inflorescence, while the highest protein content was in the lower part of that. On the other hand, there was high correlation among some quality characteristics. Oleic acid content was extremely significantly positively correlated with linoleic acid content and saturated fatty acid content, and extremely significant negatively correlated with erucic acid content. Linoleic acid content was extremely significantly negatively correlated with erucic acid content, and extremely significantly positively correlated with saturated fatty acid content. Erucic acid content was extremely significantly negatively correlated with saturated fatty acid content. Oil content was extremely significantly negatively correlated with protein content. 500 tested materials were divided into 6 groups initially by cluster analysis. Materials of excellent quality characteristics were concentrated in group I and those of high quality characteristics were in group III, IV and V, which could provide theoretical guidance in parents selection.

**Conclusions:** Branch height had significant effects on protein, glucosinolates and oil content. Considering this, seeds from these branches in which quality contents were higher should be collected only, rather than ones of the whole plant without selection. Generally, linolenic acid and saturated fatty acid differed little and the others differed greatly. It will provide a good base for new cultivars selection. Meanwhile, some correlations among quality characteristics were in contradiction with breeding goals, which could increase the difficulty in quality improvement.
Nutritional value of rapeseed oil: Alpha-linolenic acid level and a proper n-6/n-3 ratio

Rapeseed oil, a monounsaturated vegetable oil, presents a high nutritional value by its high level of Alpha-Linolenic Acid (ALA, 18:3n-3: 9%), the essential short chain precursor of n-3 family, which could be converted to Long-Chain-n-3 (LCn-3: EPA and DHA) by humans and animals. For a healthy human diet, this high level of ALA, associated to the moderate level of Linoleic Acid (LA, 18:2n-6: 20%) of this oil, induces a very low ratio n-6/n-3 (18:2n-6/18:3n-3 around 3 or less), which helps to promote the enrichment of ALA in tissues and its proper conversion to LCn-3. Based on this aspect, rapeseed is more efficient than soya oil which has a similar ALA content but more LA and a higher n-6/n-3 ratio (>10 vs 3 for rapeseed).

Populations of Western countries have a severe deficit in omega-3 intake, both in ALA (0.3% vs 0.8% of total caloric intake recommended) and LCn-3. In some cases, ALA is almost the only source of omega-3 for terrestrial animals including human. It has been shown that an adequate intake of this omega-3 precursor (ALA) in early life is able to cover the needs for LCn-3 and specifically for the brain DHA, via a proper liver conversion to LCn-3. The protective effect of ALA (rapeseed consumption) particularly during gestation and lactation of mothers, has been shown to induce a favorable effect on the brain DHA level of the offspring which is essential during early life and later, for an adequate development and protection against metabolic diseases in adulthood. Deficiency of Omega3 (precursor ALA, LCn-3: EPA; DHA) is associated to (i) Heart pathologies: thrombosis, sudden death, arrhythmia, CVD, stroke and, (ii) Brain pathologies: in the young (abnormal development, learning, behavior), in adult (depression, epilepsy) and aging populations (Alzheimer). The beneficial effect of LCn-3 has been largely reported, and despite much less studies, the beneficial impact of ALA is now well evidenced, in prevention (nutritional studies with chronic consumption) as well as an acute treatment for some pathologies.

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ALA-rich rapeseed diet for dams and mice offspring are protecting against anxiety observed with low-ALA-palm diet

Omega-3 deficiencies during gestation/lactation could have dramatic impacts on cognition and behaviour. We previously showed in n-3-deficient-rats that ALA-rich-rapeseed-diet (8%) restored brain DHA levels to normal values and was much more efficient than palm-diet.

Objectives: Comparison of rapeseed or palm diets on mother and pups behaviour.

Methods: Two groups of dams (Swiss strain) were fed during 6weeks before and during gestation/lactation with:

- (i) Deficient-ALA-palm (0.4%) diet
- (ii) Protective-ALA-rich (8%) rapeseed diet.

Post-weaning pups received diets similar to their mothers until PND40 (Post-Natal-Day40).

Results

There was no change in the behavior of pregnant dams, except in the palm-group which showed a reduced activity in a spatial memory test (Y-maze).

Pups belonging to the palm-group showed a reduced time at PND3 in the surface righting reflex test when compared to rapeseed group.

No significant differences were found between the treated groups in the geotaxis test (motor coordination and vestibular function, assessed at PND5,7,9,11), the suspension test (muscular strength, PND9&11) or in the Y-maze (assessing the short term memory post-weaning).

Three weeks after weaning, an increased anxiety was noticed in female mice of the palm-diet group when placed into an open-field (locomotor activity and anxiety).

This result was confirmed for the palm-diet group with an elevated-plus-maze test, while females born from ALA-rich-diet dams (rapeseed) showed a reduced anxiety compared to ALA-poor-palm-diet dams.

Conclusions

Females born from dams fed with rapeseed oil (8% ALA), present an absence of post-weaning anxiogenesis while those born from palm (0.4% ALA) presented an increased level of anxiety. The low n6/n3 ratio (2.3) for rapeseed (while 21 for palm) could be part of its protective effect via a better brain DHA status.
Investigation on cadmium contamination and migration from rapeseed to Oil

**Background:** China is one of the countries with longest history of rapeseed production in the world. With the rapid development of modern industry, both natural and anthropogenic sources lead to an increase of Cadmium in soils and water. Cadmium in soils and water can be more readily taken up by plants and accumulate in the food chain (Yuan et al. 2013). Investigation of cadmium level and migration in rapeseed in China is a significant guidance to rapeseed oil safety, selection of varieties and breeding of low cadmium accumulation variety of rape.

**Objectives:** The aim of this study was to investigate the Cadmium contamination and migration from seed to oil of rapeseed in the 11 main producing provinces in China and evaluate the contamination level of Cadmium in rapeseed.

**Methods:** A total of 596 representative rapeseed samples were collected from 11 main rape production provinces including Hunan, Hubei, Jiangsu and Jiangxi, where cadmium levels of soil and water are relatively high. And 30 sets of samples including rapeseed, rapeseed oil and rapeseed meal were collected from the large-scale integrated edible oil processing company in Hubei, Hunan, Guangdong, and Qinghai provinces. The cadmium contents of these samples were analyzed by atomic absorption spectrophotometry.

**Results:** Results indicated that Cadmium level in rapeseed ranged from 0.018 mg/kg to 0.142 mg/kg (P5~P95), the migration proportion of Cadmium from rapeseed to rapeseed oil was from 2% to 10%. Moreover, the migration rate negatively correlated with protein content in rapeseed with the correlation coefficient of 0.73. More importantly, the Cadmium contents of 99% samples of rapeseed were lower than 0.2mg/kg, which was significantly lower than Chinese standard maximum limit (0.5mg/kg).

**Conclusions:** In this study, we conducted investigation of the Cadmium level and migration in rapeseed in China. The results showed that the Cadmium content of rapeseed was far lower than Chinese standard maximum limit and rapeseed oils were safe and not influenced by the increase of Cadmium in soils and water.

**References:**

Improvement of iturin A production by *Bacillus subtilis* with combined shaking and static culture mode using rapeseed meal as nitrogen source

**Background:** Iturin A is a new biopesticide with low toxicity, biodegradability and environmental friendliness. But its high production costs, low productivity and difficulty in fermentation process limit its commercial production. In our previous study, the effectiveness of direct bio-utilization of rapeseed meal as a nitrogen source for iturin A production by *Bacillus subtilis* was demonstrated by using glucose as carbon source (Jin et al. 2014). Biofilm fermentation is a newly developed promising technique in fermentation technology (Zohora et al. 2009), and the production of iturin A in static biofilm fermentation was reported two times higher compared to that in the submerged culture (Rahman et al. 2007).

**Objectives:** To further decrease the production cost of iturin A, the effect of different carbon sources and static biofilm fermentation on iturin A production was investigated when using low-cost rapeseed meal as a nitrogen source.

**Methods:** A combined culture mode of shaking culture first and followed by static biofilm fermentation was proposed based on the change characteristics of *Bacillus subtilis* growth and iturin A production during single static culture.

**Results:** The results indicated that wheat bran was the best carbon source for iturin A production, and the maximum iturin A concentration was 1.6-fold higher than that with glucose as a carbon source. Thick and stable biofilm was observed when adopting static culture, and the iturin A productivity was higher than that with traditional shaking culture during the later period of fermentation. Compared to single static culture, the proposed combined culture mode could further improve iturin A production, and the maximum iturin A concentration reached 1.10 g/L, close to the highest level produced with single shaking culture.

**Conclusions:** The highest iturin A concentration produced from combined culture could reach the maximum level with single shaking culture. Importantly, this new culture strategy was more easy to implement, which can not only decrease the production cost of iturin A but can also prevent the formation of a lot of foam in the later period of fermentation.

**References:**


Nutritional value of rapeseed meal varies between oilseed rape varieties and processing conditions

Background: Rapeseed meal use in poultry has traditionally been limited because of concerns over anti-nutrients (Tripathi and Mishra, 2007). However, modern “double-zero” oilseed rape varieties have reduced glucosinolate levels, suggesting that rapeseed meal becomes more attractive for broiler diets.

Objectives: Nutritional value of rapeseed meal may be sensitive to oilseed rape variety and processing conditions (Newkirk et al. 2002). Therefore, variety and processing effects of oilseed rape on standardised ileal digestible amino acids (SID-AA) and apparent metabolizable energy (AME) content in rapeseed meals for broiler chickens were assessed.

Methods: Fifteen rapeseed varieties were obtained; eleven were hexane-extracted at CREOL producing rapeseed meal (RSM), three were cold-pressed producing rapeseed expeller (RSE), and one (var. Compass) was both hexane-extracted (RSM-Compass) and cold-pressed (RSE-Compass). SID AA was assessed using semi-synthetic diets with RSM and RSE at 500g/kg. A total of 192 14-day-old Ross-308 male broilers were fed test diets for 8 days (n=6 cages per diet; two birds per cage). AA digestibility was calculated by AA and inert marker (TiO2) quantification in diets and ileal digesta. SID AA coefficients were calculated by correcting apparent AA digestibility for basal ileal endogenous losses, and combined with diet AA to obtain diet SID AA content (g/kg dry matter, DM). AME was assessed through including RSE and RSM at 100g/kg in a maize-soybean meal reference diet, proportionally replacing all other energy-yielding ingredients. A total of 273 14-day old Ross-308 male broilers were fed the 17 resulting diets (n=7 cages per diet; three birds per cage), and excreta were collected on days 20 and 21 of age. Energy digestibility and AME content of the test diets were determined using the index method and for RSM and RSE samples through the difference method.

Results: SID coefficients across essential AA in RSM and RSE differed between varieties (p<0.05), averaging 0.84 (0.80-0.88) for RSM and 0.85 (0.83-0.87) for RSE. However, since protein levels varied between varieties and processing, larger differences emerged for total SID essential AA contents, i.e. 177 (159-198) g/kg DM for RSM and 120 (105-130) g/kg DM for RSE. RSE-Compass had greater SID coefficient than RSM-Compass for lysine (+6.2%), methionine (+4.6%), histidine (+3.2%), and arginine (+2.9%; p<0.05). AME content of RSM and RSE differed between varieties (p<0.05), averaging 13.1 (13.01-13.54) MJ/kg DM for RSM and 16.4 (15.90-17.21) MJ/kg DM for RSE. Energy digestibility and AME content were ~3% and 30% greater in RSE-Compass than in RSM-Compass (p<0.001).

Conclusions: Results suggest that oilseed rape variety and processing type can greatly influence nutritional value of resulting meals for broiler chickens. Ongoing work aims to correlate these findings with detailed biochemistry to predict nutritional value and inform breeding programs.

References:

Acknowledgements:
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Comparison on the carbohydrate metabolic enzymes and their gene expression patterns in canola differing seed oil content

**Background:** Carbohydrates are thought of as an important regulator of canola seed oil content. Although it is well known that sucrose is a starting point before fatty acid biosynthesis, understanding the physiological regulation by carbohydrates governing seed oil accumulation is fragmented.

**Objectives:** (1) compare the cleavage activity of sucrose and starch in canola high oil content line and low oil content line; (2) investigate the expression pattern of carbohydrate-related genes; (3) explore the possible roles of carbohydrate content and related enzymes on seed oil content.

**Methods:** In the present study, two canola recombinant lines differing seed oil content were used as experimental material. Activities of sucrose and starch catalytic enzymes, including neutral and alkaline invertase, sucrose synthase, and starch phosphorylase, and biosynthetic enzymes, including sucrose phosphate synthase, and AGPase, were compared in developing silique and seed of HOCL and LOCL, respectively. Sucrose and starch synthesis and catalysis genes were also compared at key stage of seed lipid deposition.

**Results:** The results showed that HOCL had significantly higher total soluble sugar concentration in the developing silique wall and seed during the seed lipid accumulation stage. Strikingly, all the enzymes showed very strong activities at 25 days after anthesis (DAA) in the silique wall of HOCL. At 25 DAA, enzyme activity was usually one-fold higher than at other stages. The result indicated that the high efficiency of cleavage of these carbohydrates in HOCL was beneficial for the rapid volume expansion and transportation of this compound to the seed for utilization. However, higher enzymatic activities were observed at the silique maturation stage in LOCL, thereby revealing the effect of these enzymes on cell wall thickening (i.e., cellulose accumulation). Similar activities of catalytic and biosynthetic enzymes suggested a homeostasis of carbohydrates in the silique wall. At seed deposition stage, all the enzymes exhibited significantly higher activities in HOCL than in LOCL, which was helpful for increased production of carbohydrates. The results of gene expression revealed that cell wall invertase was strongly expressed at late seed developmental stage in LOCL, and such expression could be closely associated with cellulose deposition. However, cytoplasmic invertase in HOCL exhibited higher levels of transcripts than in LOCL at all developmental stages. Only SUS3 showed significantly higher transcript amount in HOCL than in LOCL at all stages. The starch phosphorylase expression increased with seed development, but no significant difference was found in transcript levels between the two lines, except for SSIV at 50 DAA.

**Conclusions:** 1, extremely high activity of carbohydrate enzymes in HOCL was beneficial to produce more carbohydrates; 2, significant higher activity of carbohydrate catalytic enzymes in HOCL seed could help to produce more carbohydrate as oil synthesis substrate at seed lipid deposition stage; 3, high expression amounts of SUS3 and starch phosphorylase possibly played key roles in the higher enzyme activity in HOCL seed.

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Control effect of root-knot nematode by using rapeseed cake in continuous cultivation at greenhouse

Background: Rapeseed cake could be utilized as animal feed and fertilizer for growing horticultural crops. Rapeseed cake could be a good source for increasing farm incomes. More efficient utilization could add more value to the rapeseed production chain which in turn would raise the competitiveness of rapeseed cultivation and production. Rapeseed cake contains several glucosinolates. The glucosinolates compound is known to control nematode due to their toxicity (Ludwig-Muler et al. 1997).

Objectives: The objective of this study was to determine the effectiveness of rapeseed cake in controlling soil nematodes.

Methods: Tomato (cv. Rutger) was used as a host plant in order to study the control effect of nematode by using rapeseed cake. The tomato plants were grown for 20 days in pots filled with mixed soil (1kg) of clay loam soil (500g) and sandy soil (500g). And then the soil was supplemented with 50g of rapeseed cake from Jeju local rape varieties and ‘Sunmang’ variety, respectively. Roots and soil around roots were inoculated with egg sacs of nematodes, and then the tomato plants were grown for 60 days. The density of nematodes was investigated by separating nematodes from cultivated soils and the roots.

Results: Two different rapeseed cakes (Jeju local rape varieties and ‘Sunmang’ variety) were mixed with the soil to control nematodes environmentally. When soil physical properties in the rapeseed cake-mixed soils were analyzed, OM (organic matter), P205, Ca, Mg, and CEC (Cation Exchange Capacity) value increased. Especially, the level of OM was 3-fold higher than control soil. Glucosinolate content of rapeseed cake was higher in Jeju local rape varieties than ‘Sunmang’ variety. The major components of glucosinolates were consisted of progoitrin, glucobrassinap, glucobrassica napin, and sinigrin. These components were likely to be involved in reducing nematode density.

Conclusions: In this experiment rapeseed cake reduced the density of getting infested with nematode in the soil because the glucosinolates compound in rapeseed cake has toxicity to kill nematodes. The Glucosinolates are secondary metabolites including β-D-thioglucoside and sulphonated oxime and it is converted into isothiocyanates (ITCs) thiocyanate and indole etc (Mithen et al. 1987).

References:
Does *Camelina sativa* contamination of double low rapeseed expellers cause milk fat depression in dairy cows?

**Background:** Expellers and press cakes of double low *Brassica napus* rapeseed (RPCs) are normally recognized as valuable parts of the concentrate feed to dairy cows. However, during spring 2013 milk fat depression was observed among some high yielding Danish dairy herds fed commercial RPC's containing 13-14% fat as part of their feed. The observed milk fat depression varied, showing depression up to 0.5 % units.

**Objectives:** The objectives of the present study were identification and evaluation of possible correlations between bioactive constituents in the applied feed and the dairy cows’ milk fat depression.

**Methods:** Nine RPC containing samples from feed fed to nine different milk fat depressed herds were collected, analyzed and the analytical data were compared with corresponding data from six reference groups of herds without milk fat depression. The RPC samples were analyzed for protein, fat, fatty acids (FA’s), phenolics and glucosinolate content.

**Results and discussion:** The RPC containing samples did not differ significantly in protein content and total fat content. RPC’s in feed fed to milk fat depressed herds differed, however, from that of the reference feed with respect to FA composition. They were thus higher in c18:3n-3 (12.1% vs 8.9%), c20:1n-9 (2.7% vs 1.1 %), c22:1n-9 (0.7% vs 0.1) and lower in c18:1n-9 (51.5% vs 57.4%).

With respect to phenolics and glucosinolates, some striking differences were seen in the profiles of individual compounds; the flavonoids (especially quercetin glycosides) and ω-(R)-methylsulfinylglucosinolates.

RPC’s from milk fat depressed herds showed a characteristic content of 4 different ω-(R)-methylsulfinylglucosinolates (n=8, 9, 10 and 11) varying in total concentrations from 0.7-3.3 µmol/g RPC’s. Seeds of high quality double low *Brassica napus* only contain ω-(R)-Methylsulfinylglucosinolates with n=4 (glucoraphanin) and n=5 (glucoalyssin) and in low concentrations (0.1-0.2 µmol/g seed). Seeds of *Camelina sativa* (L.) Crantz, however, accumulate appreciable concentrations of the 4 homologues with long side chains; n=8, n=9, n=10 and n=11 (Das et al., 2014, Andersson et al., 2008) with total concentrations from 10-30 µmol/g seed depending on variety.

The observed milk fat depression was very similar to the milk fat depression caused by conjugated linoleic acid (CLA), but although the content of unsaturated FA was higher in RPC samples from milk fat depressed herds it was not considered to be high enough in the total diet to cause milk fat depression. However, based on the quantitative analysis of FA, phenolics and glucosinolates in double low rapeseed and *Camelina sativa* seeds, it seems more likely that the observed milk fat depressions are caused by RPC’s contaminated with around 15-20% of *Camelina sativa* seeds.

**Conclusions:** The cause of the milk fat depression is not completely elucidated, but it is likely that it is caused by a contamination with *Camelina sativa*, but whether it is caused by the glucosinolates or other compounds in *Camelina sativa* seeds still needs to be resolved.

**Implications:** This study shows the importance of natural product fingerprints as an important means to detect adulteration of feed impairing significantly on animal metabolism and thereby on their production yields.

**References:**

Harnessing potential of mustard glucosinolate as biopesticides

Background: Brassica (rapeseed•mustard) is the key edible oilseed crop in India after groundnut. Oil-cake contain a high-quality protein that forms an important cattle-feed and manure. However, it also contains relatively high amounts of anti-nutritive fibre compounds, phenolic acids, phytate and glucosinolates. Glucosinolates, compounds that occur in the cake, represent a viable source of allelochemic control for various soil-borne plant pests. As such, glucosinolates are commonly considered to be ultimately responsible for pest suppression (Fenwick et al., 1983). The detrimental effect of cruciferous tissues on other microorganisms has also been attributed mainly to volatile degradation products of glucosinolates released from these plants. With this background present study was undertaken to explore the potential of glucosinolate as biopesticide.

Objectives: De-oiled rapeseed meal produced in mustard oil extraction is used as cattle and poultry feed in India but due to high content of glucosinolate it has limited preference in international market. Preliminary studies show that mustard cake has anti-pestcidal potential due to its high glucosinolate content so objective of the present work is to development of the process for isolation of glucosinolate en-rich fractions and assess its potential as biopesticide.

Methods: Seed, Seed cake and oil samples were analyzed for glucosinolate content as per the method of Kaushik and Agnihotri (1999). A sequential extraction method was developed for the extraction of glucosinolate rich fraction. Samples were bioassyed for the anti-feedency and growth inhibition activity by pair choice and diet mixing bioassay respectively against Spodoptera litura.

Results: In order to determine the best source for glucosinolate extraction Indian and European Brassica species were analyzed for their glucosinolate content. Indian species of brassica (B. juncea, B. nigra, B. campestris) and European mustard (B. juncea) has very high amount of total glucosinolate. Indian mustard contains both allyl and butenyl while European mustard contains only allyl glucosinolate. Rai seed (B. nigra) is having high glucosinolate content as compared to mustard(B. juncea) and toria (B. campestris) seed. Extract prepared to isolate glucosinolate rich fractions was also analyzed and it was found that glucosinolate content of brown toria (B. campestris) extract is very high. Methanol extract of the brassica seed cake showed highest feeding inhibition whereas very high growth inhibition was obtained with brown toria formulation.

Conclusion: Glucosinolates exhibits very good antifeedency and growth inhibition against Spodoptera litura insect and the glucosinolate rich extracts can be explored for the up-scaling prospect for use as biopesticide.

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References:
The influence of variety and environment on the biochemical analysis of oilseed rape meal

**Background:** Despite major breeding advances, the use of oilseed rape meal (RSM) in pig and poultry rations remains limited by traditional concerns over their anti-nutrients (Tripath and Mishra, 2007).

**Objectives:** Biochemical analysis of a set of modern oilseed rape varieties was performed as part of a larger study to explore the potential for increasing confidence in using oilseed rape meal in monogastric diets. Using common origin material, from multiple sites, we have collected new data on variability of RSM biochemical composition, and the influences on this of genetics and environment.

**Methods:** Twenty-two varieties, from each of five locations from the 2012 UK National List variety trials program, were selected to provide maximum diversity using known data on oil content, whole-seed glucosinolate content, breeding type (hybrid/open pollinated) and origin (breeding program). This provided common origin material from a range of environments. Seed samples were milled and de-fatted by cold-hexane extraction to prepare meal samples. Using standard laboratory methodologies, samples were analysed for crude protein (CP) and amino acid composition, total glucosinolate content and composition, tannins, phytic acid and sinapine.

**Results:** Average CP content (22 varieties x 5 locations) was 34.5g/100g, with a maximum range (varieties x sites) of from 28.9 to 37.8g/100g, site and varieties effects contributing equally to this. Amino acid composition was very consistent between varieties. Glucosinolate content averaged 20.4μmol/g with a large overall range (10.8-52.5μmol/g) the variation coming principally from variety effects. This range was exaggerated by the inclusion of a new variety type with an altered oil profile and relatively high glucosinolate content. Excluding this variety reduced the overall mean value to 19.4μmol/g and the upper range limit to 36.1μmol/g. The individual glucosinolate components showed relatively little variation, the most coming from progoitrin and 4OHglucobrassicin. Tannins averaged at 1.59 mg/g catechin equivalents but also exhibited considerable variation (0.28-3.21 mg/g), largely from site effects. Phytic acid averaged 2.83 g/100g and varied less (1.32-3.78 g/100g), with the main variation again coming from sites. Sinapine averaged at 7.58 mg/g (5.10-9.30 mg/g), with similar variation observed for sites and varieties.

**Conclusions:** Levels of CP conformed closely to the 33.9g/100g published values for rapeseed meal in feed tables (Premier Nutrition, 2008) and show comparatively little variation. The principal cause of variability in glucosinolate content is genetically controlled (varieties) and levels in RSM batches for feed can continue to be improved by progressively tightening the standards set for commercial variety releases. Current differences due to variety or growing environment will be largely nullified by mixing grain loads at the crushing plants. Tannin, phytic acid and sinapine values were generally low with little potential for variety improvement in the current generation of cultivars.

**Acknowledgments:**
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Indian mustard (*Brassica juncea*) oil to combat deadly diseases

**Background:** Cancer and cardiovascular disorders remain the main cause of mortality, morbidity and health care burden worldwide. Heart disease is a leading cause of death in India. These two diseases are directly linked to diet and related factors, therefore, it is imperative to make dietary changes that could reduce the incidence of these two diseases. Indian mustard (*Brassica juncea*) is an important source of edible oil in India. Consumption of mustard oil seems to heart friendly as it has the lowest content of saturated fatty acids (SFA) among the vegetable oils. Also among the saturated fatty acids, stearic acid is considered as neutral towards heart related problems.

**Objectives:** In addition to the premium quality edible oil, seed meal obtained after extraction of oil is a rich source of many health promoting bioactive compounds including glucosinolates, phenolics, flavonoids, carotenoids, etc. Incorporating these phytochemicals in a daily food is a safe, effective and inexpensive way to guard against two of today’s most common health problems cancer and cardiovascular disorders. However, efficacy of these phytochemical is also often limited by the potential to reach the site of action as only a small fraction of intake reaches the target site. Physical instability, low solubility in aqueous medium, lower absorption rate and bioavailability are some of the factors responsible for slow pharmacological actions.

**Methods:** Under quality prospection programme, more than two thousand samples of germplasms, advance breeding and elite lines of Indian mustard grown in different agro climatic regions of India were screened for oil and seed meal quality parameters during 2000-2010.

**Results:** Total SFA content was less than four percent. Besides that, ratio of essential fatty acids content namely linoleic acid (ω-6) and linolenic acid (ω-3) was found to be very close to 1.25, the value recommended by WHO. Based on the fatty acid profiles, it was calculated that one tea spoon (about 5 g) of unrefined mustard oil could supplement about 900 and 750 mg of linoleic and linolenic acid respectively to daily diet.

**Conclusion:** Nanotechnology provides a platform to overcome these challenges and can be utilized for novel drug delivery systems with improved bioavailability as well as site specific distribution. Furthermore, value added products obtained from seed meal using low temperature drying techniques namely spray or freezing drying could be utilised for production chocolates bars, capsules, semi solid colloidal gel to enhance the acceptability of the products among the targeted groups. Besides, improving the palatability, these products will also have consistency in terms of the concentration of the targeted compounds for their site specific actions.

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Novel feed forms from canola for dairy cattle: Glycerol and high-oil canola meal

Background: Canola meal is a good protein supplement for dairy cattle. It is desirable for the canola industry to generate new high value feed products from canola and identify new markets and applications. The high level of milk production in present dairy cows creates high metabolic demand for energy (starch, fat, fiber) and protein with specific kinetic properties in digestion. Dairy producers have a need for specialty feed supplements that can be used to manage the cow’s unique metabolic needs during specific periods after calving and during lactation. Glycerol is a new feed by-product from the biodiesel industry and is a unique glucogenic, high energy source for dairy cattle (Carvalho et al., 2011). High oil canola meal (HOCM) typically is a cold press extrusion by-product from biodiesel production (Hristov et al., 2011). The fat in HOCM is a unique energy source for dairy cattle, complementary to starch. The HOCM increases polyunsaturated fatty acid content in milk fat, including Conjugated Linoleic Acid (CLA) which has nutraceutical properties.

Objectives: To determine the suitability of glycerol and HOCM, alone or in combination, as feed supplements for dairy cattle; to develop recommendations on optimal safe inclusion rates, and; to identify optimal feeding management protocols using these supplements to best support the metabolic need of high producing dairy cows.

Methods: HOCM with 10-14 % residual canola oil was from Milligan Biofuels and glycerol from Cargill Animal Nutrition. We performed four research trials at the University of Saskatchewan Dairy Research Facility. Two were in lactating cows between 2 and 7 months in lactation, where in one trial we performed a dose-response study with glycerol, and in the other studied combinations of glycerol with HOCM and distillers dried grains and solubles (DDGS), another by-product from the biofuel industry. Experimental design was a double 4x4 Latin square with periods of one month. The next two trials were performed in transition cows from -2 weeks to +6 weeks from parturition, when the cow experiences the highest metabolic stress and highest nutrient demand. Experimental design was a randomized block. In the third study we used 24 cows with three diet treatments (control standard Total Mixed Ration (TMR), TMR with glycerol, and TMR with glycerol and HOCM) and in the fourth trial 32 cows with two diet treatments (control TMR, and TMR with glycerol, HOCM and DDGS). In all trials we measured lactation performance and changes in blood metabolite composition and hormone profiles.

Results: Glycerol is a useful energy feed for lactating and transition dairy cows and acceptable up to 10% of the dry matter intake, the highest level tested, using a TMR typical for SK conditions. Glycerol feeding value is equivalent to that of corn on an energy basis. Glycerol improved lactation performance and yield of milk protein. We attribute this to the rapid availability of the energy from glycerol (high solubility) for rumen fermentation, supporting increased microbial growth and protein synthesis and improving protein supply for milk protein synthesis. Glycerol and particularly glycerol with HOCM improved energy balance and lactation performance in transition cows.

Conclusions: The canola biofuel by-product feed supplements increased milk production performance and feed efficiency of cows. Particularly, the supplementation of the TMR with a combination of glycerol and HOCM appeared most effective. The biofuel by-products have unique feeding attributes which can be used to provide increased nutrient supplementation of cows during periods with high levels of milk production and nutrient needs, such as transition dairy cows and those needing to improve body condition.

References:
Rapeseed or fish oil protects muscle mass during energy restriction in the rat

Background and Objectives: In obese subjects, the loss of fat mass during energy restriction is often accompanied by a loss of fat-free mass (mostly muscle), especially in the absence of physical activity. In other contexts leading to a muscle loss (immobilization, inflammation, cancer), several studies showed that n-3 polyunsaturated fatty acids (PUFA) modulate protein homeostasis, especially via effects on insulin sensitivity. The n-3 PUFA intake during weight loss could thus help protecting muscle mass. This hypothesis has been tested in rats with two sources of n-3 PUFA i.e. the vegetable precursor, linolenic acid (18: 3 n-3) and its long-chain derivatives (LC, 20: 5 n-3 and 22: 6 n-3).

Methods: Male adult Wistar rats (n=48) were fed for 4 weeks with a high-fat induction diet, then 3*12 rats were energy restricted during 8 weeks (50% of ad libitum intake) while the 12 remaining rats were fed ad libitum. During these two phases, the dietary lipids contained oleic sunflower oil (71% 18:1 n-9, ad libitum ADLlib and restricted ole control groups), rapeseed oil (10% 18:3 n-3, RAPE group) or fish oil (10% LC n-3 PUFA, FISH group). At the end of the restriction phase, rats were anesthetized prior to intravenous insulin injection, sampling of the Gastrocnemius muscle, and euthanasia. Post-mortem analyses were as follows: body composition, expression of muscle genes involved in proteolysis by qPCR, and activation by phosphorylation of muscle proteins involved in insulin signalling by western blotting.

Results: During the induction phase, the weight gain was similar in all groups. During the restriction phase, ADLlib group continued to gain weight while all energy- restricted rats lost weight (-20%) and fat mass (-50%). However, when compared to the ADLlib group, leg muscles significantly lost weight in the OLE group (-4 to -6%), but not in the RAPE and FISH groups. As concerns proteolysis key-enzymes, transcript levels involved in the ubiquitin/proteasome system were significantly decreased in the FISH group (-30% for MAFbx and -20% for MurF1), intermediary in the RAPE group and unchanged in the OLE group, when compared to the ADLlib group. The type of dietary fatty acids had no effect on calcium-dependent (calpain 2) and lysosomal (cathepsin D) systems. In response to insulin, phosphorylation levels of AKT and IRS1 (insulin receptor), known to stimulate protein synthesis, were significantly increased (+ 70%) in the FISH group, when compared to the ADLlib group. A similar result was observed for the transcript level of IRS1 (+ 50%), which promotes the transduction of insulin signal. The RAPE group exhibited the same activation pattern as the FISH one, with the exception of IRS1 phosphorylation level. No change was observed for OLE group.

Conclusion: Dietary n-3 PUFAs prevent the loss of muscle mass associated with energy restriction. This beneficial effect is associated with an improved activation of the insulin-signalling pathway. Most importantly, the vegetable 18: 3 n-3, supplied by rapeseed oil, has the same overall efficiency as n-3 LC-PUFA from fish oil.
Rapid determination of phenolic compounds in rapeseed oil using magnetic multi-walled carbon nanotubes as adsorbents followed by liquid chromatography-tandem mass spectrometry

**Background:** Phenolic compounds which are widely existed in edible oils, have drawn considerable interest for their antioxidant and health effects. Traditionally, the isolation and enrichment of phenolic compounds from the triacylglycerol matrix requires complicated sample treatment procedures (Bajoub et al. 2015), such as liquid-liquid extraction, dispersive solid-phase extraction (DSPE) or by solid phase extraction (SPE). Therefore, it is necessary to analyze minor constituents by a simple and rapid preparation method with high extraction efficiency and avoiding tedious steps (Zhao et al. 2012).

**Objectives:** The aim of this study was to develop a rapid and robust method for determining phenolic compounds in rapeseed oils using magnetic solid-phase extraction adsorbents (MWCNT-MNPs) coupled with high performance liquid chromatography tandem mass spectrometry (LC-MS/MS).

**Methods:** MWCNT-MNPs were simply obtained by wrapping amine-functionalized Fe3O4 magnetic nanoparticles into multi-walled carbon nanotubes. The major parameters affecting extraction efficiency were investigated, including the type and volume of desorption solvents, extraction and desorption time, washing solution, and absorbent amounts.

**Results:** The extraction procedure can be achieved by a 6 min simple vortex, and the cleanup needs only 30 s vortex without tedious concentration and derivative steps. The limits of detection (LOD) of phenolic compounds, based on a signal-to-noise ratio (S/N) of 3, were in the range of 0.05-0.40 μg kg⁻¹. The recoveries of phenolic compounds in oil samples were in the range of 85.0-110.0% with RSD less than 12%.

**Conclusions:** In the validation of MWCNT-MNPs-LC-MS/MS method in real oils, the results indicated that phenolic compounds, such as gallic acid, catechin, caffeic acid, sinapic acid and ferulic acid were widely existed in rapeseed oils, and chlorogenic acid, cinnamic acid were not detected in rapeseed oils. On average, virgin rapeseed oils have higher level of gallic acid, catechin, caffeic acid, sinapic acid and ferulic acid than refine rapeseed oils. Phenolic compounds in oils were linked to preliminary heat treatment of oilseeds and the refining process of oil. The proposed method is reliable, robust and potential for the analysis of phenolic compounds in rapeseed oils.

**References:**


Phytosterol analysis method and its application in adulteration detection of rapeseed oils

Background: Phytosterols make up the largest proportion of the non-saponifiable fraction. Due to the LDL cholesterol lowering effects and high content in edible oils, phytosterols were regarded as important nutrient components and quality parameters of edible oils (Normén et al. 2007). However, the past reports mainly focused on the several abundant phytosterols such as β-sitosterol, stigmasterol, campesterol, and brassicasterol. Therefore, it is necessary to develop a more comprehensive analytical method for the phytosterol profiles (Xu et al. 2014).

Objectives: The more comprehensive analytical method for phytosterol profiles should be developed for the subsequent quality inspection, nutrient and functional evaluation of edible oils. The aim of this study was to develop a robust analytical method for phytosterol profiles and use the method for adulteration detection of rapeseed oils.

Methods: Free phytosterol profiles of rapeseed oils were established by SPE–multidimensional gas chromatography coupled to time-of-flight mass spectrometry (GC-GC-TOF/MS) and employed to classify the rapeseed oils and other three edible oils with the help of unsupervised (Principal Component Analysis and Hierarchical Clustering Analysis, PCA and HCA) and supervised (Random Forests, RF) multivariate statistical methods.

Results: As results, 13 phytosterols were completely separated and quantitatively analyzed by GC-GC–TOF/MS. The RF model results indicated that the free phytosterol profiles of the four edible oils (rapeseed oil, soybean oil, peanut oil, and sunflower seed oil) could completely and correctly classify the oils into four groups, and therefore could be taken as effective markers for identification of rapeseed oils. Moreover, a simulated data test indicated that free phytosterol profiles could detect rapeseed oil adulterated with 10% of other oils.

Conclusions: In this study, a simple and rapid SPE method was developed for separating free sterols from edible oils, and their silylation derivatives have been analyzed by GC-GC–TOF/MS, leading to a good separation resolution. Moreover, combining with chemometric methods, this method was used to detect the adulteration of rapeseed oil. The validation results indicate this method could effectively detect the fraud rapeseed oil adulterated with more than 10% of other edible oils.

References:
Studies on volatile flavor components of cold-pressed rapeseed oils

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Background: The flavor is one of the most important sensory characteristics of edible oils, which mainly originates from volatile flavor components (VFC). Generally, different oils have its own characteristic flavor, contributing to identify oil species and analyze quality changes. Up to now, domestic and international researchers found that the main source of VFC came from oxidation products of unsaturated fatty acids in edible oils, followed by maillard reaction products between sugars and amino compounds in the process of heat treatment (Shahidi et al, 2005). Some domestic researchers analyzed the influence on VFC of rapeseed oils under different processing technologies, which demonstrated that the main VFC of rapeseed oils were glucosinolate degradation products (GSDP), oxidized volatile substances and heterocyclic carbenes. However, there were few reports on the VFC of cold-pressed rapeseed oils (CROs), and the relationship between GSDP and sensory properties of CROs.

Objectives: The VFC was the most important indicator of CROs, good-flavored CROs was directly related to the acceptable level of market. The VFC of CROs with different amounts of GSDP was measured and compared it to that of different treatments, which provided theoretical basis for developing CROs products with good tastes.

Methods: The VFC of three kinds of CROs with different GSDP contents was analyzed by combination technique of head-space solid-phase micro-extraction, gas chromatograph-mass and olfactory detection port, and the CROs were cold-pressed low-erucic rapeseed oil (LERO), cold-pressed medium-erucic rapeseed oil (MERO) and cold-pressed high-erucic rapeseed oil (HERO). In addition, we compared VFC of various kinds of rapeseed oils, including LERO, hotpressed low-erucic rapeseed oil (HLERO), solvent-extracting low-erucic rapeseed oil (SLERO), refined rapeseed oil (RRO) and microwave-pretreatment LERO (M-LERO). The sensory evaluation of different oils was carried out by several assessors with professional backgrounds of oils.

Results: The VFC of CROs was composed of aldehydes, alcohols, hydrocarbons, furans and more than 60% GSDP. By comparing rapeseed oils processed with several techniques, it was demonstrated that LERO, HERO, HLERO, SLERO and M-LERO all possessed higher contents of GSDP except for RRO, however, the types and contents of VFC of each oil were different. In addition, oxidizing volatility products of five oils were mainly aldehyde. The flavors of CROs were mainly woody, astringent, bitter, and the most intense seed-like. Low-temperature refining had no obvious influence on CROs flavor, and M-LERO presented pleasant nutty and roasted flavor.

Conclusions: Each processing technology showed great effect on the VFC of rapeseed oils, producing different contents of components. However, GSDP possessed an important part in the VFC of rapeseed oils except for RRO and aldehyde was the common oxidizing volatility products of the above studied five oils. This work contributed to the development of CROs products with desired flavors.

References:
Production of value added co-products in the seeds of the industrial oilseed crop *Camelina sativa*

**Background:** With growing concerns of diminishing fossil fuel feedstock availability and increased global climate change, oilseeds are emerging as a sustainable platform for producing fuels, chemicals and materials. Production of industrial commodities through agriculture provides an opportunity to produce these materials in large quantities at a low cost. *Camelina sativa*, belonging to the Brassicaceae family, is being targeted as an industrial crop due to its high oil content that is suitable for production of renewable industrial lipids and oleochemicals as well as aviation and other liquid fuels. However, there is currently insufficient value within Camelina to compete with other crops for acres. Options for increasing the value of the crop include engineering a value added co-product or significantly increasing its yield and oil content. Metabolix Oilseeds and its parent company, Metabolix, are currently pursuing both strategies to make *Camelina* an economically viable crop.

**Objectives:** This study focussed on seed-based production of the polymer polyhydroxybutyrate (PHB) in plastids of *Camelina* seeds as a co-product to increase the value of the seed. PHB is one member of the broad polyhydroxyalkanoate (PHA) family of renewable biodegradable materials with properties that make them suitable substitutes for many applications currently served by petroleum derived plastics. PHB is an ideal co-product for an industrial oilseed since it has multiple market applications. Besides its use as a low cost polymeric material, it has also been targeted for use as an enhanced feed supplement and as a feedstock for the production of renewable chemicals (Snell and Peoples 2013; Snell et al. 2015).

**Methods:** Multiple genetic constructs with different seed-specific promoters encoding plastid targeted PHB biosynthetic enzymes were constructed and transformed into different accessions of *Camelina sativa*. The best genetic constructs for PHB production were identified and homozygous PHB Camelina lines were produced. PHB levels were quantified and the effect of PHB production on carbon partitioning in seeds was determined.

**Results:** PHB levels of up to 15% of the mature seed weight were obtained in bulk Camelina seeds (Malik et al. 2015), the highest level of PHB produced in a seed to date. Transmission electron microscopy showed the presence of distinct granules of PHB in seed plastids. High level production of PHB had varying effects on germination, emergence and survival of seedlings.

**Conclusions:** The high level of polymer produced in seeds of *Camelina* is an important step forward for commercializing an oilseed-based platform for PHB production. Additional work is on-going to increase seedling emergence and vigor in high producing lines.

**References**


Variability of fatty acids and tocopherols in NS rapeseed collection

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Background: Because of its high-quality nutritional composition, rapeseed is a common source of edible oil. Recently, the focus in rapeseed breeding has turned to improving and altering the content and composition of salutary oil constituents, such as oleic acid and linolenic acid contents and tocopherols (Fritsche et al. 2012). One of the main goals in the rapeseed breeding program at the Institute of Field and Vegetable Crops, Novi Sad (Serbia) is to create genotypes with a specific level and combination of different fatty acids and tocopherols. Classification and characterization of rapeseed germplasm and the selection of superior genotypes for utilization in the field production or as parents in future hybridization program can be effectively performed by using multivariate analysis (Jankulovska et al. 2014).

Objectives: The main objectives of the study were to exploit the variability of fatty acids and tocopherols content within the NS rapeseed collection, to classify the genotypes based on their oil quality and to identify genotypes with desired fatty acids and tocopherols content.

Methods: Total of 49 genotypes were analysed for alpha and gamma tocopherols and oleic, linoleic, linolenic, stearic, palmitic, arachidic, behenic, eicosenoic, lignoceric and erucic acid content. Classification of rapeseed germplasm was performed using multivariate statistical methods. Principal component analysis (Revelle 2014), cluster analysis (Suzuki and Shmodaira 2013) and two-way cluster analysis (Day 2012) were applied.

Results: Principal Component Analysis revealed 5 PC components with Eigen value >1, which explained 78.70% of the total variability. Cluster analysis and two-way cluster analysis helped identify genotypes with similar fatty acid and tocopherol composition. Two main groups could be identified on the dendrogram, the first having two genotypes and the second comprising 44 genotypes. Three genotypes did not belong to any group.

Conclusions: The applied techniques can be helpful for identification, selection and optimized exploitation of rapeseed genotypes with desirable oil quality.

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Advanced calibration method for glucosinolate analysis in rapeseed seeds using a Near Infrared Reflectance spectrometer

**Background:** The fast and cheap and nondestructive analytical methods of large quantity of samples are in big need for breeding and research purposes. Today there are two instrumental methods available – NMR for fat and moisture estimation in seeds or NIR- able to measure all important chemical components. The basis of obtaining reliable results by NIR is good, robust calibration model. The “Local” method was introduced by John Schenk (Schenk and Westerhaus 1997) and was developed to evaluate large databases (many thousands of samples) of spectra and reference values using the single sample prediction concept (method of analyzing an unknown material patent US 5795826 A).

**Objectives:** For calibration purposes couple mathematical methods (multiple regression, principal component, neural network) was developed. In this study one less popular method called “Local” was investigated and compared to classical PLS method to check if this method can generate more precise results.

**Methods:** Spectrometric data were obtained by NIRS 6500 spectrometer with ISISCAn 4.6 software. All calculations were made by using the WinIsi (Foss) package. 2400 samples were used as calibration set and 600 samples as validation set. The samples were collected over 7 years from western Poland locations. Corresponded reference analysis of glucosinolate content by gas chromatography of desulfoglucosinolates (Raney and MacGregor 1990 Michalski et all 1995) was accomplished.

**Results:** The sample set of 2400 samples was used to generate PLS calibration models. The PLS calibration generates whole spectrum equation estimating the value of calculated components. It need linear data and work properly with samples similar to used in calibration. The same set was used to generate Local database. Obtained validation results for both methods were compared and the Local error was slightly less than Global error for all glucosinolates (Glucanapin 1,1-1,2, glucobrassicanapin 0,4- 0,5, Progoitrin 2,3-2,4, naplofein 0,3-0,6 Glucobrassicin0,17-0,2, 4OH-glucobrassicin1,0-1,2 and total 3,6 -3,7. All result in µM/g seed)

**Conclusions:** The comparison of prediction errors (SEP) shows that local is giving slightly better results. To make Local method work it is necessary to collect very large amount of representative samples what force multiyear collection process and high reference analysis cost. If the obtained database is truly representative it can be used to any material covered by samples it contains. It is possible to generate database using commercial software- WINISI, when Neural network calibrations need cooperation with machine vendor, as there no subsequent software is available. It was observed that database with majority of samples in the range of 5-30 µM/g glucosinolates generate calibration equations that poorly estimate the high glucosinolate samples when PCA global equations worked properly.

**References:**
421
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Evolution of high oleic low linolenic (HOLL) winter oilseed rape (WOSR) production in Switzerland: How agricultural innovations can open markets and increase production options

Background: HOLL winter oilseed rape (WOSR) produces a vegetable oil suitable for frying without partial hydrogenation, thanks to its specific fatty acid profile.

Objectives: Analyze how the production of HOLL WOSR evolved in Switzerland since its introduction in 2004, and the possible future trends.

Methods: Data of WOSR production were compiled and analyzed in terms of HOLL surface, yield, and oil quality. Surveys about crop management and quality were organized.

Results: HOLL WOSR was first grown in Switzerland in 2004. The surface allocated to HOLL constantly increased, to reach 8’000 ha for the 2015 harvest. This represents 32% of today’s total WOSR surface. This development took place without a reduction of conventional WOSR production, showing that a new market for a differential industrial purpose had opened, increasing the Swiss farmers WOSR production portfolio. Its production started with the OP-line variety Splendor. It was replaced in 2007 by the OP-line V141OL, then by the Ogura Hybrid V280OL in 2012. A new hybrid variety, V316OL, was registered in 2014 and will be grown in 2015. Its average yield is 20% higher than V280OL. Yield and quality were alternatively improved by the genetic progress of new varieties. The average yield increased from 2.5 t/ha in 2004 to 3.3 t/ha in 2014. Important progress was also observed in relation to oil quality. Linolenic acid content decreased from 3.5% on average in 2009, to 2.5% in 2014. Oleic acid increased to 79% between 2007 and 2012 (V141OL), then decreased to 77% with V280OL from 2012 to 2014. This evolution, yielding a more heat-stable oil, is not only due to a varietal progress and environmental consequences, but it is also the result of a successful partnership among all the players of the production chain. Farmers are well aware of the importance of good agricultural practices to produce a high-quality HOLL oil, with low linolenic acid content (long rotation, soil tillage to limit volunteers, avoidance of conventional-HOLL seed mixing, distance between HOLL and conventional WOSR fields, etc., Baux et al. 2013). A cost/benefit analysis, based on gross margin evolution for each sector in the value chain, showed that the economic benefits related to the HOLL WOSR segment were significant and were shared by all the players along the value chain (Pellet 2011).

Conclusions: The development of a new type of WOSR allowed for the establishment of an additional WOSR market in a relatively short period. To satisfy producers as well as the industry, continuous efforts are made to optimize quality in terms of yield and oil quality. New varieties are tested each year and efficient crop management is investigated to secure this new market.

References:
Major proteins of canola have distinct structural and physico-chemical properties

**Background:** Commercial interest in canola protein for food use has resurfaced due to the increased demand for plant proteins. Besides the nutritional indicators, the functional performances which directly relate to the protein composition determine positioning of canola protein products in the food protein market. Protein products recovered from de-oiled canola contains storage and structural proteins of the seed. Crucifer seed storage proteins cruciferin (CRU, 11S) and napin (NAP, 2S) compose the majority of recoverable proteins from canola. Limited information (Wu & Muir, 2008) is available on the structural basis of functional potential and performance of canola CRU and NAP. Most of the canola protein isolates described in scientific literature are CRU and NAP mixtures in various proportions.

**Objectives:** Comparative investigation of structural and physico-chemical properties of 11S and 2S storage proteins of canola seeds to predict functional performances.

**Methods:** *Brassica napus* seeds produced in greenhouse conditions were de-oiled with hexanes and meal protein was extracted at pH 8.5 using Tris-PO4 buffer containing 0.75 M NaCl. Cruciferin and napin isolation and purification was according to Bérot et al. (2005). Structural and physico-chemical property characterization of these two proteins was by electrophoresis (native, 1D and 2D) and spectroscopic (UV-cD, fluorescence and FT-IR) methods. Amino acid composition was determined according to official method of analysis.

**Results:** CRU and NAP obtained from this purification process had >98% protein based on total N. Hexameric nature of CRU was confirmed and it was consisted of polypeptides ranging from 56-19 kDa. Un-dissociated NAP had molecular mass of 13 kDa that resulted in 10 and 7 kDa polypeptides upon S-S bond reduction. Presence of isoforms was evident for both CRU and NAP. Amino acid profile and isoelectric focusing profile confirmed the basic nature of NAP and neutral nature of CRU. Napin showed shallow endothermic transition with peak denaturation temperature at 96°C and Cruciferin showed at 90°C. Cruciferin had very low solubility between pH 3 and 8 but become nearly 90% soluble at pH 2 and 10 while napin showed 100% solubility between pH 3 and 9 with a slight depression at pH 7.4. Secondary structure of CRU was sensitive to pH change while NAP was not. Neutral salts at 0.2 and 0.5 M concentration enhanced solubility of CRU across pH 3 to 10 but solubility of NAP was affected negatively.

**Conclusions:** Major storage proteins of canola have quite distinct structures and molecular characteristics. Therefore protein products obtained from canola will have different properties and functionalities based on the abundance of these two proteins. Separate recovery of these two protein types will enable to make use of their inherent value more effectively.

**References:**
Nitrogen value for ruminants of regular solvent-extracted rapeseed meal

**Background:** The solvent-extracted rapeseed meal (RSM), which is produced in France, presents a certain between- or within-factory variability. This variability can modify the nitrogen value for ruminants.

**Objectives:** The aim of the study realized in 2012 was to compare in sacco nitrogen effective degradability (NED) and in vitro nitrogen enzymatic degradability (DE1) of different RSM in order to verify the relation between these criteria used for the prediction of nitrogen value for ruminants.

**Methods:** In sacco and in vitro measurements were conducted on 15 samples of RSM, quite representative of the existing variability studied with 35 samples of RSM collected previously by Chapoutot et al. (2011). In sacco measurements were done as described by Michalet-Doreau et al. (1987) with a double latin square including 3 cows and 6 replicates. The data measured in this study have been integrated in the new “Systali” model described by Chapoutot et al. (2013) to calculate the new nitrogen values of RSM.

**Results:** The present values of nitrogen enzymatic degradability (DE1) are much lower than those obtained for 2 decades, which were exploited for the elaboration of the prediction model of nitrogen effective degradability (NED) nowadays used in France. The reduction of the DE1 values of RSM is certainly related to an increase in the temperature of the treatments used in the extraction processes. The results of in sacco measurements allowed to confirm the NED values proposed in the Tables INRA (2007) for this feed and to validate the accuracy of the prediction model of NED. Moreover, these new results lead to increase the precision of the estimation of NED from current DE1 values. The data measured in this study have been integrated in the new “Systali” model to calculate the new nitrogen values of RSM. Compared to INRA 2007 ones, the new Systali “Table” values are modified, with a higher PDIN/PDIE ratio (higher PDIN value and lower PDIE value). A simulation was done with the new calculator “Systool” in order to quantify the effect of the inclusion of RSM in maize silage-based diets for dairy cows upon its real nitrogen values. The digestive interactions, much more precisely taken into account in the “Systali” model, tend to slightly increase the nitrogen “Ration” values of RSM (especially the PDIN value) compared to the “Table” ones.

**Conclusions:** These results do not call into question the model validity of the prediction of NED with DE1. The mean values of the 15 samples of this study are very close to those of the INRA 2007 ones.

**References:**
Collaborative inter-laboratory study on glucosinolates analysis for ISO 9167-1 revision

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Background: Analyzing glucosinolates (GSL) in rapeseed and other Brassicaceae remains crucial to ensure the quality of the seeds for certification, trade and the meal for animal feeding. Reverse phase liquid chromatography of the desulfo-GSL was standardized (ISO 9167-1, 1995) as the reference method and revision became necessary because of new safety and efficiency requirements.

Objectives: The aim was mainly to substitute the methanol, considered toxic, used for the extraction procedure, extend the scope to other Brassicaceae, test a rapid isocratic mode of elution, and finally, determine the precision data of the revised method.

Materials and methods: Six samples of seeds (3) and meal (3) from France (3) and Canada (3) with various GSL contents (from 1.5 to 150 µmol/g) were prepared by appropriate homogenization-division, and sent to each of the eighteen laboratories from thirteen countries which agreed to participate to the collaborative. Two samples were Brassica juncea with low and high GSL contents. Duplicate analyses were required with gradient and isocratic HPLC elution modes, the gradient elution (reference method) being applied in priority. The statistical treatment was performed according to ISO 5725-2 with Cochran and Grubbs tests to detect stragglers and outliers.

Results: Eighteen laboratories sent results for the gradient elution mode and only six for the isocratic one. The ring-test was carried out without noticeable difficulties for the gradient method, especially for the revised operating mode part (extraction by ethanol 50%, no replicate, purification method for sulfatase). Some problems still remained regarding the availability of the internal standard glucotropaeolin required for the isocratic elution.

For the gradient mode, the relative standard deviations of repeatability (RSDr) and reproducibility (RSDR) ranges were 1.2 - 6.1% and 9.2 - 21.0%, respectively. For the isocratic mode, the ranges of RSDr and RSDR were 0.4 - 3.1% and 12 - 59.0%, respectively. The relative bias between results from the two elution modes ranged from -1.4 to 10.3% for the five samples with GSL contents higher than 5 µmol/g, and +41% for the sample containing less than 2.5 µmol/g GSL. The number of retained laboratories for the isocratic mode did not meet the ISO requirements (6 or 5 vs. 8) for a collaborative study.

Conclusions: Comparison with previous ring-tests performed in 1988 and 1992 indicated that precision data obtained for the present study were similar, even better in the tested range of GSL content than previous precision data. The added data for the mustard samples (B. juncea) allow now to include this type of seed in the scope of the revised standard. Although results from both elution modes were similar, the isocratic mode should be only included in the informative annex, the gradient elution mode being the reference method.
Glucosinolates and by-products in rapeseed meal related to hydrothermal processing

**Background:** For safety reasons, rapeseed meal (RSM) is usually desolventized with strong hydrothermal treatments, leading to various levels of residual glucosinolates (GSL), and protein solubility. The RSM nutritional quality may be then lowered for monogastrics, due to GSL breakdown products whose reliable and "easy to use" indicators lack.

**Objectives:** The study aims to propose and test methods for identifying and quantifying breakdown products from GSL, and myrosinase activity. The relationship between hydro-thermal treatment of RSM and the routes of the GSL degradation should also be helpful to predict the anti-nutritional potential of the meal in the diet.

**Materials and methods:** Preparation of material to be tested: 1) GSL extracts were purified from seeds of *Brassica napus*, *B. campestris* and *Crambe abyssinica* leading to batches with different GSL profiles. 2) 2 batches of 1.5 kg rapeseed seeds (one of them was previously blanched with steam to inactivate the myrosinase) were pressed and deoiled with hexane then desolventized at low temperature. The two RSM batches were treated by either dry or wet toasting at 110°C from 15 to 90 min by the means of a bench-cooker, to promote different routes for GSL breakdown.

**Analysis:** GSL according to ISO 9167-1, isothiocyanates (ITC), nitriles and oxazolidine-2-thiones (OT) by GC-MS and GC-FID, myrosinase activity by enzymatic assay and spectrophotometry. Methods were applied on purified extract of GSL first, then on the RSM samples collected in the bench-cooker after 30 min of treatment.

**Results:** 1) Methods of analysis for GSL, OT and ITC were successfully tested on purified GSL extracts under various conditions to breakdown GSL into OT, ITC and nitriles. Recoveries and balance were consistent with the reaction stoichiometry. When purified GSL were added to RSM, recoveries remained at high level for GSL (90-100%), but decreased for OT (40%), nitrile and ITC (10%). The relationship observed between recovery and quantity of added ITC indicated a matrix effect, probably due to interactions with amino-acids.

2) GSL analysis of the eight RSM samples processed on the bench-cooker did not show any effect of the blanching. The effect of the toasting on GSL breakdown was higher when steam was applied (at 30 min: 45% vs. 27%). Nitriles were detected in the blanched RSM while only ITC were detected in the no blanched RSM. These observations were in agreement with the literature data on the GSL degradation and the routes with or without myrosinase activity.

**Conclusion:** Although nitriles and ITC were detected at a low level, these results showed that the GSL degradation route in a processed RSM could be determined by chemical analysis. The residual GSL content and myrosinase activity can be accurately determined and the quantity of ITC or nitriles produced could be then predicted. Nevertheless, as ITC can be linked to amino-acids, further studies are necessary to evaluate their bio-availability, their actual anti-nutritional effects and their impact on the protein quality. The latter can also be directly reduced by the hydrothermal treatment applied to the RSM.
Evaluation of ethanol and 2-propanol for rapeseed oil extraction

**Background:** Oil extraction from rapeseed or sunflower involves preparation of the seeds including conditioning and pressing, solvent extraction and solvent elimination from oil and residue. Hexane is commonly used by industry because of high efficiency and specificity for oil extraction and low energy required for its removal from miscella and meal. Nevertheless, hexane has drawbacks like high flammability, dangerousness for health and environment deleterious effects and then, makes relevant the research for alternative solvent. Ethanol (EtOH) and 2-propanol (2POH) because of their lower toxicity and solvent properties could be candidates.

**Objectives:** EtOH and 2POH were compared to hexane for rapeseed oil extraction. Yield and specificity of the oil extraction were determined and feasibility of EtOH and 2POH recycling in continuous extractors was evaluated.

**Material and methods:** The experiments were performed with a 6 L extractor to simulate a cross flow extraction on a multistage extractor. 2 kg of rapeseed press cake (19% oil, 9% moisture) was extracted by circulating 5 L of solvent during 15 min and the operation was repeated six times with renewing the solvent (EtOH/water (90.4/9.6), 2POH/water (85.2/14.8) and technical hexane). The miscellas from EtOH and 2POH were then cooled to recover the extracted oil by dephasing and coalescence.

**Results:** The oil residue in meal was less than 2% with each of the three solvents. The extraction efficiency was strongly correlated with their hydrophobicity: hexane > 2POH > EtOH and the necessary number of washings for each solvent was 4, 5 and 7 respectively. EtOH and 2POH allowed extracting polar compounds such as glucosinolates (40 - 50% decrease). Tocopherols were better extracted with EtOH (1930 ppm) than with 2POH (818 ppm) or hexane (743 ppm). Due to the extraction of other polar compounds, the protein content of the meal was increased by 3 (2POH) and 4 points (EtOH). Recovery of the oil from the miscella with EtOH and 2POH was performed (yield around 75%) by dephasing at 4°C. Crude oil from alcohols has very low phosphorus content (12 ppm vs. 554 ppm for hexane). In contrast, the enrichment in water after several cycles due to the moisture of the extracted material was critical, particularly for EtOH which properties to extract oil were altered. The solvent also required to be purified to separate and recover polar compounds.

**Conclusion:** Compared to hexane, EtOH and 2POH needed more volume and contact time to completely extract the oil. Partial extraction of polar compounds was observed, that could lead to increase the meal quality (more protein and less glucosinolates) but requires the solvent to be purified. The moisture content in the material and the solvent should be maintained at a low level to make easier the solvent recycling. Greater tolerance for water and improved extraction kinetics were observed with 2POH, but EtOH provided more promising quality improvement for oil and meal and should receive a better acceptance by consumers.
Composition of kernel and hull fractions obtained from rapeseed dehulling

**Background:** Although dehulling oilseeds enhances the meal quality, this process was rarely applied at industrial scale to rapeseed because of the oil-losses in hulls and the possible effect of the glucosinolates concentration in dehulled meal. The knowledge of the bio-chemical composition of both hulls and kernels from current rapeseed cultivars would notably help to optimize the dehulling level to be economically profitable.

**Objectives:** This work aims to characterize the bio-chemical composition of the whole rapeseed seed, the pure kernel and hull fractions, and to study the components variability with various cultivars and cultivation areas.

**Methods:** Six rapeseed cultivars were grown in both Cher and Charente-Maritime areas in France. Dehulling was performed in a vertical-conical impactor and based on the projection by the centrifugal force of the seed to the internal wall of the machine (CETIOM dehuller). Then, kernels and hulls fractions were separated by aspiration and sieving. Seeds were frozen (24 h; -20°C) before dehulling to obtain pure hull fractions. They were previously dried (2h, 60°C), then frozen (24 h; -20°C) to obtain the pure kernel fractions. Contents of water, oil, proteins, ash, fiber, crude cellulose and glucosinolates were measured on whole seeds, pure kernel and hull fractions. ANOVA was used to determine correlations between components.

**Results:** The hull mass fractions ranged from 16.8 to 21.2% of the seed mass. The proportions of the various constituents of the seed located in the hulls were: oil: 2.9%; protein: 11.2%, ash: 30%, Neutral Detergent Fiber (NDF): 73%, Acidic Detergent Fiber (ADF): 80%, lignin: 95%, glucosinolates: 3.7%. The hulls were less rich in oil (8.4% ± 1.6%) than usually reported (10.6-16.4%).

The dried deoiled pure kernel fraction contained 47.8% of proteins, 10.8% of NDF, 6.6% of ADF, 0.5% of ADL, 5.5% of ashes and the major part of the glucosinolates (on average 93.4% of the whole seed). A strong negative correlation was observed between the proteins content of the hulls and their fibers component (R = -0.86, -0.89, -0.70 and -0.78 for the NDF, ADF, ADL and Crude Fiber, respectively). The ANOVA revealed no significant relationship between cultivar, growing conditions and hull content of the seeds, although the oil content and the protein content of the fractions were significantly affected by cultivar and to a lesser extent by the location of the crops.

**Conclusion:** The complete removal of rapeseed hulls could result in high protein meal, which content corresponds to 125% of the proteins of whole seed meal. This high protein meal would have 130% of the glucosinolates of the non-dehulled meal but only 37% of its NDF, 30% of its ADF and 5% of its ADL. Ashes would remain equal while crude fiber would be decreased to 37% of the non-dehulled meal.
Solid/liquid expression behavior of rapeseed during discontinuous pressing

Background: Solid-liquid expression or pressing is a unit operation in which liquid is separated from solid-liquid mixture by mechanical compression. This technology is widely used for rapeseed oil extraction and its control is a major issue in the efficiency of the crushing process since the characteristics of the expeller cake such as the mechanical strength and the porosity, may affect the efficacy of the solvent percolation. Although pressing is mainly performed in oil mills, on continuous flow screw presses, the study of discontinuous pressing is of interest to better understand the relationship between the pressing efficiency, the seed quality, the pretreatments and pressing parameters.

Objectives: As the mechanism of solid/liquid expression from rapeseed seeds during pressing is very complex, this work is devoted to better understand it by modelling the compression behavior of the seeds.

Methods: The impacts of seed pretreatments (dehulling, crushing/flacking and cooking) and pressing parameters (pressure, temperature) on the solid-liquid expression behavior of rapeseed were studied based on the theory of filtration/consolidation. This theory gives a comprehensive approach for description of liquid flow inside compressible porous materials based on the analogy with Fick’s diffusion theory. Indeed, the seed pressability is characterized by a consolidation coefficient \( b \) (m².s⁻¹). In this work, the applicability of a semi-empirical model to calculate this coefficient was investigated. Rape seeds were first pretreated (cooking, flacking) under different conditions. Samples (300 g) of treated or untreated seeds were then compressed using a hydraulic press for 60 min. The pressure and the temperature were varied from 20 to 150 bar and from 20 to 110°C, respectively. For each experiment, the oil yield was determined, the thickness of the press cake was recorded during pressing and the corresponding consolidation coefficient was calculated.

Results: Results showed that the highest oil yield (≈ 70 %) was obtained by pressing flacked and cooked seeds (90°C during 30 min) at 100 bar. On the other hand, it was shown that the employed model allowed the determination of the consolidation coefficient only for pre-treated seeds (cooked, flacked). The compression behavior of untreated seeds cannot be described by filtration-consolidation behavior. For treated seeds, the consolidation coefficient increased with pressure and temperature. For example, it increased from 5 10⁻⁷ m².s⁻¹ to 3 10⁻⁶ m².s⁻¹ when the pressing temperature rose from 20°C to 110°C. In fact, the faster was the cake deformation, the higher was the consolidation coefficient. Experimental and model data adjustment showed that the model reasonably well approximated the experimental data for a consolidation behavior index equal to 0.5. This result reflected that treated seeds presented creep characteristics and, both primary and secondary consolidations of tissue occurred.

Conclusion: The obtained results showed significant effects of seed preparation (crushing/flacking and cooking) and pressing parameters on the pressing efficiency and the consolidation behavior. In the case of prepared seeds, the proposed semi-empirical model allowed a good prediction of the consolidation kinetics without needing to perform long and expensive experiments. The results are profitable to extend the research on continuous pressing.
Chemical composition and nutritive value of meals from yellow-seeded canola

**Background:** *Brassica napus* is the most commonly grown canola species in Canada. In recent years, breeding attempts to increase oil in the seed and reduce fibre content in the meal, lead to the production of yellow-seeded *B. napus* canola and canola-quality *B. juncea*. Earlier research from our laboratory has demonstrated superior quality characteristics (i.e., increased protein and sucrose, and reduced dietary fiber contents) of these meals in comparison with conventional *B. napus* black.

**Objectives:** To evaluate the chemical and nutritive composition of meals derived from yellow-seeded *B. napus* and *B. juncea* canola and to determine amino acid digestibility and available energy contents needed for diet formulations in a subsequent growth performance study with broiler chickens.

**Methods:** Canola meals for this study were produced using a large-scale, pre-press solvent extraction process. Apparent metabolizable energy (AMEn) and standardized ileal amino acid digestibility (SIAAD) of *B. napus* yellow, *B. juncea*, and the conventional meal were determined with broiler chickens from 14 to 19 d of age (AMEn assay), or from 14 to 21 d of age (SIAAD assay) using 6 pens of 6 birds each per treatment. Birds were fed either basal diet (control group) or the basal diet supplemented with 30% of canola meals for AMEn, and test ingredient as a sole source of protein for SIAAD evaluation. The nutritive value of canola meals was further validated using 7 pens of 50 broiler chickens per treatment. Birds were fed wheat/corn/soybean meal-based diets containing 15% of canola meals in the starter (1-10d), grower (11-24d), and finisher (25-36d) phases of the experiment.

**Results:** In comparison with the conventional meal, yellow-seeded *B. napus* and *B. juncea* contained (DM basis) more crude protein (43.4 and 47.2 vs. 41.1%), more sucrose (10.1 and 8.0 vs. 6.6%), and less total dietary fiber (29.8 and 28.9 vs. 35.0%), respectively. The highest content of all essential amino acids (except cysteine) was observed in *B. juncea* meal. The AMEn and SIAAD values for *B. napus* yellow, *B. juncea* canola, and the conventional *B. napus* black were 1865, 2092 and 1902 kcal/kg DM, and 82.5, 83.2, and 81.8%, respectively. Enzyme (multicarbohydrase) supplementation resulted in AMEn values of 2131, 2264 and 1851 kcal/kg DM, respectively. In the growth performance study, BWG averaged 2.32, 2.30, 2.19, and 2.31 kg for the control, *B. napus* black, *B. napus* yellow and *B. juncea* meals, respectively, and no significant difference in FCR between the control and the diets containing canola meals were observed indicating that all types of canola meal could be used effectively and replace SBM in broiler chicken rations.

**Conclusion:** It would appear evident that breeding for low-fiber canola would result in quantitative rather than qualitative changes as evidenced by increased oil, protein, and sucrose contents and decreased fiber content in the seed. Canola meal could be used effectively in broiler chicken rations at 15% in all 3 phases of growth when diets are formulated based on digestible amino acids and available energy contents.

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Changes in seed quality during maturation of double-low winter oilseed rape

**Background:** Oilseed rape is an important crop in China. Over 1.000 million tonnes of rapeseed are produced annually for the extraction of edible oil and the meal is a useful source of protein in livestock feeds. In general, production of high quality seeds depends upon the appropriate time of harvest. However, it is rainy during harvest of oilseed in China, which could result in yield losses. In order to avoid the rainy season, seed should be harvested as soon as possible, when physiological maturity has been reached. This study was done to verify the development stage and to define the optimum harvest date by analysis of changes of seed quality.

**Objectives:** Five double-low conventional varieties and 3 double-low hybrids varieties were used. The samples were collected continuously once per five days and started tentatively on early May.

**Methods:** The materials were sown in multi-point areas in two years. NIR analysis of oil content and glucosinolate content was carried out using a Foss NIR Systems Series-5000. The fatty acid compositions were determined by gas chromatography.

**Results:** The glucosinolate content remained stable during maturation. However, there was a significant increase in oil content until May 16, and then no change. The change of the fatty acid components in seed was very different with each other. The content of erucic acid, stearic acid and oleic acid remained stable during maturation. There was a significant reduction of palmitic acid, but there was a significant increase between linoleic acid and linolenic acid.

**Conclusions:** There are some changes of the oil content in the different year because of the variable climates, but the oil content would reach the highest value in May 16-18 each year, and it is just time to physiological maturity (Li et al, 2009). According to fatty acid composition, seeds could be harvested after physiological maturity, but it is the optimum harvest period after the ripening stage.

**References:**
**Oil quality traits in Canola. Understanding and manipulating saturated fatty acid content**

**Background:** Although Canola oil is already a healthy oil, considered low in saturated fatty acids, further reduction of saturated fatty acid content is a trait of interest in Canola breeding. To better understand saturated fatty acid biosynthesis in the developing canola seed we generated plants with increased total saturate content through micro-RNA mi mediated gene silencing and cDNA overexpression. We also took a non-GM approach to silence individual genes encoding enzymes of fatty acid biosynthesis, generating a series of plants with reduced saturate content.

**Results:** Data indicates that targeting specific members of gene families encoding enzymes of primary metabolism can result in plants with a heritable phenotype of reduced seed saturated fatty acid content with no obvious detrimental effects. Plants producing seed containing more than 40% saturated fatty acid content were comparable to control plants in growth and seed yield, but were slow to establish at low temperature.

**Summary:** A number of examples will be presented with altered levels of seed oil saturated fatty acid content. The potential for further development of new seed quality traits will be discussed.

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Performances of rapeseed meal in dairy farms in France

Background: Zootechnical and economic results registered in farms confirm the interest of rapeseed meal (RSM) in dairy rations. However, the generalization of its use may be limited due to a number of questions that persist about this raw by-product.

Objectives: The objectives were to measure the influence of high levels of dietary RSM on the production and reproduction performances and the sanitary status of dairy herds and to identify the limitations, motivations and interests of the incorporation of RSM in Haute-Marne area.

Methods: Two homogeneous groups of 42 farms on maize silage system composed of matched pairs, were made by multivariate analysis. For the RSM group, the ration included at least 4 raw kg/d of RSM. For the other group (No RSM) diet supplementation was achieved by other nitrogen correctors. Animal performances were recorded for 3 months after herds’ selection during the month preceding the study. The measured variables were: milk production per cow, fat and protein contents, artificial insemination (AI) success, leucocytes and mastitis. The feed cost and feed margin were calculated using purchase prices of concentrates and by-products, and standardized prices for fodder (100 € / TMS for corn silage). The data were processed by variance analysis.

The sociological study was based on the realization of semi-structured interviews to collect the reasons and motivations of farmers to use, or not, RSM. Interviews were also applied to professionals that work with farmers to find out what is RSM image in farms.

Results: It appeared that the addition of a large amount of RSM (4.8 ± 0.7 kg/dairy cow/d of RSM on a total of 5.2 ± 0.7 kg/dairy cow/d of nitrogen corrector) in the ration of RSM group, compared to No RSM group, did not significantly changed the raw milk production (-0.2 kg/d) or fat content (-0.2 g/kg). However, it significantly increased the milk protein content (+0.3 g/kg, P <0.05), although the intake of the two groups was nearly identical. Reproductive performances, with the success rate of first AI on 3-month study (+1.1% success) and the health status of the udder (mammary cell count and % of infected cows/month) were not significantly modified. On the economic front, farms of RSM group compared to the Not RSM group, had a lower ration cost of 0.48 €/cow/day (P <0.001), with a difference of -15.9 €/1000l of milk, and presented a higher feed margin of +0.42 €/cow/day (P <0.05) with a saving of +17 €/1000l.

RSM, as a local and non GMO raw material with attractive price, has a good image among breeders and professionals. However, it is perceived as a less “noble” product compared to soybean meal. The sociological study reveals that feed companies are not particularly favorable to rapeseed meal.

Conclusions: This study confirms that significant amounts of rapeseed meal can be introduced into dairy rations without affecting performances and allow an improvement of production costs of the dairy sector.
Effect of rapeseed meal on milk and cheese quality

**Background:** In France, about 2.3 million tons of rapeseed meal are used in feedstuff and 60% of them for ruminants. Rapeseed meal is actually the first meal included in dairy cows diets because of its economical interest. Nevertheless we observed a lack of references on the ability of milk for cheese production.

**Objectives:** In that context of replacing soybean by rapeseed meal in dairy cows diets, a trial was done in order to compare the quality of milk and cheese of cows fed with soybean or rapeseed meal.

**Methods:** 2 groups of 7 Holstein cows were fed with whole diets composed of maize silage, sorghum and rapeseed or soybean meal for 5 weeks followed by 2 weeks of transition and 5 weeks of inversed diets. Milk of each diet was collected for cheese fabrication.

**Results:** With the use of rapeseed meal replacing soybean meal, cows produced as much milk and this milk was richer in proteins (P<0.01). As well, a significant increase in milk casein content and casein/protein ratio was observed. It was higher than the one observed by Martineau et al. (2013). Rapeseed meal induced a decrease in total and soluble calcium contents of milk without affecting colloidal calcium and phosphorus contents, and induced an increase in soluble phosphorus. Rapeseed meal decreased saturated fatty acids percentage and increased oleic and linolenic acids.

With rapeseed meal, milk coagulation and curd firming times were longer as mentioned by Mietton et al. (2004). Curd firmness and cheese yield decreased with poorer retention of fat in the curd, without any effect on physico-chemical composition of cheese at the end of ripening. Only a few volatile compounds (12.5%) were significantly affected by the diet.

Rapeseed meal significantly enhanced the concentration of ketones and decreased the concentration of esters, sulfur compounds and furans. The diet did not induce rheological differences between cheeses but their texture was slightly more grainy and less sticky with rapeseed meal. The salty and pungent tastes, the “little sour milk” and spicy aromas were greater (P<0.05) with rapeseed meal and broth, egg yolk (P<0.05), hazelnut and butter aromas (P<0.10) were greater with soybean meal.

**Conclusions:** These first results show that rapeseed meal improves the production of milk proteins but modifies the cheese production probably in relation with the increase of casein/protein ratio. Mineral correction of diets or technological modifications of cheese production have to be tested in order to try to correct times of coagulation.

**References:**


Towards understanding the genetics controlling a low saturated fat trait in *Brassica napus* and transfer into commercial inbred canola lines

**Background:** Canola oil from *Brassica napus* is promoted as healthy edible oil since it contains the lowest level of saturated fatty acids (SFA) of any vegetable oil. A shift in cultivation from Polish canola to Argentine canola in the 1990s resulted in an increase in total SFA content (Rakow and Olson 2010; Raney et al. 1999). For the past 13 years, AAFc has been developing stable lines that are low (<5%) in SFA content using a combination of methodologies including interspecific crossing and mutagenesis breeding strategies (Rakow and Olson 2010; Raney et al. 1999).

**Objectives:** 1) Investigate genetic control of total and major SFA components: dominance behavior, maternal inheritance and population distribution of Palmitic acid (16:0) and Stearic acid (18:0). 2) Examine the stability of the low SFA trait across multiple environments within segregating populations. 3) Identify highly productive lines containing the low SFA or lower SFA to use in hybrid combinations or elite crosses.

**Methods:** The low saturate (<4.5%) line was crossed with 10 advanced breeding lines. In 2 seasons, open-pollinated and/or self-pollinated F1:2 and F2:3 seed was harvested from 3 and 2 reciprocal cross combinations, respectively and F1:2 seed from 7 additional one-way crosses. From six of the cross-combinations, doubled haploid (DH) populations were developed from reciprocal F1s. A total of 545 DH lines were evaluated at two locations in 2013 and 388 in 2014. Harvested seed from all trials were analyzed for fatty acid profile including all SFA components using gas chromatography.

**Results:** Based on fatty acid profile analysis of bulked harvested F1:2 seed, the trait appears to be incompletely dominant in all 10 cross combinations. There was little evidence of maternal effect in F2:3 seed from 2 reciprocal cross combinations; however, there was strong evidence of maternal inheritance for 18:0 and total SFA in the F1:2 seed and in the DH populations. The frequency distributions of the F2:3 populations were continuous and normally distributed; however, distributions of all DH populations were bimodal for palmitic acid. Four of the DH populations fit a 1:1 segregation model for palmitic acid suggesting control by a single major gene. Frequency distributions of DH lines were continuous for 18:0 and total SFA. Transgressive segregants for total SFA lower than the trait parent were not present in any population; however, lines with lower SFA with improved agronomics compared to the trait parent were identified. Correlations between sites in 2013 were high (0.90 to 0.99) for total SFA and components across DH populations.

**Conclusions:** Based on these results, it is expected that the low SFA mutations will be useful for overall reduction of SFA within hybrid breeding programs where the trait is only incorporated into one hybrid parent, but reduction of saturates to less than 5% will require the trait to be introgressed into both hybrid parents.

**References:**

Rakow, G., and Olson, T. V. 2010. Breeding and genetic research towards the creation of yellow seeded, low saturated fat *Brassica napus* canola. in: 13th International Rapeseed Congress, Prague, Czech Republic.

Digestibility additivity in growing pigs of phosphorus from biofuel by-products

**Background:** Controlling phosphorus releases is an important environmental issue for swine production. Feed remains the most interesting way to limit its excretion by pigs. The use of Biofuel by-products from wheat and rapeseed has increased in France, but phosphorus digestibility data are little or unknown, even less the additivity when mixed in a feed.

**Objectives:** The aim was the measurement of the true digestibility of phosphorus in rapeseed meal (RSM) and bioethanol by-products from wheat (BBP), and the evaluation of the additivity for this criterion in a two by-products mixture.

**Methods:** The true faecal digestibility (tDP) and the phosphorus retention coefficient (rCP) were measured for two bioethanol by-products (BBP1 and BBP2) and for one biodiesel by-product (RSM) included at 25%, and a mixture of 12.5% BBP1 and 12.5% of RSM (BBP1/RSM) in semi synthetic feeds on five pigs per treatment for 5 days of collection after 14 days of adaptation.

**Results:** The tDP of BBP 1 and BBP 2 was similar (NS) and high (50.4 and 53.1%), but the release of urinary P was important, probably due to low dietary Ca, that induced a drop of rCP (28.7 and 34.3%). The RSM had very close tDP (33.1%) and rCP (32.3%). The association of BBP1 and RSM led to an intermediary tDP (43.7%). As the three diets were significantly different from each other (p<0.001), this result was consistent with the hypothesis of additivity of tDP. The rCP was improved (p<0.01) in the BBP1/RSM (38.4%) compared to the others, probably by balancing the Ca/Pd ratio.

**Conclusions:** True digestibility of phosphorus was evaluated in two bioethanol by-products (≈ 50 %) and in a rapeseed meal (≈ 33 %). The additivity of phosphorus digestibility of a bioethanol by-product and a rapeseed meal when associated in a same feed was confirmed.
Changes in canola seed protein structure and properties during pre-press solvent extraction process

Background: Canola (Brassica napus) meal of pre-press solvent extraction (PSE) is dark, has lower protein solubility and in vitro digestibility than laboratory prepared meal (Garla et al., 1994). Protein is the most valuable fraction of canola meal but protein recovery from PSE meal is difficult and results in protein products having poor functionalities (Pastuszewska et al., 2003). Including all the intermediate steps, pressure, temperature, and solvent environment changes associated with PSE may have direct effect on seed constituents leading to physico-chemical changes of meal components as evidenced by the altered nutritional value of PSE meal (Mustafa et al., 2000).

Objectives: Investigate the changes in the physico-chemical properties and structural details of protein fraction of canola seed during PSE in order to find out the critical processing step/s that affects protein structure and properties.

Methods: Samples from commercial PSE operation were obtained at 5 different stages; 1) feeder, 2) cooker (98-100°C), 3) press (60-100°C), 4) solvent extractor (60-70°C with hexanes), and 5) desolventizer (82°C upper and 112°C lower deck) from three different processing cycles spanned in a 3-month period. Total protein, total oil, protein dispersibility index (PDI), protein solubility at pH 4, 7 and 10, FT-IR spectral characteristics, in vitro protein digestibility with pepsin and pancreatin, available lysine content and total free sugar contents were determined.

Results: The oil-free basis protein content of the meal was at 38.6–41.8% level as the oil extraction progressed. PDI values of the meal were reduced by ~30% after cooking and ~65% upon desolventizing. Complete loss of pH 4 soluble protein fraction (~17.5% in seed) after desolventizing indicated that napin (2S) protein became insoluble. Insoluble protein content started to increase after the cooking step as soluble protein content at pH 7 and 10 was reduced by 20 and 39%, respectively. Desolventizing caused up to 70% reduction in pH 10-soluble protein content. Increases in the beta-sheet and random coil component levels and the changes in the amide I, amide II, -PO3 and C=O related functional areas of FT-IR spectra indicated destabilization of proteins at secondary structure level. Changes in the levels of available lysine and free sugar contents were evident but not the in vitro protein digestibility values.

Conclusions: Processing steps that involve temperature >70°C contributed to the changes in the properties and structural components of canola proteins indicating modifications in the proteins and their association with other seed constituents.

References:
Seed coat color and oil content in *Brassica rapa* populations

**Background:** *Brassica rapa* (B. rapa) is an important vegetable and oilseed worldwide. Most *Brassica* species are black-seeded, such as Chinese cabbage in *B. rapa*. However, there are natural yellow-seeded mutations such as yellow sarson in *B. rapa*. Yellow seeds are associated with significantly thinner seed coat, reduced cell size in all seed coat tissue and larger embryos. These characteristics lead to a low hull proportion and high oil and protein content in the seed (Stringam et al. 1974; Rahman et al. 2001).

**Objectives:** In *Brassica* breeding programs, seed coat color and oil content are valuable traits. In the current study, seed oil content of two *B. rapa* populations were analyzed for the relationship between seed coat color and oil content.

**Methods:** Two hundred recombinant inbred lines (RILs) have been developed using two crosses (yellow sarson x turnip rape [BU] and yellow sarson x Chinese cabbage [SR]). Each of the 200 RIL were analyzed for oil content and seed color. Seed color was classified into yellow, light yellow, brown and dark brown. Near-infrared spectroscopy (NIR) and solvent extraction methods were used to collect oil content data.

**Results:** Using the NIR method in the BU population, oil content of yellow-seeded RILs increased by 1.91% and 2.56% when compared to brown-seeded and dark brown-seeded RILs, respectively. When using the solvent extraction method, oil content of yellow-seeded RILs increased by 3.01% and 2.86% when compared to brown-seeded and dark brown-seeded RILs, respectively. In the SR population using the NIR method, oil content of yellow-seeded RILs showed an increase of 3.58% and 1.4% when compared to brown-seeded and dark brown-seeded RILs, respectively. Under the solvent extraction method, oil content of yellow-seeded RILs showed an increase of 3.3% and 0.85% when compared to brown-seeded and dark brown-seeded RILs, respectively.

**Conclusions:** NIR and solvent extraction results showed that yellow-seeded *B. rapa* had higher oil content compared with brown and dark brown seeds. Since *B. rapa* is one of the parental species of *Brassica napus*, yellow-seeded *B. rapa* progenies can be integrated into canola breeding for higher oil content.

**References:**


Biochemical properties of *Brassica napus* diacylglycerol acyltransferase 1

**Background:** Diacylglycerol acyltransferase (DGAT) catalyzes the acyl-CoA-dependent acylation of diacylglycerol to form triacylglycerol (TAG), which is the main component of seed oil. In *Brassica napus*, the level of DGAT activity has a substantial effect on the flow of carbon into seed oil (Weselake et al., 2008). Although membrane-bound DGATs have been studied for several decades, their mode of action and regulation remains poorly understood due to difficulties associated with purification.

**Objectives:** Studying the properties of DGAT1, a key enzyme in oil biosynthesis, will increase our understanding of the regulation of seed oil formation.

**Methods:** Recombinant *B. napus* DGAT1 (BnaC.DGAT1.a) was purified through initial solubilization in 1% n-dodecyl-β-D-maltopyranoside (DDm), followed by cobalt affinity chromatography and size-exclusion chromatography (Caldo et al., 2015). The properties of BnaC.DGAT1.a in detergent micelles were characterized including the oligomerization states, specific activities at each stage of purification and substrate specificities. The first 113 residues of BnaC.DGAT1.a corresponding to a soluble regulatory region was expressed in *Escherichia coli* and purified. The role of this domain in oligomerization was investigated as well as its ligand binding properties.

**Results:** Purified BnaDGAT1 in DDM micelles predominantly exists as dimer, which can associate further to form tetramer. The major dimeric form was purified about 126-fold over the DDM-solubilized fraction and was found to prefer different acyl-CoAs in the following order: α-linolenoyl-CoA > oleoyl-CoA > palmitoyl-CoA > linoleoyl-CoA > stearoyl-CoA. The N-terminal domain (BnaC.DGAT1.a1-113) was eluted from size-exclusion chromatography as a tetramer, which is in agreement with previous studies. Purification of truncated N-terminal domain revealed that residues 49-113 can associate to form dimer while the first 48 residues appear to be involved in tetramerization. When dissolved in a membrane-mimetic environment, BnaC.DGAT1.a1-113 assumes α-helical structure as revealed by circular dichroism (CD). This N-terminal region was implicated as an allosteric exosite for acyl-CoA as revealed by previous Lipidex-1000 binding studies. In the current study, ligand perturbation analysis monitored using CD also showed that oleoyl-CoA could interact with this regulatory domain. In addition, isothermal titration calorimetry showed that this interaction is an exothermic process and follows the sequential model for positive cooperativity.

**Conclusions:** DGAT1 appears to shift between two oligomerization states, a phenomenon that may be related to regulation of enzyme activity. This process appears to be mediated by the N-terminal region, which can bind acyl-CoAs through sequential positive cooperativity.

**References:**


Optimized rapeseed oils rich in endogenous micronutrients ameliorate risk factors of *atherosclerosis* in high fat diet fed rats

**Background:** Micronutrients in rapeseed such as polyphenols, tocopherols, phytosterols and phospholipids in rapeseed exert potential benefit to *atherosclerosis* (Szydlowska-Czerniak 2013). Some part of these healthy components substantially lost during the conventional refining processing. Thus some new processing technologies, such as cold pressing, dehulling-cold pressing and microwave pretreatment-cold pressing, have been developed to produce various endogenous micronutrient-enriched optimized rapeseed oils.

**Objectives:** The aim of this study is to determine the effects of the various endogenous micronutrient-enriched optimized rapeseed oils on atherosclerosis risk factors in rats fed a high-fat diet.

**Methods:** Rats received the high-fat diet containing 20% casein, 35% maize starch, 15% glucose, 5% cellulose, 3.5% mineral mixture (AIN-93M), 1% vitamin mixture (AIN-93M), 0.2% choline bitartrate, 0.3% DL-methionine and 20% fat. The refined rapeseed oil or optimized rapeseed oils obtained with various processing technologies as lipid source. After 10 weeks of treatment, plasma was assayed for oxidative stress, lipid profiles and inflammation.

**Results:** Micronutrients enhancement in optimized rapeseed oils significantly reduced plasma oxidative stress, as evaluated by the significant elevation in the activities of CAT and GPx as well as the level of GSH, and the significant decline in lipid peroxidation. Optimized rapeseed oil with the highest micronutrient contents obtained by microwave pretreatment-cold pressing reduced the levels of TG, TC and LDL-C as well as IL-6 and CRP in plasma.

**Conclusion:** These results suggested that the optimized rapeseed oils rich in endogenous micronutrients might contribute to prevent atherogenesis and make them very promising functional food in cardiovascular health promotion.

**References:**
Determination for aflatoxins B1 in rapeseed oil using a rapid time-resolved fluorescent test strip

**Background:** Rapeseed oil is one of the most extensive consumed oils in China, covering about 50% of plant oil. Aflatoxin B1 (AFB1), produced by fungi, can seriously contaminate rapeseed oil throughout the whole production process, including before and after harvest and storage, transportation, and consumption. AFB1 has been proved to be toxic, mutagenic, teratogenic, and carcinogenic and can cause hepatic and extrahepatic carcinogenesis of humans and livestock. Thus, it is urgent to develop rapid determination method for AFB1 determination in rapeseed oils. Although numerous of studies have focused on the AFB1 determination in rapeseed oil, such as HPLC, LC MS/MS or ELISA, etc., these methods were hampered in practice due to the requests of specific instruments, skilled operators, laboring sample preparation, or the fake positive results. It is important to develop a rapid, sensitive, on-site determination for rapeseed oils. In this abstract, a test strip-based rapid, sensitive, on-site determination for AFB1 in rapeseed oil was developed and validated.

**Objectives:** It is to establish a rapid, sensitive, on-site determination for AFB1 in rapeseed oil, along with simplification of sample preparation. This method can afford a promising alternative for rapid, sensitive and on-site determination of AFB1 in rapeseed oil. This proposal can be used in the determination of AFB1 in rapeseed oil throughout the whole production. It also can provide key technique support in the government monitoring of the food safety in rapeseed oil.

**Methods:** The monoclonal antibody against AFB1 was domestically produced using the hybridoma antibody technology[1]. The covalent coupling of Eu3+-microbead and anti-AFB1mAb or IgG were followed a modified EDC conjugation method.[2] The time-resolved fluorescent test strip was made by using the XYZ3050 dispensing Platform, CM4000 Guillotine Cutter, and LM4000 Batch Laminator (BioDot, Irvine, CA, USA). The immunoreagent concentrations on the test line and control line, along with the reaction conditions were optimized, respectively. This test strips were further evaluated, including limit of detection, linear range, recovery, precision, specificity, and agreement between the results of the test strip and HPLC method. Real rapeseed oil samples were finally tested using this proposed test strip.

**Results:** The results of Eu-based time resolved fluorescent test strip was recorded by using a portable reader within 10 min. Results found a limit of detection of 0.10 ng/ml, a considerable linear range of 0.10-10.0 ng/ml, recoveries from 85.10% to 115.25%. It was also found of excellent of precision (RSD 6.8%) and specificity (RSD below 9.7%). Little cross-reactivity was found with AFB2, AFG1, AFG2, and AFM1. Excellent agreement of results between the test strip and HPLC methods was recorded, while 50 rapeseed oil samples from local markets were determined with the test strip, finding an AFB1 content of 0.35-4.26.

**Conclusions:** This test strip can be widely applied in the rapid on-site determination for AFB1 in rapeseed oils. This method allowed the rapidness, high sensibility, and low cost. It can be widely employed in the determination of AFB1 in rapeseed oil and further used in food safety monitoring.

**References:**


Identification and quantification of glucosinolates in *Camelina sativa* seed by ultra performance liquid chromatography-photodiode array detector-tandem mass spectrometry

**Background:** *Camelina sativa* L. Crantz belongs to *Brassicaceae* family. It is heat and drought tolerant and is thought to require fewer agronomic inputs and is therefore a good alternative crop for marginal lands. Its seed contains three glucosinolates: glucoarabin (9-(methylsulfinyl)nonylglucosinolate or GS9), glucocamelinin (10-(methylsulfinyl)decylglucosinolate or GS10) and 11-(methylsulfinyl)undecylglucosinolate or GS11). The structures of these glucosinolates are similar to that of glucoraphanin (4-(methylsulfinyl)-butylglucosinolate). The degradation product of glucoraphanin is thought to be an anti-cancer compound.

High performance liquid chromatography (HPLC) is widely used to determine content of intact glucosinolates and desulfoglucosinolates. However, a typical HPLC gradient run time takes 25 to 50 minutes for intact glucosinolates and desulfoglucosinolates, hindering the camelina line screening process.

To better utilize camelina, we developed an Ultra Performance Liquid Chromatography-Photodiode Array Detector-Tandem Mass Spectrometry (UPLC-PDA-TQD) method for the analysis of desulfoglucosinolates with a reduced LC run time and solvent consumption. Then we surveyed six accessions for their glucosinolate content.

**Objectives:** Determine if UPLC can shorten the LC run time for glucosinolate analysis. Screen several Canadian camelina accessions to determine their glucosinolate profile and content.

**Methods:** Glucosinolates were extracted and converted to desulfoglucosinolates based on AOCS Official Method Ak 1-92. The analyses employed a Waters UPLC-PDA-TQD system equipped with a BEH Shield RP18 column (2.1 x 50 mm; 1.7 µm) and held the initial conditions (100% water at 0.8 mL/min) for 0.3 minutes before a linear solvent gradient of 0% to 25% acetonitrile (v/v) over the next 6.7 min. The desulfoglucosinolates were quantified at 229 nm and identified by monitoring the characteristic loss of 162.2 mass units using MS/MS constant neutral loss scans.

**Results:** UPLC reduced the typical LC run time of 25-50 minutes for camelina desulfoglucosinolates to less than 10 minutes and consumed 60% less acetonitrile. Thus, it is possible to analyze more than 140 samples in a 24 hour period. When a typical HPLC separation is used, only 30-60 samples can be analyzed a day. The camelina accessions surveyed contained 21 to 31 µmol glucosinolates per gram of seed. GS9 (4.6-7.0 µmol/g seeds), GS10 (13.7-20.2 µmol/g seeds) and GS11 (2.5-3.6 µmol/g seeds), were identified and quantified. Among them, GS10 was the major glucosinolate. Additionally, we identified a putative minor glucosinolate, 8-(methylsulfinyl)octyl glucosinolate (GS8), in all six accessions based on its mass spectrum. GS8 content ranged from 0.06 to 0.13 µmol per gram seeds. While GS8 is known in other plant tissues, this is the first report of GS8 in camelina seeds based on our knowledge.

**Conclusions:** UPLC reduced typical LC run time from 25-50 minutes to less than 10 minutes, greatly increasing our screening throughput and ability to detect minor glucosinolates in camelina and other crucifer species. The six camelina accessions surveyed contain glucosinolates at 20-30 µmol/g seeds, with GS10 being the major glucosinolate in all accessions. A putative minor glucosinolate (8-(methylsulfinyl)octyl glucosinolate) was identified in these accessions. Further work is needed to confirm the identity of the minor glucosinolate.

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Physiological enhancement for alleviation of low temperature stress in canola (*Brassica napus*) using abscisic acid

Low temperature stress severely influences canola (*Brassica napus* L.) cultivation as an important worldwide biofuel and oily plant. Seed priming is an approach for improving germination characteristics under stress. Plant hormones like abscisic acid (ABA) have significant potential as priming materials. The present study was conducted to enhance low temperature tolerance in *Brassica napus* by seed priming with ABA. In this study, the effect of seed priming with three concentrations of ABA (50, 100 and 150 μM) was investigated on germination of *Brassica napus* cv. Zarafm under low temperature stress (3°C). ABA-priming, especially with 50 μM concentration increased germination percentage and vigor index and decreased mean germination time. Higher concentrations of ABA were not as effective as low concentration. Protein content was affected and reduced by ABA treatment. The highest and the lowest level of soluble proteins was located in the embryo axis (245.2 mg/ml), and seed coat (67.3 mg/ml), respectively. ABA-priming promoted superoxide dismutase and peroxidase activity for detoxification of superoxide and hydrogen peroxide radical, respectively. However, polyphenol oxidase was not affected. Seed coat exhibited the highest activity for the antioxidant enzymes.
Overexpression of annexin, \textit{AnnBj2} confers salinity and ABA tolerance on mustard plants at germination and early seedling growth

\textbf{Background:} Annexins belong to a multigene and multifunctional group of proteins, which can bind the plasma and endo membranes in a calcium dependant manner. Gene expression studies of these proteins carried out by different groups revealed their differential expression pattern in different tissue and developmental stages of the plants. Their expression pattern significantly changes in response to different stress (biotic and abiotic) conditions and signalling molecules. Overexpression of some annexins conferred abiotic stress tolerance to the transgenic plants. In mustard, \textit{Brassica juncea}, six annexins (\textit{AnnBj1}, \textit{AnnBj2}, \textit{AnnBj3}, \textit{AnnBj4}, \textit{AnnBj6} and \textit{AnnBj7}) were reported to exhibit differential expression pattern in response to salinity, oxidative stress, wounding and signalling molecules (Jami et al. 2009). Among these, \textit{AnnBj2} showed strong upregulation at transcript level to all the four signalling molecules (abscisic acid, methyl jasmonate, ethephon and salicylic acid). This member of the annexin family may play an important role in plant signalling in response to stress condition.

\textbf{Objectives:} To study the function of \textit{AnnBj2}, we planned to overexpress it in the native system under the control of a constitutive promoter and generate several overexpression lines. These transgenic lines will be analysed under different abiotic stress conditions and compared to the wild type.

\textbf{Methods:} To study the function of \textit{AnnBj2} gene, it was overexpressed under the control of CaMV35S constitutive promoter. Overexpression lines of this gene were generated in \textit{Brassica juncea} through Agrobacterium mediated transformation using cotyledonal petiole as explant. The green shoots generated in the presence of 15mg/l kanamycin as selective agent were transferred to the rooting media. The plantlets were shifted to green house and allowed to self-pollinate. The seeds of stabilized lines in T3 generation were used for germination assays. For germination assay, seeds were sterilised and placed on 1/2 MS media supplemented with 0, 100, 200 and 300 mM NaCl. To check the ABA sensitivity seeds were placed on 1/2 MS media supplemented with 0, 4 and 8 \textmu M ABA. Emergence of radicle was used as a scorable marker for the germination. After six days of incubation, germination percentage, fresh weight, root and shoot length and proline contents were measured.

\textbf{Results:} Putative transgenic mustard plants were characterized for the constitutive expression of \textit{AnnBj2} and lines overexpressing \textit{AnnBj2} were selected for further analysis. \textit{AnnBj2} overexpression lines showed significantly higher germination percentage than the wild type (control) seeds under different concentrations of NaCl and ABA. In response to NaCl and ABA stress, \textit{AnnBj2} overexpression lines showed enhanced growth in terms of fresh weight, longer root and shoot length and increased proline content than the wild type genotype.

\textbf{Conclusion:} As the \textit{AnnBj2} overexpression lines were more tolerant to NaCl and comparatively less sensitive to ABA than the wild type genotype at germination stage, it could be a good candidate gene for genetically modifying the crop to perform better under saline conditions.

\textbf{Reference:}

The impact of sulfate restriction on seed yield, oil and meal quality of winter oilseed rape depends on the ability to remobilize sulfate from vegetative tissues to reproductive organs

**Background:** Compared with other crops such as cereals, oilseed rape is particularly sensitive to S deficiency because this crop is characterized by a high demand for sulfate. Nevertheless, our knowledge about the stages of development the more sensitive to S limitation or the physiological processes that are involved in S management by oilseed rape subjected to sulfate restriction remains largely unknown.

**Objectives:** Our goal is to investigate the response to S restriction applied at two crucial growth stages (bolting or early flowering) on (i) the S management (uptake vs remobilisation) and (ii) the seed yield, oil and meal quality in winter oilseed rape (cv Capitol).

**Methods:** Sulfate limitation was applied at bolting or early flowering stages under controlled conditions. A pulse-chase 34SO42- labelling method was carried out in order to study the S fluxes from uptake and remobilization at the whole plant level. The contribution of tonoplastic SULTR4-type transporters in the efflux of sulfate from the vacuole of source leaves was studied at the transcriptional level. Seed quality was carried out by NIRS analysis for oil composition and two dimensional gel for seed proteome.

**Results:** When S limitation was applied at bolting or early flowering stages, the leaves are the most important source organs for S remobilization during reproductive stages. By combining 34S-tracer with biochemical fractionation in order to separate sulfate from other S-compounds, it appeared that sulfate was the main form of S remobilized in source leaves. Tonoplastic SULTR4-type transporters were specifically involved in the sulfate remobilisation from leaves in case of S limitation. The seed yield and quality from S-limited plants were dramatically reduced compared to control, suggesting that the increase of both S remobilization from source leaves and root proliferation in order to maximize sulfate uptake capacities, were not sufficient to maintain the seed yield and quality. When S limitation occurred at the early flowering stage, oilseed rape can optimize the mobilization of sulfate reserves from vegetative organs (leaves and stem) to satisfy the demand of seeds and maintain the seed yield and quality as far as possible. Results also indicated that the stem may act as a transient storage organ for remobilized S coming from source leaves before its utilization by seeds. Proteomics approaches in mature seeds have revealed that the protein quality of seeds was reduced depending on the severity of S limitation and was associated with a reduction in S-rich seed storage protein accumulation (such as Cruciferin Cru4) which favoured S-poor seed storage protein (such as Cruciferin BnC1).

**Conclusions:** The impact of sulfate restriction on seed yield and quality of oilseed rape depends on the ability (i) to remobilize sulfate from vegetative tissues to reproductive organs and (ii) to maximize the sulfate uptake. Consequently, these physiological traits such as S remobilization, root proliferation, or transient S storage in stem, could be used in breeding programs to select genotypes with high S use efficiency and elevated seed yield and seed quality.

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Effect of seeding date on oil and protein content in winter rapeseed cultivars

Background: According to the total production of major oilseeds in the world, rapeseed takes the second place just after soybean (USDA 2014). It is primarily grown because of high quality oil and proteins that are used as animal feed (Vujaković et al. 2014). The seed contains 40-48% oil and 18-25% proteins. Seeding date is an important management factor in rapeseed farming as the proper seeding time allows sufficient crop growth and development to reach satisfactory yield and yield components.

Objectives: The aim of this study was to determine the effect of seeding date on oil and protein content in four winter rapeseed cultivars during two cropping seasons. In Southeast Europe such studies are scarce.

Methods: The field trial was conducted to study the response of oil and protein content of four rapeseed cultivars (Banaćanka, Slavica, Express, and Valeska) to six seeding dates (SD1-21 August, SD2-31 August, SD3-10 September, SD4-21 September, SD5-1 October, SD6-9 October). The two-year trial was carried out from 2009 to 2011 at Rimski Šančevi, near Novi Sad (Serbia) as randomized complete block design (RCBD) with four replications on 6 m² plots. Row spacing was 25 cm between rows and 5 cm within rows. Oil content (NMR method) and total proteins content (Kjeldahl method) were determined after harvest. Analysis of variance (ANOVA) via GenStat (trial version) was used for statistical analysis.

Results: Oil content ranged between 41.19% (cv. Valeska) and 42.69% (cv. Express). Significantly lowest oil content across seeding dates was found in SD6 (40.67%), and highest in SD4 (41.86%). Valeska showed significantly highest mean protein content (21.54%). Protein content was highest in SD6 (20.18%). Between years there were highly significant differences in both traits. Increased oil content in the second year (2010/2011) was related to weather conditions which were favorable for rapeseed. However, protein content was significantly higher in the first year (2009/2010). The three-way ANOVA for both traits indicated that all main effects (year, cultivar, sowing date) and first order interaction (year × sowing date) were highly significant. The cultivar gave the highest contribution to oil content (50.7%). The contribution of year to oil content was also high (34.4%), and to seeding date 4.2%. Protein content was primarily affected by year (70.5%), while cultivar contributed 9.7% and seeding date 5.3%.

Conclusion: Seeding date significantly affected rapeseed oil and protein content. Oil content decreased with delayed seeding. Study results may be helpful in recommending optimal rapeseed seeding date in this region.

References:
Ecosystem carbon balance of field-scale canola and barley in central Alberta

Background: Canola acreage is close to 8 million ha and 90% of the production is exported. This is a large environmental footprint nationally and globally. Sustainability is a market issue that impacts canola market accessibility. Central Alberta is known as a region of intensive canola and barley production, resulting in relatively high grain and seed yields. The deep, black Chernozem cropland, originally broken from grassland, with a high soil organic matter content, may lose a quantity soil carbon each year until equilibrium is attained (Malhi et al. 2010; National Inventory Report 2011). The eddy covariance system is micro-meteorological method for studying within year ecosystem carbon (C) dynamics as well as annual ecosystem C-flux which includes soil and crop organic-C sources and sinks.

Objectives: To compare the ecosystem carbon balance for hybrid canola and barley crops grown intensively at a field scale using input levels typical of the Central Alberta region.

Methods: Hybrid canola and spring barley were planted on approximately May 16 and harvested using farm-scale equipment over four years in 20 ha fields from 2011 until 2014 at Lacombe, AB. Each field was equipped with an eddy covariance system. Instrumentation and measurement were similar to Skinner (2008). Net ecosystem-C flux (NEE) was determined (365 d yr⁻¹) on a daily, seasonal and annual basis. Grain, residue and root dry matter (DM) yields were determined and converted to C (kg ha⁻¹) to investigate organic-C inputs and outputs. Thus biome-C change could be determined.

Results: Canola was an NEE-C sink in all years, while barley was a sink in 3 of 4 years. Contribution of residue and roots to the NEE-C pool averaged 3.3 and 4.4 Mg C ha⁻¹ yr⁻¹ for barley and canola, respectively. However, harvested grain and seed yield were high, averaging 4.3 and 3.3 Mg DM ha⁻¹ yr⁻¹ for barley and canola, respectively. At the biome-C level, after removal of grain and seed-C from NEE-C, both barley and canola crops were sources in 3 of 4 years averaging 0.3 and 0.5 Mg C ha⁻¹ yr⁻¹, respectively.

Conclusion: Both barley and canola managed intensively were capable of balancing NEE-C annually, however neither species balance the system-C pool after harvested grain or seed was accounted for. However, the Century model estimates that cropland recently broken from grassland, with a high organic matter content as used in this research, loses approximately 0.5 Mg C ha⁻¹ yr⁻¹ (National Inventory Report 2011) due to respiration of organic matter. Thus this intensive farming practice should be considered sustainable.

References:
Fertilization of winter oilseed rape with urea with inhibitors

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Background: Fertilization with urea without inhibitors may in some cases be less effective (Watson et al., 1990). Due to hydrolysis, grow losses of nitrogen to the atmosphere, which may be 5 to 20%, in extreme conditions of up to 50% (Harrison and Webb, 2001). For the stabilization of urea is used nitrification inhibitors - Dicyandiamide (DCD) (e.g. fertilizers Alzon*46 and Ensin) and urease - N-(n-butyl) thiophosphoric triamide (NBPT) (e.g. fertilizer UREAstabil). Urea with nitrification inhibitors or urease inhibitors has a high assumption for the application in oilseed rape nutrition. This will increase the effectiveness of nitrogen fertilization and also limit the pollution of groundwater and air (Šimka et al., 2010).

Objectives: The aim of the experiment was to determine the effect of urea with nitrification inhibitor (Alzon) and urease inhibitor (UREAstabil) after autumn and spring nitrogen fertilization in relation to yield, oil content, and TSW.

Methods: Field experiments were established in the years 2009/10 - 2011/12 on Research Station of Czech University of Life Sciences Prague in the locality Cervey Ujezd (GPS 50.0718794N, 14.1701372E, altitude 398 m asl). The average annual air temperature is 7.7 °C and an average total annual precipitation is 549 mm. Autumn dose of nitrogen was 45 kg N/ha and was applied in fertilizers: Alzon, UREAstabil and urea. At the spring fertilization we applied fertilizer Alzon and UREAstabil only twice (90 + 65 kg N/ha) in spring, while the control LAV fertilizer (ammonium nitrate with limestone) four times (40 + 35 + 50 + 30 kg N/ha). Fertilizers Alzon and UREAstabil contain 46% of nitrogen, same as urea. Fertilizer LAV has 27% of nitrogen.

Results: Autumn application of urea with inhibitors increased the yield by 1 – 2% compared to unstabilized urea (Alzon - 3.65 t/ha, UREAstabil - 3.70 t/ha and urea - 3.62 t/ha). Ureas with inhibitors decreased slightly oiliness (by 0.2 to 0.5%) and increased TSW (0.5 – 0.7%). Spring combined application of urea with inhibitors decreased slightly yield (by 1 – 4%) compared to the standard LAV (Alzon - 3.46 t/ha, UREAstabil - 3.55 t/ha, LAV - 3.60 t/ha). But the advantage is saving of two applications of urea with inhibitors compared to the standard LAV (four spring applications). Fertilizer Alzon increased oiliness by 0.7% and TSW by 1.7%. Fertilizer UREAstabil increased only TSW by 0.4%.

Conclusions: Oilseed rape nitrogen nutrition using urea with inhibitors proved very useful. In autumn application is evident increase of yield. Due to the gradual release of nitrogen, it is possible to combine doses in spring and thus save the number of passes over the field.

References:
Brassica carinata germplasm development as an industrial oilseed

Brassica carinata is a blackleg resistant, stress tolerant oilseed brassica that has compared favorably with other brassica species in tough conditions such as the historically hot, dry, and windy southernmost regions of the Prairies and extending into the Northern Great Plains of the United States. Very good shatter and lodging resistant characteristics are native to this species. Carinata produces an oil that has been found to be highly suited as a feedstock in the manufacture of Bio Jet fuel and biodiesel. The meal component, obtained after the oil has been extracted, is protein rich and has potential to serve as an excellent animal feed additive. Agrisoma Biosciences Inc., in partnership with Agriculture and Agri-Food Canada, have commercialized adapted varieties of B. carinata as a sustainable source of industrial oil feedstock. In response to increased demand and interest from new markets, Agrisoma has established a carinata breeding program seeking to develop high yield potential, high oil content, and earlier maturing varieties. As part of this development process, Agrisoma Biosciences has collected and evaluated a large number of Brassica carinata accessions and lines from various world collections. Germplasm evaluation has been focused on agronomic adaptation to geographies with potential for commercial acreage, seed quality characteristics, and genetic relationships among the germplasm collection. This work has required a number of partnerships and collaboration of effort. For example, headed by a progressive University of Florida extension team in Northern Florida, suitable carinata backgrounds and agronomic practices are being evaluated for its use as a winter crop option in the Southeast United States. Further, working in close partnership with Agriculture and Agri-Food Canada, germplasm has been evaluated on a molecular basis using GBS methodology, to estimate genetic distance among the various original accessions and breeding lines. The information generated in these studies have been useful in understanding the plasticity and genetic diversity available in this species.
New oil seed crops for new French cropping systems

Background: A review was made in 2013 on different seed oil species including Brassica carinata and Camelina sativa. The objective was to describe for each species the agronomic requirements and to focus on the production of molecules which could have some interest for industrial uses. This work showed that the most interesting species at both agronomic and quality point of view was B. carinata and camellina, because of their short cycles and composition of their seeds.

Objectives: A research program called ANOI* aims to study new oil seed crops with short crop cycle which can produce molecules that can have interest for industrial targets to introduce them in French cropping systems, in particular to develop crop rotations with 3 crops in 2 years. The objective of the project is to collect information on phenology, accumulation of reserves during seed filling and on yield potential.

Methods: Two oil seed crops (B. carinata and Camelina) were tested by CETIOM in 2014 in experimental field plots in comparison to spring rapeseed in two experimental trials located in the north of France: the first one to explore the variability of nitrogen supplies and the second one to analyze the genetic variability of camellina. Another trial was sown in the south of France, in order to study the behavior of the three species in limiting water availability situation. An experiment was also conducted in glasshouse in 2014. In all these experiments, development stages and composition of the seeds (oil, fatty acids and protein concentration) of the three species was measured.

Results: The first results indicate that the total length of the cycle for B. carinata and camellina exceeds 100 days with the varieties tested. B. carinata, such as spring rapeseed, is very sensitive to insect attacks (especially blossom beetle Meligethes aenus) which affect greatly yield while C. sativa reaches more promising yields under French northern conditions. At a quality level, B. carinata can be a source of erucic acid and C. sativa can provide high levels of alpha-linolenic acid, complementary to fatty acids found in rapeseed oil.

Conclusions: The field and glasshouse experiments indicate that the length of the cycle is too long to make 3 crops in 2 years. It will be useful to have earlier varieties. Experiments will be renewed in 2015, in which genetic variability of camellina will be extend in order to identify behavior of specific lines for yield or composition of the seed.

References:
PERISCOPE: A new phenotyping experimental device for individual root and shoot investigations in reconstructed canopy until harvest, under field-like conditions

Background: Cultivars with improved Nitrogen Use Efficiency (NUE) are central to face economic competitiveness and environmental sustainability of rapeseed. Under low N input, a higher NUE can be achieved by increasing N uptake efficiency (NuPe) and/or by improving N reserve use. NuPe results from root development and specific nitrogen uptake. Exploring the GxN variability of this efficiency remains challenging, as a reliable quantification of fine root fraction and N uptake remains difficult in the field, and as alternative systems such as rhizotrons are not suitable to manage rapeseed plants until harvest under field-like conditions.

Objectives: Our aim was to design and test a new semi-controlled culture system for growing rapeseed plants, able to fit the following requirements: i) quantitatively access to each plant fraction, including fine roots, ii) be relevant until harvest to estimate final yield components, iii) generate individual plants in a reconstructed canopy, with phenotype and yield similar to those of field grown plants, and iv) combine measurements at plant and crop scales.

Methods: The PERISCOPE system consisted of individual columns of 1m high and 0.16m diameter, grouped by 24 into boxes of 1m³, placed outside and therefore submitted to field climate. Each column was filled with substrate and regularly supplied with nutrient solution. In boxes, the space between columns was filled with soil, in order to ensure the thermal insulation of root parts and the implantation of two rows of border plants around the columns and thus to mimic environmental conditions of the field. Six seeds were sown on each column. After thinning out, a single plant was kept per column, leading to an homogeneous canopy of 35 plants/m², which was grown for the whole crop cycle until harvest. Various combinations of substrates and N treatments were experienced in 2 locations. Plants were harvested at 7 sampling dates during the crop cycle and divided into tuberized roots, fine roots, leaves, stems, pods and seeds. Biomass and N content of the different plant fractions were measured and the leaf area index (LAI) was assessed.

Results: This new system was successful at collecting plant fractions, including fine roots, until harvest under field-like conditions. In addition, the canopies generated were similar to those obtained in the field, showing equivalent aerial DM, N content and harvest index. Results showed a good repeatability between plants and revealed a significant contribution of fine roots to total plant biomass (up to 23 %). The PERISCOPE system was also adequate to discriminate substrate and nutrient effects on plant traits.

Conclusions: This new experimental device proved to be effective to characterize the phenotypic responses of rapeseed to N limitation and should be helpful to screen large genotypic variability for NUE traits, including contribution of fine roots to NuPe. A comparison of 8 contrasted genotypes is currently under progress using the PERISCOPE system. This device could also be useful to study root interactions in associated cropping combining several species whose location can be modulated by columns positioning.
Canola establishment – How important is seed size?

**Background:** ‘Moisture seeking’ seed is a common practice in some regions in Australia. It involves the placing of seed into soil moisture below dry topsoil, often at depths below what is generally optimal for a particular variety or crop. The main aim of this is to ensure a crop is planted and germinates within the optimum planting window, with producers often prepared to accept a reduced emergence to ensure timely crop establishment.

**Objective:** The aim of this research was to evaluate the seed characteristics (seed size and variety type; hybrid or open-pollinated) that improve crop emergence, especially in challenging soil conditions, such as when planted relatively deep.

**Methods:** Six canola varieties were planted at three seeding depths (25, 50 and 75 mm) in five plot experiments over the 2012 and 2013 seasons in central-west New South Wales (NSW), Australia. Emergence data were collected for each sowing depth and plots were harvested for grain yield. The varieties included three hybrids and three open-pollinated (OP) varieties, with one triazine tolerant (TT) variety and two non-TT varieties within the hybrid and OP groups. Seed of the hybrid varieties was generally larger than seed of the OP varieties.

A pot study was also conducted where seed of each variety was graded into two separate size classes; large (2.0-2.4 mm) and small (1.0-1.4 mm), and planted at 25, 50 and 75 mm to determine the relative importance of seed size and hybridity on observed emergence differences.

**Results:** In all five small plot experiments deeper sowing reduced canola emergence, however emergence of the hybrid varieties was significantly better than emergence of the open-pollinated varieties at all seeding depths. Grain yield was reduced by deep sowing to a lesser degree than plant emergence, with the hybrid varieties being higher yielding at all seeding depths.

In the pot study, there were only small treatment effects at the 25 mm sowing depth. As seeding depth increased from 25 to 75 mm canola emergence was reduced, however the effect was greatest for the small seeded OP varieties (94% reduction) and least for the large seeded hybrid varieties (18% reduction). Planting large seed improved canola emergence within both the hybrid and OP varietal groups.

**Conclusions:** This research has shown that planting relatively large canola seed will improve canola emergence, especially when conditions at planting are challenging. Growers can capitalise on these findings by either purchasing large commercial seed or by grading their own retained OP seed for larger seed size.
**SuMoToRI**, a model to simulate growth and sulfur content in rapeseed (*Brassica napus* L.) until the onset of pod formation

**Background:** There is an increasing demand for oilseed rape to meet worldwide needs for the food and biofuel industries. Its high sulfur (S) requirements during the vegetative phase can drastically impact yield and oil quality. In a context of S oligotrophy, S fertilization management before the onset of pod formation has become a major issue. Modelling S requirements and allocation within the main plant compartments could be a helpful approach to correct deficiencies occurring during these early stages.

**Objectives:** In this study we developed a predictive model of plant growth until the onset of pod formation in relation to S availability (SuMoToRI, Sulfur Model Towards Rapeseed Improvement).

**Methods:** SuMoToRI is a compartment model that distinguishes green leaves, fallen leaves and the rest of the plant. Effective organ growth is calculated by taking into account air temperature, photosynthetically active radiation and S availability. The model works with a daily time increment and at the plant level. SuMoToRI was calibrated and evaluated with independent datasets from greenhouse experiments in ample and limiting S supply conditions until the onset of pod formation.

**Results:** SuMoToRI predicts the dynamics of the leaf area, the distribution of both dry biomass and the amount of S within the plant compartments. It can also simulate the fractions of S that is used for growth defined as structural and metabolic functions (S organic compounds) and of S that is stored as sulfate (major mineral form) within these compartments. Key processes were introduced with a mechanistic approach: (i) the growth S demands for the green leaves and the rest of the plant match the critical demands required for organ expansion and are estimated by construction of the critical S dilution curves; (ii) the daily S offer was estimated as the sum of daily S uptake and a pool of mobile S shared by all organs, which is enriched by S remobilized from senescing leaves. SuMoToRI is run with few parameters. The model gave satisfying predictions of growth dynamics and took into account an S-organic pool required for growth and an S-mobile pool used for remobilization towards growing sinks. The parameter values were not dependent on S uptake except for variables underlying carbon (C)-related processes, that is, radiation use efficiency, specific leaf area and C leaf allocation, which required specific calibration in severe S limitation. These results suggested that S restriction might affect C assimilation and that the imbalance in the nitrogen to sulfur (N:S) ratio might also affect photosynthesis.

**Conclusions:** The results also bear out the crosstalk between N, S and C metabolisms that deserves to be further underpinned. SuMoToRI introduces novel features compared to other mineral-driven crop models because it considers the specificities of S nutrition in a crop that undergoes leaf senescence during the vegetative phase. It can be a relevant framework not only to analyse early deficiencies in oilseed rape but also to model the responses to S nutrition of other crops.

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Pod shatter resistance in WOSR hybrids improves yield reliability and can bring additional benefits in the rotation

Pod shatter (a seed dispersal strategy inherited from the wild ancestors of Brassica napus) has long been regarded as a problem in cultivated varieties of Winter Oilseed Rape. Adverse weather conditions at maturity, delayed harvest and diseases which can affect the pods such as Phoma, Sclerotinia, Verticillium and Alternaria all have the potential to increase seed loss, as can pre-harvest and harvest operations themselves. Historically, many techniques have been used to try to reduce (or compensate for) these losses; including Chemical ‘stickers’, Insurance against bad weather, slow combine speeds and special headers. If reduction is unsuccessful then specific volunteer management strategies may be required. All of these things give rise to additional expense and trouble for the grower.

In 2011 at the International Rapeseed Congress in Prague we presented the first results of breeding for seed pod shattering resistance in WOSR hybrids. These hybrids were developed from inherited resistance linked to the Ogura restorer segment.

In this poster we will show supportive data on the effectiveness of this resistance and examine the breadth of information relating to the value and use of the pod shatter resistance trait from first commercialisation to the present day. Data is included from laboratory tests, field trials and experiences with commercial crops, and demonstrates how this trait is adding to the future sustainability of the crop.

In addition to the direct yield benefit for the grower, we will examine the impact on reduced inputs, increased harvest flexibility, lower slug populations throughout the rotation, better control of plant populations due to lower volunteer numbers and the specific management advantages when using Clearfield™ varieties.
The effect of variety, fertilization and sowing date on overwintering of oilseed rape

Background: Diversifying cereal monoculture (maize, wheat), main cropping system in Serbia, can provide numerous agronomic and environmental benefits, particularly with winter crop, like oilseed rape. Despite the advantages of autumn-seeded oilseed rape, winter survival present a big constraint in our continental climate with cold winter. Leaf number and plant development before winter may be a limiting factor for overwintering of winter rapeseed (Waalen et al. 2013). Cessation of growth and sufficient levels of photosynthates are necessary for the cold acclimation and freezing tolerance during the. Beside above plant characteristics also some extreme soil and weather conditions have large influence on it, and this is reason why is winter survival a very complex trait (Rapacz et al. 2014).

Objectives: There are many laboratory procedures in research work for measuring freezing tolerance: plant tissue water content; ion leakage from plant cells after a freezing stress or meristem regrowth after plants are subjected to freezing temperatures. But, in commercial production field survival is the only one and final decision method concerning winter survival and freezing tolerance of oil seed rape. So, we decided to investigate multiyear influence of some of cropping practices (sowing date, fertilization and varieties) on winter survival of oilseed rape.

Methods: The field trials with four replication were conducted at Institute of field and vegetable crops, 45°19’N, 19°49’E, altitude 70 m, on a chernozem soil. It present four year data, from two trials: planting date with (20.VIII, 01.IX and 10.IX) and autumn fertilization (0, 20 and 40 kg ha⁻¹, equal amount of each N, P₂O₅ and K₂O) both experiments were a split-plot design with sowing date or fertilizations as the main plot and four varieties (Jet neuf, Banacanka, Samuraj and Falcon) as the sub-plot (4m *1.7m).

According to numbers of plants per row in the beginning of November, after plant growth had ceased, and in the beginning of March, when growth had resumed, was calculated percentage of winter survival. Data processed by ANOVA, using software MSTATC, Model 20, combined over year. The effect of each treatment /interaction was assessed by its partitioning in their total sum of squares.

Results: According to F-test in both trials significant effect (p<0.01) on winter survival had year and variety and interaction year*variety. Their partitioning in total sum squares was 47, 10 and 30% respectively in fertilization trial and 27, 21 and 37% in planting date trial. Planting date, fertilization and all other interaction had no significant effect. Overall average survival was 83.7 and 85.2% respectively for fertilization and date trial. The lowest was in 2002/03 around 73.0% and the highest in 2005/06 93.7% and 91.0% respectively. The lowest winter survival had variety Samuraj 76.4 and 74.3% and the highest variety Falcon 87.2 and 89.3% respectively.

Conclusions: Field winter survival of oilseed rape mainly depended of weather fluctuation and crop variety, while cropping practice had no significant influence on it.

References:
Screening of germplasm with resistance to pod shattering in rapeseed

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**Background:** Shattering resistance is an important trait for rapeseed varieties suitable for mechanical harvesting, therefore screening of germplasm with pod shattering resistance is the basic work for the breeding of shattering resistance. Although there is some genetic variation in *B. napus*, the degree of resistance is considered inadequate to reduce loss of shattering in harvest management. However, Wen et al screened two lines (Chinese origin) with shatter resistant, which could potentially be used as parents to develop new varieties for improved this trait.

**Objectives:** To screen available germplasm with good resistance to pod shattering among semi-winter accessions from China in *B. napus*.

**Methods:** In the present paper, we employed two methods, including random impact test (RIT) and shattering percentage test in the field, to evaluate pod shattering resistance of 75 accessions of *B. napus* in three years.

**Results:** These accessions tested displayed wide variation in shattering resistance index (SRI) and shattering percentage (SP) in the field, which ranged from 0.01 to 0.70 with the variance coefficient (CV) of 70.70% for SRI, and from 1.58% to 55.51% with CV of 62.53% for SP in the field. The simple correlation analysis showed there was no correlation between pod SRI and SP in the field when all accessions included. However, for the accessions with strong or weak shattering resistance, there was no different between two methods, except for the accessions with the shattering resistance between strong and weak ones. The SP and the wall thickness of pod had significantly negative correlation (*r* = −0.429), pod SRI and the wall thickness of pod had significantly positive correlation (*r* = 0.687). Thus, the pod wall thickness can be used as an auxiliary index to screen shatter resistant germplasm.

**Conclusions:** A germplasm, Ny with high pod shattering resistance was identified in this study. Ny has thicker wall and smoother surface of pod. The shattering percentage of Ny was 7.74% under adverse condition (two weeks after the maturity), and 1.58% under normal condition (one week after maturity). The pod SRI of Ny was 0.70 in 2011 and 0.48 in 2013, higher than that of rest accessions. Ny will be a valuable resource for rapeseed breeding for pod shattering resistance in the future.

**References:**
Organic winter oilseed rape response to N fertilisation and preceding agroecosystem

**Background:** Increased knowledge on how to use organic amendments will increase crop productivity in organic farming and reduce nutrient losses to the environment. In order to obtain better estimates of the optimum nitrogen (N) fertilisation rate (OptN) in spring to organic winter oilseed rape (WOR), the yield response to organic fertilisers as affected by different previous crops and sites need to be determined.

**Objectives:** The overall objective of this work was to contribute to a better synchronisation of spring N fertilisation to soil N supply and crop N requirements in order to improve yield level and N use efficiency in organic WOR. The specific objectives were to a) determine the effect of autumn and spring application of organic fertilisers on yield of organic WOR grown after various preceding crops; and b) investigate whether OptN in spring can be determined by various factors.

**Methods:** The effect of autumn and spring application of organic fertilisers on the yield of organic WOR with various preceding crops was studied at eight organic farm sites in southern Sweden in 2008/2009 and 2009/2010, in a two-factor experiment. Autumn N fertilisation (F1) comprised Biofer (meat meal pellets), applied at 0 and 50 kg N ha⁻¹, and spring N fertilisation (F2) comprised increasing rates of Vinasse (liquid by-product from yeast industry): 0, 50, 100, 150, 200 kg N ha⁻¹. How soil mineral N, N uptake in late autumn and early spring, plant available soil N during spring and summer (SoilNplant) and yield level was associated with OptN in spring was investigated.

**Results:** At three of the eight field sites, autumn N application resulted in significantly higher yield than no fertilisation in autumn (140, 320 and 400 kg ha⁻¹ yield increase). At OptN in spring, yield increased significantly at five of the eight sites, by on average 780 kg ha⁻¹. The greatest yield increase was 1393 kg ha⁻¹ at OptN= 190 kg N ha⁻¹, at a site with grass ley (14-years old) as preceding crop. The lowest yield increase, 240 and 390 kg ha⁻¹ at OptN=41 and 61 kg N ha⁻¹, respectively, was obtained at two sites with white clover and red clover as preceding crop.

**Conclusions:** Autumn N application of Biofer cannot be recommended after white and red clover as preceding crops since it had no impact on yield. Autumn N application can be justified and small yield increases can be expected after previous crops such as grass leys. Late sowing and autumn fertilisation in this study resulted in high levels of soil mineral N in late autumn and thus an increased risk of N losses. Spring N fertilisation with Vinasse can be recommended when the preceding crop is grass ley, since the yield increase and N use efficiency were the highest. Spring N fertilisation does not increase yield after a leguminous preceding crop, as pasture or clover. Regression analysis showed that soil mineral N, N uptake in late autumn, SoilNplant and yield level must be considered when estimating spring N fertilisation.

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Examining the feasibility of several oilseed crops for production in the Inland PNW

**Background:** The USA remains highly dependent on fossil fuel imports and interest in producing bio-jet fuel from vegetable oils has increased. *Brassicaceae* oilseed crops have high oil content that make them suitable for biofuel production. In addition, *Brassicaceae* crops have shown rotational benefits when grown with small grain cereals that predominate in the dry land Pacific Northwest (PNW). However, few studies have examined species adaptability to PNW growing conditions, the physiological growth pattern, basic plant morphology, reaction to biotic and abiotic stresses, or rotational effects.

**Objectives:** In this study we examined yield and oil content of three fall-planted *Brassicaceae* species (*Brassica napus*, *B. rapa*, and *Camelina sativa*) and six spring-planted species (*B. napus*, *Sinapis alba*, *B. juncea*, *B. carinata*, *B. rapa*, and *C. sativa*) to evaluate the adaptability of these oilseed crops in our region.

**Methods:** Field trials were grown at two locations in Idaho, in 2013 and 2014. Winter and spring planted trials were grown adjacent, although they were not inter-randomized. Weeds and pests were controlled throughout by application of appropriate herbicides and insecticides. At maturity, crops were swathed (winter only) and combine harvested and a subsample was taken from each plot for oil content analysis. After harvesting the oilseed crops, the complete trial area was planted to winter wheat to determine the rotational effect of the previous oilseed crop.

**Results:** Highest seed yield was obtained from the two winter canola (*B. napus*) cultivars (‘Wichita’ 4,491 kg ha⁻¹ and ‘Amanda’ 4,186 kg ha⁻¹). Winter industrial rapeseed cultivars, ‘Durola’ and ‘Dwarf Essex’, also produced high seed yields of 3,780 kg ha⁻¹ and 2,732 kg ha⁻¹, respectively. Yields of spring cultivars and species were significantly lower than the winter ones. The highest yielding spring cultivar was DKL 30-42 1,954 kg ha⁻¹ (*B. napus*) followed by ‘Oasis’ 1,632 kg ha⁻¹ (*B. juncea*). The highest oil yield was produced by Wichita, 2,142 L ha⁻¹, Amanda, 2020 L ha⁻¹, and Durola 1983 L ha⁻¹; all winter *B. napus* cultivars. Oil yield of spring crops was markedly lower, particularly from the low oil content mustard species (*S. alba* 346 and 336 L ha⁻¹) and camelina (*C. sativa* 467 L ha⁻¹).

Estimating simple farm returns at harvest 2014 prices showed highest returns from winter canola or rapeseed at $2273 ha⁻¹ ($906 acre⁻¹). Farm returns from spring canola or rapeseed was $842 ha⁻¹ ($341 acre⁻¹). Farm returns on mustard were equal to spring canola due to traditionally higher seed value for condiment spices. Over all cultivars and species there was no significant difference in following winter wheat yield potential.

**Conclusions:** Winter canola or winter rapeseed (*B. napus*) cultivars show best potential for producing bio-jet fuel feedstock crop in the PNW. Highest potential spring crops include canola or rapeseed (both *B. napus*), although one *B. carinata* line (AAC-A110) also performed well. Note that the winter crops were planted on fallow ground (common in many PNW areas), and the yield is realized over two years.
Isolation of MYC2 gene from *Brassica napus* and analysis of its expression in response to salt stress

**Background:** The *Arabidopsis* gene MYC2 encodes a key transcription factor in an abscisic acid dependent pathway in *Arabidopsis* (Nakashima, and Yamaguchi-Sh, 2005). The expression of this transcription factor is induced by salt and drought stress. Moreover, Myc2 has been shown to be involved in various plant responses to biotic stresses (Lorenzo et al., 2004).

**Objectives:** Isolation of MYC2 gene from *Brassica napus* (*BnMYC2*) and analysis of its responsiveness to salt stress

**Methods:** Using the specific primers designed from the exon of *Arabidopsis* MYC2 (*AtMYC2*), a cDNA fragment of *BnMYC2*, with the size of 962bp, was cloned and sequenced from the rapeseed cultivar SLM046. Using the new primers and RACE technique, the 5’ and 3’ cDNA ends of *BnMYC2* were isolated and then sequenced. Alteration in the expression of *BnMYC2* was monitored upon exposure of Canola to salt stress.

**Results:** The sequence of the cloned fragment of *BnMYC2* showed great homology to *AtMYC2*. Application of RACE technique helped us to obtain the 5’ end fragment with the length of 574bp and the 3’ end fragment with the length of 687bp. The sequenced fragments were assembled to attain full length cDNA of *BnMYC2* proximate to 2047bp. The coding regions of *BnMYC2* cDNA showed 81% identity to those of in *AtMYC2*. Moreover, bioinformatic analysis revealed that the protein encoded by *BnMYC2* is a hydrophilic protein which contains a bHLH domain. Furthermore, quantitative analysis showed an upregulation in the expression of *BnMYC2* in the leaves and roots of canola under salt stress conditions. In roots, maximum expression of *BnMYC2* was observed after three hours of salt stress, while in leaves, *BnMYC2* expression reached the highest level after six hours of salt stress.

**Conclusion:** This study provides the first insight into the responsiveness of a MYC2 transcription factor of canola in stress response of the plant to salt. Here we show that Myc2 expression would be elevated upon salt stress, and the response could be monitored in the roots as well as shoots. Our results suggest that MYC2 may play a crucial role in the response of canola to abiotic stresses.

**References:**


Calculation of N fertilizer rates in winter oilseed rape in order to maximize farmer incomes and to minimize greenhouse gas emissions

The Decision Support System (DSS) “Réglette azote colza” provides an estimation of the optimal spring N application rate in winter oilseed rape (WOSR) based on a N balance sheet method. This DSS is used by about one third of farmers cultivating WOSR in France. The parameterization of the DSS was made to achieve an expected yield without accounting for economical nor environmental issues, and did not accurately account for organic fertilizers nor for the effects of legumes in crop rotation. The objective was to develop a new version of this DSS, in order to maximize the farmer incomes, minimize the greenhouse gas emissions, and to better account for organic fertilizers and legumes.

Methods:
The N rates (X) are calculated at the end of winter as follows:

\[ X = (b \times y + Rf) - (Pi + Ri + M + Mha + Mpro1 + Fleg + Fass), \]

Where b is the amount of N uptake per unit of grain yield, y is the target yield, Rf and Ri are the mineral N in the soil at harvest and at the end of winter, Pi is the N uptake by plants at the end of winter, M, Mha and Mpro1 are N mineralization parameters, and Fleg and Fass are the effects of grain legumes respectively as a previous crop or as an intercropped green manure. In soils where the measurement of mineral N is not possible, this equation is adapted.

The users of the DSS must estimate y and Pi, while all other terms of the equation are parameters that were either determined in this study or taken from the previous version of the DSS. Pi is calculated from fresh biomass measurements and from parameters that estimate the amount of N per unit of fresh weight (Cbw and Cew, respectively for the beginning and for the end of winter).

Results and conclusions: For all the French regions, the same median values of b, Cbw and Cew were obtained: respectively 6.45 kgN/100kg grains\(^{-1}\), 57.5 and 70.0 kgN/ha\(^{-1}\), compared to the previous regionalized values that ranged from 6.5 to 7.0 for b and from 65 to 75 for Cbw and Cew. In our study, a great variability of values was observed between experiments. For instance, 10% of values were under 5.30, 46.2 and 55.3, respectively for b, Cbw and Cew, and 10% above 7.37, 73.9 and 93.8. Hence, the use of the median values may over- or underestimate the rate of N fertilizer. Thus, a range of values was tested. The best combination, resulting to the maximum gross margin and to the lower greenhouse gas emission among the combinations that maximized the gross margin, was 7.0, 50 and 65, respectively for b, Cbw and Cew, which corresponded approximately to a 30% risk of underestimation of the N rate.
Uniform plant stands enhance the synergy of pod formation and seed yield in canola

**Background and Objectives:** Canola (*Brassica napus*) productivity is often limited by non-uniform plant establishment, especially in areas with short growing seasons, such as western Canada, where crop plants usually have a limited time span to adapt and compensate for yield losses due to poor plant establishment. It is unknown how the uniformity of canola plant stands would affect pod set and seed yield. This study quantified the impacts of uniformity of canola plant stands on pod formation, seed set, and seed yield.

**Methods:** Field experiments were conducted at 16 site-years across the different soil-climatic zones of western Canada. At each site-year, a hybrid canola cultivar was seeded at various seed rates to achieve plant densities of 20, 40, 60, 80, and 100 plants per m², each with uniform stands in comparison with non-uniform stands. To determine the nature of the treatment by site-year interactions in a quantitative manner, we used the Nonmetric Multidimensional Scaling test to group site-years with different treatment effects. As a result, Lacombe 2010, Lacombe 2011, Lacombe 2012, Melfort 2010, and Melfort 2011 were grouped as the “high-yielding sites,” and the other 11 site-years were grouped as the “low-yielding sites.”

**Results:** At both high- and low-yielding sites, uniform stands had significantly higher seed yields than non-uniform stands at the same plant density. At low-yielding sites, uniform stands increased seed yield by 32%, 21%, 8%, and 7% at the plant densities of 20, 40, 60, and 80 plants m⁻², respectively, compared with the corresponding non-uniform stands. At high-yielding sites, the uniform stands increased seed yields by 21% versus the non-uniform stands at plant densities lower than 60 plants m⁻². There was a linear relationship between the number of fertile pods per m² and seed yield, with the uniform stands having a greater intercept (Y = 1329.6 + 0.144X) and higher adjusted R² (0.74) compared with non-uniform stands (Y = 742.8 + 0.191X, R² = 0.63). The relationship between fertile pod number and seed yield was altered by plant uniformity. An evenly distributed plant community increased the synergies between pod formation and seed set, and promoted seed development, whereas non-uniform stands increased interplant competition, and reduced the distribution of optical radiation limiting the development of fertile pods.

**Conclusions:** Across the diverse climate-soil zones on the Canadian prairies, canola stand uniformity has a significant impact on canola productivity, with uniform stands increasing seed yield by up to 32% at the low-yielding sites and by 20% at the high-yielding sites compared to non-uniform stands. Thus, achieving uniform plant stands may serve as a major approach to maximize the yield potential in canola.
Rapeseed-legumes intercropping: A new strategy to improve *Brassica napus* crop

**Background:** *Brassica napus* L. requires high level of Nitrogen (N) fertilization to achieve significant yield. The improvement of agricultural practices is crucial to decrease N inputs and reduce their negative environmental impacts. Intercropping with legumes offers an environmentally sustainable source of N through the process of biological N fixation which can complement or replace N fertilizers (Garg and Geetanjali, 2007). Several studies have shown that cereal-legumes intercropping has a positive impact on cereal yield and grain N content under low N inputs (Malézieux, 2009). However, the management of brassica-legume intercropping requires more studies taking into account different legume species and/or N-fertilization levels.

**Objectives:** Using additive and substitutive designs, objective of this work consists to test if different legume species (contrasting in their above and belowground architecture) could have benefit effect on rapeseed crops. The aim of this study is to characterize intercropping effects on rapeseed (and legume) dry weight production, N and S nutrition, N soil reserves and N derived from atmosphere (%Ndfa).

**Methods:** Rapeseed and three legumes species (*Lupinus albus* L., *Trifolium incarnatum* L., *Vicia sativa* L.) were grown in monoculture and in rapeseed/legume intercrops (RL) in a greenhouse under low N conditions. One (M1) or two (M2) rapeseed monoculture were grown to study additive and substitutive designs, respectively. Plants were grown in pots filled with a mixture of soil and sand, watered exclusively with demineralized water. After three months, plants were harvested to determine root and shoot biomass (DW). N, S contents and the δ15N of plants were determined using an IRMS spectrometer linked to a C/N/S analyzer. The %Ndfa of legume was estimated according to Shearer and Kohl (1986).

**Results:** The DW and N amount of rapeseed were similar between M1, M2 and RL. N amount in soil increased in RL compared to M1 and M2. Moreover, the %Ndfa of legumes was significantly higher in RL than in monoculture legumes (LL). No difference between the S content of rapeseed was observed in M1, M2 and in RL whereas the S content of legumes is significantly lower in RL than in LL.

**Conclusions:** This study shows that legumes enhance the soil-N pool in RL. In addition, we have shown that the %Ndfa of legumes was significantly increased in RL conditions than in LL while their biomass and S content were lower. These results suggest that in intercropping conditions, soil S resource is preferentially used for rapeseed growth with a negative impact on legume biomass. To verify this hypothesis, effect of different levels of S fertilizer on RL growth will be perform in field conditions.

**References:**
 Prediction of rapeseed yields is improved by combining several climate variables using linear regression

Background: Pre-harvest yields predictions are important for development organizations in order to improve the supply chain. At the present time, it is impossible to know the crop yield before the harvesting operations.

Objectives: We plan to build a statistical model to predict the crop yield each year. This model is built at regional scale, based on climatic data.

Methodology: Based on monthly climate variables, a statistical model is fitted to the time series of yield and climate from 1976 to 2014 (Michel and Makowski, 2013). The model is used for main French regions of rapeseed production (Burgundy, Lorraine, Centre, Picardy, Poitou-Charentes). Monthly weather data are provided by the French Organisation of Weather Forecast (Météo France). Yields data are provided by the French Ministry of Agriculture (AGRESTE). The accuracy of the yield predictions obtained with the models was then assessed by cross-validation.

Results: Depending on regions, the uncertainly varies from 1.5 to 3.2 q.ha-1. We are going to improve the yield predictions by combing the model results, assessment with field measurements.

References:
AZODYN-rapeseed: A biophysical model for decision support in nitrogen fertilization and harvest prediction

**Background:** The precise adjustment of nitrogen fertilization is difficult on rapeseed because the crop can absorb high amounts of nitrogen during autumn, a part of plant nitrogen is lost when the leaves fall during winter and the Nitrogen Use Efficiency (NUE). The dynamic crop model AZODYN (Jeuffroy and Recous, 1999) rapeseed computes nitrogen dynamic supply in the plant and the soil, but it is necessary to test the model on various conditions in France and improve the model.

**Objectives:** We develop a methodology to improve the AZODYN model based on the comparison between field observations and simulations. Then, we test the model in various situations of soil (deep or superficial), of climate (Western or Eastern region) and plant growth.

**Methods:** AZODYN-rapeseed is built up and assessed on twenty situations. AZODYN simulates the aboveground biomass and the yield linked to nitrogen and water dynamic supply in the plant. This model is based on soil nitrogen at sowing, soil type, climate and nitrogen application (date and amount). First, we present the improvements of the model (mineralization in the ground, N plant dynamic…). Second, the model was compared with measurements for biomass, nitrogen amount in the plant and in the soil on eighteen sites situated in different regions of France from 2007 to 2011.

**Results:** The accuracy of the model AZODYN-rapeseed is 20% for plant biomass, nitrogen content and nitrogen soil.

**References:**
The study of natural variability of winter oilseed rape at vegetative and reproductive stages allows to propose an ideotype adapted to restricted nitrate supply

Background: Only 50% of N fertilizer is recovered in seeds of winter oilseed rape at harvest, reflecting its low nitrogen (N) use efficiency (NUE). Previous studies based on N balance suggested that NUE at vegetative stage is limited by N utilization efficiency (NUe), especially N remobilization efficiency (NRE), while N uptake efficiency (NUpe) seems crucial after bolting, when N availability is reduced.

Objectives: Our goal was to identify the limiting component of NUe at vegetative and reproductive stages to propose an ideotype (virtual genotype) adapted to reduced N inputs.

Methods: To define the limiting factors of NUe, a long-term pulse-chase 15N-labelling method was performed to precisely determine (i) the fluxes of N remobilization and N uptake at the whole plant level and (ii) accurate indexes of NUe component. The N fluxes were determined (i) at vegetative stage in 4 genotypes showing contrasted leaf NRE and biomass production in response to a nitrate limitation (Aviso, Oase, Californium and Samourai; Girondé et al., 2015) and (ii) at reproductive stages (from bolting to mature seeds) in the genotypes Aviso and Oase.

Results: At vegetative stage, the genotype Aviso produced the same leaf biomass in ample and restricted nitrate supply, reflecting its higher NUe compared to Oase, Samourai and Californium. This higher NUe is associated with a higher leaf NRE, which leads to a reduced N lost by leaf drop, but not to a higher amount of N distributed to the growing leaves. Indeed, the higher leaf biomass production of Aviso is due to a higher NUe in young leaves (expressed as the fresh matter production by mg of total N distributed to the growing leaves, i.e. from remobilization and uptake). After bolting, the genotype Aviso also presented the highest NUe under restricted nitrate supply. As the monocarpic senescence during the final reproductive stages was efficient, the better seed N filling of Aviso is due to a higher N remobilization from leaves to stem, reducing the N lost by leaf drop and increasing the N availability for seed filling.

Conclusions: These results suggest an ideotype adapted to low N inputs, characterized by a reduced N loss by leaf drop consequently to a higher leaf NRE during all the growing cycle. At vegetative stage, a higher NUe of the remobilized N in the growing leaves is required to produce a higher biomass before bolting. After bolting, the N from leaves has to be transiently stored in stem during the transition between vegetative and reproductive stage to keep the N from leaves until its remobilization for seed filling during monocarpic senescence.

References:
Non-chemical control of volunteers of clearfield oilseed rape

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**Background:** The introduction of imidazolinone-tolerant oilseed rape/canola varieties (Clearfield system; CL) raises questions of non-chemical control of CL volunteers. These volunteers can be partly or fully tolerant to ALS-inhibiting herbicides, depending on cross-pollination and on the active ingredients. The capacity of seeds to fall dormant and the mode of tillage are crucial for the soil seed bank and volunteer emergence (Gruber et al. 2009, 2010). A comparison of CL-varieties with different levels of dormancy for the capacity to build a soil seed bank under different tillage was not yet performed until now. This information would allow to develop strategies for non-chemical control of CL volunteers.

**Objectives:** Seed survival and flowering volunteers of two CL oilseed rape varieties with high and low seed dormancy should be determined under different tillage systems.

**Methods:** Two CL winter oilseed rape varieties were grown with six different modes of tillage from August 2012 – July 2013 in SW Germany. The varieties have been selected for their varietal disposition to low or high seed dormancy, respectively (Weber et al. 2010). Tillage treatments were deep inversion and deep non-inversion tillage, each with or without preceding stubble tillage in the first week after harvest, shallow non-inversion tillage, and no-till. The soil seed bank was determined in February 2014, and volunteers were counted in spring 2014 in winter wheat, the first following crop to oilseed rape. No herbicides were applied at all.

**Results:** Seed loss at harvest accounted for 1500 seeds m-2 on average. Across tillage treatments, the soil seed bank of the high dormancy variety was significantly larger, comprising 2.4 times as much seeds m-2 (131) as the soil seed bank of the low dormancy variety (54). The soil seed bank across both varieties ranged from 47 _ 200 seeds m-2 (soil inversion without stubble tillage _ deep non-inversion tillage with stubble tillage). The number of flowering volunteers in winter wheat ranged across varieties between < 0.01 plants m-2 (soil inversion without stubble tillage) and 4 plants m-2 (no-till). This number depicts a worst case situation without any herbicides; less volunteers are expected in practical farming with use of herbicides.

**Conclusions:** Low dormancy varieties seem advantageous in the long run; soil inversion several weeks after harvesting could most efficiently reduce the soil seed bank and volunteers.

**References:**


**Effect of cattle manure/compost type and application frequency on canola yield and nutrient uptake from an acid soil under greenhouse conditions**

**Background:** In addition to higher recommended N and P fertilizer rates than cereal crop; canola production also requires S fertilizer input. With increased availability of dried distillers’ grain and solubles (DDGS) as feedstuff to replace a portion of barley grain in livestock diets, manure from DDGS diets is generally high in available S content. It is well known that manure releases nutrients slowly for crop uptake. However, manure behaves as a canola nutrient source is not well understood.

**Objectives:** (1) investigates the effect of cattle manure/compost type and application frequency on canola yield and nutrient uptake under controlled greenhouse conditions and (2) compares the amount of N and P nutrient removed by canola to that of barley.

**Methods:** Two types of manure were collected from the Lethbridge Research Centre feedlot: REG from cattle on a typical finishing diet and DG from cattle fed a diet similar to REG, except with 30% corn DDGS replacing an equal amount of barley grain. The REG and DG manure were used as is, after being stored for 100 days, or after composted for 100 days. Canola was grown and harvested in amended soil for four 7-week cycles. An un-amended CK was included for comparison. Amendments were applied at three frequencies: FRE1 at the start of every cycle at 60 g/kg soil; FRE2 at the start of cycles one and three at 120 g/kg soil with no amendment application for cycles two and four and FRE4 at the start of cycle one at 240 g/kg soil with no application for cycles 2 to 4. The three application frequencies simulate manure/compost applications of 60 Mg/ha every year, 120 Mg/ha every other year and 240 Mg/ha once every four years. Barley was also grown in amended soil, but only with FRE1 application frequency for comparison.

**Results:** Cumulative canola biomass yield and N and P uptake were generally highest in DG manure, followed by DG compost and REG manure with values from REG compost being the lowest. Canola yield and N and P uptake from manure/compost amended treatments were all higher than un-amended CK. Applying amendments at the start of cycles one and three generated the highest biomass yield and N and P uptake than application at the start of every cycle and only once at the start of cycle one. The higher yield and nutrient uptake associated with DG manure/compost reflects its higher N and P content than REG manure/compost. Compared to barley, canola biomass was 1.7 times, N uptake 1.4 times and P uptake 1.5 times greater under the FRE1 application frequency.

**Conclusion:** DG cattle manure/compost had higher yield potential than REG manure/compost, while applying once every two cycles (years) could enhance canola yield and N and P uptake. The greater canola N and P uptake than barley suggests feedlot producers should grow canola to increase the nutrient export from soil with excess nutrient accumulation often found in heavily manured soils near the livestock operations.

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Sustainable canola production increases with cropping system diversity

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**Background:** Canola (*Brassica napus L.*) production has been steadily increasing in Western Canada. High frequency canola rotations increase canola production risks.

**Objectives and Methods:** From 2008 to 2013, two direct-seeded experiments were conducted at multiple western Canada locations. In the first experiment, continuous canola and all rotation phases of wheat (*Triticum aestivum L.*) and canola or field pea (*Pisum sativum L.*), barley (*Hordeum vulgare L.*) and canola were studied to determine the effect of canola rotation frequency on canola seed yield, quality and associated pest species. In the second experiment, continuous canola including sequences of different herbicide-resistant canola and two-cultivar mixtures of herbicide-resistant canola from different sources were compared to wheat and field pea rotations with canola. Fertilizers, herbicides, and insecticides were applied as required for optimal production of all crops.

**Results and Conclusions:** When compared to continuous canola (first experiment), canola yield increases ranged from 7 to 18% in wheat-canola rotations and from 14 to 34% in field pea-barley-canola. For each annual increase in the number of crops between canola, canola yield increased from 0.20 to 0.36 Mg/ha. In the second experiment, rotating herbicide-resistant canola types over years or mixing two cultivars of the same herbicide-resistant type provided no pest management, yield or seed quality advantages compared to planting the same herbicide-resistant cultivar type each year. In both experiments, decreased blackleg (*Leptosphaeria maculans* (Desmaz). Ces. & De Not.) disease and root maggot (*Delia spp.*) damage were associated with greater canola yields as rotational diversity increased. Long-term sustainable canola production increases with cropping system diversity.

**Reference:**
**Isolation and sequence analysis of a BnCSDP3 gene in Brassica napus L.**

**Background:** Rapeseed is one of the most important oil-producing crops in China and worldwide. The yield and quality of rapeseed is frequently threatened by environmental stresses including heat, drought, cold and high salinity. Cold shock domain proteins (CSDPs) are highly evolutionarily conserved nucleic acid-binding proteins, which have large-scale regulatory effects on plants development and stress responses. Although CSDPs are proved to be involved in plant cold stress responses in Arabidopsis and wheat, little is known about their functions in rapeseed.

**Objectives:** To understand and study the functional roles of BnCSDP3 gene in response to cold stress, cold shock domain protein BnCSDP3 in Brassica napus was cloned, and its sequence characteristics as well as biological functions were analyzed and predicted.

**Methods:** According to the sequences of the AtCSDP genes obtained from NCBI, candidate sequences of BnCSDP genes were obtained by searching homologous sequences in Brassica napus database. Specific primers were designed based on the candidate sequences for reverse transcription-polymerase chain reaction (RT-PCR) to clone the full-length cDNA of BnCSDP genes. The bioinformatic methods were used to analyze the sequence characteristics and biological functions of BnCSDP genes using online service.

**Results:** BnCSDP3, a novel gene from Brassica napus was isolated and characterized. The full-length cDNA was 870bp, containing one complete 870bp open reading frame (ORF), which had no introns and encoded 289 amino acid residues. The predicted molecular weight of BnCSDP3 encoded protein was 28.78 KD with the isoelectric point of 7.42. Any signal peptides were found in amino acid residues of BnCSDP3, which indicated that BnCSDP3 was not a secretory protein. Subcellular localization prediction showed that BnCSDP3 might localize in cytoplasm without chloroplast transit and mitochondrial targeting peptides. The sequence alignment demonstrated that N-terminal and C-terminal of deduced BnCSDP3 exhibited a typical cold shock domain (CSD) and a glycine-rich region interspersed with CCHC-type zinc fingers respectively, which were high similarity with other CSDP3 from other species. Accordingly, it was named the BnCSDP3 and might own the function of cold resistance.

**Conclusions:** The gene BnCSDP3 was isolated from Brassica napus and its sequence characteristics were analyzed in detail. The data presented here will future promote our understanding of the biological functions of BnCSDP3 and provide a basis for function verification of BnCSDP3.

**References:**


Environmental impacts of high intensity oilseed rape cropping systems

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**Background:** In recent decades the high technology agriculture increases yields due to the application of fertilizers, pesticides, growth regulators and the use of new varieties. However, the requirements to our modern agriculture increased. In addition to meet the food demand of a growing world population, the agriculture should also contribute to the energy demand and concurrently produce in an environmental friendly manner. In Europe environmental policy strategies associated with the use of renewable energy sources and economic benefit for farmers lead to an increase of the oilseed rape (OSR) production area. Especially in Germany the cultivation area of OSR almost doubled from the 1990 and now amounts to approximately 1.5 Mio ha. During the same period the total crop area remained on a constant level (FAO Stat, 2015).

**Objective:** A field rotation experiment established in 2002 in the Hercynian dry region of central Germany was evaluated with the REPRO software to quantify the environmental impacts in dependency of preceding crop combination before OSR and N-fertilization level.

**Methods:** The field trial was established at the experimental farm Etzdorf (Saxony-Anhalt, 11° 45.443'E, 51° 26.095’N) of the University Halle. For the evaluated experimental period the precipitation averaged 499 mm per year and the mean air temperature is 9.2 °C (2005-2014). The trial was based on the rotation winter wheat - winter wheat - winter wheat - OSR - OSR and on an OSR-monoculture. Thus resulting for the first time in the year 2005 in four different preceding crop combinations before OSR: winter wheat - winter wheat (first OSR-crop), winter wheat - OSR (second OSR crop), OSR-OSR (third OSR crop) and the OSR-monoculture. Additional to the preceding crop combination fertilizer treatments with 120 kg N ha-1 and 180 kg N ha-1 were established in the field trial design in the year 2012/2013. An analysis of the environmental impacts of the greenhouse gas emissions, the energy intensity and the N-balance was carried out with the software REPRO.

**Results:** The results show, that the first OSR has the highest and the OSR-monoculture the lowest yields in most of the years. As a result the greenhouse gas emissions and the energy intensity per kg rapeseed were lowest when OSR followed the winter wheat-winter wheat preceding crop combination. Also the N balance is low in the first OSR-crop. The highest environmental impact was observed, when OSR is cultivated in monoculture. A reduction of the N fertilization level leads to better environmental performance independent of the preceding crop combination.

**Conclusions:** To achieve high yields and to meet the requirements of an environmental friendly production cropping intervals in OSR cropping systems are crucial. A high OSR proportion in the rotation is not favourable under consideration of greenhouse gas emissions, energy intensity and N-balance. Furthermore the reduction of the N-fertilization level could be an opportunity to mitigate the environmental impacts.

**References:**
Are corn, soybeans and canola compatible: Is there a niche for canola in the lower Midwestern US - a case for frost seeding canola

**Background:** Cropping systems in the lower Midwestern United States are primarily corn/corn/soybeans, corn/soybeans, or corn/wheat/soybeans. Much research is underway to move canola into this cropping rotation. Breeding of winter survivable canola varieties adapted to the region is progressing. The challenge is, within the region, full season corn and soybean varieties are not harvested in time for optimal winter canola planting. Corn and soybean harvest may not be complete until mid-November, sometimes far later. Optimal winter canola planting time in the region is prior to September 15th. Wheat fits into the corn/soybean system because it should not be planted in parts the region until after early to late October due to concerns with the Hessian fly. There are many potential benefits to including canola in the cropping system, but it must fit the cropping system. Could late winter sowing encourage lower Midwestern farmers to try canola? Frost seeding is commonly used in the region to sow clover seeds into pastures. Might frost seeding work for canola too?

**Objectives:** The objectives for this study were to determine if frost seeding canola was agronomically viable and if so, when should it be sown in Missouri. A secondary objective was to determine if frost seeded canola yields similarly to fall seeded winter canola.

**Methods:** Seven canola cultivars were broadcast seeded onto prepared 3m X 3m plots on February 14th and March 9th, 2015. Three winter and four spring canola cultivars were trialed. Each cultivar was broadcast by hand on both dates at the rate of 5.38 kg/ha. Fall weed control was not applied. Fertilizer was applied in the fall and 89.3 kg/ha N as urea was applied on April 13, 2015.

**Results:** The weather in late winter and early spring 2015 in Missouri was characterized by slightly lower than normal temperatures and limited rainfall. Rainfall in late March and early April was normal. Each of the seven cultivars germinated at both of the planting dates. As of April 10th, there appeared to a slight difference in the growth of canola sown in February versus that sown in March, and between spring and winter cultivars, although all are in stage 2 growth. Spring cultivars appeared to be growing a bit faster than the winter cultivars planted at the same time. As a comparison, winter canola planted as a variety trial in an adjacent plot was in late stage 3 or early stage 4 on April 10th. The secondary objective will be determined at harvest. Weeds were problematic; henbit (*Lamium amplexicaule*) and cereal grasses were the main weedy species, the former a winter annual weed and the latter volunteer seed from a cover crop trial that went to seed. Large weeds were removed by hand between April 14th and 16th.

**Conclusions:** Objectives 1 and 2 were met. Frost seeding of canola is agronomically viable. It appears from this study that canola can be frost seeded in Missouri between mid-February and early March. It also appears that either spring or winter canola can be established through frost seeding. Broadcast seeding of canola seed may not be the most economical means of seeding high value seed, but for frost seeding it may be the best method. Early morning seeding (before 8 a.m.) may be necessary to ensure that the ground is frozen for seeding and to prevent excessive soil rutting and soil damage from equipment. Thus, commercial producers may have to sow canola seed over several days or even weeks. Freeze thaw cycles seemed to be effective in working the seed into the soil sufficiently to create good seed to soil contact necessary for germination. Fall weed control is necessary to reduce competition on the canola. The application of spring nitrogen fertilizer should favor canola growth over the growth of henbit, but the cereal grasses will remain a concern. Future studies will consider the use of a grain drill for frost seeding compared to broadcasting the seed, and if a fall cover crop of spring oats aids in frost seeded canola establishment. Objective 2 results will be reported on at a later date.

**References:**


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Effects of specific organs on seed oil accumulation in *B. napus* L.

**Background:** Seed oil content is an important agricultural characteristic in rapeseed breeding. Genetic analysis shows that the mother plant and the embryo play critical roles in regulating seed oil accumulation. However, the overwhelming majority of previous studies have focused on oil synthesis in the developing seed of rapeseed.

**Objectives:** In this study, to elucidate the roles of reproductive organs on oil accumulation, silique, ovule and embryo from three rapeseed lines with high oil content (zy036, 6F313, and 61616) were cultured in vitro.

**Methods and results:** The results suggest that zy036 silique wall, 6F313 seed coat, and 61616 embryo have positive impacts on the seed oil accumulation. In zy036, our previous studies show that high photosynthetic activity of the silique wall contributes to seed oil accumulation. Herein, by transcriptome sequencing and sucrose detection, we found that sugar transport in 6F313 seed coat might regulate the efficiency of oil synthesis by controlling sugar concentration in ovules. In 61616 embryos, high oil accumulation efficiency was partly induced by the elevated expression of fatty-acid biosynthesis-related genes.

**Conclusions:** Our investigations show three organ-specific mechanisms regulating oil synthesis in rapeseed. This study provides new insights into the factors affecting seed oil accumulation in rapeseed and other oil crops.
Yield adjustment to planting date in *Brassica napus* L.: An implication of floral organ genesis

**Background:** Canola yield component consists of silique number, seed number per silique, and seed weight. Biologically, silique number depends on effective flower numbers and branches. However, the effective flower number is usually environmentally sensitive, i.e., nitrogen supply and planting date. Delayed planting occurs in current rice-canola rotation production system in China due to the global warming and postponing of rice harvesting time, which always results in the reduction of silique numbers and hence yield. However, little information is available on planting date regulates canola flower bud differentiation.

**Objectives:** 1. To illustrate the process of canola flower bud differentiation; 2. To grasp the dynamics of flower number at main reproductive stages under different planting dates.

**Methods:** A consecutive three-year field experiment was conducted with a split plot design, where three planting dates (early, optimal, and late) served as main plot and three varieties differing in maturity, 1358 (early), Zhongshuang 11 (middle), and Zheshuang8 (late) employed as sub-plot. Flower bud differentiation process was visualized by stereoscopy. Initiation of flower bud differentiation and dynamic of flower numbers were recorded as well under different planting date.

**Results:** Delayed planting date reduced seed yield mainly due to the reduction of silique numbers. The process of flower bud differentiation was similar in all planting dates and genotypes. However, initiation of flower bud differentiation and duration were largely varied under different planting dates and among genotypes. Flower numbers among genotypes and planting dates showed increased from budding to middle flowering stage and then decreased to the level of budding stage. Flowers at delayed planting date were significantly lower than that optimal planting date in all genotypes, which averagely reduced by more than 10%. Genotypic variation of flowers was markedly. Zheshuang 8 produced more flowers than other genotypes under all planting dates. The ratio of effective siliques to total flowers ranged from 60 to 70% in genotypes at all planting dates.

**Conclusions:** 1. High activity of flower bud differentiation kept from budding stage and ended at end of flowering under all planting dates; 2. Flowers at budding stage is a key point to determine the final effective silique numbers; 3. Large quality of flowers after budding stage degenerated; 4. Insufficient growth under delayed planting date caused reduction of flowers.

**References**


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Characterization of winter oilseed rape response to density and initial conditions

**Background:** Plant plasticity is the capacity for a plant to adapt to its environmental constraints (Sultan, 2000). A better understanding of winter oilseed rape plasticity may help to explain plant functioning and to predict plant behaviour in a changing environment.

**Objectives:** Due to this plasticity, there is a great variability between plants within a crop (Behrens and Diepenbrock, 2006), expressed by different features of architecture, among which the number of leaves and branches. Our objective is to understand how this variability takes place. In this article, we focus on the vegetative part of the crop cycle and simple architectural variables.

**Methods:** One cultivar (Pollen) have been grown in ten square containers in order to control growth conditions (Grignon, France, 2012-2013). Plants were sowed in buckets and transplanted two weeks later at two densities (49 and 35 pl/m²) with a regular distribution pattern. At the highest density, there was two treatments: homogenous (plants with similar sizes) and heterogeneous (plants were divided into three categories depending on their initial size: Small, Medium, Big). The pattern of distribution of heterogeneous plants was predefined in order to study different types of neighbourhood. At the lowest density, there was only the homogenous treatment.

Once a week from September 2012 to April 2013, the number of leaves by plant was counted and plants were photographed to compute green surface area. Besides, four destructive measurements have been carried out: initial stage, end of winter, end of branch appearance, flowering stage. At each date, all the plants of each container were weighted, organ-by-organ. Results are derived from statistical methods applied to the experimental data (software R 2.15.2).

**Results:** A great variability in the leaf dynamics was observed even within a pot, whatever the treatment. The number of final leaves ranges from 14 to 32. Considering only the heterogeneous experiment, initial plant size explained 30% of the variability of final leaf number. There is a significant effect of the initial size on phyllochron too. The ranking of plant phyllochrons remained constant over time, meaning the heterogeneity was maintained during the growth, without visible rebalancing effects between plants.

**Conclusion:** Next step of this work is to study neighbourhood effect between plants using photographies. This study may help to better understand interactions between plants within the crop. These results will be used to validate a functional-structural plant model of WOSR and extend it from individual plant to population of plant level.

**References:**

Branching regulation by red to far-red ratio in *Brassica napus*

**Background:** Branching is a key process of plant architecture and yield because it determines the number of inflorescences and pods. Its regulation is highly complex (Jansen et al., 2014) and is part of the so-called shade avoidance answer (Casal, 2012). In the field, the main identified factors that trigger or repress branching are the competition for light and red to far-red ratio (ζ, Chelle et al., 2007). If many studies can be found on other species, these processes have been scarcely studied on Winter Oilseed Rape (WOSR).

**Objectives:** The objective of the study is to characterize the answer of the plant to a reduction of ζ. Two different devices were used: blue filter and far-red diodes, both reducing ζ. One of the questions was: One of the questions was: is it possible to make a plant isolated to resemble a crop plant by only modifying the red to far red ratio in the environment? A major concern was to disentangle the effect of light quality (ζ) and light quantity (PPFD, Photosynthetic Photon Flux Density).

**Methods:** Two experiments were carried out. In the first one, plants in pot were placed at a density of 20 plants m\(^{-2}\) into mini-greenhouses made of blue filters that reduces the ζ and PPFD (ζ\(^{-}\)). Thus two different Controls were added: one with a filter reducing the PPFD but not the ζ (PPFD\(^{-}\)), and a second one without any filter. Filters were applied at different periods of times and for different durations. In the second experiment, isolated plants in pots were placed outside. The base of the stem was lighted with far-red diodes to reduce ζ. Plants were compared to Control plants without diodes. In the third experiment, plants grown in the field were placed isolated (by destruction of their neighbours) from the end of winter phase.

**Results:** We observed no reduction in the number of branches in the plants grown under ζ\(^{-}\)- and PPFD\(^{-}\)-. However, plants presented characteristics of the shade-avoidance response: higher stem, and longer ramifications. Plants with far-red diodes did not bear branches at the base of their stem that was longer than the one of the Control plants. Isolated plants grown in the field presented a bushy port.

**Conclusions:** Results confirmed that ζ triggers or represses branching in WOSR. The plant response seems global with filter device, while more focused on branching using diode device. Interactions with PPFD and thus with carbon resources was also evidenced. Further characterisations on biomass allocation within the different architectures obtained could help understanding plant response and the interaction with the C functioning.

**References:**

Strategies to augment oilseed brassica production in India through systematic evaluation of land resources

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**Background:** India is the world’s fourth largest edible oil economy with total consumption of 18.94 mT against domestic availability of 8.96 mT during 2011-12 (DAC, 2013). The gap between demand and domestic supply is expected to widen with annual compounded growth rate of 2.7% in demand in comparison to 2.2% in domestic availability. The gap between production and consumption could be reduced through augmenting productivity, building favourable infrastructure and policy support. And assessment of natural resources and prevailing land use conditions is a pre-requisite for building sustainable production system.

**Objectives:** Oilseed Brassica (OSB) has enormous production potential to meet edible oil demand of the country (Kandpal et al 2001). But, matching requirement of OSB varieties to existing landuse conditions is essential. This calls for systematic evaluation of all land resources to identify existing and potential high OSB production zones in the country. With this hypothesis an attempt has been made to identify dominant land utilization types (LUTs) under OSB production system and suggested suitable strategies. To develop efficient resource and input based dynamic strategies for enhancing mustard production in the country.

**Methods:** Twelve attributes (Kandpal et al, 2001) from climate normals of 103 observatories and 13 landuse attributes of 242 districts under OSB cultivation were calibrated and statistically analyzed. Various thematic maps were generated and subsequently superimposed to generate LUT map. The final map was used to generate separate strategies using socio-economic conditions, infrastructure and institutional support in respective LUTs.

**Results:** The important OSB producing districts has been grouped into five LUTs. Subsistence farming is prevalent in 78 districts covering 0.28 mha area. Low adoption of technologies and varieties resulting poor seed yield (<0.78 t/ha). The productivity in this LUT could be improved to 1.35 t/ha through adoption of latest varieties, balance use of NPK fertilizers, soil-water conservation techniques and extensive extension activities. Almost 0.97 mha area of 93 districts with average seed yield of 0.98 t/ha is under transitional phase from subsistence to commercial farming. The productivity potential of 1.8 t/ha has been assessed through adoption of latest varieties, biofertilizer application, ensure use of NPKS fertilizers, protective irrigation, extensive extension activities, promotion of small-scale industries and market support. OSB is one of the important commercial crop in 43 districts occupying 1.26 mha area with average yield of 1.02 t/ha. The LUT has potential to produce seed >2.5 t/ha through assured supply of quality seed, biofertilizer application, soil-test-based application of micro-nutrients, management of biotic stress and market support.

OSB is produced as high acreage-high yielding cash crop in 12 districts occupying almost 12% GCA (1.14 mha) with average yield of 1.32 t/ha. While it is most dominant crop in 16 districts covering 1.52 mha area with average seed yield of 1.42 t/ha. These two LUTs has potential to achieve 3.0+ t/ha yield targets through addressing various soil-health issues and adoption of LUT based efficient production technologies.

**Conclusions:** There is significant variability among the OSB cultivation conditions and hence in strategies to enhance production. The technologies for different LUTs are already available but needs refinement for easy adoption. A strong support from extension agencies, policy planner and other stakeholders is also required to achieve the targets.

**References:**
The effect of temperature stress on heterosis in spring *Brassica napus* L.

**Background:** Early planting date has been identified as a critical component of achieving increased seed yield in spring *Brassica napus* L. (canola) in Canada (Kirkland and Johnson, 2000). Heat stress has been implicated in limiting seed yield of spring canola grown in Canada (Kutcher, et al., 2010). Heat is especially problematic in Canada compared to other canola growing regions because the most sensitive growth stage in spring canola (floral development/reproduction) often occurs during the highest temperatures of the growing season. It is imperative that parental heat tolerance is examined to determine the interaction and impact in the resulting hybrid.

**Objectives:** To determine the effect of temperature stress on heterosis in a set of elite spring canola inbreds and the corresponding hybrid combinations.

**Methods:** A group of 10 inbreds (5 females and 5 restorers), 25 hybrids and 2 control cultivars were planted at four locations (2 Manitoba, 2 Ontario) in a split plot randomized complete block design with four replications. The main plot was planting date and the sub plot was genotype. Inbreds and hybrids were blocked together to minimize neighbor effects and the plots were 1.5m x 6m with treatments and replications included in all rows and columns. Temperature was recorded from planting date to physiological maturity. Data was collected on early growth, days to first flower, days to last flower, flower number, pod number, plant height, maturity, seed yield, thousand kernel weight and seed oil, seed protein, glucosinolate and saturate content. Best linear unbiased predictions were calculated for inbred per se yield, hybrid yield and inbred general combining ability.

**Results:** There was a significant effect of treatment on yield and yield components across three of the four locations tested. Hybrids exhibited heterosis across treatments at all locations and the effect of heterosis increased when the planting date was delayed. A second field season will be conducted in 2015.

**Conclusions:** The effect of planting date significantly reduced yield and yield components. Heterosis significantly increased the yield and yield components. Subsequent field seasons are required to elucidate the true effect of heat stress and minimize confounding factors that invariably occur in field environments.

**References**


Soil nitrogen availability affects nitrogen distribution in spring oilseed rape (*Brassica napus* L.)

**Background:** Rapeseed has a high capacity to take up mineral nitrogen (N) from the soil, accumulating large amounts of N in vegetative tissues (Rossato et al., 2001). The N content declines with cumulative leaf area index assessed from the top of the canopy, indicating that N distribution is driven to some extent by light distribution within the canopy, which is the main driver of carbon assimilation. However, how environmental factors such as light, N availability and their interaction exactly govern these N gradients, is still largely unknown. This understanding is necessary in any attempt to improve the N-use efficiency of rapeseed, manipulating it by means of plant breeding or optimizing fertilization strategies.

**Objectives:** The aim of this study was to evaluate the effect of soil N availability and leaf light interception on N content per unit of leaf area and their physiological determinants in rapeseed.

**Methods:** On April 17th 2013 spring rapeseed (Solar CL) was sown in Wageningen, the Netherlands, under an open-sided shelter. The treatments were two rates of N fertilization (50 and 150 kg ha⁻¹) and two plant population densities (50 and 150 plants m⁻²), arranged in a factorial design with three replications. Photon flux density was measured at canopy level. Phenological growth stages were recorded and leaf photosynthesis measurements were carried out on five plants. A sampling was performed 7 days before flowering to determine leaf area as well as dry matter weight and N content of individual leaves. With this information N content per leaf area (Narea), leaf mass per unit of area (LMA) and N per unit of leaf mass (Nmass) were calculated. The remaining leaves and stems were also collected to estimate N uptake. Soil samples were collected at six stages to estimate N content.

**Results:** Narea was affected by leaf position in the plant showing variation in response to N availability and plant population density. This trait was asymptotically related to light saturated photosynthesis rate per unit of area. At high N availability, photon flux density was correlated with LMA, which is the physiological trait most associated with Narea. Under N shortage, N availability was closely related to N uptake, which, in turn, was linearly related with Nmass.

**Conclusions:** Leaf area and total leaf N content were affected by N availability and plant population density.

LMA, the physiological trait most associated with Narea, was clearly associated with photon flux density at high N availability. Under N shortage, N availability became the limiting factor for N allocation. This suggests that manipulating traits associated to LMA (i.e., leaf thickness, density) are the most feasible ways to improve leaf N content. For breeding programs with a focus on low N input, besides LMA, traits associated with Nmass can be manipulated to increase carbon assimilation.

**References:**
The impact of *Rhizoctonia* spp. on crop stand establishment in canola and new seed treatment solutions

**Background:** Over the years canola in Canada has become a key crop. Introduction of new genetics, hybrid varieties, excellent weed control technology, soil conservation ensuring soil moisture at planting, and precision planters have all led to higher yields and productivity. In addition, canola planted hectares have increased significantly since the introduction of canola which has resulted in changes in agricultural practices such as shorter cropping rotations and continuous cropping. The impact of shorter cropping rotations and crop residue from the previous year have the potential to increase the prevalence and severity of soil-borne pathogens such as *Rhizoctonia* spp. The direct impact is seed rot, pre- and post-emergence damping-off, and seedling blight resulting in poor crop stand establishment.

**Objective:** A three year survey was initiated across the prairie provinces of Canada to assess the distribution and prevalence of *Rhizoctonia solani* in canola, barley, beans, corn, pea soybean and wheat crops and to characterize recovered isolates for anastomosis grouping and pathogenicity. The second objective also evaluated new seed treatment technology in canola to control early season infection from *R. solani* to protect seedlings from poor establishment early after planting.

**Methods:** In 2009, 2010, and 2011, field crops were surveyed in Alberta, Saskatchewan, and Manitoba at the early seedling stage. A diamond–shaped pattern of 50 m per side was used for sampling. Twenty seedlings were collected per field with one seedling sampled every 10 m around each side of the diamond. Seedlings were shipped to the University of Guelph for processing. Isolates of *R. solani* recovered from plant tissue were characterized for number of nuclei, anastomosis groups, and pathogenicity. Isolates found to have pathogenicity were used to evaluate seed treatment activity in field.

**Results:** Of the 61 fields surveyed, *R. solani* was recovered from 35 (57%) of the field samples. A total of 130 isolates of *R. solani* were found belonging to anastomosis groups (AG) 2-1, 4, 5, 9, and 11. Eight binucleate isolates were also recovered. In growth room studies isolates belonging to AG 2-1 were virulent primarily on canola while isolates of other anastomosis groups tended to have a wider host range. Symptoms included pre and post-emergence damping off, crown rot, and to a lesser extent root rot. The majority of binucleate isolates ranged from non-pathogenic to low virulence on the hosts tested. Field trials evaluating seed applied fungicides for early in-season protection demonstrated excellent control of *R. solani* by preventing pre- and post-emergence damping-off in canola. A mixture of various seed- applied fungicides with different modes of action and biokinetic properties proved to be most effective in ensuring strong stand establishment.

**Conclusions:** Collectively, the survey results are important for documenting the widespread occurrence of *R. solani* in field crops. There is an important need for effective seed treatments in canola to maximize stand establishment to realize high yield potentials. However a holistic approach that includes agronomic practices like crop rotations is key to a sustainable and successful stand establishment in canola.
Does canola influence soil-emitted nitrous oxide emissions from crop rotations on the Canadian prairies?

**Background:** Crop production is an important source of nitrous oxide (N\textsubscript{2}O) and contributes directly to the increasing concentration of atmospheric N\textsubscript{2}O. Research underpinning estimates of soil-emitted N\textsubscript{2}O from cropping systems on the Canadian prairies is based overwhelmingly on data collected from spring wheat-based cropping systems (Rochette et al. 2008). Canola is now seeded to over 30% of the total annual cropped acreage on the Canadian prairies, but limited research has quantified the potential influence that crop type may have on soil-emitted N\textsubscript{2}O.

**Objectives:** 1) Compare soil-emitted N\textsubscript{2}O emissions from canola, wheat and field pea 2) compare the influence of preceding crop type (canola, field pea and cereal) on soil emitted N\textsubscript{2}O emissions.

**Methods:** Soil-atmospheric exchange of N\textsubscript{2}O was measured approximately weekly from spring thaw to fall freeze-up using nonflow-through nonsteady-state chambers over a three year period. Measurements were made on selected treatments in a long-term rotation study near Scott, Saskatchewan, Canada. The study was established on a loamy textured (Orthic Dark Brown Chernozem) soil. Crops represented in the study included pea (Pisum sativum L.), spring wheat (Triticum aestivum L.) and canola (Brassica napus L.). Specific rotations considered included continuous pea, wheat-pea, pea-canola-wheat, canola-wheat, continuous wheat and, continuous wheat without N fertilizer (check). Measurements on the pea-canola-wheat rotation were taken on the canola phase only. Canola and wheat crops received recommended applications of fertilizer N, with the exception of the wheat “check” treatment.

**Results:** Annual estimates of direct soil-emitted N\textsubscript{2}O ranged from 0.16 to 0.93 kg N\textsubscript{2}O-N ha\textsuperscript{-1}. Emissions from the pea phase of rotations were low and comparable to the unfertilized wheat check (no N applied). Emissions were similar for wheat or canola crops grown on wheat or pea residues. Over the 3-years, the fraction of fertilizer N lost as N\textsubscript{2}O ranged from about 0.1% to 0.2% on the latter plots. Wheat grown on canola residues had significantly higher emissions, with the fraction of fertilizer N lost as N\textsubscript{2}O averaging about 0.5%.

**Conclusions:** Nitrous oxide emissions from the pea phase of the rotations were lower than the N-fertilized wheat or canola phases, and were not different from the unfertilized check. Emissions were similar for wheat or canola phases when grown on wheat or pea residues, but N\textsubscript{2}O emissions were significantly higher when wheat was grown on canola residue. Further research is required to determine why fertilizer-induced N\textsubscript{2}O emissions are higher in the presence of canola compared to wheat or pea residues.

**References:**

**Consistency of different waterlogging-tolerance indexes in canola (Brassica napus L.) after submerging seeds in the room and plants in the field**

**Background:** Canola (Brassica napus L.) is one of the three most important oil crops in China. It is mainly grown in Yangtze River Basin, where the winter oilseed rape is often followed by a paddy rice crop which is flooded for several weeks during spring and summer and it rains often in autumns. The waterlogging stress happens during the seedling stage, bolting stage and flowering stage of canola. It is extremely important to enhance its waterlogging-tolerance and efficiently breed and screen out the tolerant cultivars.

**Objectives:** The objective of this study is to determine the consistency of different waterlogging-tolerance indexes in canola after submerging seeds in the room and plants in the field.

**Methods:** A total of 15 canola cultivars with different levels of waterlogging tolerance were selected for the study. Both indoor anoxic germination and artificial waterlogging under field conditions were carried out to determine several waterlogging related morphological and physiological indexes. For indoor study, germinated canola seeds were submerged in distilled water for 14 hours before further growth. Several morphological indexes were measured to evaluate waterlogging tolerance of these canola cultivars. For the field study, canola plants were subjected to successive artificial water flooding for 10 days at seedling stage. Morphological and physiological indexes after waterlogging treatment, yield traits at mature stage were measured. Correlation analysis between waterlogging-tolerant indexes of germinating seed in the room and those physiological and morphological data in the fields was performed.

**Results:** The results showed significant variation between 15 canola cultivars. Anoxic germination under room condition resulted in great differences in kinds of waterlogging-tolerant indexes, such as vigor index, survival rate, relative seedling length, relative root length and fresh weight; in the fields, plant growths were repressed seriously under waterlogging stress: root fresh weight, root length, aerial parts fresh weight, plant height, plant fresh weight, aerial parts dry weight, root dry weight and root/shoot ratio were decreased by various degrees. Meanwhile, physiological indicators like contents of soluble sugar, soluble protein, malondialdehyde (MDA) and proline, as well as activity of superoxidase (SOD) increased; on the other hand, plant height, number of effective branches, number of pods per plant, number of seeds per pod, yield per plant decreased significantly. Especially, the number of effective branches decreased by 31.81%~78.02% compared with control. 1000-seed weights were increased in some materials. Waterlogging tolerance capabilities varied between materials, but generally, waterlogging-tolerant plants showed less genotype reduction. Correlation analysis showed that waterlogging-tolerant indexes of germinating seed in the room were significantly correlative to those physiological and morphological data in the fields, as well as the final yield characters.

**Conclusions:** This consistency demonstrated that different methods of determining waterlogging tolerance in canola came to the same conclusion. Moreover, it provides a potential possibility to screen out or predict the waterlogging-tolerant canola cultivars in the lab conditions so that the breeding of the waterlogging-tolerant cultivars can be accelerated in the future.

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Improved simulation of growth and development of canola in Australia using the APSIM-Canola model

Background: The canola (Brassica napus L.) module in the Agricultural Production Systems Simulator (APSIM) was developed in the late 1990s. APSIM-Canola simulates crop development, growth, yield and nitrogen accumulation in response to temperature, photoperiod, soil water and nitrogen supply, using a daily time-step, with widely-accepted approaches. The model was tested across a wide range of environments in the Australian grains industry and has been applied in studies of numerous agronomic and crop adaptation issues. However since that time no module improvement has occurred while significant change has occurred in canola production systems including new cultivar types (e.g. hybrids, herbicide tolerant), earlier sowing in some environments and inclusion of winter canola in high-yielding environments.

Objectives: To update the APSIM-Canola model parameters to account for the phenological and physiological characteristics of new cultivars and management systems.

Methods: We gathered phenology data (sowing and flowering dates), for a range of cultivars sown at several sowing dates and up to 16 locations across Australia (Lilley et al 2015). APSIM phenology parameters for cultivars were derived from the observed values using the optimisation process described in Robertson et al. (2002). The optimisation process used daily values of daylength and temperature between sowing and the start of flowering to account for the response of a cultivar to vernalisation, photoperiod and thermal time. The APSIM model was then run to investigate optimal management strategies for expansion of dual-purpose canola into high rainfall zone environments of Australia.

Results: Time to flowering ranged from 80 to 189 days across the range of cultivars, locations and sowing dates. The relationship between observed and predicted time to flowering showed minimal bias, with the data for each of the cultivars falling near the 1:1 line. RMSD values were between 8.5 and 12.4 days, 5 to 10% of the mean period from sowing to flowering. A simulation analysis using newly developed parameters showed that in the high rainfall zone of Australia winter canola cultivars could be sown in early autumn to provide significant forage (>2000 sheep grazing days/ha) and mean annual grain yields of 3.3-5.0 t/ha if stock were removed prior to bud-visible stage. Later sowing of spring cultivars produced similar yields but much lower grazing potential.

Conclusions: The APSIM parameters derived in this process enabled simulation analysis of farming systems to address contemporary farming systems issues with current and relevant cultivars. This included an analysis of several management factors to optimise productivity of dual-purpose canola in Australia’s high rainfall zone.

References:
Have your canola and eat it too - European winter canola for dual-purpose (grain-graze) use in Australia

**Background:** Dual-purpose crops grazed by livestock during the vegetative stage and then grown on to produce grain are an effective way to intensify agricultural production. Though Australia mostly grows spring crops in low to medium rainfall areas (350-500 mm), cropping has recently expanded into the colder, higher rainfall zones (>600 mm) (Zhang et al 2006, Riffkin et al 2012). Dual-purpose cropping of winter cereals has been practiced in the area for some time, while the idea of using winter canola (*Brassica napus*) as a dual-purpose rotation crop is relatively new (Kirkegaard et al 2008; Sprague et al 2013), but adds flexibility and profitability to the farm business.

**Objectives:** We investigated the feasibility and profitability of using dual-purpose European winter canola, as both autumn-sown annual crops, and spring-sown biennial crops.

**Methods:** European winter canola varieties were sown in a number of replicated agronomic experiments in the high rainfall zone of southern Australia. The experiments were designed so that areas of the plots could be grazed by sheep during the vegetative stage with un-grazed plots excluded by fencing. Experiments included autumn-sown plots (March/April) that were grazed in winter (June-August) and harvested in December, and spring-sown plots (sown November) that were grazed from January to May and also harvested in December. Grain yield was measured at maturity.

**Results:** From 750 to 2500 sheep grazing days/ha could be achieved during the May to August period for March-sown crops, with little impact of grazing on seed yield (range 1.9 to 4.5 t/ha). These data are consistent with earlier studies that used mechanical defoliation or “crash-grazing” (Kirkegaard et al. 2008) and confirm excellent performance of winter canola under more prolonged commercial grazing. The spring-sown crops also provided a significant amount of summer and autumn forage for grazing and recovered to produce high grain yield, although the impacts of grazing on the spring-sown crops was more variable.

**Conclusions:** The European winter canola types provided large amounts of forage that was highly valuable to the livestock enterprise in winter, and recovered to produce high grain yield. The concept is an excellent avenue for sustainable intensification of food production on mixed farms in Australia’s high rainfall zone.

**References:**


Evaluation and selection criteria of drought tolerance in Chinese semi-winter type rapeseed at different developmental stages

**Background:** Water deficit has long been considered as the top environmental factor limiting crop productivity worldwide (Boyer 1982), and tolerance to drought is definitely one of the most complex biological traits. It is of great interest to understand the architecture of biological traits that contribute to seed yield under stressed condition, since it is vital for the breeding of drought tolerant variety. So far, little is known about the yield structure and selection criteria for semi-winter type rapeseed varieties adaptable to the drought-prone environments.

**Objectives:** The present study was conducted to (1) clarify whether drought tolerance is stage-specific in Chinese semi-winter type rapeseed; (2) evaluate the impact of exceptionally long and severe climatic drought on seed yield and its component traits; and (3) establish selection criteria for the breeding of drought tolerant varieties.

**Methods:** Thirty seven newly bred Chinese semi-winter type rapeseed varieties were used as plant materials in this study. This panel includes 35 F1 hybrids and 2 open-pollinated varieties. The method of sand cultivation was employed (Hu et al. 2013). At seedling stage, nutrient solution plus 14% (w/v) Peg6000 was used to stimulate an osmotic stress. The field experiments included two treatments, i.e. rainfed (drought stress) and irrigated (moisture condition). A randomized complete block design was used, each with three replications. Plants were harvested when they ripened in April. Thirteen biological traits were measure both under stress and control conditions.

**Results:** Severe osmotic stress has great impact on seed germination and caused a reduction in Seeds vigor by 33.1%. Seed yields of 37 genotypes ranged from 45 to 2339 kg/ha, with an average of 759.5 kg/ha under drought stress. Drought susceptible index for yield was significantly associated only with relative water content (r=0.622). Seed weight, height of first branch, plant height, and number of pods per plant were all markedly reduced under drought condition and thus seemed to be sensitive to drought. Two (i.e. 08SH09 and Mianza03-33) out of the top three tolerant genotypes at germination stage were also shown to be tolerant based on seed yield drought susceptibility index. To maintain higher seed yield under drought environment, a plant should grow shorter and faster but with higher seed weight and more pods per plant.

**Conclusions:** Tissue relative water content is a good indicator for early screening of drought tolerance. The important contributors to seed yield under drought environment were listed, in descending order, as: 1000-seed weight, days to maturity, number of pods per plant, and plant height; however, height of the first branch and number of primary branches are more vital for seed yield in moisture condition.

**References:**
Identification of drought stress-responsive genes in leaves of *Brassica napus* by RNA Sequencing

**Background:** Drought is the uppermost natural stress factor causing reduction of crop yield. The major rapeseed (*Brassica napus*) production region is often threatened by drought disaster, severely affecting the supply security of edible oils.

**Objectives:** To identify the candidate genes involved in drought stress response in leaves of *Brassica napus* thereby exploring the molecular mechanism of drought stress adaptation of it, the transcriptomes of *B. napus* seedlings leaves under two different conditions were compared using RNA sequencing (RNA-Seq).

**Methods:** Total RNA were extracted from leaves of *B. napus* cultivar ZY821 at six-leaf stage under normal (ZY) and natural water loss (ZY8D) conditions, and then were used for RNA-Seq analysis on the Illumina Hiseq 2000 platform. Ambiguous reads and low-quality reads were filtered using NGSQCToolkit v2.3.3. The TopHat2-Cufflinks-Cuffmerge-Cuffdiff standard pipeline was applied to identify the differentially expressed genes (DEGs), taking the *B. rapa* chromosome v1.5 and *B. oleracea* Scaffold v1.0 as reference. In order to perform GO term and KEGG enrichment analyses, the up- and down-regulated DEGs were further analyzed using the BINGO plugin in Cytoscape v3.1.0 and KOBAS2.0, respectively. Subsequently, the qRT-PCR assays were implemented to verify the expression patterns of three representatives of the up- and down-regulated DEGs, respectively.

**Results:** After filtration, a total of 26192312 and 28378899 high-quality reads were acquired in ZY and ZY8D for screening DEGs, 86.6% and 85.8% of the filtered reads derived from ZY and ZY8D could be accurately mapped to the reference sequence, demonstrating the high confidence of the RNA-Seq and the reference. Of the 3657 DEGs, 1431 and 2226 genes were detected to be up- and down-regulated, respectively. GO enrichment analysis indicated that the up-regulated genes were mainly enriched in response to abiotic stress and chemical stimulus, and 127 and 141 out of these DEGs were involved in response to water deprivation and ABA stimulus, respectively. However, down-regulated DEGs were mainly overrepresented in defense response to plant pathogen, protein kinase activity and response to SA stimulus. KEGG enrichment analysis showed that up-regulated genes were significantly associated with phenylpropanoid and carotenoid biosynthesis pathways, and starch and sucrose metabolism, while the down-regulated DEGs mainly enriched in plant-pathogen interaction and signal transduction pathways of ABA, SA and jasmonic acid (JA). The results of qRT-PCR analysis of six DEGs were consistent with those of RNA-Seq data, further confirming the reliability of RNA-Seq results.

**Conclusions:** In total, 3657 drought stress-responsive genes were identified using RNA-Seq, GO and KEGG pathway analyses identified the overrepresented molecular function categories and pathways of DEGs.
The transitional cultivation patterns of winter oilseed rape in China and the corresponding nutrient management strategies

**Background:** Both direct-sowing and transplanting are the dominated methods to establish winter oilseed rape (Brassica napus L.) in the current Yangtze River Basin, China, which play important roles in the development of national oilseed rape industry and edible oil supply security. Direct-sowing was the main cultivation pattern at the beginning and transplantation get started from 1960s with direct-sowing as primary, and then transplantation became get full-scale adoption during 1980s to 1990s. Nowadays, direct-sowing and transplantation cultivations have co-existed in winter oilseed rape production of China. Correspondingly, nutrient management practices had progressed from farmyard manure application to nitrogen (N) and phosphorus (P) chemical fertilizers application, and then converted to advocate the balanced application of N, P, potassium (K), and borax (B) fertilizers, and now formed the high-yielding and high efficiency technique system for transplanted oilseed rape.

**Objective:** There were significant differences in the cultivation characters and growth progresses between direct-sown winter oilseed rape (DOR) and transplanted winter oilseed rape (TOR), their growth stages, plant density, population structure and individual morphology were emphatically compared. Furthermore, the differences in nutrient responses, absorption and distribution, requirement and utilization between DOR and TOR were emphatically compared and discussed.

**Results:** The DOR showed generally shorter growth stages, weaker plants and lower individual yield, compared with TOR. However, DOR played group effects under the higher plant density and had stronger abilities to absorb nutrient and water due to the greater root group, and therefore showed the potential of high yield and high efficiency. DOR were more sensitive to nutrient deficiency and insufficient nutrient supply leaded to weak individual growth and population decline. DOR had lower biomass and nutrient transport efficiencies, and it showed higher requirement for P and K than TOR. The nutrient management strategy of "promotion in early and stabilization in late" for DOR was presented according to the existing researches, and it included: ensuring P and K supply based on the rational and balanced nutrients application, applying organic fertilizer and returning straw, and paying more attention to the role of N fertilizer in the development of individual and population. The N fertilizer as basal should be reduced and seedling topdressing need to be increased to improve plant growth in the early stage, and appropriate topdressing before flowing are also required to ensure population density and yield formation. The recommended N fertilizer application practice of DOR is basal fertilizer: seedling fertilizer: overwintering fertilizer: bolting fertilizer = 40%:30%:15%:15%.

**Conclusions:** Nutrient management should work with other cultivation practices, e.g. selecting early-maturing and density-resisting variety, adopting mechanization precision sowing technique, increasing appropriately plant density to reduce fertilizer rate, compensate late-sowing, and improve sink and source, and enhancing plant protection to control diseases, pests and weeds. Furthermore, the key points in future studies for DOR were also proposed in this paper, in hope of providing references for further improvement of cultivation practices and scientific fertilization technique.
Ideotype population exploration: Growth, photosynthesis, and yield components at different planting densities in winter oilseed rape 
(\textit{Brassica napus L.})

\textbf{Background:} Rapeseed is one of the most important edible oil crops in the world and the seed yield has lagged behind the increasing demand driven by population growth. Winter oilseed rape (\textit{Brassica napus L.}) is widely cultivated with relatively low yield in China, so it is necessary to find the strategies to improve the expression of yield potential. Planting density influences the yield by regulating growth, yield components (Diepenbrock 2000), and photosynthesis, which are the target traits closely related to the ideotype of crops (Donald 1968). Few studies have described the ideal population structure in winter rapeseed.

\textbf{Objectives:} The objectives of this study were to optimize the yield and yield components of two elite winter varieties that were commonly grown in the Yangtze River basin under several planting densities and to identify the physiological mechanisms that contribute to the high yield.

\textbf{Methods:} The field experiments were conducted in Wuhan in the Yangtze River basin with one conventional variety (Zhongshuang 11, Zs11) and one hybrid variety (Huayouza 9, HYZ9) at five planting densities (27.0 × 10^4, 37.5 × 10^4, 48.0 × 10^4, 58.5 × 10^4, 69.0 × 10^4 plants ha⁻¹) to investigate the yield components. The physiological traits for high-yield and normal-yield populations were measured.

\textbf{Results:} Our results indicated that planting densities of 58.5 × 10^4 plants ha⁻¹ in Zs11 and 48.0 × 10^4 plants ha⁻¹ in HYZ9 have significantly higher yield compared with the density of 27.0 × 10^4 plants ha⁻¹ for both varieties. The higher leaf net photosynthetic rate (Pn) and water use efficiency (WUE) were observed in the high-yield populations. A significantly higher level of silique wall photosynthesis and rapid dry matter accumulation were supposed to result in the maximum seed yield.

\textbf{Conclusions:} The ideal morphological traits of the two varieties were moderate number of siliques and primary branches per plant as well as high number of siliques and primary branches per unit area. Furthermore, higher LAI (~5.0), Pn, and WUE were observed in the high-yield population, whereas they decreased more rapidly after anthesis compared with the normal-yield population. It was suggested that the higher SAI (~7.0) and longer duration of high silique wall photosynthesis likely resulted in a significantly higher biomass at the seed-filling stage and a subsequently higher seed yield.

\textbf{References:}
Leaf-pod senescence, root morphology and seed yield of winter oilseed rape 
(*Brassica napus* L.) at varying plant densities

**Background:** Oilseed rape is one of the most important sources of edible oil in human diet. Winter oilseed rape (*Brassica napus* L.) is widely cultivated along the Yangtze River in China, which represents about 30% of total seed yield worldwide and 89% of that national wide. With the increasing demands driven by population growth, it is necessary to increase the seed yield of rapeseed crops. Plant density is an important factor affecting seed yield and yield components of oilseed rape as well as creating a difference between individual and group performance that can be utilized. Photosynthate supply plays an important role in pod and seed development (Alex et al. 2006). The root is the most important organs of plants for uptaking water and nutrients (Gersani et al. 2001).

**Objectives:** The objective of the present study was to (i) investigate leaf-pod senescence and the root morphological parameters and their effects on seed yield at different plant densities by using two elite winter rapeseed varieties that were commonly grown in the Yangtze River basin; and (ii) determine possible mechanisms behind this effect.

**Methods:** The conventional winter rapeseed variety Zhongshuang 11 (ZS 11) and the hybrid variety Zhongyouza 12 (ZYZ 12) were used. The assay of leaf and pod wall photosynthesis, chlorophyll and lipid peroxidation, root morphology and dry matter biomass and nitrogen use efficiency in high yield population (HYP) and normal yield population (NYP) were determined.

**Results:** HYP showed a rapid decrease in GLAI, leaf photosynthetic rate and chlorophyll content after peak anthesis. The high yield highlighted the rapid increase of PAI and longer duration of high pod wall photosynthesis accompanying with the accelerated leaf senescence after peak anthesis. Moreover, the larger reduction of root morphological parameters (root length, root tips, root surface area and root volume) showed the availability of more assimilation from vegetative organs to the pods and seeds.

**Conclusions:** The high yield highlighted the rapid increase of PAI and longer duration of high pod wall photosynthesis accompanied with the accelerated leaf senescence after peak anthesis. Moreover, the larger reduction of root morphological parameters showed the availability of more assimilation from vegetative organs to the pods and seeds.

**References:**
Mineral nutrient efficiency remobilization during leaf senescence and modulation by nutrient deficiency

Background: As sessile organisms, higher plants have to cope with permanently fluctuating mineral resource availability. Besides strategies such as stimulation of root growth (Gruber et al., 2013), increased transporter activities (Gojon et al., 2009) and biotic interactions, nutrient storage and further mobilization have been mostly studied for only a few macronutrients, nitrogen being the most described (Avice and Etienne, 2014).

Objectives: The aims of this study were firstly to monitor the apparent remobilization of macro- and micronutrients from senescing leaves in 5 crop species (Brassica napus, Triticum aestivum, Hordeum vulgare, Pisum sativum, Zea mays) with the aim to describe contrasting patterns of remobilization. Finally, the putative remobilization of each nutrient was quantified using B. napus subjected to 13 individual nutrient deficiencies in order to maximize remobilization and decipher whether remobilization is also triggered by senescence and/or nutrient deficiency.

Methods: Leaves of Brassica napus, Triticum aestivum, Hordeum vulgare, Pisum sativum, Zea mays grown under field conditions were harvested regularly during their life span and analysed to evaluate the net mobilization of 13 nutrients during leaf senescence. Further experiments were performed with rapeseed plants subjected to individual nutrient deficiencies.

Results: While N was remobilized in all plant species with different efficiencies ranging from 40% (maize) to 90% (barley), rapeseed has intermediate remobilization efficiency. Other macronutrients (K-P-S-Mg) were mobilized in most species. Ca and Mn, usually considered as having low phloem mobility were remobilized from leaves in wheat and barley. Leaf content of Cu-Mo-Ni-B-Fe-Zn decreased in some species, revealing that they can be remobilized. Overall, wheat and barley appeared to be the most efficient at remobilization while maize and rapeseed were the least efficient. In rapeseed, compared to field conditions, remobilization from leaves was similar (N-S-Cu) or increased by nutrient deficiency (K-P-Mg) while nutrient deficiency had no effect on Mo-Zn-B-Ca-Mn, which seemed to be non-mobile during leaf senescence under field conditions. However, Ca and Mn were largely mobilized from roots to shoots.

Conclusions: Following 13 different nutrients suggests that rather different mechanisms will need to be considered: remobilization from organic storage forms (such as for N) tightly linked to senescence, mineral storage that requires more or less specific transporters (S-Mg-K-P), the effect of deficiency that increased remobilization compensating under reduced root uptake, restricted transport, and finally remobilization from shoots or from roots (Mn-Ca).

Using 5 plant species suggests that remobilization efficiency is probably affected by previous plant breeding, the plant development scheme (source sink ratio or environmentally induced senescence), and plant evolution.

References:
Some aspects of intercropping fall-sown rapeseed with annual legumes for fresh forage production

**Background:** Fall-sown brassicas, such as fodder kale (Brassica oleracea L. var. viridis L.) or rapeseed (Brassica napus L.) are considered the first sources of fresh forage in the spring in many temperate regions, such as South Eastern Europe, and are highly esteemed in feeding milk cows. Despite considerably high fresh forage yield and prominent earliness, rapeseed usually contains about 10% of forage dry matter and thus is often regarded as being less overall productive than other annual forage crops.

**Objectives:** In order to enhance the agronomic performance of rapeseed, a model was developed of intercropping rapeseed with annual legumes, such as pea (Pisum sativum L.) and vetches (Vicia spp.), aiming at increasing the total fresh forage and forage dry matter crude protein yields, decreasing the weed infestation and demonstrate the superiority of rapeseed-legume intercrops over their sole crops. Such models were previously demonstrated as beneficial for the mineral nutrition of both components (Mikić et al. 2015).

**Methods:** A series of small-plot trials was carried out from 2010/2011 to 2012/2013 at the Experimental Field of IFVcns at Rimski Šančevi, in the vicinity of Novi Sad. It included four intercrops at a ratio of 50%: 50% of rapeseed, playing the role of a supporting crop, with four annual forage legume cultivars, namely pea and Hungarian (V. pannonica Crantz), common (V. sativa L.) and hairy (V. villosa Roth) vetches, as the supported crops, and the sole crops of all five cultivars. Rapeseed was cut in full budding, while annual legumes were cut in full bloom. Forage dry matter (FDmy, t ha\(^{-1}\)) and forage dry matter crude protein yields (FCPy, kg ha\(^{-1}\)) were determined in all cultivars and their intercrops, with land equivalent ratio (LER) calculated for both parameters.

**Results:** The average FDmy ranged between 7.4 t ha\(^{-1}\) in the intercrop of rapeseed with Hungarian vetch and 9.4 t ha\(^{-1}\) in the intercrop of rapeseed with hairy vetch. In the first of these two intercrops, rapeseed contributed more to the total FDmy, while in the second one it was rather dominated by hairy vetch. All the values of LER for FDMY were higher than 1, proving an economic reliability of all four intercrops. Regarding the average FCPY, it was highest in the intercrop of rapeseed with hairy vetch (1996 kg ha\(^{-1}\)). This intercrop also had the highest average LER for FCPY of 1.18.

**Conclusions:** The overall agronomic performance of intercropping rapeseed with annual legumes for forage production proved to be superior over the sole crop rapeseed cultivation for the same purpose in both higher forage dry matter yield and forage dry matter crude protein yield, suggesting it as a potential novel component in farming systems in temperate regions.

**References:**
The accelerated aging of *Brassica napus* seed under salt stress

**Background:** In the previous decades, the problem of salinization has become more prominent due to reduced availability of fresh water and increased use of inadequate irrigation water higher in salt. Under such conditions, seed germination is particularly difficult due to the high concentration of salt in the sowing zones after capillary action of soil solution. There are various endogenous (plant) and exogenous (external environment) factors affecting seed germination in saline conditions, but the most important are seed viability and seed aging. At the same time, due to the high content of oil, seed of rapeseed is very sensitive to the storage and deterioration.

**Objectives:** Considering the growing demand for food production on the one hand and increasingly growing areas of saline soils on the other hand, studying the tolerance of seed to increased salt content in soils becomes greatly important. The aim of this study was to investigate the effect of different NaCl concentrations on germination after exposing the seed to stressful conditions that contribute to the accelerated aging.

**Methods:** The seed germination and the intensity of lipid peroxidation were measured in three rapeseed genotypes: Banačanka, Jasna and Kata. These parameters were determined in fresh seeds and repeated after exposing seeds to double stress conditions of high temperature and high humidity (100%). The accelerated aging test was performed according to the Hampton & TeKrony (1995) method in which seeds were kept in a water bath at 39°C for a period of 72h. To determine whether different concentrations of NaCl (control, 100, 150, 200 mM) cause oxidant damage in seeds, the intensity of lipid peroxidation was measured by malondialdehyde (MDA) content (Ng et al., 2000) as the most important product of lipid peroxidation.

**Results:** The results showed that the seed germination and intensity of lipid peroxidation depended both on the level of salt stress and on the genotypes. With increasing NaCl concentration the lipid peroxidation intensity gradually increased until the percent of seed germination decreased. Although genotype Banačanka showed the most significant increase of lipid peroxidation intensity at the highest level of salt stress (338.1% compared to control), the lowest decline in seed germination (31.8% compared to control) was also observed.

**Conclusions:** The negative correlation relationship between the intensity of lipid peroxidation and seed germination indicates that the degree of peroxidation of lipid membranes is of great importance for normal processes in cells under stress conditions during the process of germination. In oilseed species autoxidation of lipids during storage is the main reason for the sudden deterioration of seeds.

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Brassica carinata as a winter cover crop in the southeastern United State

Our goal is to deliver crop oils and bio-based products that are priced competitively and are commercially viable. Taking advantage of underutilized winter fallow land is a primary focus of this project. Row crop land is often some of the most fertile lands in Florida and the Southeastern United States. If winter cover crops are not grown nutrients leach out of the root zone during the off season, possibly impacting the environment. Our farm plan will demonstrate how crop rotation with a biofuel crop, Brassica carinata, during the dormant season of food and fiber crops can increase farmer revenue and reduce farm risk through crop diversity. In the 2014-2015 winter season over 1500 ha were planted in Florida, Georgia, and Alabama. This planting was based on 3 years of research conducted at the University of Florida’s North Florida Research and Education Center in Quincy, FL. Specific projects include development of advanced breeding lines appropriate for the region, determining optimum planting dates, planting density, fertilizer requirements, tillage practices, and row spacing. The impact of B. carinata on soil microbial activity, plant pathogenic nematodes, and soil health is also being investigated. Crop rotation aspects are being considered as well, especially the impact of carryover herbicides and the potential impact on the following summer crops including soybean, peanuts, and cotton. Thus far we have found that a mid-November planting date using 35 cm row width and a seed planting density of 8 kg/ha is optimum. With 80 kg/ha of nitrogen (25 at plant and 55 in late January) we expect a minimum yield of 1500 kg/ha. In our studies we obtain a mean yield of 3000 kg/ha with some advanced varieties being over 5000 kg/ha. With present varieties a mid-November planting can be harvested in mid-May. Major efforts are underway to select for genetics that are cold tolerant, resistant to common herbicides, shorter season, and high quality and quantity of oil.

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Incidence and severity of foliar diseases in *Brassica juncea* L. (Czern. & Coss.) under elevated CO$_2$ is affected by plant defense chemistry

**Background:** The atmospheric concentration of [CO$_2$] has increased by at least 35%, since the start of industrial revolution (IPCC, 2007). With increasing concern about the effects of global climate change on food production, it is important to devote greater effort towards studying the impact of various aspects of elevated CO$_2$ on the development of plant disease epidemics and the specific plant-pathogen interactions under field conditions (Chakraborty et al., 2008). *Brassica juncea* (L.) Czern. and Coss. (Indian mustard) is an important oilseed crop cultivated on about six million hectares of land in India. The crop accounts for nearly one-third of the oil produced in India and is considered to be country’s second most important edible oil (Damodaran and Hedge 2005). Most of the previous studies reported the effect of elevated CO$_2$ on agronomic traits of *Brassica* spp. but none of them have analyzed its effects on plant-pathogen interactions.

**Objectives:** Natural incidence and severity of foliar diseases on mustard plants grown under free-air CO$_2$ enrichment (FACE) were examined. Effect of elevated CO$_2$ was also studied on plant’s structural, biochemical and defense parameters and correlated with disease expression.

**Methods:** *B. juncea* plants were grown under FACE supplied with CO$_2$ concentration at a level of 550 ppm. The control plants were grown in an open field under natural conditions where concentration of CO$_2$ was 390 ppm. Disease incidence was recorded in mustard plants through visual inspection beginning one week after seedling emergence till inception of leaf senescence. Investigation of various structural and biochemical changes in leaves under elevated CO$_2$ were performed using scanning electron microscopy (SEM), spectrophotometry, and high pressure liquid chromatography (HPLC).

**Results:** The study revealed that there is increased incidence and severity of White rust caused by *Albugo candida* while decreased incidence and severity of *Alternaria blight* caused by *Alternaria brassicae* and Donwny mildew caused by *Hyaloperonospora brassicae* in mustard plants grown at elevated CO$_2$. Leaves of mustard plants grown under elevated CO$_2$ possessed more amount of epicuticular wax which, together with higher concentration of total phenols and phenylalanine ammonia lyase activity, may have increased the ability of mustard plants to resist infection by *A. brassicae* and *H. brassicae*. Mustard plants grown under elevated CO$_2$ showed a decrease in stomatal density and pore size, and consequently also in stomatal conductance. This might explain the decrease in disease index of downy mildew caused by stomata-invading pathogen *H. brassicae*. There was three times higher concentration of total sugars in leaves of plants grown under FACE. A significant increase in the concentration of total glucosinolates (GSS) was also observed in plants grown under elevated CO$_2$ but a decrease in their diversity. Higher sugar availability and lower GSS diversity may account for higher incidence and severity of white rust caused by an obligate biotroph, *A. candida*.

**Conclusion:** This is the first report on altered plant-pathogen interactions under FACE from India. Our results signify the impact of elevated CO$_2$ on disease development in *B. juncea*, model crop, enabling to conjecture its performance in projected scenario of elevated CO$_2$ regime. The study highlights the fact that different pathogens of a crop may respond differently to elevated CO$_2$ depending on how the host itself is affected by the changed conditions. Studies on one crop cannot be extrapolated to another.

**References:**


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Effects of solar radiation and temperature on alpha-linolenic acid content of High Oleic Low Linolenic (HOLL) Oilseed Rape

**Background:** Temperature and solar radiation are known to affect fatty acid profile of crop species. Alpha-linolenic acid (C18:3) content of oilseed rape (OSR) is negatively correlated to average minimum temperature during grain filling (Baux et al. 2013). Solar radiation is also negatively correlated to OSR linolenic acid (Izquierdo et al. 2009).

**Objectives:** As low C18:3 content is a major quality parameter for HOLL oil, we intend to assess the effect of temperature and solar radiation on the fatty acid profile of HOLL winter OSR, in field experiments and under semi-controlled conditions.

**Methods:** Two HOLL varieties were grown in growth chambers from the onset of flowering until physiological maturity. Temperature was set to 10/18 °C or 15/23°C (14 hours daylight), and artificial light was either total or shaded. Fatty acid content was analyzed after hand-harvest. This trial was repeated for two growing seasons. In a field trial, we hand-harvested grains of HOLL mature plants from four different height levels. We aimed to test the hypothesis that lower pods receive less solar radiation than those from upper pods, and therefore contain seeds with higher C18:3 content. Lastly, the relationship between linolenic acid content of the HOLL hybrid variety V280OL and average minimum temperature during seed filling (as described by Baux et al. 2013) was studied by means of linear regression using data from four years of field trials.

**Results:** Under controlled conditions, light and temperature were both negatively correlated with linolenic acid content. The lowest C18:3 percentage was found in the treatment with highest temperature and light intensity. In the field trial, we measured more solar radiation on the top of the plant, and observed significantly lower levels of linolenic acid compared to the lowest part of the plant. The regression analysis showed that linolenic acid content of HOLL OSR was negatively correlated with average minimum temperature, even if the effect was not as strong as for conventional varieties. The parameters obtained for V280OL (y = -0.13x + 4.06) could be introduced in a crop model, to predict the linolenic acid content depending on the temperature during seed filling.

**Conclusions:** Field trials as well as experimentation in growth chambers confirmed that for HOLL OSR, temperature and solar radiation have a significant effect on linolenic acid content. To guarantee heat-stability of HOLL oil, linolenic acid content has to be as low as possible. Our results show that C18:3 content is minimized in the upper part of the plant, and with high solar radiation and warm temperature during the seed filling.

**References:**
Nitrogen fertilizer rates impacts on agronomic and environmental performance of ethiopian mustard (*Brassica carinata*) in South Dakota

**Background:** The introduction of non-food oilseed crops can aid in diversifying biofuel sources and lessen the demand of producing biofuels from food-based crops such as corn and soybean. Ethiopian mustard (*Brassica carinata*) has been identified as a potential non-food biofuel crop suitable for production in South Dakota. However, the best fit for the crop in current cropping systems, best management practices, and environmental impacts remain to be determined.

South Dakota State University has ongoing research projects involving carinata, including variety evaluation, fertility requirements and greenhouse gas emissions, on-farm demonstration plots, oil extraction and meal recovery, meal processing and utilization, economic and insurance analyses, and life cycle analysis. This presentation will report on this ongoing work with emphasis on N fertility requirements and environmental impacts.

**Objectives:**

a) Evaluate the response of carinata to various nitrogen fertilizer rates in South Dakota.

b) Evaluate soil and environmental impacts associated with carinata production managed with nitrogen fertilizer in South Dakota.

**Methods:** Separate nitrogen fertility trials were conducted in 2013 and 2014. In 2013, trials evaluated the response of one carinata variety to five nitrogen rates (0, 34, 67, 101, and 134 kg/ha) at four locations (Brookings, Pierre, Wall, and Bison) in South Dakota. Plot size was 1.5 m x 7.6 m, with no border rows between plots. The variety 080814EM was used at Brookings and AAC110 was used at the other three locations. In 2014, trials evaluated the response of two carinata varieties to four N rates (0, 28, 56, and 84 kg/ha) at two locations (Brookings and Pierre). Plot size was 1.5 m x 7.6 m, with border plots between treatment plots. The nitrogen treatments were broadcast over the top of the plots immediately after planting. Soil samples were collected at the start of the experiment and at harvest from 0-10, 10-20 cm depths for analyzing soil organic carbon (SOC), soil inorganic carbon (SIC), pH, EC, and total nitrogen (TN). Soil surface greenhouse gas (GHG) fluxes were also monitored.

**Results:** Seed yields in 2013 were highest at Brookings, likely owing to better stands and more precipitation during the seed-filling period. In 2013 there was no yield response to applied nitrogen at any of the locations, despite early visual differences between low and high levels of applied N. Lack of border rows between treatment plots may have confounded the response to N. In 2014, both carinata varieties showed a positive, statistically significant response to applied N at the Brookings location. The best yields were observed at the N rate of 84 kg/ha. The trial at Pierre was abandoned due to herbicide drift injury. Soil parameters such as SOC, SIC, TN, pH and EC were not impacted by N rate treatment. Whereas soil surface CO₂, N₂O and CH₄ fluxes were highly variable in all the treatments and did not provide any clear trend, work is ongoing to address these further.

**Conclusions:** The trials showed that good yields of carinata are possible with adequate plant stands and precipitation. In 2014, the greatest carinata yields were observed at the highest nitrogen fertilizer rate used in these studies.
Relative importance of pre- and post-flowering growth of juncea canola hybrids in water-limited environments in Australia

**Background:** Canola quality *Brassica juncea* (juncena canola) has been developed as an oilseed crop for low rainfall environments. Studies on *B. juncea* have also shown that there is exploitable heterosis in seed yield. Breeding programs to develop juncea canola hybrids have been initiated by SeedNet Australia. However, there is lack of knowledge on the plant traits that promote yield heterosis under low rainfall environments.

**Objectives:** Field and glasshouse research aim to compare the importance of pre- and post-anthesis growth in maintaining seed yield of hybrid juncea canola in water-limited environments of Victoria, Australia.

**Methods:** Replicated experiments were conducted in the Mallee (low rainfall) and Wimmera (medium rainfall) regions of Victoria. Glasshouse experiments were conducted at Parkville, The University of Melbourne. The experiments were conducted for two years (2012 and 2013) and consisted of juncea canola hybrids, some of the parental lines and control cultivars of juncea canola and canola in fields. The glasshouse experiments included juncea canola hybrids, parental lines and a juncea canola control cultivar. The glasshouse experiments had two treatments: well-watered and drought imposed after first flower open to maturity.

**Results:** In the field trials, biomass at flowering consistently had stronger positive relationship with seed yield at low yielding sites than at higher yielding sites in both the years. Biomass at flowering had a significant and positive relationship with final biomass and harvest index at low the yielding site only. In contrast, post-anthesis growth (growth from anthesis to maturity) and final biomass had stronger positive relationship with seed yield at the high yielding site than low yield sites. At the low yielding site, biomass at flowering was more strongly related than post-anthesis growth to seed yield whereas post-anthesis growth had a stronger relationship than biomass at flowering with seed yield at the high yielding site. In the glasshouse experiments, number of pods per plant consistently had positive correlations with seed yield under well-watered condition while seeds per pod and thousand seed weight consistently had positive correlations with seed yield under drought.

**Conclusions:** The field results indicated that early vigour with high harvest index was desirable for seed yield at low rainfall site; and higher post-anthesis growth leading to higher total biomass is desirable for relatively high rainfall site. The glasshouse results suggested that higher seed yield under water deficit came with relatively lower number of pods but better filling leading to higher seeds per pod and thousand seed weight. In favourable moisture condition, seed yield came from higher number of pods. Breeding for low rainfall environments should target for good early growth with higher harvest index and relatively few pods allowing better seed filling under water deficit.

**References:**


Impact of water-deficit during seed development on seed yield and yield components of juncea canola

**Background:** Canola quality *Brassica juncea* (juncea canola) has been developed as an oilseed crop for low rainfall environments. However, yield needs to be improved to commercialize it as an alternative crop for the low rainfall environments. Several studies have shown that *B. juncea* has exploitable yield heterosis indicating that hybrid cultivars could be a means of yield improvement. There is limited research on hybrid *juncea* canola. In particular, there is lack of understanding on yield components and other traits that help maintain yield heterosis under low rainfall environments.

**Objectives:** The glasshouse trails aim to determine most important yield components in maintaining seed yield of juncea canola hybrids under water deficit during reproductive stage.

**Methods:** Two glasshouse trials (one planted in February and the other in July) were conducted at the University of Melbourne, Parkville in 2014. The trials consisted of juncea canola hybrids, their parental lines and a juncea canola control cultivar. The trials had two treatments: well-watered and water-deficit after first open flower to maturity. Data were recorded for phenological traits (days to first open flower and physiological maturity), biomass at harvest, seed yield per plant, yield components (number of pods per plant, number of seeds per pod and thousand seed weight) and harvest index.

**Results:** In the first trial, thousand seed weight and seeds per pod were the only two traits not significantly affected by water stress. There were significant (p<0.05) differences between genotypes for all other traits measured. Effects of treatments were significant for days to maturity, biomass at harvest, seed yield per plant and number of pods per plant. None of the traits measured showed significant effects for genotypes x treatment interaction.

In the second trial, there were significant differences between genotypes for all the traits measured. Effects of the treatments were significant for days to maturity, biomass at harvest, seed yield per plant, number of pods per plant and thousand seed weight. Genotypes x treatment effects were significant for biomass at harvest, seed yield and number of pods per plant.

Number of pods per plant had consistently strong positive relationship with seed yield per plant in both the experiments. Thousand seed weight was not associated with seed yield in both the experiments whereas number of seeds per pod was positively associated with seed yield per plant only in the first experiment. In the both the experiments, earlier flowering and maturity was positively associated with higher harvest index and higher number of seeds per pod.

Consistent effects of treatments on number of pods per plant indicated that number of pods per plant was the major yield component responsible for seed yield variation.

**References:**

Improving grain quality by exploiting Genotype – Environment – Management Interactions (GEMI)

Background: Genotype – Environment – Management Interactions (GEMI) are widely studied for grain yield, but not so much for quality parameters like oil and protein contents, fatty acid profiles, amino-acids composition, vitamins etc… Yet, with such knowledge it would be possible to define agronomic recommendations which would favor quality stability, and to complete some stakeholder's needs for quality rapeseed grains in view to produce enriched products.

Objectives: The aim is to identify the factors which affect grain quality parameters such as oil and protein contents, and to determine if these parameters generate GEMI. If GEMI are observed on winter rapeseed multi-environment trials (MET), they will be analyzed as it is usually done on the yield, and cultivar resistance to stress will be characterized.

Methods: First, statistical methods such as multiple regression or PLS regression were achieved on data from rapeseed MET, to assess the effects of environmental conditions and crop management practices on grain quality. Then, GEMI for grain quality parameters were considered and quantified by a common approach often used for grain yield. GEMI were described through an Analysis of Variance (ANOVA) model, partitioning oil or protein content variability into components linked to different sources of variation: genotype, environment, and GEMI. If GEMI are revealed to be significant, they will be explained by environmental covariates, using the DiagVar approach, in view to characterize cultivars. The used model expresses GEMI as the sum of the cultivar’s specific responses to each of those stresses and resources. In that case, estimated coefficients quantify genotypes resistance to the main environmental stress.

Results: There is a negative correlation between oil and protein concentration, which is why factors affecting oil content should be opposite to those affecting protein content. These factors are mainly climatic stresses during the seed filling stage. Protein and oil contents generate significant GEMI, but remain difficult to understand entirely.

References:
Allelopathic effects of winter wheat stubble on winter canola germination and biomass accumulation

Background: Winter canola (*Brassica napus* L.) is an important rotational crop for Oklahoma winter wheat (*Triticum aestivum* L.) as a tool to clean up weed infested fields. Many growers in this region are relying more and more on no-till systems to improve soil moisture holding capacity and prevent soil losses to runoff and erosion. However, establishing winter canola into no-till systems following wheat can be challenging.

Objectives: Wheat is known to have allelopathic exudates which can inhibit weed germination (Zhang et al. 2015). We hypothesize here that certain wheat varieties exert an allelopathic effect on winter canola survival in no-till systems where crop stubble is not removed.

Methods: Wheat straw samples were collected from 2 locations of Oklahoma State University’s 2014 wheat variety trials, Chickasha and Lahoma, OK. Experiments were initiated as a complete 2 x 42 factorial with canola variety as factor one and wheat variety as factor two. Straw was chopped to 5cm lengths and a “tea” was made from the straw simulating a 35 bushel wheat crop with 2.5 cm of rainfall between harvest and canola planting. Wheat straw was “brewed” 48 hours and vacuum-filtered. Ten canola seeds of each variety were treated with three mLs of tea and subsequently watered as needed with distilled water. Digital images were taken at 3, 5, and 7 days after treatment (DAT). Fresh and dry weights were taken for each plot at the conclusion of the study. Digital images were evaluated for plant biomass pixel counts.

Results: One-third of the 42 varieties tested across two locations significantly decreased winter canola fresh weight 7 DAT. Wheat stubble samples from Chickasha had greater allelopathic effects than those sampled from Lahoma. The following straw samples affested canola germination and biomass accumulation as much as 50% regardless of collection location or canola variety: ‘Endurance’, ‘Pete’, ‘Armour’, ‘OK Rising’, ‘WB-Grainfield’, and ‘Doublestop CL+’. The following wheat straw samples from Chickasha, OK significantly reduced biomass accumulation for both canola varieties: ‘Deliver’, ‘OK Bullet’, ‘CJ’, ‘WB-Redhook’, ‘LCS Mint’, and ‘Centerfield’; however, samples of the same varieties from Lahoma did not reduce canola germination and biomass. ‘Doans’ was the only wheat variety to reduce canola biomass accumulation collected from Lahoma, while Chickasha samples of the same variety had no effect.

Conclusions: It is important to further investigate the capacity for wheat straw from particular varieties to impact canola germination and biomass accumulation in the fall, as this is vital to establishment and winter survival. Remaining wheat straw samples from this experiment which had an effect on canola germination will used to investigate effects in the field in Fall 2015.

References:
Enhancing soil resilience and productivity of Indian mustard through green manuring and residue management in semi-arid tropics

Background: Oilseed brassica (OSB) is an important edible oil crop of Indian subcontinent and shares one-third of domestic edible oil production. However, yield stagnation has been experienced in last decades. Multiple soil health and climate change issues are major physical reasons for the yield stagnation which needs to be addressed immediately. And building soil organic carbon (SOC) content is most reliable but challenging option to make OSB production system sustainable in semi-arid tropics.

Objectives: Mustard residue due to poor fodder value is usually burnt to clear the field but its incorporation into the soil system could pave ways to improve the soil organic pool (DRMR, 2011). Additionally, green manuring during rainy season is advocated for improvement of fallow-mustard sequence. Therefore the purpose of the study was to evaluate the effects of mustard residue and green manure on soil health and mustard productivity.

Methods: The long term replicated experiment keeping conventional practices (CS), Sesbania Green Manuring (SGM) and 2.5 t/ha mustard straw recycle + SGM (MSGM) in main plot and eight combinations of NPK fertilizers in subplot was started in 2004-05 at Bharatpur. The crop and soil health attributes on each treatment were recorded at regular intervals over the study period. The actual yield was transformed to relative yield to minimise the temporal effect on the data. The standard ANOVA was performed to compare the treatments in a year and pooled information to draw logical conclusions.

Results: SGM significantly improved the SOC, soil organic microbial biomass, infiltration rate, available NPK status, but decreased bulk density over CP. MSGM further augmented the soil health attributes. The increase in fertilizer levels from N40P8.7K0 to N80P17.4K33.3 also improved the soil attributes gradually. This gradual improvement in soil health was clearly visible in yield attributes and seed yield from 4th year of experimentation. Overall, mustard seed yield was increased by 40.6% due to SGM and by 61.1% due to MSGM over CP in 9 years. Increase in fertilizer levels from N40 to N80 and P8.7 to P17.4 also improved the seed yield significantly while results of K application were inconsistent. The combined application of N80P17.4K33.3 synergistically increased the seed yield by 53.6% over N40P8.7K0. The growth in relative yield of mustard over years followed logarithmic function and predicted the achievement of plateau yield in 11 years under MSGM and 18 years under SGM in comparison to 33 years under CP.

Conclusion: OSB is important source of edible oil and has great potential to make India self reliant in edible oil. But the yields are presently stagnating with negative growth in area and production. The trend could be reversed through application of these findings and double the seed yield and profit margin to bring back the crop to the path of sustainable production and thereby the country to reduce the import bills.

References:
Coefficient of correlation in winter rapeseed

Background: Rapeseed profitability and market demands determine whether farmers will choose to grow it or not. Stable rapeseed production depends on many factors (Radić et al. 2011a). Considering that only rapeseed cultivars and hybrids with high genetic potential are grown today, for yield (over 5t per acre), oil (45-50%) and protein content (18-20%) in seed, as well as that serious growers apply adequate and opportune agricultural practices, it can be concluded that stable rapeseed production is significantly affected by various climate factors (Radić et al. 2011b). Some seed characteristics can be positively or negatively correlated, and the correlation can largely affect rapeseed seed yield.

Objectives: The aim of this study was to determine correlations between observed parameters and effect of locality on the tested winter rapeseed cultivars.

Methods: Testing took place on two localities in the Republic of Srpska, Bosnia and Herzegovina (Bijeljina and Brčko) in 2009/2010. Five different rapeseed genotypes (G-1 to G-5) were tested. The trial was laid out in three replications. Seed yield of certain genotypes was determined on an experimental plot, while 1000 seed weight, seed germination, oil and protein content were determined in the laboratory of Oil Crops Department of the Institute of Field and Vegetable Crops, Novi Sad (Serbia). The study used these parameters: climate factors, seed yield, seed germination, 1000 seed weight, seed oil and protein content. GENSTAT computer program was used for the analysis of variance of two-factorial experiments.

Results: Yield ranged between 1,838 kg (G-5) and 2,543 kg (G-3). Thousand seed weight showed similar results with lowest 3.5 g (G-5) and highest 4.3 g (G-3). Seed germination ranged between 75% (G-5) and 91% (G-3), while oil content ranged between 44.64% (G-2) and 48.15% (G-4). Protein content ranged between 17.27% (G-5) and 20.03% (G-4). Based on the results we concluded that production location significantly affected all observed parameters. There was a significant correlation between seed yield, 1000 seed weight and oil content. Observing all five cultivars and parameters, genotype G-3 was the best cultivar in the production conditions. On the other hand, G-5 had the lowest results for almost all observing parameters.

Conclusion: Based on the obtained rapeseed yield results, it can be concluded that there was a significant effect of locality on the choice of cultivars and hybrids. Therefore, it is necessary to choose appropriate cultivars and hybrids for certain localities.

References:
Study on heat stress traits tolerance in rapeseed/canola (*Brassica napus* L.)

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**Background:** Heat stress is an alarming threat for crop production including rapeseed/canola (*Brassica napus*). It causes early abortion of flowers and causes the pollen mother cells to rapidly enter into meiotic prophase and finally causes pollen grain death which leading to pollen sterility (Parish et al., 2012). Seed yield contributing traits, such as pods per plant, pod length, seeds per pod, seed weight are suffered by heat. High temperature during flowering time significantly reduces the seed oil and seed protein content. Seed production reduced sharply at 7d 350C and 150C HTS during early flowering stage (Angadi et al., 2000).

**Objectives:** To identify canola germplasm containing heat stress tolerant trait through natural and artificial screening.

**Methods:** A total of 160 previously genotyped *Brassica napus* germplasm of spring type canola were naturally screened in the field. Data on pollen sterility, racim height, number of pod per racim, flower abortion per racim, pod length, seed weight were taken. Different degrees of pollen sterility, flower and pod abortion, seed weight were observed in the germplasm in the field. Screening of the same germplasm under artificial heat stress simulating condition in a walk-in plant growth chamber is in progress. The heat stress simulation condition in the growth chamber is designed as 180C for 8 hours, temperature ramped up from 180C to 350C in 6 hours, followed by a constant 350C for 4 hours, and then the temperature will be ramped down from 350C to 180C in 6 hours. The heat treatment will be given for 5 day, and then will bring back the treated plants into a normal greenhouse growing conditions. A controlled experiment has set with the same germplasm without the heat stress treatment. The phenotypic data will be aligned with the SNP genotyping data to identify the genomic region controlling the heat tolerant gene(s) in the wide accessions of *B. napus* through a genome-wide association mapping.

**Results:** Variable pod abortion and pollen sterility were identified among the germplasm screened in the field. Under heat stress simulation condition in the growth chamber, high variation on pollen sterility, flower abortion, yellowing desiccated pod, pod abortion were observed compared with the control experiment conducted in normal greenhouse conditions. Several germplasm were identified tolerant to heat stress in growth chamber heat simulation conditions.

**Conclusions:** The heat stress tolerant accessions will be used in breeding program to introgress the trait into elite breeding lines. Genome-wide association mapping will be conducted to find the genomic region controlling the trait.

**References:**


Genotype by environment interactions for seed yield and physiological traits in Indian mustard (*Brassica juncea L.*) under heat stress and normal environments

**Background:** Among the ever-changing components of the environment, the constantly rising ambient temperature is considered one of the most detrimental stresses (IPCC, 2007). Physiological traits are limited in number and often do not reliably portray genetic relationships since genotype by environment (G x E) interaction reduces the rate of genetic improvement. This makes it necessary to test selections over several environments. Indian mustard, because of its resilience to diverse agro climatic conditions and sustainability towards abiotic stresses (heat), occupies the premier position as an important oil producing crop in India. However, the sensitive behaviour of the existing varieties of Indian mustard to different growing environments in Rajasthan leads to fluctuations in its yield, which is mainly attributable to the occurrence of G x E interaction.

**Objectives:** To evaluate the G x E interaction on seed yield and physiological traits and to isolate the promising and stable genotypes based on their stability parameters.

**Methods:** The experiments were conducted at the experimental farm, ICAR-DRMR, Bharatpur, India during rabi 2012-13 and 2013-14 under heat stress and normal temperature situations. Two hundred seeds of each genotype including two checks (BPR-543-2 and RH-30), were sown under heat stress and normal temperature conditions in crbd with three replications. The crop was raised strictly under conserved moisture conditions. All genotypes were grown in two rows of five meter length. Growth and physiological characters, including, percent population survival at 10 days after sowing (PPS 10DAs) and percent population survival at 25 days after sowing (PPS 25DAs), percent membrane stability index (PMSI), percent excised leaf water loss (PEWL), percent relative water content (PRWC), percent water retention capacity of leaves (PWRC), seed yield per plant (g), 1000-seed weight (g) and percent oil content were recorded from five randomly selected plants of each genotype. Percent leaf membrane stability index (PMSI) was determined following the method of Premachandra et al., (1990) as modified by Sairam (1994).

**Results:** The mean sum of squares due to genotype and environment interactions was found highly significant for all the traits under study except oil content. Stability analysis was carried out as per Eberhart and Russell (1966) model for all the observed characters in order to verify the presence of variance due to component of G x E interaction. The genotype CS-54, Kanti, DRMR-1826 and RGN-48 attained more percent population survival at 10 and 25 days after sowing alongwith regression coefficient equilant to unity and S2di near to zero considered as stable. The stability parameters for PRWC revealed that genotype JN-031 (mean = 67.14, bi= 0.69, S2di=0.25), DRMR-1346 (mean = 66.67, bi= 1.03, S2di=1.26) and DRMR-1351 (mean = 67.95, bi= 0.98, S2di=0.78) recorded high mean PRWC to population mean alongwith regression coefficient near to unity and S2di near to zero exhibiting the stability, therefore their performance was stable and desirable. The genotype GM-3 (mean=20.94, bi= 0.90, S2di=0.40) and DRMR-541-41 (mean=21.59, bi= 0.92, S2di=0.29) recorded maximum mean seed yield per plant to population mean alongwith regression coefficient near to unity and S2di near to zero considered as stable. While the genotype JN-031 had attained maximum seed yield per plant alongwith regression coefficient near to unity, exhibit average stability.

**Conclusions:** By genotype by environment interactions for seed yield and physiological traits it was concluded that genotype JN-031, DRMR-541-41, GN-3, JN-032, CS-54, Kanti, RGN-48, DRMR-1826, DRMR-1346 and DRMR-1351 were found as stable. Additionally these genotypes may be included in any breeding programme where objective is to develop high-yielding stable genotypes under heat stress situation.

**References:**

Water use efficiency, productivity, gas exchange traits and sustainability of Indian mustard (*Brassica Juncea L.* Czernj & Cosson) under micro irrigation and fertigation system

**Background:** Indian mustard (*Brassica juncea*) is an important edible oilseed crop in Indian subcontinent. The area, production and productivity of rapeseed-mustard were 6.6 Mha and 8.3 mt and 1250 kg/ha. India holds 9% of world’s arable land and only 4% of the water resources. Rapeseed-mustard is mainly grown under limited irrigation water conditions. The limited water can be suitable and efficiently utilized through proper irrigation scheduling under micro irrigation and fertigation of soluble major elements. Adaptive advantages of mustard to water stress also reported, but the effects of use of limited water through micro irrigation system (MIS) and different levels of N fertigation on growth, physiology, yield attributes, yield and water use efficiency had not reported adequately.

**Objectives:** In this background an experiments was conducted to assess the impact of irrigation methods on growth, gas exchange traits, yield attributes and yield of mustard crop, to evaluate the moisture dynamics of soil during crop growth period and the economics MIS and the check basin irrigation (CBI) on mustard based crop.

**Methods:** The experiment on MIS and fertigation methods for Indian mustard was conducted at DRMR farm located at 77.300 E longitudes, 27.150 N latitude (2009-12). The treatments consisted of five irrigation system in main plot viz. micro-sprinkler system (MS), MS followed by CB, drip irrigation system (DS), DS following by CB and CB alone. In sub-plot four levels of N, control (0 kg/ha N), 40 Kg/ha N, 80 Kg/ha N and 120 Kg/ha N doses were taken.

**Results:** The outcomes revealed that MS, DS significantly influenced dry weight, chlorophyll concentration, photosynthetic rate, photo synthetically active radiation (PAR), internal CO₂ concentration (ICC), primary and secondary branches, main shoot length, total siliquae and 1000 seed weight. The accumulation of soluble nitrogen, sugars, starch, proline and increased internal CO₂ concentration and net photosynthesis might be the plausible reason for increased photosynthetic rate and biomass under these micro irrigation and fertigation with higher levels of N (Sharma and Ramachandra 1990, Singh). The mustard seed yield was increased by 24 % due to micro-sprinkler irrigation (MS) and by 18 % due to drip irrigation. Increase in N-fertigation levels from N0 to N80 had significantly improved mustard yield attributes, seed and oil yield while results of N120 application were inconsistent. The higher photosynthetic rate was observed with higher internal CO₂ concentration. The sustainability index MIS was > 0.50 and higher production efficiency (9-13 kg/ha/day) was also recorded under MIS.

**Conclusions:** Micro irrigation sytems (MIS) at every level of N fertigation out yield the effect of check basin irrigation (CBI), save up to 50 % of irrigation water and enhanced the seed and oil productivity of Indian mustard.

**References:**
N-Efficiency in winter oilseed rape and prediction by hyperspectral reflectance

**Background:** Nitrogen efficiency of oilseed rape is low and high amounts of fertilizer are applied (Pouzet 1995). This causes environmental problems. Due to EU-legislatve restrictions N efficiency has moved into focus of breeders. N use efficiency (NUE) is the product of N uptake efficiency (NupEff) and N utilisation efficiency (NutEff). They contribute to different portions to genetic variation in NUE (Kessel et al. 2012). Direct selection for N efficiency parameters is laborious. Indirect selection methods would be very helpful. Hyperspectral reflectance correlates with several N efficiency parameters (Müller et al. 2008).

**Objectives:** Genetic variation in N uptake (Nup), NupEff, NutEff and NUE is analysed. The contribution of NupEff and NutEff to genetic variation in NUE is estimated. Hyperspectral canopy reflectance is validated for its applicability as selection tool for N efficiency. Therefore calibrations for several N efficiency parameters are developed.

**Methods:** A diverse set of 29 genotypes was tested in 5 environments in Central and Northern Germany in two parallel trials. One was harvested at end of flowering (eof), the other one at maturity. Both trials were conducted at 2 N-levels (N0: no N fertilizer, N1: 160–180 kg N/ha). Aboveground biomass at eof and seed and straw at maturity were harvested. N content was determined. Nup, NupEff, NutEff and NUE were calculated. Hyperspectral canopy reflectance was measured in the field before flowering and during fruit development using a HandySpec Field spectrometer (tecS, 2 sensors MMS1 305–950 nm, PGS-NIR2.2 951–2215 nm). Unscrambler 10.3 (Camo) was used to calculate calibration models. Several approaches were tested.

**Results:** Seed yield, Nup, NupEff, NutEff and NUE showed significant genetic correlation. Heritabilities ranged between 0.76 (NupEff) and 0.92 (NutEff). NupEff, NutEff and NUE were significantly higher at N0 than at N1. Except NupEff the traits were significantly affected by the interaction between genotype and N level. NutEff contributed to a higher portion to genetic variation in NUE than NupEff. For Nup at maturity, Nup at eof and seed yield promising calibrations based on hyperspectral canopy reflectance were developed.

**Conclusion:** Significant genetic variation was found for N efficiency parameters and heritabilities were high. It can be concluded that N efficiency is a selectable trait which can be implemented in breeding programs. Hyperspectral canopy reflectance can be used as selection tool for N efficiency.

**References:**


Electrical capacitance: A selection tool for root traits and N-efficiency?

**Background:** N-efficiency of oilseed rape (OSR) is low. High amounts of N-fertiliser are applied (Pouzet 1995). This causes environmental problems. EU-legislative restrictions have moved N-efficiency into focus of breeders. Root characteristics are crucial for N-uptake (Nup), but direct selection is difficult. Electrical capacitance (EC) has been discussed as in-situ measurement for root traits (Dalton 1995)

**Objectives:** EC of winter OSR is analysed for genetic variation and its relationship to root traits and N-efficiency.

**Methods:** 29 genotypes were tested at 2 N-levels in 5 environments in Germany (EC29). Nup, N-uptake efficiency (NupEff), N-utilisation efficiency (NutEff) and N-use efficiency (NUE) were determined. EC was measured at end of flowering and during fruit development. Ten genotypes differing in EC were tested in field trials (2 locations, EC10f) and under controlled conditions (EC10c). In EC10f EC, root mass and stem diameter were determined. In EC10c EC and stem diameter measurements were followed by root sampling. Root masses were determined. An image-based platform (Bucksch et al. 2014) was used to capture projected root area, root density, root system width and tip diameter.

**Results:** EC showed significant genetic variation and heritabilities of 0.65–0.95. EC29 revealed genetic correlations (N=29) between EC and NupEff (r=0.64), NutEff (r=0.40), NUE (r=0.58) and N-content (r=0.54). In EC10f correlations on plot level (N=40) were significant between EC and root masses (r=0.33–0.46) and stem diameter (r=0.78). The latter was also significant for single plants. In EC10c EC genetically (N=10) correlated to stem diameter (r=0.91), lateral root mass (r=0.67) and root area (r=0.59). For single plants correlations were significant between EC and stem diameter, root mass, root system width, tip diameter and projected root area.

**Conclusions:** EC showed significant genetic variation and high heritabilities. It has to be considered as a genetic trait. It remains open if EC measures root traits. Significant correlations were found. But correlation coefficients to stem diameter were higher. As genetic correlations to root traits and N-efficiency parameters are only medium (r=-0.54–0.64), EC should not be considered as appropriate selection tool.

**References:**


Effect of seed size and suitable cleaning on seed viability, vigour and quality of rapeseed (*Brassica napus L.*) cultivars

Inspection on seed physical purity in order to produce seeds with suitable size, good viability and free of inert matter is the most important step in the process of seed quality control. According to the effect of seed size on seed quality, this study was conducted in the laboratory, greenhouse and the field of Seed and Plant Certification and Registration Institute (SPCRI) in 2009-10, based on completely randomized design and randomized complete block design with three replications. The four factors experiment with 16 treatments that consisted of two canola’s cultivar (Okapi and Talaye), four separated seed size (1.4, 1.6, 1.8 and 2 mm) and two levels of using and none using of gravity separator tool were applied. So for conducting of this project 48 samples were provided randomly from each land race and placed to seed analysis laboratory of the SPCRI and samples were affected by treatments and finally rate of seed, seed purity, noxious weed seeds and whole number of weed seeds was recorded. Some part of the seed samples was planted on the germination paper and final germination (7 day after planting), seedling dry weight was recorded and also some indices as mean time to germination, germination speed and seedling vigor was calculated by using of seedling vigor index (SVI). The second part of the seed samples was used for carrying out the greenhouse experiment and also seedling emergence speed, final green percentage of seedling and some related characteristics was determined. Based on the results using of 1.6 mm sieve size accompanied to gravity separator tool is recommended for cleaning these two varieties while germination percent and seedling emergence was improved and also higher seed loss percent was attained by using of sieves with 1.8 and 2 mm size. Whatever lower seed loss percent was attained using 1.4 mm sieve size but percentage of seed purity was decreased and also number of weed seeds increased which resulted in rejection at standard seed certification process. So the best treatment was related to the sieve with 1.6 mm size that was standard in the aspect of physical purity and germination percent.
Developing production technology of canola quality rapeseed-mustard for north-west India

**Background:** India has to import about 50% of its requirement of edible oils and there is not much scope in the country for area expansion under oilseeds (Paroda 2013). To meet the increasing demand of edible oils, alternate strategies through superior cultivars and matching agronomic management are required. Availability of rapeseed-mustard varieties/hybrids with canola traits is anticipated to enthuse farmers towards cultivation of these crops.

**Objectives:** To assess hybrids and cultivars of canola Indian mustard (B. juncea) and oilseed rape (B. napus) to nitrogen applications and row spacings; and to find out optimum age of seedlings for transplanting of current canola oilseed rape cultivars.

**Methods:** Study comprised of three field experiments. In experiment 1, two hybrids (PHR 1, PHR 2) and three cultivars (mcP 633, rlc 3, rlc 1) of Indian mustard and in experiment 2, three hybrids (PGSH 52, PGSH 53, PAC 401) and one cultivar (GSC 6) of oilseed rape were evaluated. In both the experiments genotypes were allocated to main-plots whereas combinations of two nitrogen (100 and 125 kg/ha) doses and row spacings (30 and 45 cm) comprised the sub-plots. Third experiment comprised combinations of two transplanting dates (25 November, 10 December) and two cultivars (GSC 7, GSC 6) of canola oilseed rape in the main-plots and three ages of seedlings (30, 35 and 40 days old) in sub-plots.

**Results:** Indian mustard hybrid PHR 2 produced 2.4, 11.6, 6.1 and 32.2% higher seed yield (2494 kg/ha) and 5.2, 13.0, 20.8 and 38.6% higher oil yield (1041 kg/ha) than PHR 1, RLC 3, RLC 1 and MCP 633, respectively. Oilseed rape hybrid PAC 401 produced 6.8, 9.8 and 14.4% higher seed yield (2727 kg/ha) and 6.8, 19.5 and 15.4% higher oil yield (1160 kg/ha) than PGSH 52, GSC 6 and PGSH 53, respectively. Differences in yields and quality due to nitrogen and row spacing in both Indian mustard and oilseed rape were marginal and non-significant. Oilseed rape transplanted with 30 days old seedlings produced 5.8 and 12.1% higher seed yield and 7.4 and 13.3% higher oil yield than 35 and 40 days old seedlings, respectively. Quality seed, oil and seed meal remained unaffected by dates of transplanting, cultivars and age of seedlings.

**Conclusions:** The study demonstrates higher yield potential of hybrids than cultivars of canola Indian mustard and oilseed rape. These promising hybrids and cultivars respond to application of 100 kg N/ha and 30 cm row spacing. Transplanting of younger seedlings (30 days old) of oilseed rape up to second week of December performed better than older seedlings.

**References:**
Genetic variation for heat tolerance in *Brassica juncea*

**Background:** Heat stress causes adverse alterations in plant growth, physiological processes and productivity (Sharma 2014). Exigencies of the intensive/multiple cropping systems results in delayed planting of rapeseed-mustard crops in north-west India. This can cause significant yield losses due to forced maturity. Heat stress during seed filling stages results in low yields and poor seed quality. Development of new cultivars tolerant to heat stress is a major challenge and thrust area for rapeseed –mustard research.

**Objectives:** Documenting differential tolerance of Indian mustard genotypes to terminal heat stress. Morpho-physiological traits and stress indices associated with thermo tolerance were also investigated.

**Methods:** A set of 44 advanced breeding lines/varieties from different agro-climatic zones of India were sown at optimum (third week of October) and late sown (3rd week of November) conditions. Seed filling periods under delayed plantings normally coincide with rising end season temperatures. Each genotype was sown in paired rows with three replications in random block design. Photosynthesis was recorded on 3rd and 4th fully expanded top leaf (90 DAS), yield components and SY at maturity. Heat tolerance indices and correlations (SSI, STI, HTE and YSI) were computed for biomass and yield.

**Results:** Normal sown cultivars had higher photosynthetic rates (Pn, 14.1-26.8 mean 21.3 umol/m2-s-1), lower average stomatal conductance (Cs, 0.892 mol/m2-s-1) and Tr (7.09 mol/m2-s-1). Late planting down regulated Pn (9.92-16.7 mean 14.3) but upregulated Cs (1.01) and Tr (8.29). Pn estimates were comparable for RB50, NRCR701 and DRMR537-40 across two planting dates. WUE was lowest in DRMR541 (2.42 umol/mmol) and maximum in RH-555A (3.87). Overall, average Pn declined by 32.8%, WUE by 42.4% while Cs and Tr increased by 10.8 and 16.9% with delayed planting. Terminal heat stress significantly influenced most productivity related traits except siliquae on main shoot and seed weight. Trait depreciations were observed @ 6.3% for plant height, 2.9% for main shoot length, 32.0% for primary branch number, 39.5% in secondary branches, 28.8% for total siliquae and 9.5% for silique length over normal planting. NRCDR-02 showed the least (37.3) number of siliquae on main shoot and BPR549-9 the highest (60.2). Seed weight varied from 3.88g (EJ17) to 6.4g (BPR-543-2). Lower biomass (14.7-45.2 mean 31.1) and SY (2.58-9.25 mean 6.45) were recorded under late planting. Decline in biomass was 27.6 % and SY 25.4% over timely sown crop. Genotypes namely, PBR331, RGN197, Parasmani, RRN631, CS54, BPR541-4, RB50 and SKM 301 were considered heat stress tolerant. Positive correlation existed between yield under timely sowing (Yts) and HSI, while negative correlation existed between Yts and HTE for yield and biomass. Under late planting, yield (Yls) was negatively associated with HSI.

**Conclusions:** Modified tolerance indices revealed PBR331, RGN197, Parasmani, RRN631, CS54, BPR541-4, RB50 and SKM 301 as promising for terminal heat stress tolerance.

**References:**
Allelic variation for shp-1 gene in crop Brassicas

Background: Unsynchronized pod shattering in Brassica leads to significant yield losses (Wang et al. 2007). The appropriate approach to solve this problem is by revealing the genes involved in the regulation of pod dehiscence. There are many genes involved in the mechanism of pod dehiscence that have not been identified in Brassica. So far only the orthologue of shp-1 has been reported in Brassica napus and is available at NCBI repository. Based on this information attempts were made to isolate and clone putative gene shp-1 from different species of Brassica.

Objectives: To characterize the natural variation in shattering in Brassica by determining the allelic differences in the gene/coding sequence of the shp1 gene clones among the six species viz, B. rapa (2n=20, AA), B. nigra (2n=16, BB) and B. oleracea (2n=18, CC), and three amphidiploid species, B. juncea (2n=36, AABB), B. carinata (2n=34, BBCC), and B. napus (2n=38, AACC).

Methods: A set of primer pairs (shp-1F 5’-ACAGGTACGCTTCTCTACTC-3’ and shp-1R 5’-TGAAGGGAGGTGGTCTTGA-3’) were designed from the functionally characterized shp-1 gene sequence of the B. napus cv Bridger (BnSHP1 gene, Accession no. AF226865) for amplification of a putative shp-1 in diploid and amphidiploid species of US’s triangle. The amplified PCR products were eluted and ligated into pGEM®-T Vector System I and bacterial strain JM109 of E.coli was used for the maintenance of all recombinant plasmids. Colonies carrying the recombinant plasmids were confirmed by restriction analysis. The positive clones were sequenced using M13-Universal primers through Sanger Sequencing. The identities of the clones were compared to known sequences using BLAST. The sequences reads were analyzed in Geneious software v5.5.7. Sequence features were identified in all sequences using Arabidopsis model in FGENESH and GeneMarkHMM. Phylogenetic and molecular evolutionary analyses were conducted using MEGA6.

Results: Multiple sequence alignment of shp-1 gene of various Brassica subspecies models the informative and non-informative sites that have occurred over the evolution. Such allelic variation of shp-1 gene showed significant effect at structural and functional aspects of proteins. Extensive shatterproof1 sequence-based analysis of Brassica species genome have provided the evidence of shp-1 gene existence and similarity between three separate but closely related diploid species (AA, BB, CC) and creation of three new amphidiploids (AABB, AACC, BBCC) genes derived from the genes of ancestral diploid species.

Conclusions: The complete full length gene sequence of Brassica subspecies is under further investigation and more detailed examination will be presented during the conference, highlighting the relationships between the members of US’s triangle.

Reference:
Full sib progeny selection for genetic improvement of Indian mustard (*Brassica juncea* L.) under moisture stress conditions

**Background:** Indian mustard (*Brassica juncea* L.) is considered as self pollinated. However, out crossing of 7-18% (Abraham, 1994) has been reported which indicates certain level of heterozygosity and suggests use of population improvement strategies to expose hidden variability. Success of any population improvement approach depends upon genetic gains per cycle of selection. The information on this aspect is almost lacking in Indian mustard.

**Objectives:** Study was carried out during 2006-07 to 2013-14 to study the effect of different cycles of full sib progeny selection in creating genetic variability for yield and its components under moisture stress conditions.

**Methods:** Experiment was started during rabi 2006-07 involving Varuna and BPR-148 in crossing programme. From F2 variable population, 5 sets of five plants were selected randomly. This constituted the male plants. Each male plant in each of the set was crossed to a set of 4 randomly selected plants from the population using them as females. Total 100 crosses were made to develop biparental progenies (Comstock and Robinson, 1948). Full sib progenies were evaluated during rabi 2009-10 in augmented design under rain fed conditions with check varieties. Significantly superior progenies for seed yield/plant in comparison to checks and base population were selected and equal amount of seed from these progenies was mixed and sown in crossing block (2010-11). From this variable population, 124 full sib progenies as per North Carolina Design-I (NCD-I) were developed and evaluated (2011-12) in augmented design under moisture stress conditions. Again significantly superior progenies were selected from second cycle of selection and equal amount of seed from these progenies was mixed and sown in crossing block (2012-13) for development of full sib progenies. 120 full sib progenies so generated were evaluated (2013-14) in augmented block design under moisture stress conditions with four checks. Data were analyzed statistically as per standard procedure.

**Results:** Genetic gains were compared between 1st and 3rd cycle of selection and it was observed that genetic gain has increased appreciably from 1st cycle to 3rd cycle of selection for seed yield/plant (from 57.16 to 85.47). This indicates accumulation of favourable alleles in enhancement of yield per se through full sib progeny selection. There were 18 progenies selected in first cycle of selection, 17 progenies in second cycle of selection which showed significant superiority over checks and base population. In third cycle of selection 25 progenies not only showed significant superiority and selected over general mean of the progenies.

**Conclusion:** Population improvement revealed significant genetic gain in each cycle of selection. Therefore, it is advocated to follow selected sib-mating between selected progenies plant for accumulation of favorable allele(s) as well as breaking the undesirable linkages.

**References:**
Analysis of characteristics of winter oilseed rape (*Brassica rapa* L.) growth and development in cold and arid areas of Northern China

**Background:** With the application of winter rapeseed cultivars with ultra cold-tolerance, the growing area of winter rapeseed in China has shifted northerly from Tianshui (Negative Accumulated Temperature, NAT, -151.0°C) to Zhangye (NAT -729.55°C), Jiuquan (NAT -746.83°C) and Urumqi (NAT -1092.0°C) etc. Thus, it is necessary to characterize the changes of the growth and development of winter rape under severe natural conditions in Northern China.

**Objectives:** This study sought to analyze the characteristics of winter oilseed rape (*Brassica rapa* L.) growth and development in its northern adapted area, aiming at offering a theoretical basis for breeding new winter oilseed rape cultivars (*B. rapa* L.).

**Methods:** Field tests were conducted during 2006-2013 in eight locations in China including the original growing area and seven northern adapted areas of *B. rapa* L. Analyses were conducted based on the major climate factors of the testing locations, overwintering ratio, changes in the agronomic traits of the selected winter oilseed rape cultivars (*B. rapa* L.) during their growing period. Comparing with the original growing area.

**Results:** This study shows that the overwintering ratio of the selected cultivars in the northern adapted area reduced from 93-100% to 40-95%, while their growing period increased from 280-284 days to 287-289 days. The life cycle of winter *B. rapa* L. could be characterized as the long overwintering stage, the short growing period before winter, in the northern adapted area. Compared with those grown in the original growing area, winter oilseed rape grown in the northern regions exhibited phenotypic differences including a shorter plant height and smaller thousand seed weight. Yield of Longyou No. 6, a cultivar with strong cold-tolerance, had increased significantly from 0.74 t/ha (in Tianshui) to 3.33 t/ha (in northern adapted area).

**Conclusions:** Winter oilseed rapes grown in the cold and arid areas of Northern China suffer from a relatively adverse weather conditions. Thus, cultivars used for agricultural production in these areas are required to have excellent tolerance with cold conditions. Seeding has to be done at an appropriate time to ensure enough accumulation of vegetative growth before winter.

**Reference:**

Effect of seeding date on production of organic and conventional rapeseed

**Background:** Being the third-leading source of vegetable oil in the world, rapeseed is popular oil crop for various purposes, while cultivated Brassica species are generally regarded as excellent rotation crops. Rapeseed is also organically grown for oil production, as green manure and is a useful cover crop. Brassica species can also be used in pest management though the efficiency of their application is variable and relatively low. Considering appropriate rapeseed cultivation practices, seeding date (SD) can significantly affect plant vigor and the ability of plants to compensate damage by biotic or abiotic stress (Valantin-Morison and Meynard 2008). Due to rising interest in organic rapeseed, additional information on appropriate agricultural practices could be useful.

**Objectives:** The performance of conventional rapeseed varieties at various SD in organic growing was tested. Results should be useful to both breeders and farmers to determine the need for specific organic breeding programs or agricultural practice improvement. The objectives of this study were to: (1) compare conventional rapeseed cultivars performance in conventional and organic farming systems, and (2) investigate the effect of SD on some basic rapeseed production traits like emergence, survival rate, seed, oil and protein yield.

**Methods:** Five winter rapeseed 00 type cultivars were sown in two farming systems, each with three seeding dates and four repetitions. The trials were organized in a randomized block design and the effect of cultivar and farming system on emergence, yield, oil and protein content was evaluated. The fields were kept free from weeds, insects and diseases according to the recommended practices. In organic field, weeds were removed mechanically and manually, while insects were treated with an insecticide used for organic production. The seed samples for analysis of oil and protein content were taken during harvest.

**Results:** We found that some conventional rapeseed cultivars like Slavica could be successfully used in organic farming systems, and concluded that developing new cultivars specifically for organic farming is not necessary. Yield and oil content were lowest in SD3 in both farming systems. There was no significant effect on the protein content. It was found that late sowing date and shallow soil tillage are related to high ratio of weed biomass, especially in organic production.

**Conclusions:** Rapeseed can be used in organic agriculture, but further improvement of agricultural practices is needed. The biggest problem is the complete lack of chemical options for organic weed management, which makes good agricultural practices such as farm hygiene more important, to prevent the spread of pests, diseases, weeds and that may reduce production. Preliminary results will be used to further improve the initial organic agricultural practice and select the most appropriate cultivars for further testing and organic production.

**Acknowledgements:** This research is a part of the project TR 31025 financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia

**References:**

Using mason bees (*Osmia* spp.) for rapeseed yield improvement

**Background:** Pollinators can play a significant role in rapeseed production. Honeybees are excellent pollinators, but in the previous decades the number of bee hives has dramatically decreased due to the yet unexplained colony collapse disorder. Due to these reasons, alternative pollinator species and their use come into focus for research.

**Objectives:** Orchard bees, or mason bees, *Osmia* spp. (Hymenoptera: Megachilidae) are used for pollination of orchards and certain berry crops worldwide (Krunić and Stanisavljević 2006). The aim of this study was to evaluate the effectiveness of *Osmia cornuta* and *O. rufa* pollination in rapeseed production and their effects on the yield.

**Methods:** The effects of orchard bees on rapeseed yield and their value as pollinators were studied during spring 2013 at Rimski Šančevi site, near Novi Sad, Serbia (N 45° 20' 22" E 19° 50' 9.62")

Fine netted isolation cages of 48 m² each were used for two trials set up under the same field conditions. In the first trial, the efficiency of *O. cornuta* and *O. rufa* was compared on four rapeseed varieties. In the second, the effect of rapeseed genotype was tested using only *O. cornuta*. A total of 20 males and 10 females were introduced into every cage at the beginning of the flowering stage. Every tested rapeseed variety had a control cage without bees. Statistica 12, StatSoft was used for statistical analysis.

**Results:** Average yield for all cages without bees was 1.710 kg/ha, for *O. cornuta* cages 1.941 kg/ha and *O. rufa* cages 2.116 kg/ha. The only significant yield differences were found due to the effect of genotype in the second trial where genotype variability was higher. Considering oil content, thousand seeds weight and hectoliter weight, only hectoliter seed weight was significantly affected by genotype in the first trial, while the presence of bees had no significant effect. In the second trial, the only significant effect was that of genotype on oil content and hectoliter weight.

Average oil content was 39.14% for all cultivars in the control cages, 39.72% for cultivars with *O. cornuta* and 39.95% with *O. rufa*. Thousand seeds weight was the lowest for seeds from *O. rufa* cages (4.83 g), followed by *O. cornuta* (4.95 g) and control cages (5.04 g). Hectoliter seed weight varied from 69.50 in cages with bees to 69.73 kg in control cages.

**Conclusions:** Obtained data suggest that *Osmia* bees could be a useful addition, if not a substitute for honey bees. Interactions between rapeseed varieties and solitary bee species should also be taken into account when planning further long-term trials. The genotype and environment effects could be prevailing due to variable genotype attractiveness to bees, insecticide treatments or unfavorable climatic conditions. Nevertheless, the results are promising and encouraging for further research.

**Acknowledgements:** This research is a part of the project TR 31025 financed by the Ministry of Education, Science and Technological Development of the Republic of Serbia

**References:**

Background: Drought is one of the greatest worldwide environmental constraints for agriculture. Therefore engineering drought tolerance in plants has huge economical importance. Molecular and genomic analyses facilitate gene discovery and enable genetic engineering using several functional genes to activate drought tolerance. Molecular chaperones are key components contributing to cellular homeostasis in cells under adverse growth conditions. The binding protein (BiP) is an endoplasmic reticulum (ER) resident molecular chaperone, assists in the folding of proteins and also acts in the ER quality control mechanism. Water stress disrupts ER homeostasis and promotes the accumulation of misfolded or unfolded proteins in the ER lumen caused ER stress. BiP plays a major role as a sensor of disturbances in protein folding. The ER resident molecular chaperone BiP mediates an increase in drought tolerance and delays drought-induced leaf senescence in soybean and tobacco (Valente et al, 2009).

Objectives: The overexpression of BnBiP in Brassica napus can increase tolerance to water deficits, osmotic stress and tunicamycin treatment. The detail molecular mechanism of BnBiP conferring drought tolerance will be discovered.

Methods: Plant expression cassette containing the BnBiP gene was transformed into Brassica napus. Water stress and tunicamycin treatment are applied to untransformed (WT) and overexpression (OE) transgenic line plants. Drought-tolerance parameters are measured. Subcellular localization of BnBiP is performed by Arabidopsis thaliana protoplast transformation. Expression of BnBiP protein is induced in E.coli.

Results: BnBiP is localized in the plasma membrane. 73.8 kD BnBiP protein is soluble. The observation that enhanced accumulation of BnBiP prevents dehydration. Fluctuations of BnBiP levels correlate inversely with the activity of oxidative stress-induced enzymes. BnBiP overexpression correlated with the decreased oxidative damage and a delay in leaf senescence under water deficit conditions. BnBiP OE transgenic lines show increased resistance to the cell death-promoting effect of tunicamycin in germination and further growth. qRT-PCR analysis suggests BnBiP overexpression delays UPR (unfolded protein response) and NRPL (N-rich proteins)-pathway mediated chlorosis and appearance of senescence-associated markers.

Conclusions: the prosurvival effect of BiP was associated with the modulation of the ER and osmotic stress-induced NRP-mediated cell death signaling. The enhanced expression of BnBiP prevented BnNRP-mediated cell death. The results implicate BnBiP as a negative regulator of stress-induced NRP-mediated cell death response. Thus, it is not surprising that the overexpression of BnBiP delays drought-induced senescence in Brassica napus OE lines, and confers the increased adaptation of these transgenic lines under water deprivation conditions compared with the wild plants. The dynamic competition model for BnBiP competed with misfolded proteins will be confirmed in the future.

References:
Model-based analysis of potentials to increase nitrogen use efficiency of winter oilseed rape

**Background:** Winter oilseed rape (WOSR) is characterized by high nitrogen (N) uptake efficiency and lower N utilization efficiency (Malagoli et al. 2005). Early leaf senescence due to self-shading and incomplete N translocation to pods and seeds cause low N use efficiency (NUE) and N harvest index (NHI) (Schjoerring et al. 1995).

A model could provide scenarios to quantify the effects of improvements of physiological processes like N translocation on NUE and NHI.

**Objectives:** The aim of this work is to quantify the effects of changing dry matter partitioning, N dilution or improved N translocation on NUE and NHI by means of a validated dynamic crop model.

**Methods:** A dynamic crop growth model based on the light use efficiency approach was developed. It contains dry matter production and partitioning, N uptake and partitioning, leaf and pod area growth, as well as senescence and translocation processes and yield formation. In addition, drought stress and N depletion effects on growth of WOSR are included in the model.

The model was parameterized with data from field experiments conducted in northern Germany and validated with independent data sets from several sites in Germany as well as Châlons, France. Long-term scenarios (1980-2010), calculated for different sites include variation of allometric relations between leaf, stem and pod dry matter, as well as improvement of N translocation processes.

**Results and Discussion:** Parameterization and validation of the model showed its capability to simulate dry matter and N dynamics as well as leaf, stem and pod area growth. Effects of management practices like sowing date and N fertilization level are well represented by the model. Therefore, the model can be used to calculate scenarios, e.g. genetic improvements by a decrease of remaining N concentration in stems and roots at harvest. Scenarios of decrease of remaining N concentration in stems and roots from 1% to 0.5% indicate additional 30-40 kg N/ha available for translocation into pods but the sink capacity of pods for N during seed filling has to be considered.

**Conclusion:** The crop growth model is able to simulate physiological crop development under varying conditions. Scenarios simulated with the model could be helpful to estimate potentials to increase NUE. Further improvements of the model may be achieved by implementing a layered canopy representation, which would allow an analysis of effects of N distribution and canopy architecture on WOSR productivity and NUE.

**References**


Genetic variation and inheritance of seed longevity in the oilseed rape DH population Sollux x Gaoyou

Background: Volunteer oilseed rape plants may emerge in succeeding crops in high numbers. This appearance is based on the seed capability to survive for 10 years or longer in the soil. Seed survival is related to dormancy and to seed longevity. Seed longevity in crop species is in part genetically determined (Nagel et al. 2014). The half-viability period is estimated to be 7.3 years for different cabbage (Brassica spp.) varieties under ambient conditions (20 °C, 50% RH; Nagel et al. 2014). Artificial ageing methods are usually used to mimic seed behavior in storage, but real data of long term stored seeds are rarely available.

Objective: The aim of this study was to investigate the germination rate and the germination vigor of seeds of the DH population Sollux x Gaoyou after thirteen years of storage in a semi-conditioned seed storage room.

Methods: The DH population was developed from a cross between the German winter cultivar Sollux and the Chinese semi-winter cultivar Gaoyou and consisted of 292 lines. The DH population was grown in 2000/01 in the field at two locations in western Germany and at two locations in China (Xian and Hangzhou; Zhao et al. 2005). Seeds harvested from open pollinated plants were ever since stored in Göttingen in a seed storage room with temperatures varying from about 7°C to 25°C depending on the season. Germination test was conducted from July to Dec. 2014. 50 seeds per line and location were placed on filter paper in Petri dishes, watered with 12 ml of deionized water, and kept in dark condition at 16-17 °C. The percentage of fully germinated seeds as well as the hypocotyl length was determined after 10 days.

Results: There were large and highly significant differences in the germination rate and the germination vigor of the DH population after 13 years of storage. As a mean over 4 locations the germination ranged from 0 to 70% (mean 19%), while hypocotyl length varied from 0 to 3.88 cm (mean 0.99 cm). There were also highly significant effects of the locations for both seed germination and germination vigor. Seeds harvested in Hangzhou germinated to 26%, while seeds from Reinshof, Xian and Weende germinated to 15, 17 and 19%, respectively. Heritabilities ranged from 72% to 77% for germination and germination vigor. Spearman’s rank correlation between seed germination and germination vigor was positive (0.74**).

Conclusions: Results show large and significant differences in seed germination and germination vigor. QTL analysis is in progress to identify genomic regions involved in seed longevity.

References:
Oilseed rape yield evaluations under controlled conditions: Not a load of rubbish!

**Background:** Evaluation of yield performance and other relevant agronomical traits under variable environmental conditions is a major aim of crop breeders. However, efficient selection for genetic variation of yield traits in connection to diverse abiotic stress factors, such as drought or nutrient efficiency, is still challenging. Results from controlled pot and greenhouse trials for complex traits are generally poorly transferable to field experiments. In winter oilseed rape (WOSR, *Brassica napus* L.) one reason for this is the great potential for environmental fluctuation during the long, 11-month lifecycle. Another reason is the restriction of adequate root growth by small pots, which strongly influences the response to water and nutrient availability.

**Objectives:** To improve the field transferability of greenhouse pot experiments, we established a plant growth system comprising large refuse containers (120 L “wheelie-bins”) that allow detailed phenotyping of field crop populations under semi-controlled growth conditions with minimal constriction of root growth. We tested the system to assess traits related to drought stress and nitrogen use efficiency in highly diverse WOSR cultivars.

**Methods:** Genetically diverse WOSR cultivars were grown at field densities throughout the entire crop lifecycle in “wheelie-bins” with a quadratic planting area of 0.16 m², which were filled to a depth of 90 cm with a dried soil mixture that was prepared according to the requirements of the specific experiments: For drought experiments a sandy soil was separated in topsoil and subsoil fractions and the well-watered control was held at a level of 60% Wc, whereas the drought control was only watered to a level of 30% Wc. For nitrogen experiments a clay-loam soil mixed with sand at a ratio of 1:1 was filled in the containers and the low nitrogen (LN) treatment received an equivalent of 40 kg N ha⁻¹, while the containers with the high nitrogen (HN) treatment received an equivalent of 200 kg N ha⁻¹ (split into two applications). The different experiments were carried out over two years to compare seed yields from individual containers to plot yields from multi-environment field trials.

**Results:** Comparisons of yield data from the containers to full-plot field trials demonstrate that results from controlled-environment pot experiments are highly transferable to field conditions. In particular, we were able to accurately predict yield levels in the field from measurements taken on container-grown plants. Furthermore, since the variation in root morphology is an important aspect in consideration for enhanced abiotic stress tolerance, the container system builds a compromise in terms of root growth between field and pot experiments and allows the assessment of root morphology and biomass. In this regard we found a huge genetic variation of root morphology, particular under contrasting N-fertilization. The large-container system represents a highly promising platform to evaluate breeding material for physiological and yield-related abiotic stress responses.
Effects on cold tolerance of winter 
*Rapa* under ABA spraying on leaves

**Background:** By the changes of the global temperature, northern China becomes the new district for winter oilseed rape. But this district is very cold in winter, the lowest temperature is always reach to -30°. There only *Brassica rapa* can survive. The *Rapa* leaves will gradually become yellow and dry from 5 leaf stage before wintering. Until the end of November, the whole seedling is yellow and dry only leave the root in the soil from which the new leaves will sprout next year. So roots plays a very important role in this time. ABA is currently recognized as the important plant hormone to cope with water stress and it always start from root.

**Objectives:** Spraying ABA on leaves to analysis the effects of exogenous ABA on cold tolerance of winter *Rapa* before the wintering, then make sure the best time and spraying concentration on them.

**Methods:** Setting the test in the field, Longyou 8(*Brassica rapa*) as the material, sprayed ABA on leaves by different concentrations(5, 10, 15, 20, 25 mg·L-1) and sprayed in different periods (3 leaf stage, 5 leaf stage, 6 leaf stage, 7 leaf stage) by 15 mg·L-1 before the wintering. Spraying distilled water was as the CK. Determined the overwintering rate, the activities of SOD, POD, CAT and content of soluble protein, soluble sugar.

**Results:** Spraying ABA can significantly improve overwintering rate and physiological and biochemical substances content. The effect is extremely significant when the concentration is 20mg·L-1. The overwintering rate improve 26.7%, and the activities of SOD, POD, CAT and content of S protein, soluble sugar, were significant increased 32.3%, 71%, 451.8%, 78.3%, 78.3%, 22.1% than CK respectively. It also can reduce the content of MDA, lower 42.5% than CK. At the 6 leaf stage, the MDA content of winter *Rapa* decreased the most, and the content of soluble sugar, the content of soluble protein and winter survival rate increased 4.4%, 35%, 10% than CK respectively.

**Conclusions:** Exogenous ABA can decrease the damage under low temperature and improve the cold tolerance of winter *Rapa* in north China. It is suggested that the best time to spray ABA on winter *Rapa* is 6 leaf stage, and the best concentration should be 20 mg·L-1, so it can effectively improve the cold tolerance of winter *Rapa*.

**References:**

Evaluation of four *B. juncea* genes for pod shattering resistance in *B. napus*

**Background:** Silique dehiscence (pod shattering) in canola (*Brassica napus*) can result in significant yield losses. Therefore, one of the goals in canola breeding is to develop pod shattering-resistant cultivars. Although many attempts have been made to increase shatter resistance through conventional breeding, so far traditional breeding methods have been unsuccessful. On the other hand, transgenic manipulation of *Arabidopsis* genes, such as SHP1/2, FUL, ALC and IND, resulted in transgenic lines where silique fails to split; breaking of the silique with excessive force is needed to release the seeds (Liljegren et al., 2000; Liljegren et al., 2004; Østergaard et al., 2006). Pod shattering resistance in *B. juncea* is generally greater when compared with *B. napus* (Kadkol et al., 1984). Transgenic manipulation of canola with *B. juncea* genes which are involved in silique dehiscence (Jaradat et al., 2014) may increase resistance to pod shattering to a reasonable extent that excessive force would not be needed to release the seeds.

**Objectives:** To engineer *B. napus* with four putative dehiscence genes from *B. juncea* and evaluate these transgenic lines for resistance to pod shattering.

**Methods:** Standard molecular biology methods were used to produce transgenic canola plants through Agrobacterium mediated transformation. To assess pod shattering, an optimized method was used where the siliques were shaken with metal rods or metal beads in polypropylene tubes on a reciprocating shaker.

**Results:** Homozygous lines with single insertion were identified. Several transgenic lines displayed greater resistance to pod shattering than controls. The pod shattering range is 35%-80% for controls, but 5%-40% for transgenic lines. Thus, the four *B. juncea* genes may be useful to develop pod shattering resistant canola.

**Conclusions:** Laboratory tests showed that manipulation of *B. napus* canola with *B. juncea* genes improved pod shattering, however, field test is necessary to validate this.

**References:**


Influence of straw mulching on cold resistance and yield of rape

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Background: The damage of low temperature and freezing is one of the main limitations of the production of winter rapeseed in the Yangtze River region of China. The extreme cold weather occurs frequently in the Yangtze River region of China and has seriously affected the safety of the winter rape's overwintering of safety in the region. In recent years, the workers who research rape breeding of cold resistant varieties have done lots of work and cultivates a series of new cold resistance Brassica napus L, while we seldom see the study on cold resistance of cultivation measures of the late sowing rapeseed.

Objectives: This paper mainly studies the effects of different agronomic measures on the regulation of late sowing rape field soil temperature and the freezing injury degree, yield, effect. It can not only put forward the corresponding technical measures for freezing winter rape in Yangtze River region of China, but also provides scientific basis for the development of antifreeze cultivation of late sowing rape.

Methods: (1) Soil temperature was determined with long pole soil thermometer. Soil temperature in 10 cm and 20 cm layer in each plot was recorded in 15:00pm after treatment. Mean soil temperature of every continue 10 days made as the temperature of these duration.

(2) Plant cold injury investigation standard referred to Liu Houli's method. 50 plants samples were surveyed randomly on each plot on 5-7 days after severe frost. Cold injury index is calculated by formula (1) with surveyed each plant.

Cold injury index (%) = \left(\frac{1\times S1+2\times S2+3\times S3+4\times S4}{N\times 4}\right) \times 100 

*S1, S2, S3 and S4 mean plant numbers with 1-4 grade cold injury; N means total surveyed plant number.

(3) Plant height, first branch number, effective silique number, seeds per silique, 1000-seed weight and some other characters were determined from these plant samples. Yield was determined from a subplot in each plot.

Results: The result shows that Straw mulching (T1) can affect the soil temperature and cold resistance of Qing you 19. When the ambient temperature is lower than the ground temperature, Straw mulching (T1) play a better role of resisting the low temperature than the treatment of heaping soil around roots before winter (T2), whereas the T2 is better.

Conclusions: If the Straw mulching (T1) can be widely used, it can improve canola yield and solve the problem of straw burning. Besides, although T2 (heaping soil around roots before winter) can increase yield for rapeseed, it needs too much money and labor. How to make the maximum benefit of the T2 should further research.

References:
Analysis of chloroplast ultrastructure, stomatal characteristic parameters and photosynthetic characteristics of chlorophyll-reduced mutant in *Brassica napus* L.

**Background:** Chloroplasts are unique and important organelles in the plant cell, which capable of carrying out photosynthesis, but also, the place of synthesis chlorophyll, starch, lipid and amino acid. The development of chloroplast and chlorophyll biosynthesis play crucial role for normal photosynthesis in plant. Abundant leaf color mutants, the ideal material to carry out the research of structure of photosynthesis system and function of gene and its regulation mechanism, which is excellent germplasm resource of heterosis utilization.

We found the chlorophyll-reduced mutant NY in 2005 had been bred stable recessive genic male sterile two-type line was less reported spontaneous mutant in *Brassica napus* L., to study its chloroplast ultrastructure, stomatal characteristic parameters, photosynthetic pigment content, photosynthetic characteristics, agronomic traits and the relationship between each other is of important theoretical value and practical significance.

**Objectives:** In order to discuss the yellowing mechanism and provide a theoretical basis in rape breeding practice, the relationship between chloroplast ultrastructure, stomatal characteristic parameters, photosynthetic pigment content and photosynthetic characteristics of the spontaneous chlorophyll-reduced mutant NY in *B. napus* L. were studied.

**Methods:** Taking the rape mutant NY, wild type NG, F1 (NY×NG) and rF1(NG×NY) as research materials, the heart-leaf and the flatten-leaf at five-leaf stage were used for chloroplast ultrastructure observation, stomatal characteristic parameters investigation, photosynthetic pigment content determination, photosynthetic characteristics measurement and agronomic traits investigation.

**Results:** In general, the chloroplast development degree in yellow heart-leaf and yellow-green flatten-leaf from mutant NY was worse than that of wild type NG, F1 (NY×NG) and rF1(NG×NY) from chloroplast ultrastructure; the chloroplast numbers of heart-leaf in a lower epidermis stomal guard cell from mutant NY was decreased about 40%, whereas the number in yellow-green flatten-leaf was close to that of the wild-type NG in stomatal characteristic parameters; the Chla, Chlb, Chl(a+b), carotenoids and the net photosynthetic rate of mutant were significantly lower than those of the same period of wild-type and F1, rF1 in content and composition of photosynthetic pigment as well as photosynthetic characteristics. Correspondingly, the growthperiod delayed, economic traits deteriorated and grain yield per plant decreased, which happened in the mutant, but the above agronomic traits and photosynthetic characteristics from F1 and rF1 were restored to normal levels.

**Conclusions:** The mutant NY is one of total chlorophyll deficiencitype caused bychloroplast structure developmental defects. Abnormal chloroplast structure, less grana and grana lamellelaand lowerchlorophyll contentare main reasons for mutantlower net photosynthetic rates andworstagronomic traits.

**References:**


High temperature stress studies in Indian mustard (*Brassica juncea* L.) tolerant genotypes under rainfed conditions

**Background:** Indian mustard is the major crop of winter season in southern Haryana state of India and is grown also as a rainfed crop in some pockets. High temperature at seedling stage causes seedling mortality and also increases painted bug population, thereby, reduces proper plant stand in the field. At terminal stage, high temperature shuts down photosynthetic machinery causing shriveled seeds that ultimately reduces crop yield.

**Objectives:** Early sown crop faces seedling mortality upto 100 percent due to high air temperature prevailing at that time. If this crop is sown too late, it faces high temperature stress later on causing heavy yield losses due to forced maturity. Therefore, it is imperative to breed varieties that are tolerant to high temperature stress.

**Methods:** The trial was conducted under three dates of sowing [(30th September (early sown), 22nd October (normal sown) and 11th November (late sown), 2012 and 2013)] with 57 identified high temperature tolerance genotypes received from different centers in India (CCSHAU, Hisar; RAU ARS, Ganganagar; IARI, New Delhi and DRMR, Bharatpur) under rainfed conditions. These genotypes were sown in two rows each under rainfed conditions. One hundred seeds of each genotype were sown in each row. Seedlings were allowed to grow under natural conditions in the field. All the agronomic and plant protection measures were followed as per recommendations.

**Results:** (a) High temperature tolerance at seedling stage: Germination % and seedling mortality (%) were recorded at 7 DAS and 10 & 20 DAS, respectively. Maximum temperature range between 7 DAS (recording of germination %) and 10 DAS (recording of mortality %) was 31.00 C to 34.50 C. Some of the genotypes survived high temperature (34.50 C) at seedling stage. Genotypes showing <20% seedling mortality at 10 DAS were categorized as thermo tolerant.

(b) High temperature tolerance at terminal stage: These genotypes were sown in the field at two dates of sowing i.e. Ist optimum, D1 (22.10.2012 & 13) and IInd late, D2 (11.11.2012 &13) to allow the crop to experience the high temperature at terminal stage viz. grain filling stage (100 DAS-maturity) under natural conditions. Observations were recorded on seed yield/plant (g) and 1000-seed wt. (g). Reduction % in these traits under D2 over D1 was computed. The genotypes having <20% reduction in seed yield and 1000-seed wt. were rated as high temperature tolerant at terminal stage.

**Conclusions:** Mean of data of 2 years on plant mortality at 10 DAS showed that only 5 genotypes viz. Pusa mustard-27, RH-1003, Pusa mustard-25, RH-0748 and Pusa Bold were identified as High temperature tolerant at seedling stage. whereas mean of 2 years data on % reduction in seed yield & 1000-seed wt. of mustard genotypes RH-725, DRMR-659-49, RB-55, BPR-543-2, RH-673, RH-819 and RB-100 were identified as High temperature tolerance at terminal stage. These identified genotypes are being used in crossing programme (13 x 13 Partial diallel) at Bawal during 2014-15 season.
Screening for canola (*Brassica napus* L.) accessions with contrasting early vigor for genetic studies and breeding

**Background:** Seed germination and successful seedling establishment are two independent events of major concern in crop production which affect crop yield (Jain and Staden 2007). Selecting accessions with rapid germination and good establishment which are recognized as two useful parameters of early vigor is important for improving crop production (Pinthus and Kimel 1979; Yamauchi and Winn 1996).

**Objective:** The objective of this study was to evaluate germination speed of canola accessions from a world-wide collection to identify accessions with high and low early vigor. In addition, the relationship between germination speed and seed weight, and how smoke water, ABA and GA affect germination speed were assessed.

**Method:** A total of 137 accessions originating from 17 countries were used to conduct germination trials in petri dishes at 25 °C in darkness. Comparisons of germination speed among the various accessions were based on T50, i.e. the time at which 50% of seeds germinated (Limami et al. 2002). The accessions were classified into three categories fast (F), medium (M) and slow (S). The fast and slow accessions were identified and validated in repeated petri dish and pot experiments, and also treated with four chemicals i.e. deionized water (control), smoke water, gibberellic acid (GA) and abscisic acid (ABA).

**Results:** The 137 accessions were grouped into three categories: F, M and S and each category accounted for 6.6%, 84.6% and 8.8% of the accessions, respectively. Finally, 9 fast and 12 slow germination accessions were identified. Although accessions in the F category showed significantly faster germination and emergence than those in the S category, seedling growth parameters did not differ greatly. Based on germination speed and seedling characteristics, 4 accessions with high early vigor and 4 with low early vigor were identified. Seed germination speed was not affected by seed weight and was not directly influenced by the application of GA and ABA, but 10% smoke water significantly delayed seed germination.

**Conclusions:** Early seedling vigor can be reliably estimated through a combination of germination speed with seedling vigor index, as these represent germination, emergence and seedling growth. The identified accessions for high and for low early vigor can be used to look into the genetic and molecular mechanism that determines early vigor. Breeding of superior canola cultivars with high early vigour has the potential to be of great significance in canola production.

**References:**


Research on easy and reduced cultivation techniques of rapeseed in Yangtze River Basin of China

**Background:** In China, conventional high-yield cultivation in rapeseed overly depended on manual work leading to high yield not high-efficient, so comparative benefit of planted rapeseed reduced. And, too much water and chemical fertilizers influence the quality of rapeseeds and increase the cost of cultivation, but pollute farmland and water system. So it is very important to discuss new cultivation techniques for rapeseed.

**Objectives:** To study easy and reduced cultivation techniques of rapeseed according with china's national condition in Yangtze river basin of China, in order to save labour and reduce cost and increase benefit of cultivation.

**Methods:** From 2010 to 2014, double-low rapeseed variety Qinyou 19 was tested for the cultivation. After harvesting rice planted in heavy viscosity soil in WuHu of Yangtze river basin of China, the two techniques of mechanized tillage and sowing and harvest and mechanized tillage and soil preparation for broadcast sowing and harvest were compared with conventional seedlings transplantation technique.

**Result:** After continuous test of four years, the results showed that the output value of rapeseed only increased 3.88% and 4.00% respectively to compare the two new cultivation techniques with conventional technique, but from cost saving on fertilizer, pesticide and expense for mechanized tillage and labors, pure profit increased 6794.10 RMB/hm²and 11422.2 RMB/hm² respectively, and ratio of output and input both increased 1.25 times.

**Conclusion:** Easy and reduced cultivation techniques of rapeseed combined agriculture traits with agricultural machinery may save cost greatly and improve economic benefit, so it is a development direction of rapeseed cultivation techniques in China.

**Conference:**


Effects of nitrogen fertilizer types and application times on the nutrient uptake and yield of winter oilseed rape under high density and direct-sowing conditions

Background: China is an important country of winter oilseed rape production in the world. The traditional way of rapeseed cultivation in China consists of seedling raising and transplantation, manual field management, and manual harvest, with no or little mechanization. Thus it consumes a lot of labor and requires a high production cost in per unit area (mainly labor cost) (Zhang C L et al. 2010; Guan C Y 2011). In recent years, new cultivation ways by machineries with high density and direct-sowing in winter rapeseed are accepted by more and more farmers because large quantity of farm labors are shift to cities, causing decrease in manual labor availability and increase in labor cost in the rural areas (Wang H Z 2005; Guan C Y 2006; Ma N et al., 2011). Chemical fertilizers (especially nitrogen fertilizer) are usually applied for 3~4 times in the traditional cultivation system of winter oilseed rape, whereas little information about the types of nitrogen fertilizers and times of fertilization is available for the management of winter rapeseed under high density and direct-sowing conditions.

Objectives: Field experiments were carried out to investigate the gain of grain yield, amounts of nutrient uptake and efficiency of nutrient utilization under high density and direct-sowing with different N fertilizer types and different times of fertilization. Suitable, feasible and labor-saving fertilizer application methods were expected in this study.

Methods: Three field experiments were carried out for the study in the Chengdu Plain under a rice-rapeseed rotation system from 2011~2014. The first experiment included 3 different modes of N fertilizer application times (one time as a basal fertilizer, two times as a basal fertilizer and a top dressing, three times as a basal fertilizer and two times of top dressing), using the same rates of fertilizers (225kg N/hm2, 112.5kg P2O5/hm2, 112.5kg K2O/hm2) under high density (36×104/ hm2) from direct-sowing. The second experiment consisted of 4 different treatments of N fertilizer types applied only once as a basal fertilizer (normal urea, coated urea, compound fertilizer, 50% normal urea +50%coated urea) and 2 different treatments of two time fertilization (50%normal urea as basal fertilizer +50%normal urea as top dressing, 50%coated urea as basal fertilizer + 50% normal urea as top dressing) at the same fertilizer rates (189kg N/hm2, 90kg P2O5/hm2, 90kg K2O/hm2), plus one contrast treatment(without N fertilizer). The third experiment included 3 different N fertilization times only (as same as in the first experiment), but lower fertilizer rates (180kg N/hm2, 90kg P2O5/hm2, 90kg K2O/hm2) were applied.

Results: The differences in plant nutrient uptake, grain yield, oil content (%) and nutrient utilization efficiency (grain yield/plant available N) were not significant among different N fertilizer application times under high density and direct-sowing conditions. Grain yields from different fertilization times varied in different years and showed a tendency of relatively higher yield with higher fertilizer rates. Grain yields and amounts of nutrient accumulation were significantly different between the treatments with N fertilization and without N fertilization. The types of N fertilizers showed no significant effects on grain yield, oil content (%), nutrient uptake and nutrient utilization efficiency. Overall, the slow-releasing type of N fertilizer (coated urea) resulted in a relatively higher grain yield or nutrient accumulation under the same fertilization times.

Conclusions: Based on the above results of experiments in winter oilseed rape under high density and direct-sowing, we concluded that the times of N fertilization could be reduced to one time as a basal fertilizer or two times as a basal fertilizer and a top dressing. Moreover, the slow-releasing N fertilizer type (coated urea) could be applied to get higher yield, higher nutrient accumulation and to alleviate the pressure on environment.

References:

Stress-responsive gene ICE1 from winter oilseed rape (*Brassica campestris* L.) confers cold tolerance in transgenic tobacco

**Background:** ICE1 acts upstream of the CBFs in the cold-response pathway. Arabidopsis ICE1 activates CBF3 expression during cold treatment. The activated CBF3 binds to the CRT/DRE cis-acting element in the promoter regions and induces the expression of downstream cold-responsive genes, thereby improving freezing tolerance (Chinnusamy et al. 2003). Overexpression of ICE1 in transgenics resulted in improved freezing tolerance, supporting an important role for ICE1 in the cold stress response.

**Objectives:** Winter oilseed rape (*Brassica campestris* L.) cultivar Longyou 6 seedlings can survive winter at −32 °C in the cold and arid regions in Northern China. Longyou 6 was used as experimental materials. ICE1 gene was isolated and characterized from Longyou 6. The ICE1 gene was transferred into tobacco and the effects of ICE1 transformation on the cold tolerance of tobacco under low temperature was studied.

**Methods:** The full-length ICE1 cDNA sequence from *Brassica campestris* (rape) was obtained by the technology of rapid amplification of cDNA ends (RACE). The recombinant plasmid, pBI121-ICE1, was introduced into the *Agrobacterium tumefaciens* strain GV3101. Transformation of tobacco was performed using Agrobacterium-mediated leaf disc transformation method. Transgenic lines and non-transgenic plants were planted on MS medium to allow the seeds to germinate under greenhouse conditions. Six-week-old tobacco seedlings were treated with 2 °C for 7 d, and then leaves was collected for physiologic parameters measurements.

**Results:** A novel gene, ICE1 (GenBank accession number: JF268687), was isolated and characterized from *Brassica campestris* (rape) cultivar Longyou 6. The cDNA length of ICE1 is 1737 bp with an open reading frame of 1500 bp. The ICE1 contains the conserved bHLH domain. The ICE1 gene, with the CaMV35S promoter, was introduced into tobacco. When transgenic and non-transgenic plant stressed by 2 °C with seven days, the transgenic lines were characterized by increased levels of chlorophyll content, photosynthetic rate, stomatal conductance, relative water content, proline content, soluble sugar content, SOD and CAT activities, and decreased levels of electrolyte leakage and MDA content.

**Conclusions:** These results indicate that the ICE1 from winter oilseed rape cultivar Longyou 6 is a positive regulator of cold tolerance, which may play an important role in the regulation of the cold stress responses in plants.

**References:**
The effect of waterlogging on yield at the early flowering stage in *Brassica napus* L.

**Background:** Different from those waterlogging-tolerant species, rapeseed (*Brassica napus* L.) is sensitive to waterlogging stress due to the lack of aerenchyma and high rate of radial oxygen loss from the root base. In China, 80% of rapeseed is planted along the Yangtze River, as a rotation crop in rice paddy fields. Seeds are sown in rice paddies in autumn directly after the rice harvest. Young seedlings are exposed to the still-humid paddy soil and often encounter rainfall during the flowering stage in spring, which is a very critical stage in whole-plant development for ultimate yield.

**Objectives:** The aim of this study is to answer the following questions: (1) what are the adverse effects of waterlogging on yield and seed quality at the early flowering stage? (2) which morphological traits play a key role in yield loss? and (3) are there any differences in the evaluated traits among these varieties?

**Methods:** A field experiment was conducted using 20 rapeseed varieties to evaluate the effect of waterlogging at the early flowering stage on yield and seed quality. The field experiments were conducted in three different environments in China. 10 agronomic traits were evaluated including plant height (Ph), branch number (Bn), branch height (Bh), main inflorescence length (Mil), siliques on the main inflorescence (Smi), siliques on branches (Sb), siliques per plant (Spp), seeds per silique (SpS), main root length (Mrl) and thousand seed weight (Tsw). Finally, the plants in each plot were harvested for yield evaluation. In addition, some traits related to seed quality were also assessed using FOSS-NIR systems. The waterlogging tolerance coefficient (WTC) was used to evaluate waterlogging tolerance.

**Results:** The results showed that waterlogging stress affected rapeseed growth and caused yield loss. Except for Bh and Tsw, all other traits were significantly affected by waterlogging. A correlation analysis revealed that the WTCs of all the morphological traits were significantly correlated with that of yield, except of the WTCs of Bh and Bn. However, the WTCs of seed weight and seeds per silique were not found to be significantly correlated with that of yield. Additionally, waterlogging affected the oil quality by increasing erucic acid and glucosinolate content. Waterlogging also caused an increase in linolenic acid and a decrease in linoleic acid, indicating that waterlogging might affect metabolic pathways involving lipid biosynthesis.

**Conclusions:** The reduction in the number of siliques per plant after waterlogging is mostly due to the decrease of siliques on branches, which governed the final yield after waterlogging. Our study reveals the effects of waterlogging on different varieties of rapeseed at the early flowering stage and provides some data that may be useful for breeding more tolerant varieties.

**References:**

Changes of endogenous hormones in seedling influence on flower bud differentiation for early maturing rapeseed

**Background:** Early maturing rapeseed benefit adapting multi-plant system and escaping hot harm season in China. Early-maturing and early flowering is significantly correlated (r≥0.90), and flowering time is an important factor that determine maturation time (Amiri-Oghan et al. 2009). The bud differentiation of the rape flower is regulated comprehensive by a variety of hormones; the effects of the GA on flower buds differentiation and flower development play a dominant role (Ruth et al. 1992). Hormone changes in rapeseed plant tissue regulate and control flower buds differentiation.

**Objectives:** Investigate the impact of endogenous hormones changes on flower bud differentiation process and the development of floral organs for early mature rape under different sowing date, to do so can guide early maturing rapeseed breeding and high efficient cultivation practices.

**Methods:** 1358, Zhongshuang11 and Zhesuang8 represents early maturing, mid-maturation (CK1) and late-maturing variety (CK2) separately. Test was conducted in testing base located in Wuhan (30°34'N, 114°20'E) in 2013/14. Three sowing date with a 7d interval, planting density 150,000 plants per hectare. Endogenous hormone was determined during seedlings.

**Results:** The earlier they were sowed, the sooner flower bud differentiation. 1358 takes the shortest time at all the three sowing date. By sowing delaying, the flower bud differentiation time for 1358 is getting longer and longer. As for the flowering time, early-maturing variety 1358 is earlier than CK. Its differentiation, squaring and bolting for 1358 required less active accumulated temperature then CK. GA3 and ZR average content in 1358 seedlings of each sowing are significantly higher than CK, IAA and ABA cont is significantly lower than CK, higher GA3 and ZR and lower IAA and ABA could promote flower bud differentiation early.

**Conclusions:** Duration time for flower bud differentiation is shortest for 1358 than CK. By the sowing delaying, early varieties prolong flower bud differentiation. And early maturing variety rape bloom earlier and has longer flowering period. High GA3 is benefit for bud differentiation. ZR had the same function with the GA3.

**References:**
Effects of paclobutrazol on yield and mechanical harvest characteristics of direct-seeding winter rapeseed

**Background:** In order to control the problem of lodging, plant growth regulators (PGRs) (stem shorteners) were applied to control plant height, which could help to meet mechanical seed harvesting demands. However, there was not a precise recommendation of paclobutrazol application time and concentration. Besides, little research was available about paclobutrazol affected on pod shatter resistance in oilseed rape to date.

**Objectives:** The aim of this research was to study the effects and mechanism of paclobutrazol (PP333) treatments on rapeseed yield and mechanical harvesting.

**Methods:** Paclobutrazol was foliar sprayed at the concentrations of 0, 150 and 300 mg L–1 to canopy closed stage and the early bud stage, on two cultivars of rapeseed Yangguang 2009 and Fengyou 520. The degree of lodging was measured for 20 plants per plot at maturity. Lodging was assessed by measuring the angle of inclination of the stem base from the vertical; severe root lodging was recorded when the stem base was at an inclination of > 45° whilst the stem remained undamaged and stem lodging was recorded when the stem had buckled locally. Lodging rates (lodging %) was scored with the formula [(the lodging in plot/the plot area)× 100%] at maturity of three replications, as described by Peng et al. (2014). The snapping resistance and culm lodging resistance index (CLRI) were measured at maturity stage according to the methods Islam (2007) and Wang (2014) with some modifications. Silique shatter resistance was determined according to Morgan (1998). After random impact tests, dry weight of the pod was recorded.

**Results:** Our results demonstrated that: 1) PP333 treatment significantly increased the rapeseed lodging resistance, silique shatter resistance and yield. 300 mg L–1 PP333 at the bud beginning significantly more enhanced lodging resistance and silique shatter resistance, but 150 mg L–1 PP333 at closure period more significantly enhanced yield of two rapeseed varieties. 2) PP333 treatment reduced rapeseed seed numbers per pod, while pods per plant, 1000-grain weight and yield were all enhanced. At the same time, increased thickness of rhizome, root-top ratio (fresh) and snapping resistance, reduced plant height and culm lodging index resuled in reduced angle of plant lodging, which indicated an improvement in the ability of root and stem lodging resistance. Silique shatter resistance increased as increased silique water content and silique dry weight, and delayed pod maturity.

**Conclusions:** In summary, closure period sprayed with 150 mg L–1 of PP333 is the best time and concentration, it significantly enhanced ability of lodging resistance and silique shatter resistance and yield, which could meet rapeseed mechanized production model.

**References:**


Rape seed value creation from the perspective of industry chain - Survey data from Yangtze River Basin

**Background:** With the deepening of market reform and opening to the world, to improve core competitiveness has been top priority for China's seed industry to cope with the increasingly fierce competition caused by multinational seed enterprises. Under market environment, core competitiveness of an enterprise or industry is embodied in value creation ability. How is the value creation ability of each industry chain segment? How much is each segment’s contribution to total seed value? Which is the strategic segment in seed industry chain value creation? The current literature has not systematically studied on these issues.

**Objectives:** This research examines the contribution of each segment of industry chain and explores the value creation rule of seed industry chain through construction and evaluation of value creation model, which provides reference for China's seed industry to improve seed value creation ability and value management level to enhance the overall competitiveness.

**Methods:** Based on farmers’ seed purchasing behavior and research, six main rape seed value elements have been selected, including internal core value, internal general value, appearance and image, brand, advertisement, and service. Seed industry chain includes breeding, production and development, agent and retailing. Taking value elements and their contribution to total value and value creation segments and their contribution to value elements as the main themes, the questionnaire consists of two aspects, respondent’s basic information and respondent’s evaluation on factors accounting for the proportion of total seed value and each segment’s contribution to value elements.

**Results:** (1) The contribution rate of internal core value, internal general value, seed appearance, brand, advertising and service to total seed value is 35.17%, 18.13%, 9.76%, 14.23%, 8.80%, 13.92%. (2) Breeding, production and development, agent, and retailing create 50.14%, 31.00%, 9.82%, 8.76% of internal core value; 43.08%, 40.92%, 8.33%, 7.67% of internal general value; 22.15%, 51.10%, 15.48%, 11.27% of seed appearance; 22.11%, 26.22%, 31.97%, 19.70% of brand; 10.35%, 30.11%, 33.31%, and 26.23% of advertisement promotion; 10.13%, 25.58%, 30.57%, 33.72% of service. (3) The contribution rate of breeder, manufacturer, agent and retailer to rape seed industry chain value creation is 33.70%, 33.85%, 17.63% and 14.82%.

**Conclusions:** According to the matching principle of value creation and profits, profits gained by each segment bearing value creation activities should be generally consistent with its contribution to value creation. Therefore, seed industry chain should adjust the existing distribution pattern and construct reasonable interest distribution mechanism to guarantee the benefits of breeders, developers and farmers, making them have enough capital and power input into value creation activities especially seed breeding and development, strategic segment of rape seed industry chain.

**References:**
Parable of the rapeseed seed

Background: The Parable of the Mustard Seed is one of the most renowned stories from the New Testament. The economic significance of rapeseed today may justify an attempt of interpreting this parable with this crop as its main object. The parable of the mustard seed and the kingdom of heaven appears in three Gospels: in verses 31 and 32 of Chapter 13 of the Gospel of Matthew, ‘Another parable put he forth unto them, saying, The kingdom of heaven is like to a grain of mustard seed, which a man took, and sowed in his field: Which indeed is the least of all seeds: but when it is grown, it is the greatest among herbs, and becometh a tree, so that the birds of the air come and lodge in the branches thereof;’ in verses 31 and 32 of Chapter 4 of the Gospel of Mark and verse 19 of Chapter 13 of the Gospel of Luke. These three versions have common crucial elements.

Analysis: From a theological viewpoint, the mustard/rapeseed seed may be considered a single grain of faith, that, if tended well, may produce countless fruits of a spiritual nature to the benefit of one’s soul and being. There is also an emphasis on the contrast between the size of a mustard seed and the plant it produces: the mustard/rapeseed plant, if sown and nourished well, grows into ‘a great tree’, ‘greater than all herbs’; indeed up to 2 m at their full physiological development. The second part of the parable carries its moral impact: it is faith that opens one’s soul to spiritual benefits, here in the form of ‘the birds of the air’, with this ‘great tree’ of a mustard/rapeseed plant depicted as providing them with dwelling. Again, it may produce more than 15 lateral branches per plant, with a length up to half that of the stems, more than 30 large and broad leaves per plant and the fresh aboveground biomass yields of more than 20 t ha⁻¹. Ultimately, the alighting of ‘the birds of the air’ on the mustard/rapeseed crop may be a token of all positive changes within a person of firm faith. The mustard/rapeseed plant may therefore be regarded as a human body, within which faith may find its lodging, but also as a crop with a substantial inner potential, such as a high seed and biomass oil and protein content.

Conclusions: Learning more about our past may assist us in better contemplating our future: by this reason, such broad and complex interdisciplinary studies, touching upon both the spiritual and earthly spheres of everyday human lives, indeed may result in the casting of more light on the very dawn of agriculture, where both mustard and rapeseed surely have their deserved position.

References:
Mikić, A. Reminiscences of the cultivated plants early days as treasured by ancient religious traditions - The mustard crop (Brassica spp. and Sinapis spp.) in earliest Christian and Islamic texts. Genet Resour Crop Evol (submitted)
Pest Surveillance Initiative (PSI)-Advancing research into grower practice

**Background:** Pest Surveillance Initiative (PSI) is a grower led molecular detection laboratory focused on identification of risks to successful canola production. In partnership with government and public sector researchers, PSI plays a key role in advancing the research discoveries off the lab bench, optimizing methodology for high throughput analysis and providing commercial testing services for crop pathogens.

**Objective:** Using an 'orphan drug' strategy, provide growers access to molecular assays that under normal market conditions the testing industry has shown little interest in marketing due to high cost per sample or a small number of end users. The first project of the Initiative is to optimize technologies for the detection and quantification of low concentrations of clubroot (*Plasmodiophora brassicae*) in Manitoba.

**Methods:** To date, Manitoba has reported very low number of plant positive samples for clubroot. In order to establish a benchmark of clubroot status, soil samples from the field access points of representative fields from 450 township-range coordinates across southern Manitoba were collected, dried and ground. Subsamples were analyzed for presence of clubroot DNA at low levels using modifications to the quantitative polymerase chain reaction (qPCR) technology (Cao et al 2007). Standard dilution curves from clubroot galls as well as artificially infested soils were used as controls.

**Results:** While individual field results are provided to the submitter only, the aggregate data is posted to a dynamic map tool (www.mbpestlab.ca) that provides an up to date status of the occurrence as well as severity (measured by number of spores per gm soil) of clubroot infection in Manitoba. Results form the foundation of extension and field management programming.

**Conclusions:** DNA-based surveillance provides a powerful early warning system of pending crop pests before visible symptoms of damage occur. Field level information using advanced technology, coupled with training tools and extension programs, work together to provide growers information they need to make informed management decisions. The surveillance strategy model is being applied to other crop-pathogen combinations.

**References:**

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Food losses and waste in the French oilseed sector

Background: Recently published reports suggest significant amounts of food losses and waste across the world, but reliable data is scarce. In industrialized countries, little attention has been put so far on losses and waste at the upstream stages of food supply chains (primary production, post-harvest operations, processing). A general food losses and waste approach was initiated by INRA on most relevant vegetable and animal sectors in France, from primary production to food supplying. This study is a contribution to close knowledge gaps on food losses and waste in the French oilseed sector.

Objectives: The overall objectives of this study were to i) identify causes and determinants of oilseed losses and waste at the different stages, ii) quantify them, and iii) discuss potential measures and research perspectives for reduction.

Methods: In this study, food losses and waste are considered all edible parts of food intended for human consumption which are removed from the food supply chain and are not used for animal feed. All process steps in the oil supply chain were described from harvest to supplying, including storage, transportation, crushing, refining and packing to identify the key steps impacting losses. For tofu, all process steps from harvest to supplying were described, including soy milk and tofu production. Data were collected mainly from professionally qualified experts, due to a lack of publications on the topic.

Results: Whilst oilseeds and vegetable oils are adapted to long-term storage compared to other products, total vegetable oil losses from harvest to supplying remain very significant (around 71,4 kT equal to 10% of the oil consumption in France). It was established that the main determinants for oil losses are harvest and refining. The total food oil losses for rapeseed, sunflower and soybean were 9.8%, 7.0% and 6.0% of the potential French oil production, equal to about 50.6, 14.3 and 6.5 M€ or equal to the annual consumption of around 3.029.000, 856.000 and 389.000 people, respectively. For tofu losses, harvest is the main factor. The total tofu losses were 8.2% of the potential soybean production.

Conclusions: The total of food losses from harvest to supplying come out as substantial in the French oilseed sector. Losses are higher for rapeseed than for soybean or sunflower mainly due to cutting and threshing steps. Measures to improve the efficiency of the oilseed system at the key steps are discussed: genetic traits (resistance to shattering in rapeseed or soybean, resistance to lodging in sunflower, upper basal pods in soybean), design of the combine harvester or refining conditions (soft refining). Our study recalls that besides oilseed loss reduction, measures to improve the oilseed system efficiency should also target yield losses (in extreme years, up to 12% of the multiannual mean yield).

References:
**Orbitide metal binding**

**Background:** Orbitides (Linorbitides/LOs) are circular peptides, linked via an N-to-C terminal peptide bond, consisting of 5 to 12 amino acid residues with molecular masses of approximately 1 kDa, exhibiting a wide range of biological activity (Shim et al., 2014). Previous studies showed peptide binding with metals. For example, LOs form complexes with Tb3+ and the metal ion positioned proximally to the Phe residue (Chatterji et al., 1987). In addition, LOs bind Ba2+ more tightly than the other cations studied namely K+, Na+, Mg2+ and Ca2+ (Tancredi et al., 1991). The conformational (circular) structure of orbitides makes them suitable candidates for metal binding.

**Objective:** The current research focuses on the study of metal binding of orbitides in solution. In this study, we choose four metal salts including zinc acetate, zinc sulfate, lead acetate, and cadmium nitrate. These orbitides might act as chelators that reduce toxicity of dietary heavy metals consumed with food or encountered in the environment.

**Methods:** Nuclear magnetic resonance (NMR) and mass spectroscopy (MS) were employed to detect LO metal complexes and determine their structures. Peptide-metal solutions were prepared in deuterated methanol (CD3OD) and methanol for NMR and MS studies, respectively.

**Results:** The proton NMR spectra show metal peptide interaction of LOs with various metal salts. The 1H-methyl signal of methionine S-oxide (SOCH3) shifted downfield in all LOs when compared to control solutions. The spectra of LOs mixed with Zn(OAc)2, ZnSO4 and Pb(OAc)2 were very similar. The alpha proton of Phe (4.6 ppm) was altered while an amide proton at 7.7 ppm disappeared. The methyl peak of methionine S,S-dioxide (SO2CH3) in related LOs does not show the same shift in presence of Zn(OAc)2 and Pb(OAc)2 observed in their methionine S-oxide analogs. Singly charged [LO+M-2H]+ and doubly charged [LO+M-2H]2+ were produced in peptide metal solution (10-2 M, methanol). Cd(NO3)2 shows strong binding at 10-2 M as compared to other metals and Zn(OAc)2 least. Metal complexes were observed forming at 10-2 M to 10-4 M but not at lower concentrations (10-5 M to 10-8 M).

**Conclusion:** Based on the NMR and MS data results, it is confirmed that the binding strength of metals tested were as follows: Zn(OAc)2 < Pb(OAc)2 < ZnSO4 < Cd(NO3)2. At lower concentrations, there might be competitive binding of metal and sodium ions which make it difficult to detect cadmium binding products.

**Reference:**
Albumin complexes with flaxseed gum

Background: Coacervation of protein and polysaccharide is a fundamental physicochemical phenomenon that can affect processes such as encapsulation, protein separation and recovery, enzyme immobilization, gelation, emulsification, and foam stabilization (de Kruif et al., 2004). Flaxseed (Linum usitatissimum L.) gum (FG) contains anionic polysaccharides that occur mainly in the outermost layer of the flaxseed hull (Cui & Mazza, 1996). It has been used as food ingredient and has been reported to interact with proteins, such as salt-soluble meat protein, porcine myofibrillar protein, and whey protein isolate (Chen et al., 2007). However, mechanisms underlying the interactions in aqueous environments has not been systematic studied.

Objectives: The driving forces involved in complex coacervation between FG and bovine serum albumin (BSA) should be understood. Findings from this study will help to advance the understanding of mechanisms underlying associative phase behavior between BSA and FG in solution.

Methods: Turbidimetric analysis and Zeta potential were employed to monitor coacervation between BSA and FG as a function of pH, biopolymer mixing ratio (R), and destabilizing agents (NaCl and urea). Changes of particle size in solution were studied and phase diagrams were established.

Results: Critical pH dependent phase transitions (pHc, pH1, and pH2) associated with the formation of soluble and insoluble complexes of a BSA-FG mixture (R = 1:1) with a total biopolymer concentration (CT) of 0.05% (w/w) were observed at pH 5.4, pH 4.8, and pH 2.0, respectively. The maximum interaction (OD600 = 0.818 ± 0.005) was found at R = 2:1 and pH 3.4 (pHmax) in the absence of destabilization agents. As R increased from 1:15 to 15:1 the critical phase transition pH also increased (pH1 from 4.2 to 5.2, and pH2 from 1.8 to 2.8). The shift of pHmax from 2.80 to 4.80 was consistent with the isoelectric point of BSA-FG mixture while the pHc was independent of R. NaCl significantly suppressed biopolymer interactions and decreased the pHc, pH1, and pHmax, while the pH2 was increased. An overall shift of turbidity curve towards more acidic pH was observed in the presence of urea with less suppression of maximum OD600 than NaCl. Particle size distribution of BSA-FG (R = 1:1, CT = 0.05%, w/w) by dynamic light scattering at different pH provided further insight into the association obtained and disassociation processes during complex coacervation.

Conclusions: Findings from this study demonstrate that electrostatic attractive forces primarily stabilized complex coacervate formation between BSA and FG while secondary stabilization is contributed by hydrogen bond formation. Results obtained provide essential background knowledge to introduce the application of protein-FG interactions in food, pharmaceutical, and cosmetic products.

References:


Commercial oilseed dehulling of oilseed

**Background:** Dehulling, prior to oilseed extraction, is commonly practised in soybean processing but it has only been introduced recently for commercial rapeseed (canola) processing. Dehulling of *Brassica carinata* (A.) Braun and *Camelina sativa* (L.) Crantz is challenging as the small seed size and high oil content lead to poor dehulling efficiency (Mulder et al., 2012).

**Objectives:** The status of commercial dehulling of rapeseed and the quality of products arising from rapeseed dehulling will be presented.

**Methods:** Dehulling of *B. carinata* (L.) and *C. sativa* (L.) was conducted by roller mill and disc mill followed by fractionation in a grain aspirator. The whole seed was ground in either roller or disc mill at two different temperatures (room temperature and freezing temperature) to study the effect of temperature in grinding behaviour of oil seeds. Grinding at freezing temperature was conducted to avoid oil flowability from the cotyledon to the hull during grinding and to improve hull fractionation. Particle size distribution of ground seeds was determined. Geometric mean diameter and geometric standard deviation were calculated.

**Results:** After removal of oil from oilseed the remaining oilseed meal is an excellent source of protein. Seed coats of oilseed are high in soluble and insoluble fibre, which limits both the utility and inclusion rate of oilseed meal in some animal feeds.

**Conclusions:** Freezing grains before grinding had a marked effect on dehulling specially on the carinata. Utilization of the hull fraction of rapeseed and dehulling of *B. carinata* and *C. sativa* will also be discussed.

**References:**
Protein concentrates from brassica meal

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Background: Thin stillage (TS) is usually concentrated to thick syrup by evaporation (Monceaux & Kuehner, 2009). However, the cost of evaporation is expensive and large amounts of energy are required. If the ethanol, oil and protein processing plants are physically close together, the TS from the ethanol production could be used for protein extraction. The ethanol industry would save energy from avoiding evaporation. The protein industry would not have to purchase water for the process or energy to heat the water for protein extraction. Biodiesel industry could supply defatted meal and biodiesel glycerol for protein industry.

Objectives: This research explored the utilization of waste and co-products of ethanol industry, oil seed plant, and biodiesel industry in an integrated process for protein extraction and energy conservation.

Methods: Brassica juncea (L.) Czern meal was defatted prior to utilize for protein extraction. TS compounds, ion contents, and types and amounts of salts were determined (Ratanapariyanuch et al., 2011). Protein and oil contents of defatted meal, TS, and extracted protein were analyzed (AOCS, 1990). Isoelectric point of protein extracted from ground defatted meal using NaCl solution was examined using titration method. Protein extraction efficiency from ground defatted meal was conducted using TS, with a range of final concentrations on NaCl 0.0344-1.1656 M and pH 7.6-10.4 using biodiesel glycerol and 1 N HCl. Comparison of the relative efficiency of protein extraction using TS, model TS or NaCl solution was also studied with NaCl concentration 1 M and pH 10. Extracted protein using TS was tested for SDS-PAGE, peptide sequencing, amino acid composition, In vitro digestibility, and lysine availability.

Results: Both pH and salt concentration of the extraction medium affected protein extraction efficiency. Optimum extraction efficiency was observed at pH 10 and a salt concentration of 1.0 M. The extracted protein isolates from ground defatted meal were approximately 100 percent protein and had an isoelectric point of pH 6.4. SDS-PAGE and peptide sequencing showed that napin and cruciferin were the most prevalent proteins in extracted fractions at molecular weight approximately 14, 18-20, 20-22, 34 and 55 kDa. Amino acid composition of extracted protein was comparable to those of other researchers and met the requirement of FAO standard and human requirement. The extracted protein had a high lysine content (5-6 g/100 g of protein). Lysine availability and In vitro digestibility of the concentrate were approximately 40 g/kg of protein and 75%, respectively.

Conclusions: It was discovered that isoelectric point of extracted protein was 6.4. The highest protein extraction efficiency occurred at a pH 10 and a salt concentration of 1 M. The compounds present in TS did not have any effect on the efficiency of protein extraction. Only napin and cruciferin were found in extracted proteins. No differences in quality of proteins extracted from the meal using NaCl solution, model TS, or TS were found (gel electrophoresis, amino acid composition, In vitro digestibility and lysine availability). The quality of amino acids available from isolates was sufficient to meet FAO standard and human nutritional requirements.

References:
Virginia, USA: Association of Official Analytical Chemists, Inc.
Understanding the root traits in rapeseed/canola (*Brassica napus* L.)

**Background:** Plant roots are equally important as above ground plant parts and have a major contribution to yield and sustainability of plant by increasing nutrient uptake and better establishment in stress condition. Rapeseed/canola has both winter and spring form available. The winter type has a vigorous root system whereas the spring type has a weakly developed root system.

**Objectives:** Limited information on genetic control of root system in *B. napus* is available. This study was conducted to investigate the genetics of root vigor in rapeseed/canola and to identify the quantitative trait loci (QTL) associated with root vigor and days to flowering.

**Methods:** Three different populations were used to study the inheritance of root vigor. Crosses and reciprocal crosses were made between spring type parent Regent and winter type parent ARC97018, Regent and winter parent Lagoda, and spring type parent Legend and winter type parent Lorenz. Root vigor was scored on a scale 1-5, described by Rahman & McClean (2013). One of the F2 populations of winter and spring cross was used to identify QTL controlling the root vigor and days to flowering in canola (*B. napus*). About 3k SNP markers were derived from the population through genotyping by sequencing technology and were used in constructing a linkage map.

**Results:** Dominant natures with at least three genes are responsible for vigorous root system in *B. napus* was identified. Root length showed a positive significant correlation with both seed yield per plant and late flowering. Linkage map was constructed with 673 SNPs with a LOD threshold four. One QTL, NRV was identified on chromosome A01 (24.7 Mbp) for root vigor, and explains 16.3% of the total phenotypic variation. GBF Interacting Protein 1 (*GIP1*) and Small Auxin-Up RNAs (SAUR)-like family proteins are the two candidate genes, related to root growth and development were identified within this QTL region. Two QTL for days to flowering, DTF1 and DTF2 were identified, accounting for 21.7% and 15% of the total phenotypic variation, respectively. The QTL DTF1 was assigned on chromosome C08 (9.43 Mbp), and DTF2 was assigned on chromosome C04 (14.56 Mbp).

**Conclusions:** Further research is in progress to understand the complex nature of different root traits in canola and to find out the genomic region controlling the traits through genome-wide association mapping approach.

**References:**
Evaluation of rapeseed-mustard genotypes from India, Australia and China for resistance to shatter

Background: *B. juncea* accounts for >80% of production while *B. napus/B. rapa* have limited acreage in India. The Australian *B. napus/B. juncea* and Indian *B. napus* suffer yield losses due to pod shatter.

Objectives: Genetic enhancement for shattering resistance/tolerance by combining rapeseed-mustard genotypes from India, China and Australia, supported by ACIAR.

Methods: Australian, Indian and Chinese cultivars/breeding lines of *B. napus* (155) and *B. juncea* (95) were evaluated at TERI research field (NCR, India). About 50% plants of each genotype were left standing in the field four weeks post maturity. Two methods were used; visual assessment prior to harvesting on a scale of 1-9 (1–high shattering, 9–low shattering), and percent pod shatter \[
\text{[(No. pods shattered /total no. pods on main shoot) x100]} \]
Six exotic *B. napus* genotypes; Surpass, Trilogy, Trigold, Tranby, Monty, RR005 and 3 Indian double low genotypes; TERI(OO)GS17, TERI(OO) EM05,TERI(OO)R9903 [IC 405232] were utilized to generate breeding populations for shatter tolerance.

Results: Seedling emergence/vigor were good/excellent in all genotypes. Exotic genotypes (Australian/Chinese) had an extended flowering period, and maturity duration was almost similar/exotic lines were late compared to Indian genotypes. The data for pod shatter from both methods showed a good correlation. Indian *B. juncea* genotypes/*B. napus* TERI genotypes were tolerant to shatter. Twelve accessions of *B. juncea* (JN033, JM018, JO009, CBJ001, CBJ002, CBJ003, CBJ004, TABP15, MIPR, XINYOU4, XINYOU9, XINYOU8) and 14 of *B. napus* (Lantern, Rainbow, RQ011, AV Sapphire, RQ001-02M2, RR009, Surpass, Trilogy, Trigold, Tranby, Monty, RR005 and 3 Indian double low genotypes; TERI(OO)GS17, TERI(OO) EM05,TERI(OO)R9903 [IC 405232] were utilized to generate breeding populations for shatter tolerance.

Conclusions: The most significant are the early maturing breeding populations with good plant type from crosses involving Trilogy/TERI(OO)R9903 (BC3, shattering 24%), Tranby/TERI(OO)R9903 (BC2, shattering 14%), TERI(OO)R9903/RR005 (BC2, shattering 7%) and Surpass/TERI(OO)R9903 (BC3, shattering 8%). The selected accessions are being utilized for breeding shatter tolerance in Australian genotypes.

References:


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