Opening lecture: Plant functional genomics: from data to crops.

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The global population is predicted to reach more than 9 billion by 2050, while at the same time, food production will become more challenging due to climate change. A new agricultural revolution, based on genomic knowledge and applied bioinformatics, is required to meet this demand for food security. I will outline some of the recent advances in plant genomics and bioinformatics and describe how these technologies are being applied to revolutionise the breeding of improved plant varieties, with greater yield and improved tolerance to the impacts of climate change.
SESSION 1: Genome organization and genome editing

S1-O-01. Population genomic analyses identify signatures of selection and loci associated with agronomic traits in *Brassica napus*.

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*Brassica napus* is one of the world’s most valuable oilseed crops. Natural and artificial breeding selections of *B. napus* have resulted in numerous genetically diverse morphotypes and ecotypes with optimized traits and ecophysiological adaptation. To further understand the influence of selections on important agronomic traits, we conducted a comprehensive genomic assessment of genomic selection loci and their corresponding phenotypic traits based on the genome-wide resequencing of 800 diverse *B. napus* accessions. We detected 160 domestication-selective sweeps through comparisons of whole-genome genetic diversity between the different groups of oilseed rape accessions and between 120 loci associated with 30 agronomic traits after genome-wide association study (GWAS). There are 70 loci associated with 22 agronomic traits overlapped with selective sweeps. We found that six favourable alleles of low seed glucosinolate and erucic acid content, taken as examples, had been under strong selection in the process of ‘double low’ breeding (canola). Our results provide a genomic basis for improving *B. napus* cultivars and for further evolutionary analysis of oilseed crops.

**Keywords:** Oilseed crops, genomic selection, GWAS, ‘double low’ breeding.
S1-O-02. Cytonuclear interactions remain stable during allopolyploid evolution despite repeated whole-genome duplications

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Plant cells arose through the endosymbiotic engulfment of a cyanobacterium that subsequently formed the chloroplast genome, enabling plants to develop new critical functions. Almost all chloroplast proteins are now encoded in the nucleus, but some chloroplast protein complexes are jointly encoded by both nuclear and chloroplast genes, which interact to facilitate essential plant functions, such as the photosystems. Allopolyploidy, resulting from the hybridization and genome doubling of two divergent species, can disrupt these fine-tuned cytonuclear interactions, as newly formed allopolyploid species confront biparental nuclear chromosomes with a uniparental organelle inheritance. Such unequal genome inheritance may affect the conformation of the five cytonuclear complexes in allopolyploids. We used Brassica as a model to study the effects of paleopolyploidy and dichotomic divergence in parental species, as well as the effects of recent allopolyploidy in Brassica napus, on genes implicated in cytonuclear complexes. Because the B. napus parental diploid species are paleohexaploids, we first identified paleologous copies of cytonuclear complex genes. We found that these genes are preferentially retained in duplicates, are nearly all transcribed and are undergoing strong purifying selection, in accordance with the ‘gene balance hypothesis’. Subsequently, we compared expression patterns of cytonuclear complex homoeolog genes between resynthesized B. napus individuals and their respective diploid parents. The neo-polyploids showed neither biased sub-genome expression nor homogenization of homoeologs, due to highly conserved parental chloroplast genomes. These findings provide new insights and an innovative framework to understand the impact of cytonuclear interactions on interspecific hybridization and allopolyploid speciation.

Keywords: chloroplast, interspecific hybridization, allopolyploidy, intergenomic conflicts, genome inheritance, duplicated genes, Brassica sp.
S1-O-03. Application of Oxford Nanopore Technologies sequencing to Brassica genome improvement

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Plant genome assembly has been developing rapidly with costs declining and scaffold size and genome coverage improving; however, with short read technologies it is still inevitable that regions will be missed and duplicated or repetitive regions are often collapsed. Concomitant with these improvements there is a growing appreciation that copy number variants, presence/absence variants and structural rearrangements have played an important role in the adaptation of phenotype. Long read sequencing technologies offer a unique opportunity to capture these often elusive genome differences. In order to study a large number of lines the technology needs to be both cost effective and preferably accessible to many labs. A de novo genome assembly was generated for Brassica nigra using only Oxford Nanopore Technologies (ONT) sequence reads to assess the utility of ONT long read for deciphering a polyploid genome. The resultant assembly was error corrected using Illumina short reads (x17 coverage), HiC data was added to generate pseudomolecules, and the result was compared to an assembly generated using a more traditional Illumina based sequencing approach. The ONT assembly extended the original reference assembly by 91 Mb, covering ~94% of the expected genome size. The majority (85%) of the additional assembled sequence represented repetitive DNA, yet almost 10,000 additional genes were added to the new assembly. The long read assembly provided a novel insight into the repetitive genome structure, access to previously hidden genes (including much of the disease repertoire), and could span non-recombinant regions. This technology offers many opportunities for accessing some of the structural variation in Brassica species and we are currently testing its efficacy for tackling the further complexity of the Brassica napus genome.

Keywords: Brassica B genome, nanopore sequencing, genome structure
S1-O-04. An easy and efficient CRISPR/Cas9 platform for targeted mutagenesis in allotetraploid oilseed rape.

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With the rapid evolution of sequence specific nucleases (SSNs) based genome editing technology, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) is emerging as effective strategy for functional genetic researches, as well as genetic improvement in crop plants. However, the gene redundancy feature within the allotetraploid rapeseed genome is one of the major challenges for simultaneous modification of different homologs in the first generation. In addition, large scale screening to identify mutated transgenic plants is very time-and labor-consuming using the conventional restriction enzyme-based approaches. In this study, we developed an easy and efficient rapeseed CRISPR/Cas9 genome editing vector through synthesizing a premade U6-26 promoter driven sgRNA expression cassette. In our experiment, a sgRNA was constructed to target five rapeseed SPL3 homologous gene copies. Meanwhile, a polyacrylamide gel electrophoresis (PAGE)-based screening approach was established for rapid identification of CRISPR/Cas9-induced mutagenesis events in all targeted genomic site of five BnSPL3 homoeologs. The effectiveness of PAGE-based screening strategy was further validated by high-throughput sequencing analysis, which demonstrated that the proportion of CRISPR/Cas9-induced mutagenesis ranged from 96.8% to 100.0% in plants with obvious heteroduplexed PAGE bands, otherwise this proportion was only 0.00%-60.8%. Consistent with those molecular analyses, Bnspl3 mutants exhibited developmental delay phenotype in the first generation. In summary, our data suggest that this set of CRISPR/Cas9 platform is qualified for rapidly generating and identifying simultaneous mutagenesis of multiple gene homologs in allotetraploid rapeseed.

Keywords: allotetraploid, Brassica napus, CRISPR/Cas9, genome editing, SPL3, PAGE
S1-F-01. Visualising genome structural variation in *Brassica napus*

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The genomes of plants have evolved through cycles of polyploidy, principally as a consequence of hybridization, and diploidization. This cycle is relevant for diversity and performance of crops. The identification, visualization and association with traits of structural genome changes resulting in gene copy number variation, including the outcomes of homoeologous exchanges and introgression of genetic material from related species, has been difficult. To enable characterization of these events on transcriptomic and genomic datasets, we developed the new tool Transcriptome/Genome Display Tile Plots (TDTPs / GDTPs). Using these, we have showed that genome instability in the form of homoeologous exchange occurs frequently in the polyploid crop species *Brassica napus* and such events segregate widely in the germplasm used by breeders. In addition, we have identified and delineated the radish genome segment introgressed with Rfo in a range of Ogura hybrid material. Most recently, we have applied this visualisation approach to our genome re-sequenced radiation mutagenesis panel in order to identify multiple-gene-scale deletion and duplication events.

**Keywords:** Genome evolution; Visualization; Genomics; Transcriptomics; Homoeologous exchanges; Alien introgressions; Radiation mutagenesis
S1-F-02. Structural and functional evolutionary dynamics of duplicated genes and genomes in nascent and natural B. napus.

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Polyploidy or whole genome duplication is a widespread phenomenon in plants that has played a key role in their evolution, adaptation and diversification, leading to the current astonishing biodiversity (Jiao et al. 2011). This evolutionary success of polyploids is considered to result from the important evolutionary plasticity of their duplicated genes and genomes, which begins immediately after the allopolyploidization event. Presently, a model system to study this phenomenon is Brassica napus (oilseed rape) since its whole genome sequence as well as those of its diploid parents have been recently sequenced (Wang et al. 2011; Chalhoub et al. 2014; Parkin et al. 2014). In order to detect the short and long term (after human selection) impacts of allopolyploidy on the structural and functional evolutionary dynamic of B. napus (2n=4x=38), we analysed resynthesized B. napus individuals (from two different crosses), their diploid progenitors, as well as B. napus varieties using NGS (DNA and RNA Seq). Using these data, we investigated the roles of interspecific hybridization, genome doubling, short term and long term evolution on global (both homoeologs) and homoeospecific gene transcription. More specifically, we observed that interspecific hybridization is at the origin of a transcriptomic shock that is highly buffered after genome doubling. After a few generations, important homoeologous non-reciprocal translocations were also detected in these polyploid genomes. These homoeologous exchanges, also occurring to a lesser extent in natural B. napus, play an important role in the evolution of gene transcription, and sometimes influence quantitative trait variation.

Keywords: interspecific hybridization, polyploidy, Brassica napus, structural variation, and functional dynamic of duplicated genes.

References:
Parkin et al.(2014) Genome biology, 15(6), R77.
Wang et al. (2011) Nature Genetics, 43(10), 1035–1039.
S1-F-03/P-113. High quality de novo assembly of the cauliflower genome.

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Accurate sequence information, genome assemblies and annotations are the foundation for genetic and genome-wide studies. *Brassica oleracea* comprises many important word-wide vegetable crops with extreme morphological diversity. Here we report the *de novo* assembly of a cauliflower genome Korso through the integration of single-molecule sequencing [1], optical mapping [2] and chromosome interacting mapping [3] technologies. The 552.8Mb assembly with contig N50 4.97Mb and 97.8% anchoring to chromosomes is more continuous and accurate than the current reference genomes [4,5] of *Brassica oleracea*. We annotated protein coding genes in Korso combining RNA-seq and full-length transcriptomes sequencing results and analyzed genome-wide distribution of different kinds of transposable elements (TEs). Repeat sequences represent over half of the assembly. Alternative splicing, fusion genes and non-coding RNA were identified by full-length transcriptome sequencing. Insight in the evolution of cauliflower is obtained through cataloguing of unique genes and the expansion/contraction of gene families in comparison with related *Brassica* species. The A/B compartments of Korso were revealed by high-throughput/resolution chromosome conformation capture (Hi-C) analysis to illustrate open and closed chromatin, which reveals heterochromatin regions such as centromeres and telomeres, and sheds light on the relationship between genome structure and transcriptional regulation. The Korso genome will serve as a reference for the discovery of genes and structural variations between *B.oleracea* morphotypes, as well as further promote evolutionary and functional studies.

**Keywords**: *De novo* assembly, Cauliflower, Single-molecule real-time sequencing, Optical mapping, High-throughput/resolution chromosome conformation capture, Full-length transcriptome, A/B compartment.

**References:**
SESSION 2: Genetic diversity, epigenetics, breeding and biotechnology

Keynote lecture S2-1: Ancient polyploidy, genetic variation, and domestication of *Brassica rapa* crops

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Many crops are polyploid or have a polyploid ancestry. Recent phylogenetic analyses have found that polyploidy often preceded the domestication of crop plants. One explanation for this observation is that increased genetic diversity following polyploidy may have been important during the strong artificial selection that occurs during domestication. Despite the long interest in the connection between domestication and polyploidy, this hypothesis has not been formally tested. *Brassica rapa* crops are renowned for their outstanding morphological diversity that includes oil, root, seed, and leaf crops domesticated during the past 5,000 years. Like all “diploid” vascular plants, *B. rapa* has a diploidized paleopolyploid genome and experienced many rounds of whole genome duplication (WGD). The most recent WGD was a hexaploidization that occurred ~15 MYA. Here, we analyzed transcriptome data of more than hundred cultivated *B. rapa* accessions. Using a combination of approaches, we identified more than 3,000 candidate genes associated with the domestication of four major *B. rapa* crops. The candidate gene lists were significantly enriched with genes derived from the ancient hexaploidization event. Further, we found that genes derived from this paleopolyploidy contained significantly more genetic diversity than the non-polyploid derived genes. These results suggest that genetic variation from ancient polyploidy may have contributed to the diversity of domesticated *B. rapa* crops. Given the distribution of polyploidy throughout the history of flowering plants, our results suggest that the genetic legacy of these WGDs may significantly contribute to adaptation even millions of years later.
Keynote lecture S2-2: Exploiting big (sequence) data for understanding trait genetics in rapeseed

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The age of omics allows us to study biological systems on an unprecedented scale and speed. Although a draft genome sequence has been available for the allotetraploid Brassica napus (AACC) for some time, it has become clear that rapeseed accessions differ remarkably in gene content and genome organisation. Understanding the relationships between variation in the genome and variation of traits in a species with such a dynamic and, at ~1.2 Gb, relatively large genome involves very large sequence datasets. We aimed to develop a genomics platform for rapeseed that was designed from the outset to accommodate the variability of the B. napus genome and enable computational approaches designed to elucidate associations between genome variation (sequence, functional and structural) and traits. First we developed a new AC pan-genome reference sequence, derived from gene models annotated in the Brassica A and C genomes of the progenitor species (B. rapa and B. oleracea, respectively), into which accession-specific genes were interpolated. We then built a full-scale Associative Transcriptomics panel comprising 383 B. napus accessions to better understand the genetic architecture of a wide range of traits. Newly-developed genome visualization tools using Transcriptome or Genome Display Tile Plots (TDTPs/GDTPs) have enabled us to uncover and visualize genome structural variation in B. napus resulting from traditional breeding, re-synthesis, alien introgression and radiation mutagenesis.

Keywords: Brassica napus, pan-genome, association genetics, alien introgression, radiation mutagenesis
S2-O-01. Brassica allotriploids, a solution to deal with hampered meiotic recombination

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Meiotic recombination by crossovers is the main mechanism used by breeders to shuffle the genetic diversity. Nevertheless, loci separation is often hampered due to the strict regulation of meiotic recombination, which results in a few number of crossovers formed per pair of homologs (≤ 3) primarily located on chromosome extremities (Mercier et al., 2015). In Brassica genus, which contains many economically important crops (from vegetables and fodders to oilseed), ploidy level of hybrids has been linked to hugely modify crossover frequency. The higher crossover frequency was observed in AAC allotriploid hybrids (2n=3x=29), resulting from the cross between the rapeseed (B. napus, AACC, 2n=4x=38) and its B. rapa progenitor (AA, 2n=2x=20), with a two to four-fold increase compared to diploid AA and allotetraploid AACC hybrids (Leflon et al., 2010). However, the distribution of these extra crossovers in AAC allotriploids as well as the situation in CCA allotriploids (2n=3x=28), deriving from B. napus x B. oleracea (CC, 2n=2x=18), were still unknown. To answer the first question, we developed several populations and assessed the frequency and distribution of recombination events in diploid AA and allotriploid AAC hybrids, using SNP markers well distributed along A chromosomes (one SNP each 1.2 Mb). Based on about 3.000 crossovers detected per progeny, we showed that the presence of the C genome always leads to a very substantial increase of crossovers all along the A chromosomes, especially in the vicinity of centromeres that are normally deprived of crossovers (Pelé et al., 2017). Our preliminary analysis of CCA hybrids seems to indicate similar modifications between homologous CC chromosomes. Altogether, these findings provide new insights on the crossovers reshaping occurring in Brassica allotriploids and offer a unique opportunity for Brassica breeders to efficiently combine new loci of interests by modifying the linkage disequilibrium.

Keywords: Brassica, allotriploid, meiosis, recombination, crossovers

References
S2-O-02. From diploids to a huge diversity ready-to-use for oilseed rape breeding

Sophie Paillard, Maryse Lodé, Gwenn Trotoux, Frédérique Eber, Marie Gilet, Jérôme Morice, Olivier Filangi, Fabrice Legeai, Anne Laperche, Mathieu Rousseau-Gueutin, Anne-Marie Chèvre

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A low genetic diversity is available in the allotetraploid oilseed rape (Brassica napus, AACC, 2n=38), whereas a large variability exists among its diploid progenitors, B. rapa (AA, 2n=20) and B. oleracea (CC, 2n=18). An efficient use of recombination should enable to only introgress genomic regions of interest in oilseed rape. However, genetic reshuffling during meiosis is strictly controlled, with a limited number of crossovers per pair of homologous chromosomes and a gradient of CO from centromere to telomeres. We showed that this regulation can be broken down between A homologous chromosomes in AAC allotriploid hybrids, with three times more COs and CO formation all along the chromosomes (Pelé et al. 2017). As first results indicated that the recombination modifications are the same in ACC hybrids, we used ten diverse populations of each diploid progenitor and crossed them under cages with the same male sterile oilseed rape, resulting in 1178 AAC and 365 CCA F1 hybrids. We showed that the stability of their meiotic behavior depends on the initial diploid population used as pollinator. Thereafter, all F1 hybrids from each population were cultivated under different cages but with the same oilseed rape variety, heterozygous for the restorer gene. The analysis of these progenies revealed that AAC hybrids were significantly more fertile than ACC hybrids (404 vs 109 seeds/plant). As 1 to 5% of the F1 progenies showed 38 chromosomes as expected for oilseed rape, we screened more than 32 000 plants by flow cytometry to select 80 B1 plants with 2n=38 chromosomes per diploid population. Male sterile and male fertile plants were grown under cages for two generations to ensure intercrosses between the introgressed plants. The genotyping of 1600 plants from either B. rapa or B. oleracea populations using a 15K SNP array revealed that genetic diversity from diploid progenitors were introgressed all along the chromosomes. The prebreeding populations revealed a huge variability available for breeders.

Keywords: Homologous recombination, crossover, genetic diversity, ploidy level, Brassica napus

Reference
Pelé et al, 2017, Plos Genetics https://doi.org/10.1371/journal.pgen.1006794
S2-O-03. Translational research into meiotic recombination: more than mere validations

Adrian Gonzalo¹, Aurélien Blary¹, Nicolas Christophorou¹, Marina Pfalz¹, Aurélie Bérard², Nadia Bessoltane³, Hélène Bergès³, Delphine Charif³, Catherine Charpentier¹, Laurence Cromer¹, Frédérique Eber¹, Joelle Fourment³, Anne-Marie Chèvre⁴, Marie-Christine Le Paslier², Marie-Odile Lucas⁴, Nathalie Nesi⁴, Andrew Lloyd¹, Eric Jenczewski¹

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Sustainable crop improvement largely depends on exploiting meiotic crossovers, i.e. the reciprocal exchanges of large DNA fragments between chromosomes. In addition to their mechanistic role in ensuring correct chromosome segregation (essential for fertility), meiotic crossovers are responsible for reshuffling pre-existing patterns of genetic variation between individuals and between species. Great strides have been made in elucidating the basic molecular mechanisms of meiosis and meiotic recombination in model plants over the past 20 years (Mercier et al., 2015). In this talk, we will present the results of our first attempts to translate this improved knowledge into diploid and polyploid Brassica crop species. Our reverse genetics approach has consisted in the functional characterization of meiotic recombination mutants isolated from EMS-mutagenized B. rapa and B. napus populations, that we have evaluated using a combination of cytological and genetic approaches. Our results show that it is possible to increase crossover rate significantly, by knocking-down the activity of an anti-crossover protein, FANCM (Blary et al., 2018). Then, we will demonstrate that regular meiosis is maintained in B. napus even when the number of functional copies of another essential meiotic recombination gene (MSH4) is reduced to a minimum. We have also evaluated the consequences of duplicate MSH4 gene loss on inter-homoeologue crossover and we will discuss implications.

Altogether, our results highlight the benefit of translational research into meiotic recombination, which unravels new properties for already known meiotic actors and opens new avenues to make a wider range of genetic diversity accessible to crop improvement.

Keywords: Meiotic recombination, polyploidy, translational biology, genetic diversity

Reference
**S2-O-04. Transcriptome and organellar genome sequencing elucidate the origin and diversification of allotetraploid *Brassica napus***

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*Brassica napus* is an allotetraploid species derived from *Brassica rapa* and *Brassica oleracea*. It includes three subspecies which are important economic crops: rapeseed, rutabaga and Siberian kale. However, the genome-wide phylogenetic relationships, genetic structure and diversification among the different growth habits of *B. napus* remains unclear. We performed RNA-Seq and genome survey sequencing on 183 *B. napus*, 112 *B. rapa*, 62 *B. oleracea* / wild C accessions, and five outgroups. Using this data, we constructed phylogenies from transcriptomes and organelles. With the same data, we identified six genetic clusters of *B. napus* and investigated their genetic diversity and subgenome variance. The nuclear and organelar genomes of *B. napus* show varying patterns of inheritance which provide insight into the origin and subsequent introgression of *B. napus*. We also identified a list of regions with signatures of selective sweeps and 8,187 differentially expressed genes. They reveal known and candidate genes and gene regulation networks associated with different diversification processes. This study elucidates the origin and diversification process of *B. napus* and provides new insights that can further facilitate *B. napus* breeding and germplasm preservation.

**Keywords:** *Brassica napus*, phylogenomics, diversification, selection, DEGs
S2-O-05. Towards a dynamic model of the floral transition in Brassica

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Flowering time is an important trait in crops (Irwin et al. 2016). To determine the key regulatory elements underpinning the floral transition we developed a kinetic model of the floral transition in Arabidopsis (Jaeger et al. 2013). This model captures some of the main features of the floral transition such as noise-filtering, memory and irreversibility. Our aim is to transfer this understanding from Arabidopsis to develop a predictive model of flowering in Brassica napus.

A main challenge in transferring a gene regulatory network model from Arabidopsis to B. napus is polyploidy and the resulting number of copies of genes. A first step is therefore to determine which B. napus genes (Chalhoub et al. 2014) behave similarly to Arabidopsis homeologues. To investigate the retention and regulation of duplicated genes that are predicted to control flowering in Brassica napus and their expression dynamics, we collected and analysed a transcriptomic time series during development (Jones et al. 2017).

We find preferential retention of flowering time genes relative to the whole genome. In line with the gene balance hypothesis, we find that transcription factors tend to be present as multiple gene copies but that this does not fully explain the overall pattern of retention for flowering time genes. Expression divergence is observed in between 64% and 74% of retained gene homologues. A case study of BnaTFL1 genes reveals differences in cis-regulatory elements that could explain this divergence. We present our work-in-progress on network inference and analysis from these transcriptomic data and discuss key differences between species. Such differences in the regulatory dynamics of duplicated genes highlight the challenges for translating gene networks from diploid models to more complex polyploid crop species.

**Keywords:** Brassica napus, flowering time, transcriptomics, network inference, dynamic modelling

**References**

Jaeger et al. (2013) Plant Cell 25 820-33
S2-O-06. Natural occurring epimutation is involved in *Arabidopsis* quantitative resistance to clubroot

Benjamin Liégard¹, Antoine Gravot¹, Leandro Quadrana², Yoann Aigu¹, Juliette Benejam¹, Christine Lariagon¹, Yoann Aigu¹, Juliette Benejam¹, Christine Lariagon¹, Jocelyne Lemoine¹, Vincent Colot², Maria Manzanareès-Dauleux¹, Mélanie Jubault¹

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Plant phenotypic variations are generally triggered by genomic sequence variations; however, some plant traits were recently described as controlled by epialleles. Clubroot caused by the protist *Plasmodiophora brassicae* is a major disease of *Brassicaceae* including the three most economically important *Brassica* species: *B. napus*, *B. rapa*, *B. oleracea* and the model plant *Arabidopsis thaliana*. Both qualitative (major genes) and quantitative (QTL) clubroot resistance have been identified and studied in *Brassicaceae*. Here we describe a natural epimutation controlling one moderate-effect QTL involved in *Arabidopsis* clubroot resistance. Fine mapping of the QTL, previously identified in a biparental population from the cross between one susceptible and one partially resistant accession, was first carried out using 2400 progenies from Heterogeneous Inbred Families. We thus identified a 25kb-region containing eight genes with a reduced number of SNP between the parental accessions. Transcriptomic and methylation data analyses highlighted in this region a causal 6kb epimutation spreading two genes. Significant correlation found between high methylation levels, gene repression of both genes and susceptibility to clubroot was then validated in 127 natural *Arabidopsis* accessions. Moreover, further analyses showed that epiallele maintenance was carried out by the RNA independent pathway in the susceptible accessions. These results revealed the implication of a natural epimutation in the variation of plant response to clubroot infection. Combination of favorable epiallelic variations to allelic ones could therefore constitute new opportunities to bred plant resistant varieties to biotic stress

**Keywords:** *Plasmodiophora brassicae*, Brassicaceae, QTL, methylation, epimutation
S2-O-07. Characterization of epigenetic states in *Brassica rapa* L.

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Epigenetic regulation is defined as changes in gene activities that are inherited through cell divisions without alteration of the DNA sequence. Epigenetic regulation is crucial for the development and adaptation of plants to a changing environment. DNA methylation and histone modification are the best examples of epigenetic modifications. The alteration of chromatin structure, which causes changes in transcription, is regulated by various post-translational modifications such as methylation or acetylation of the N-terminal regions of the histone proteins. Genome-wide profiles of epigenetic information (the epigenome) are available in plants using technologies such as high-throughput sequencing.

In this study, we performed whole genome bisulfite sequencing (WGBS) and chromatin immunoprecipitation sequencing (ChIP-seq) using H3K27me3 antibody. We identified that DNA methylation in the upstream and downstream regions of genes was negatively associated with expression levels, especially DNA methylation in the 200-bp upstream and downstream regions. Heavy methylation in genes is not conserved between species and heavy methylation occurs after speciation and is caused by transposon insertion that can drive variation of DNA methylation between species. By contrast, there was a significant correlation in gbM between orthologous genes in *Brassica rapa* and *Arabidopsis thaliana*. Significant correlation in gbM between paralogous genes was also found in *B. rapa*.

We identified genes having H3K27me3 and compared H3K27me3 levels between stages or between lines. Between two lines, a small number of genes showed a difference of H3K27me3 levels, while more genes showed difference of H3K27me3 levels between different stages. These results indicate that a difference of developmental stage rather than a different between lines results in a difference in H3K27me3.

**Keywords:** DNA methylation, histone modification, epigenetics
S2-O-08. Investigating the involvement of histone modifications in the control of effector gene expression in *Leptosphaeria maculans*, the fungus causing stem canker of oilseed rape

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*Leptosphaeria maculans*, a fungus causing stem canker, colonises oilseed rape in two stages: an early stage of leaf colonisation and a late stage of systemic stem colonisation without visible symptom before stem canker appears. *L. maculans* produces at least two waves of effectors, key elements of pathogenesis facilitating host invasion. *L. maculans* presents a bipartite genome structure alternating gene-rich and transposable element (TE)-rich regions. While TE-rich regions are enriched in putative effector genes strongly over-expressed during early infection, gene-rich regions contain putative effector genes specifically expressed during late infection. Here, we investigated influence of reversible histone modifications affecting genomic regions sheltering different sets of effector genes on their concerted expression. We analysed nucleosome positioning, location of histone modifications and gene expression at the genome scale combining MAINE-seq, ChIP-seq and RNA-seq data during axenic growth and performed functional analysis of two chromatin modifiers (KMT1 and KMT6). We analysed in vitro ChIP-seq data targeting two heterochromatin modifications, H3K9me3 and H3K27me3, and a euchromatin modification, H3K4me2, and found that gene-rich regions are associated with H3K4me2 and H3K27me3 while TE-rich regions are associated with H3K9me3. Analysis of in vitro MAINE-seq data showed distinct nucleosome organization for genes located in TE-rich or gene-rich regions. While RNAi silencing of KMT1, which encodes a protein involved in H3K9me3 deposition, induced an over-expression of genes located in TE-rich regions, particularly ‘early’ effector genes, silencing of KMT6, involved in H3K27me3 deposition, leads to a deregulation of genes not only associated with H3K27me3 in the wild type strain, suggesting a relocation of different histone modifications.
S2-O-09. Investigating the vernalisation requirement of oilseed rape: a tale of three genes

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Many plant species have evolved to overwinter before flowering. In *Arabidopsis thaliana* variation for a requirement for cold to flower is determined primarily by two genes *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*). *FRI* upregulates the expression of *FLC*, which in turn inhibits the expression of floral promoting genes like *FLOWERING LOCUS T* (*FT*). Prolonged cold temperatures overcome this inhibition through a process called vernalisation. Rapid cycling spring flowering accessions do not need cold to flower due to mutations at either *FRI* or *FLC* (Bloomer and Dean 2017).

Winter, spring and biennial varieties of *B. napus* are grown for seed and vegetable production and variation for vernalisation requirement is present. Four orthologues of *FRI* and nine orthologues of *FLC* have been described (Wang et al. 2011, Zou et al. 2012). The aim of our research is to understand how *FRI* and *FLC* contribute to the vernalisation requirement of *B. napus*.

Molecular characterisation of *FRI* from a panel of *B. napus* accessions revealed the presence of non-synonymous allelic variation at all four genes. A QTL-seq approach (Takagi et al. 2013) was used to determine the functional significance of this allelic variation. We have developed a segregating population from a bi-parental cross between two oilseed rape accessions that exhibit variation for vernalisation requirement and carry allelic variation at *FRI*. Illumina sequencing was performed on pooled DNA from this population and two major QTLs were identified. These QTL regions encompass orthologues of *FLC* and *FT*, but not *FRI*. Characterisation of the *FLC* and *FT* orthologues revealed the presence of both DNA sequence and gene expression differences between the parental oilseed rape accessions and we hypothesise this contributes to the flowering time differences observed. This knowledge has enhanced our understanding of the vernalisation requirement of oilseed rape, informing the generation of new varieties with adapted flowering times and improved yields.

**Keywords:** Flowering time, vernalisation, *Brassica napus*, oilseed rape, QTL-seq

**References**
S2-F-01/P-222. Expanding the gene pool of Brassica napus and exploiting its genetic diversity

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Brassica napus (A"A"C"C") is an important oilseed crop, but a young species with limited genetic diversity and short history of cultivation. To expand the gene pool and exploring intersubgenomic heterosis in B. napus, a novel dynamic gene pool of B. napus (A'A'C'C') was constructed with introgression of the A' and C' subgenome from hundreds of accessions of B. rapa (A'A') and B. carinata (B'B'C'C'). Strong intersubgenomic heterosis on seed yield of the hybrids between these new-type B. napus lines and traditional B. napus testers has been evaluated by field trials in different environments. Revealed by genetic analysis with the technique of SSR, Brassica Infinium SNP array, genotyping by sequencing and deep re-sequencing on this gene pool, the new-type B. napus population presented rich genetic diversity and abundant novel genomic alterations with substantial reconstructed genome, along with novel traits contributed by the introgression of subgenomic variation. The strategy and results of creating and evaluating the novel gene pool of B. napus would provide insights for expanding and utilizing plant gene pool based on genomics.

Keywords: Brassica napus, gene pool, introgression, genome, recurrent selection
S2-F-02. The Brassica A, B and C genomes: interspecific hybridisation, chromosome pairing, and the potential for crop improvement

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The Brassica “U’s Triangle” consists of the three diploid species B. rapa, B. nigra and B. oleracea (A, B and C genomes respectively), and the three allotetraploid species B. juncea, B. napus and B. carinata (AB, AC and BC genomes respectively). The importance of this relationship has long been recognized for studies of genome evolution and for crop improvement in this agriculturally significant genus. In particular, interspecific hybrids between the “U’s Triangle” species can transfer or combine useful genetic and phenotypic variation into crops. Since the availability of genomic resources for these species, it is now possible to identify genetic and genomic factors affecting chromosome pairing and recombination in interspecific hybrids. The degree of non-homologous recombination that occurs between and within subgenomes is critical in a) introgressing useful variation between species and genomes and b) producing stable resynthesized or novel crop types in Brassica. Genetic and genomic factors facilitating and preventing non-homologous recombination between the A, B and C genomes in Brassica allopolyploid species and interspecific hybrids will be discussed, as well as the potential to exploit these processes for crop improvement.

Keywords: Interspecific hybridisation, recombination, meiosis, polyploidy
S2-F-03/P-224. Genome wide association mapping for traits related to winter hardiness and vernalization requirement in winter oilseed rape

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Despite climate warming, in some years extreme weather conditions may occur that dramatically affect seed yield of winter oilseed rape (Brassica napus L.). Abiotic stress tolerance, particularly frost tolerance, remains an important breeding aim. Frost periods without snow cover and temperatures below -15 °C during otherwise mild winters (e.g. 2012, 2016) have led to winterkill in central Europe. Shoot elongation before winter has been identified as a key factor for winterkill. Furthermore, reduced vernalization requirement has been suggested as causative for shoot elongation before winter. The aim of this study was to assess the genetic variation and inheritance of winter hardiness in a genetically diverse set of n = 312 winter oilseed rape genotypes. The plant material was phenotyped for winterkill in field experiments in Poland, Ukraine and Finland. Morphological traits reflecting plant development and vigor were assessed in field trials before and after winter, while vernalization requirement was assessed both in spring sown field trials and greenhouse trials. Results showed significant differences in the extent of winterkill and stem elongation before winter as well as for vernalization requirement. Vernalization requirement as assessed in greenhouse trials was closely negatively correlated with stem elongation in spring sown experiments ($r_S = -0.75^{**}$). Lower but statistically significant positive correlations were found for stem elongation before winter with stem elongation after spring sowing ($r_S = 0.34^{**}$) and winterkill ($r_S = 0.27^{**}$). Genome wide association mapping was performed for the above traits using marker data from the Brassica 60K Illumina Infinium SNP array. Results from these analyses will be reported. Findings so far show that vernalization requirement and shoot elongation before winter are not as closely linked as presumed, with the latter indeed facilitating winterkill. In conclusion, results indicate that increased winter hardiness and reduced vernalization requirement are not exclusive and that such genotypes, with optimal development and flowering time in spring, may be achieved through breeding.

Keywords: winterkill, vernalization, association mapping, abiotic stress

Acknowledgments

Financial support of the BMEL (FKZ 22406012), through FNR e.V., and of the oilseed rape breeders of the GFPI e.V., especially DSV AG, KWS Saat SE, Limagrain GmbH and NPZ Lembke KG for performing field trials, is acknowledged.
S2-F-04/P-223. Characterisation of disease resistance genes in the *Brassica napus* pangenome

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With the advent of high throughput sequencing technologies and progress in bioinformatics analyses, crop genomics studies have entered into a new era. In particular, pangenomics has been developed towards a better understanding of genomic diversity for the continued improvement of crops. In addition, methods based on single nucleotide polymorphism (SNP), copy number variation (CNV) and presence/absence variation (PAV) discovery provide a valuable resource to study gene structure and evolution. In this study, we characterised resistance gene distribution and variability in the *Brassica napus* pangenome, consisting of 50 lines. The RGAugury pipeline was used to automate resistance gene analogs (RGAs) prediction. The number of core and variable RGAs in the pangenome were determined and compared with the reference genome. Furthermore, the number and distribution of RGAs in synthetics and non-synthetics lines were studied. We also identified SNPs on R-genes in the pangenome and performed physical clustering to identify those groups of genes that consistently clustered together. The results constitute a significant resource for researchers involved in Brassica genomics and breeding as characterization of diversity of resistance genes is essential for the development of new resistant varieties and their association with agronomic traits.

**Keywords:** *Brassica napus*, pangenome, resistance genes, structural variation
SESSION 3: Nutrient use efficiency, abiotic stress tolerance

Keynote Lecture S3: Natural variation in nutrient homeostasis in Arabidopsis and beyond

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Understanding the control of mineral nutrient homeostasis is a key pre-requisite of attempts towards reducing the dependence of crop yields on high levels of fertilizer. Among the most promising approaches is the exploitation of natural variation. We have used QTL analysis to find loci responsible for variation in sulfate content in Arabidopsis Bay-0 x Shahdara population. Two of the underlying genes encode isoforms of consecutive enzymes in sulfate assimilation pathway ATP sulfurylase and APS reductase. The APR2 isoform of APS reductase has been independently found to be responsible for high accumulation of total sulfur and sulfate in several other Arabidopsis accessions. Genome wide association mapping of anion contents in Arabidopsis and in Brassica napus revealed number of other candidate genes potentially responsible for control of nutrient homeostasis. The power and the pitfalls of this genetic approach will be discussed.

Keywords: Arabidopsis, sulfate, genome wide association mapping, QTL
S3-O-01. Metabolic profiling and functional metabolomics of leaf senescence and abiotic stress responses in oilseed rape. (*Brassica napus* L.)

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Winter oilseed rape, the main European oleaginous crop, is mainly grown for its seeds and extraction of edible oil and biofuel, while the residual protein meal is also used for animal feeding. It is a very high nitrogen (N)-fertilizer consuming species characterized by a rather low N use efficiency (NUE). To meet the current challenges of agroecology and sustainable agriculture through input limitation, improvement of N remobilization efficiency during leaf senescence is likely to improve significantly the overall plant NUE, particularly in oilseed rape where organic N recycling between source and sink tissues is rather inefficient. Thus, metabolic profiling explorations have been undertaken in relation to leaf development and resource allocation for the comprehensive understanding of sink and source metabolome and senescence-associated metabolic adjustments under different environmental regimes. Our study focused on quantitative analysis of primary C and N polar metabolites, as they are fundamental attributes of nutrient assimilation and recycling. Concurrently a holistic non-targeted mass spectrometry-oriented metabolomic approach has been realized to decipher leaf ageing imprinting on leaf metabolic networks. The communication will relate and discuss recently acquired results after metabolite profiling data were integrated into exploratory multivariate statistical analyses. Leaf development-dependent metabolic signatures have been pointed out and clusters of metabolic indicators could be assigned to sink/source statuses, senescence progression and culture conditions. Moreover low N-input together with water shortage impacts have been assessed on leaf metabolic fingerprints. Metabolic adjustments to drought stress have been shown to be strongly affected by preliminary N availability and N leaf status. The well-known stress-induced proline accumulation syndrome has been considered as a perfect illustration of nutrient and water stress cross-talks on metabolism.

**Keywords:** Oilseed crops, genomic selection, GWAS, ‘double low’ breeding.
S3-O-02. Partitionning of sulfur between primary and secondary metabolism. Partitionning of sulfur between primary and secondary metabolism

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Sulfur is an essential nutrient for all organisms. Plants are able to take up inorganic sulfate and assimilate it into a range of bioorganic molecules either after reduction to sulfide or activation to 3'-phosphoadenosine 5'-phosphosulfate (PAPS). While the regulation of the reductive part of sulfate assimilation and the synthesis of cysteine has been studied extensively in the past three decades, much less attention has been paid to the control of synthesis of sulfated compounds. Only recently the genes and enzymes activating sulfate and transferring it onto suitable acceptors have been investigated in detail with emphasis on understanding the control of partitioning of sulfur between the two branches of sulfate assimilation. These investigations brought a range of interesting new findings, such as common regulatory network of sulfate assimilation and glucosinolate synthesis, and identified new components of the pathway, e.g. PAPS transporter or the 2'(3'),5'-diphosphoadenosine phosphatase. The new findings will be reviewed and put into context of primary and secondary sulfur metabolism.

Keywords: Arabidopsis, sulfate assimilation, glucosinolates
S3-O-03. Genome-wide identification and analysis of Hsf gene family in *Brassica oleracea*

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*Brassica oleracea* is one of the important crops belonging to the genus *Brassica*. This *Brassica* species consists of crops like cabbage, cauliflower, broccoli, Brussels sprout, kohlrabi and kale. The sequenced genome of *B. oleracea* (Liu et al. 2014; Parkin et al. 2014) provides a resource for the identification and characterisation of important gene families such as the heat shock factor (HSF) gene family. The Hsfs acts as the terminal components of signalling networks involved in abiotic stress responses (Ohama et al. 2017; Saidi et al. 2011). However, a study on the Hsf gene family in *B. oleracea* is lacking. Here, we performed a genome-wide identification and systemic analysis of Hsf genes in *Brassica oleracea*. 35 identified genes encoding Hsf proteins were classified into A, B and C classes and their evolution, physical location, gene structure, domain structure, promoter analysis and tissue-specific expression patterns were investigated. We observed that *B. oleracea* did not have any *HsfA06a* gene. The phylogenetic analysis showed the presence of new sub-sub-classes in *Brassica* species. The subclasses A1, A4, A6, A7, B1, B2, B4 and C1, in *Brassicas* (*B. oleracea*, *B. rapa* and *B. napus*) had more genes than in Arabidopsis and the new suggested sub-sub-classes contained genes from all three *Brassica* species and no representative gene from Arabidopsis. Gene expression analysis revealed that *BoHsf* genes showed a higher mean expression in vegetative tissue (root, stem and callus) than in reproductive tissues (bud and flower). Also, a number of stress-related cis-acting elements were found in the promoter regions of *BoHsfs*. Further studies investigating the roles of *BoHsf* genes will help in improving the understanding of stress tolerance in *B. oleracea* and thus may be useful for developing crops that are resilient to climate change.

**Keywords:** Hsf genes, Abiotic stress, Heat stress, *Brassica oleracea*

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S3-O-04. Involvement of oilseed rape PI-WSCPs "Protease Inhibitors - Water Soluble Chlorophyll Binding Proteins" in nitrogen management and stress tolerance

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Winter oilseed rape is a high nitrogen (N)-fertilizer consuming crop characterized by rather low N use efficiency (NUE). In the context of climate change and N inputs regulation, attention is focused on improving NUE under abiotic stress to secure yield. A high proportion of assimilated N remains immobilized in senescent leaves and is returned to the soil failing to contribute to seed yield. Enhancement of nutrient recycling and partitioning performance from senescing tissues to the growing and reproductive organs is likely to improve overall NUE. Effective remobilization of N and C requires a fine-tuning between sink demand, associated with a photosynthetic efficiency and source supply related to proteolysis activity. In this context, PI-WSCPs, described as bi-functional proteins acting as both chlorophyll carriers and protease inhibitors, should be involved in maintaining the integrity of the photosynthetic apparatus and controlling the reallocation of proteolytic N. Thus, our work aims at investigating PI-WSCPs during leaf development and senescence in relation with N fertilization levels and water availability. PI-WSCP are duplicated genes with 36 copies identified in B. napus genome grouped in structural clusters. Proteins display in silico parietal and vacuolar subcellular localizations and exhibit divergence between PI activity and chlorophyll binding. To investigate specific contribution of these copies, B. napus plants have been grown under greenhouse conditions with two N regimes. Moreover, water stress was induced by stopping irrigation for 10 days. PI-WSCPs show sub-functionalization trends related to leaf developmental status and response to abiotic stresses. Results are discussed in regards to source-sink relationships and N management.

Acknowledgements
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Keywords: Nitrogen recycling management, drought stress, leaf senescence, source-sink relationships
S3-F-01. Simulations for optimizing sulfur fertilization in oilseed rape in the context of increased spring temperatures with the model SuMoToRI

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For the last few decades, environmental policies have led to drastic reduction in sulfur (S)-containing industrial emissions leading to reduced soil S depositions (Scherer, 2001; Schnug, 1998). This is of concern for oilseed rape (Brassica napus L.), which like most Brassica species is a high S-demanding crop (Oenema and Postma, 2003). In this context, monitoring S-fertilization has become a central issue. Moreover, ongoing and projected climate change will affect crop yield and quality worldwide, thus justifying the prediction of climate effects via modelling approaches to adjust crop management and fertilizer practices. In this modelling study, the growth and S status of winter oilseed rape (WOSR) were investigated from the end of winter until the onset of pod formation under contrasting S supplies in a range of climatic conditions acquired for seven major WOSR-producing northern countries and under the four Representative Concentration Pathway (RCP) scenarios (i.e. RCP2.6, RCP4.5, RCP6.0, and RCP8.5). Simulations were performed with the process-based model SuMoToRI for past datasets (1948-2005) and projections (2015-2099) (Brunel-Muguet et al., 2015). Simulation results indicated decreased plant biomass (mainly leaves) as temperatures increased (as expected under the increasingly negative scenarios as the century progresses) and as daily incident radiation decreased in contrast to the mobil S of leaves (mainly sulfate), which tended to accumulate as a consequence of reduced S sink (i.e. leaves) size. These simulations highlighted the increased risks of S over-fertilization, which can lead to environmental issues such as S-leaching due to high mobile-S in leaves that senesce. Overall, this in silico study raises questions about the most suitable S fertilization strategies and associated farming practices for dealing with the expected adverse climatic conditions.

Keywords: oilseed rape; temperature; radiation; sulfur; modelling; climate change

References
S3-F-02/P-307. Exploring the interactive behavior of reduced glutathione and chromium toxicity in allopolyploid *Brassica napus* as reveal by cell structural insights, protein kinases, and membrane transporters

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Despite the fact that chromium (Cr) is a toxic metal/carcinogenic to human as well as plants. However, it is extensively used for commercial purposes such as tannery industry and thus, its residues severely enriching the soil. Protein kinases (PKs) among protein-based enzymes are the promising regulators that can respond well to hazardous environmental cues. The membrane transporters (MTs) not only channelize the minerals from one organ to organ but can also control the Na*/K* balance in the cell, hence create the defense shield. Among heavy metals (HMs) remediators, *Brassica napus* is a potential candidate plant that can adsorb a handsome amount of HM from soil and can translocate into cell majorly in the vacuole, hence protect the other vital organs from the subsequent stress. In this study, we used two different cultivars of *B. napus* cvs. ZS 758 (metal-tolerant) and Zheda 622 (metal-susceptible) and seeds of these cultivars were grown in control conditions under different concentrations of Cr and GSH, i.e. 0, 400 µM Cr, and 400 µM Cr + 1 mM GSH for 6 days. Data showed that Cr had deteriorated the fresh biomass of leaf, stem, and root significantly compare than control (Ck). To explore the physiological cell-structural changes majorly in the cytoplasm, mitochondria and nucleus we deployed the transmission electron microscopy and noticed that Cr severely damaged the ultrastructure of above-mentioned organs. We also survey the whole genome PKs and MTs responsive to Cr and GSH influence. Results advocated that plants response to Cr toxicity as by up-regulated the expressions level of gene coding the nucleic acid and transition metal ion binding proteins, protein kinase activity (PKA) and phosphotransferase activities in both cultivars than respective controls. Similarly, in the case of MTs, genes related to water transmembrane transporter activity had shown the increased expression rate. Besides, Brassica plants under the combined treatments had upregulated the genes coding for PKA, signal transduction, and oxidoreductase activities as PKs and for MTs, increased the gene expression of secondary active transmembrane transporter and protein transporter activities than Ck or Cr-treated plants. As a whole, *B. napus* plants showed the tolerant behavior against the Cr toxicity by translocating the Cr ions in the less critical parts of the cell as well as upregulated the genes related to PKs and MTs when subjected to Cr and GSH environments.

**Keywords:** *Brassica napus* L., chromium, protein kinases, membrane transporters, reduced glutathione, transmission electron microscopy
SESSION 4: Next generation phenotyping, plant growth and development

Keynote lecture S4-1: 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

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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.
Keynote lecture S4-2: The impact of flowering time genes on crop productivity: more than when to flower

Dr Judith Irwin

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Agriculture and horticulture are facing the crucial challenge of adapting crop productivity to changes in the climate. More variable weather patterns require the development of crops able to perform more robustly under a wider range of environmental conditions. Understanding the environmental sensitivity of the underlying regulatory pathways controlling crop productivity is therefore central to optimising crop performance. Elements of the gene regulatory pathways first described as controlling the time to flowering have now been revealed as pleiotropic regulators of many transitions in plant development. An excellent example is the floral regulator *FLOWERING LOCUS C* (FLC), which confers the need for a plant to experience winter prior to flowering. As well as regulating time to flowering, FLC and its target gene *FT* have been shown to affect inflorescence branching, seed development and progeny seed vigour.

In this talk I will illustrate how an in-depth understanding of a key flowering time gene such as *FLC* in both *Arabidopsis* and *Brassica* could contribute towards our aim: high-yielding varieties that flower predictably and uniformly under fluctuating climatic conditions.
S4-O-01. Population structure and phenotypic diversity of a *B. oleracea* collection to study the genetic architecture of leaf variation in the diverse vegetable crops

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*Brassica oleracea* is an extremely diverse species, encompassing very diverse vegetable crops, like cabbages, cauliflower, broccoli, kohlrabi, collard greens, Brussels sprouts, and different kale crops. Previously we identified selective sweeps for the domestication trait leafy head formation and found that the ancient genome triplication, shared by all Brassica’s, facilitated this diversification (1). Several molecular pathways involved in leafy head formation and few causal genes were identified (ad/ab axial leaf patterning, auxin and cytokinin pathways). Interestingly, allelic variation of these causal genes showed that as expected >90% of the cabbages carried the heading alleles, however in non-heading types also 30-40% carried the heading alleles. We aim to study the quantitative nature of the leaf heading trait, by dissecting heading in its components, or sub-traits, and identify genes involved in leaf development and heading. We chose an association mapping approach and first collected 920 accessions including all crop types, modern hybrids and landraces, wild accessions and several C9 species. These accessions were all genotyped using Sequencing Based Genotyping (SBG). SNPs were called and used to study diversity and determine population structure. Leaf and leafy head formation was evaluated in multi-year field experiments using digital imaging and HALCON scripts. The combined data were used to identify marker trait associations and to identify causal genes for diverse leaf traits. The analysis gave insight in the diversity of the *B. oleracea* collection, the relation between modern hybrids and landraces, and resulted in identification of many QTL, with some further studied as candidate genes were identified. We conclude that this GWAS approach, combined with bioinformatic analyses of selective sweeps and knowledge leaf development in model species results in identification of genes with allelic variation involved in diversity of *B. oleracea* leaf traits.

**Keywords:** *B. oleracea*, population structure, leaf development, phenotyping, GWAS

**Reference**

Cheng et al. (2016) Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in *Brassica rapa* and *Brassica oleracea*. Nature Genetics 48:1218–1224, DOI 10.1038/ng.3634
Winter oilseed rape is characterized by low nitrogen (N) use efficiency due to a low capacity of N remobilization from senescing leaves to growing organs, especially at spring regrowth period and further flowering. Characterization of the natural genotypic variability of leaf remobilization efficiency should allow providing relevant background for crop improvement under low nitrogen input. Low field Nuclear Magnetic Resonance (NMR) has been used in several studies for investigation of leaf water status. An original interpretation of the multi-exponential transverse relaxation signal of leaf tissue taking into account subcellular compartmentation and heterogeneities at the tissue level has been recently proposed for the monitoring of oilseed rape leaf development. Applied on a wide leaf panel collected from plant grown under controlled conditions, the NMR relaxometry was shown to detect slight variations in senescence associated structural modifications of leaf tissues. The aims of the present study was to demonstrate the potentiality of NMR relaxometry to provide robust indicators of structural changes associated to leaf development for plant grown directly in field and to investigate the impact of N deficiency on these changes and its link with yield reduction. In this study, two genotypes of winter oilseed rape (Aviso and Express) with different tolerance to N deficiency were assessed. Plants were grown under field conditions and two N regimes and studied after the winter period. Field measurements were performed by a mobile NMR lab specially set up for this purpose equipped with a 20 MHz spectrometer (Minispec PC-120, Bruker, Karlsruhe, Germany). Eight discs of 8 mm in diameter were cut from each leaf rank of the plant studied without derooting the plant and then placed in NMR tubes. Transverse relaxation was measured using the CPMG (Carr-Purcell-Meiboom-Gill) sequence. The genotypic response to N depletion was evaluated through dry mass production and seed yield. The results obtained on well fed plants of Aviso genotype showed that the NMR relaxation method was able to discriminate vegetative sequential senescence occurring at regrowth and stem elongation stages from monocarpic senescence occurring during seed filling. At spring regrowth, lower tolerance to N depletion was associated with higher impact on senescence associated structural modification pattern. These results demonstrated that despite a great variability of the environmental factors, the NMR relaxation method can provide robust indicators of structural changes associated with leaf development and that it could be used under field conditions in order to evaluate the effects of N deficiency on leaf development and nutrient recycling performance.

**Keywords:** Nutrient remobilization efficiency, NMR relaxometry, field phenotyping, mobile lab

**Reference**
S4-O-03. Phytochrome A signal transduction 1 and CONSTANS-LIKE 13 coordinate orchestrate vegetative branching and flowering in leafy Brassica juncea

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Branching is a major determinant of crop yield enabling vigorous vegetative growth and dense canopy. Phytochrome A signal transduction 1 (PAT1) is a positive regulator of phytochrome A signal transduction in response to light, but the involvement of PAT1 underlying vegetative branching remains unknown. In this study, we mapped a phytochrome A signal transduction 1, PAT1, and discovered its new role of counteracting vegetative branching and flowering in the featured leafy Brassica juncea crop. Earlier branching time and intensive branching numbers were significantly displayed when PAT1 was down-regulated in expression indicating a negative regulator of vegetative branching. Down-regulated expression of PAT1 removed branching suppression induced by far-red light. Furthermore, the PAT1 gene negatively regulating of branching occurred only after bud initiation suggesting PAT1 functioned in bud out-growth and branching phases rather than bud initiation. Both biochemical and genetic evidences indicated that PAT1 directly interact with COL13, a negative regulator of flowering, in which PAT1-COL13 complex tunes vegetative branching and flowering. Taken together, our findings provide a new crosstalk modality between phytochrome signal and flowering pathways in regulating vegetative branching and flowering. Promisingly, this will greatly help to enhance crop productivity or earlier harvesting via genome editing of the GRAS-family transcription factor PAT1.

Keywords: Brassica juncea, branching, phytochrome A signal transduction 1, CONSTANS-LIKE 13, flowering
S4-O-04. How does allelic variation of the *Brassica rapa* domestication gene ARF3-1 affects leaf development

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Brassica rapa displays enormous phenotypic variation, such as leafy heads, turnips and oilseeds. In Chinese cabbage, a candidate domestication gene BrARF3-1 (auxin response factors 3-1) was identified that related to leafy head formation (1). Several genes and their feedback regulation and protein interactions involved in the leaf adaxial–abaxial pathway in *A. thaliana* are described. These are (1) the TAS3–ta-siRNA–ARF3/ARF4 pathway which promotes adaxial cell fate determination, (2) the miR166–HD-ZIPIII pathway that specifies abaxial cell fate and (3) the KANADIs, YABBY and ARF3/4 proteins that interact to promote abaxial cell differentiation. The heading and non-heading ARF3 alleles differ in amino acids changes at the auxin response super family domain (Val to Ala, Lys to Arg) and the C-terminal end (Gln to His). We will present that this allelic variation in BrARF3-1 induces different leaf phenotypes in “35S BrARF3-1” Arabidopsis plants. We also show that this variation changes the interaction with the leaf polarity proteins YABBY and KAN. Using chimeric BrARF3 genes we study the effects of amino acid changes on both protein-protein interaction and on transgenic over-expression Arabidopsis phenotypes. These chimeric proteins revealed that the Gln to His C-terminal amino acid change only influenced the interaction with the YABBY-1 protein with the pakchoi ARF3-1 genetic background.

**Keywords:** BrARF3-1, leaf development, allelic variation, protein-protein interaction

**Reference**

*Cheng et al. (2016) Subgenome parallel selection is associated with morphotype diversification and convergent crop domestication in Brassica rapa and Brassica oleracea. Nature Genetics 48:1218–1224, DOI 10.1038/ng.3634*
S4-F-01. Leveraging field phenomics for advancing Brassica crop improvement

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The federal Canada First Research Excellence Fund awarded Cdn$37M to the Global Institute for Food Security (GIFS) at the University of Saskatchewan (U. of S) in 2015 to establish the Plant Phenotyping and Imaging Research Centre (P2IRC). The seven-year initiative is based on the U. of S campus in Saskatoon and involves researchers at variety of University departments, the Canadian Light Source (synchrotron), National Research Council Canada and Agriculture Canada & Agri-Food Canada. More details about the initiative can be found at https://p2irc.usask.ca/.

The funding was awarded based upon the depth of multi-disciplinary expertise on campus to establish high throughput phenotyping in multiple crops with the inclusion of new imaging technologies and strong computational support. Research in the P2IRC is broken down into four themes: Theme 1 - Phenometrics; Theme 2 - Image Acquisition Technologies; Theme 3 - Computational Informatics of Crop Phenotype Data and Theme 4 - Societal and Developing World Impact. A platform of genomics and bioinformatics is also provided to support projects.

A key component of Theme 1 is to develop and utilize mobile platforms for data acquisition that will improve our understanding of, and efficiency in, plant breeding. This includes the application of unmanned aerial vehicles (UAVs or “drones”) and mobile platforms that move through the field to collect imaging data from canola trials. Instrument payloads provide a range of high-resolution data sources, including imagery in the ultra-violet, visible, near-infrared, and thermal spectral regions, laser-scanning, and ultrasonic sensors. A description of the status of research within the project and the implications of the application of phenomics in breeding will be presented.

Keywords: Digital plant phenotyping, UAV, phenomobile, phenomics
Oilseed rape is one of the most important oilseed crops worldwide, providing oil and proteins for food, feed and industrial uses. Faced with the challenges of adapting agriculture to climate change, seed production should have increased resilience to multiple stress factors. This concerns yield and the quality of the seed production. Seed quality includes both the nutritional quality (oil/protein content and composition) and the physiological quality (seed vigor). Both qualities are acquired during seed development, but how environment influences the molecular determinisms of these qualities remains to be elucidated. In addition, there is interplay between the regulatory factors and mechanistic actions of both qualities but their precise nature needs to be identified. Here we will review recent advances about impacts of a nitrogen or water limitation on the different components of seed quality. In addition to the environmental impacts, intense selection for specific seed components has largely influenced the seed quality. In particular, reduction in seed glucosinolates content has negatively altered seed storage protein composition as well as germination related traits. Genome-wide association studies revealed co-localizations between the corresponding loci, suggesting genetic linkage or pleiotropic effects. Nevertheless, we demonstrated that there is still space to improve these latter seed quality traits in modern grown varieties.
SS-O-01. Does “yellow” make sense in oilseed rape?

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Yellow flowers are a standard in oilseed rape cultivation. However, seeds of most cultivars are still black. Improvements in seed quality are expected from the development of yellow seeded types. Yellow seeded types were reported to have a lower fibre and higher protein and/or oil content than black seeded types. In the past various attempts have been made to develop yellow seeded cultivars. However, those cultivars have not yet been able to gain significant market shares. The reason for this remains unclear. It has been discussed that yellow seeded genotypes may suffer from lower germination and vigor and increased pre-harvest germination. Since competitive yellow seeded cultivars have not yet been developed, existing quantitative variation in seed fibre content in black seeded types may contribute to the development of oilseed rape with enhanced seed oil and protein content. The objective of the present work was to study the quantitative variation for seed fibre content in black seeded oilseed rape and to analyze correlations to other seed quality traits. In a doubled haploid population derived from the cross of the DH line SGDH14 with the cv. Express (inbred line 617) a major QTL for Acid Detergent Lignin (ADL) content was detected which collocated with QTL for oil and protein content of the defatted meal with opposite additive effects, suggesting that the reduction in lignin content resulted in an increase in seed oil and protein content. The developed plant material along with the detected QTL allele represents a first step towards the development of an improved oilseed rape quality.

Keywords: Oil, fibre, ADL, protein
S5-O-02. Mapping loci controlling fatty acid profiles and contents of oil and protein content by genome-wide association study in *Brassica napus*

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Optimal profile and quantity of fatty acids in rapeseed are very critical for maximizing the value of edible oil and biodiesel. Genome-wide association study (GWAS) could efficiently identify the genes and their network underlying complex traits and provide molecular markers to accelerate breeding. In this study, we performed GWAS to dissect the genetic variations of eight seed quality traits in a rapeseed population comprising 370 diverse accessions. Seven common SNPs for the contents of seed oil and protein were detected on five chromosomes, and five genes orthologous to function-known Arabidopsis genes were predicted as candidate genes. Genomic region of reduced variation around FAE1 loci on A08 and C03 chromosomes controlling the content of erucic acid showed that these two regions were strongly selected during breeding. With the re-sequencing data, we found, except the known haplotypes combination of the variations of BnaA.FAE1 and BnaC.FAE1, three accessions contained an additional haplotype combination controlling the moderate content of erucic acid. This study reveals the genetic variations and complex correlations of eight quality traits, which may inform breeding selection strategies for traits in crops.

**Keywords:** Association study, fatty acid profiles, candidate genes, *Brassica napus*
S5-O-03. Rapeseed meal as alternative to soybean protein for feeding monogastrics

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Poland as well as all EU countries is not self-sufficient in terms of protein production for the feed and food uses. Development of new varieties of rapeseed with canola like quality has made these species important source of oil for edible and technological purposes and protein for fodder purposes. This plant gives unique opportunities as a native alternative protein source for poultry and pigs however the problems associated with high fiber content in seeds and other anti-nutritional factors should be eliminated (Bell 1995, Kozlowska et al. 1990, Shahidi and Naczk 1992, Slonimski et al. 1999).

Primarily, an enhancement can be achieved by increased breeding effort, targeting nutritional value of seed yield. These investigations have been undertaken in the “ProRapeSeed” project, in which three Polish and three German teams are participating. The first step is evaluation, identification and categorization of varieties /lines of different origin and quality traits based on content of nutritional and antinutritional components. This broad collection will be used for association mapping for identification of candidate regions in the genome responsible for different quality traits influencing low digestibility of rapeseed protein.

Project “ProRapeSeed”, supported by NCBR due to decision no DZP/CORNET-22/87/201

Keywords: Rapeseed meal, protein, nutritional value, anti-nutritional factors

References
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S5-F-01. Importance of nutritional components and enrichment in crop breeding

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The *B. rapa* subspecies has wide genetic and morphological diversity which grown as leafy vegetables, vegetable oils, turnip greens, turnip roots, turnip tops and as a fodder crop. In general plant secondary metabolites play vital roles during different stages of growth and development. These functional compounds like glucosinolates, anthocyanins, vitamin C, total sugars, and calcium add high nutritional value to humans. We have generated double haploid (DH) lines through microspore culture from the collected germplasm accessions with high functional compounds. For glucosinolates we have performed a conventional QTL analysis using F2/3 mapping population of *B. rapa* combined with candidate gene association approach by using natural population in order to identify the genomic region and genes regulating glucosinolates biosynthesis in *B. rapa* crops. Results suggest several alleles with very high association for important compounds. Additionally the comparative analyses of several association results were completely matching with previous analyzed QTL maps. The further analysis will be done to study the identified candidate genes related to glucosinolates enhancement. Similarly anthocyanins, the most prevalent flavonoids in red/purple crops, are known to improve immune responses and reduce chronic disease risks. The anti-inflammatory activities were tested based on its inhibitory effects in cultured endothelial cells and hyperlipidemic apolipoprotein E-deficient mice using anthocyanin-rich extract from red Chinese cabbage. The results suggest that the consumption of anthocyanin-rich red Chinese cabbage is closely correlated with lowering the risk of vascular inflammatory diseases.

**Keywords:** *Brassica rapa*, glucosinolates, anthocyanins
Brassica plants are commonly attacked by multiple herbivores above and below the ground. They defend themselves by producing constitutive and induced defences, in particular glucosinolates. Most studies focus on responses to shoot herbivores, whereas relatively little is known about root–herbivore interactions. Moreover, the signalling pathways involved in induced plant responses may interact when root and shoot herbivores attack the same plant. Indeed, plants infested with root herbivores may respond differently to shoot feeding herbivores than uninfected plants. In addition, optimal defence allocation patterns may change when root herbivores are feeding. Our aim is to elucidate the mechanisms underlying the interactions between root and shoot herbivores, as well as the ecological consequences for the interaction partners. I will present our most recent work on local and systemic responses induced by Delia radicum, the cabbage root fly. I will particularly focus on the interactive effects of root-induced responses on shoot feeding specialist and generalist caterpillars. Moreover, I will discuss our latest findings on glucosinolate induction, allocation as well as transportation upon root feeding. The results of our research provide us with a better understanding of how plants optimize their defences under attack by multiple herbivores.
S6-O-01. Stress Effects on Immunity in *Brassica oleracea*

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*Brassicas* are major agricultural and horticultural crops grown throughout the world, and include a diverse range of crop types. Their production is threatened by plant diseases and abiotic stresses such as drought and adverse temperatures which cause significant losses to farmers. Recent studies have highlighted the overlap between signalling mechanisms for biotic and abiotic stresses [1]. This study aims to improve our knowledge on the signalling crosstalk in the immune system of *Brassicas* to improve their resilience to abiotic and biotic stresses.

The first layer of active defence in plants is based on the perception of pathogen (or microbe) associated molecular patterns (PAMPs/MAMPs) leading to PAMP-triggered immunity (PTI). Increasing evidence suggests that PTI contributes to quantitative disease resistance (QDR), a desirable breeding trait that potentially provides durable control of diseases in plants [2]. However, PTI is also linked to plant growth and is influenced by abiotic stresses [3].

This work is focused on understanding the effect of abiotic stress on PTI and QDR in *Brassica oleracea*. Induction of PTI by drought stress was determined by quantifying the production of reactive oxygen species (ROS) in a biparental mapping population of *B. oleracea* ssp *alboglabra* (A12Dhd) and *B. oleracea* ssp *italica* (Green Duke GDDH33). ROS production after application of various PAMP molecules was measured for individual lines of the A12xGreenDuke population following drought stress and compared to non-stressed control plants. The mapping results revealed transgressive segregation for drought inducible ROS phenotype and for resistance to *Botrytis cinerea*. Transcriptional comparison of lines with differential ROS induction is in progress. The work will provide new insight into the effects of abiotic stresses on PTI, enabling more reliable QDR to be developed in *Brassicas*.

**Keywords:** Cross talk, Abiotic stress, Biotic stress, Quantitative Disease Resistance, PAMP-triggered immunity

**References**

S6-O-02. Genetic Analysis of Immune Responses in *Brassica* Species: Mechanistic Analysis of Quantitative disease resistance in *Brassicas* by Associative Transcriptomics (MAQBAT)

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With imminent changes in agrochemical legislation and an increasingly unpredictable climate, crop disease resistance needs to be considered in a wider context than before. Utilising multiple genomic resources available to the research and breeding community, we have developed a novel approach to improve disease resistance in *Brassicas*. Our research addresses the challenges facing agriculture whilst providing new insight into the nature and regulation of defence gene function.

The plant immune system influences resistance to multiple microorganisms and is modulated by environmental conditions. The recognition of pathogen-, or microbe-, associated molecular patterns (PAMPs/MAMPs) by receptors and co-receptors in plant cells results in the activation of defence and PAMP-triggered immunity (PTI). Increasing evidence indicates that PTI contributes to quantitative disease resistance (QDR) [1]. Using methods we developed in *B. oleracea* and *B. napus* [2], we investigated whether PTI is active in *Brassica* crops and how variation in PTI-responses can be exploited for resistance breeding. We have determined *Brassicas* show multiple responses, including the production of reactive oxygen species (ROS) and gene-induction, to PAMPs such as flg22 and chitin, ultimately leading to induced resistance. The intensity and speed of responses varies between species and varieties. We found 100-fold differences in the ROS-responses within a diverse set of 192 *B. napus* lines, which we are using for Associative Transcriptomics (AT; [3]) to identify causative gene loci.

This PTI-work contributes to a multi-partner study on Mechanistic Analysis of Quantitative disease resistance in *Brassicas* by Associative Transcriptomics (MAQBAT), co-ordinated by the John Innes Centre. The aims of MAQBAT are to apply new knowledge and techniques in PTI and improve deployment of QDR to achieve more durable control of the most important pathogens of *Brassicas*.

**Keywords:** PTI, PAMP-triggered immunity, Quantitative Disease Resistance, Associative Transcriptomics, GWAS, *Brassica napus*, diversity panel, Genome Wide Association Studies (GWAS).

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S6-O-03. Analysis of the determinants of adaptation of *Leptosphaeria maculans* ‘*Brassicae*’ to a new host species

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Plant-pathogenic fungi present extreme adaptive abilities, allowing them to circumvent any new control methods. The Dothideomycete fungus *Leptosphaeria maculans* ‘*Brassicae*’ causes stem canker of cruciferous species and exhibits a hemibiotrophic lifecycle on oilseed rape (*Brassica napus*). So far, the most effective control method against *L. maculans* consists in breeding naturally resistant cultivar of *B. napus*. The disease resistance follows gene-for-gene relationship: a product of a host resistance gene (*R* gene) interacts directly or indirectly with the product of a matching avirulence gene (*Avr* gene) of the pathogen stimulating host immunity and resistance response. These monogenic, cultivar-dependant, resistant sources are rapidly overcome. In particular, in *L. maculans*, this adaptive ability relies notably on the location of *AvrLm* genes in repeat-rich genomic regions of *L. maculans*. Contrary to host resistance, nonhost resistance confers complete resistance of all genotypes of a plant species to all genotypes of a pathogen species. Plant pathogenic fungi can also adapt to a new host following a host range expansion or a host jump; in the latter case, the pathogen loses ability to infect the ancestral host. Recently, an isolate of *L. maculans* was identified as partially adapted to *Brassica carinata*, a plant species considered as nonhost for *L. maculans* so far; this isolate cannot infect *B. napus*. A cross was performed between this isolate and an isolate infecting *B. napus*. Symptoms caused by the progeny show a continuous phenotypic variation on both hosts. This suggests a complex determinism, involving many genes, of the adaptation of *L. maculans* on these hosts. The integrative analysis of comparative genomic, transcriptomic, genetic and phenotypic data will help shed light on mechanisms underlying adaptation to different hosts in *L. maculans* and on resistance mechanisms in two *Brassica* species.

**Keywords:** Nonhost, *Leptosphaeria maculans*, *Brassica napus*, *Brassica carinata*, omic analyses, genetics
Plants are often infected by more than one pathogen and there are many factors affecting co-existence of these pathogens on their host which lead to changes in their predominance [1]. Phoma stem canker is a damaging disease of oilseed rape (Brassica napus) and Brassica vegetables. This disease is caused by two closely related sibling pathogens, Leptosphaeria maculans and L. biglobosa [2]. Since L. maculans has generally been associated with stem base canker while L. biglobosa has been associated with upper stem lesions, L. maculans was considered more damaging than L. biglobosa [1, 3]. Therefore, previous work has mainly focused on L. maculans and the importance of L. biglobosa in phoma stem canker epidemics has been ignored. However, results of our recent work show that L. biglobosa can cause both damaging upper stem lesions and stem base cankers, leading to yield losses [4]. Furthermore, L. biglobosa is less sensitive to some triazole fungicides than L. maculans [5, 6, 7]. The need for effective host resistance to control this disease is greater than ever. However, previous breeding for cultivar resistance has targeted only L. maculans; there is no information about cultivar resistance against L. biglobosa. Recent studies have shown that cultivars resistant against L. maculans are often more susceptible to L. biglobosa [8]. For effective control of phoma stem canker, there is a need to target both L. maculans and L. biglobosa. The reasons why L. biglobosa has recently increased in importance in phoma stem canker epidemics in the UK will be discussed, using results from field experiments over three cropping seasons and controlled environment experiments.

Keywords: co-existence, Leptosphaeria biglobosa, Leptosphaeria maculans, oilseed rape, phoma stem canker, sibling pathogens

References
Session 6: Pathogen and insect resistance, biocontrol, crop protection

S6-O-05. Blackleg fungus and adaptation to resistance genes in canola: epidemiology and evolution from landscape to field plots and back

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Agricultural landscapes create ideal environments for plant pathogens to proliferate and rapidly evolve. Thus, a critical challenge is to design durable and effective strategies to protect crops from damage caused by pathogens [1]. Theoretical studies suggest that spatio-temporal variation in the diversity and distribution of resistant hosts across agricultural landscapes may have strong effects on the epidemiology and evolutionary potential of crop pathogens [2]. However, we lack empirical tests of spatio-temporal deployment of host resistance to pathogens can be best used to manage disease epidemics and disrupt pathogen evolutionary dynamics in real-world systems. In a field experiment [3], we simulated how differences in Brassica napus resistance deployment strategies and landscape connectivity influence epidemic severity and Leptosphaeria maculans pathogen population composition. Host plant resistance, spatio-temporal connectivity [stubble loads] and genetic connectivity of the inoculum source [composition of canola stubble mixtures] jointly impacted epidemiology (disease severity) and pathogen evolution (population composition). Changes in population composition were consistent with directional selection for the ability to infect the host (infectivity), leading to changes in pathotype (multilocus phenotypes) and infectivity frequencies. We repeatedly observed decreases in the frequency of unnecessary infectivity, suggesting that carrying multiple infectivity genes is costly for the pathogen. From an applied perspective, our results indicate that varying resistance genes in space and time can be used to help control disease, even when resistance has already been overcome. Furthermore, our approach extends our ability to test not only for the efficacy of host varieties in a given year, but also for durability over multiple cropping seasons, given variation in the combination of resistance genes deployed.

Key words: Phoma stem canker, Blackleg, Brassica napus, Resistance deployment strategy, ecology, virulence, infectivity, Adaptation

References
S6-O-06. Dissection of gene expression networks involved in *Verticillium longisporum* resistance in *Brassica napus*

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*Verticillium longisporum* is a soil-borne fungal pathogen that causes serious yield losses in winter oilseed rape (*Brassica napus*). The fungus can survive in the soil for many years as microsclerotia and since the fungal hyphae grow through the roots into the xylem, fungicide based control is not effective for this pathogen. Therefore, breeding for resistant varieties is the most promising approach for controlling the damage inflicted by *V. longisporum*. However, narrow gene pool of *B. napus* combined with rigorous selection for seed quality traits such as zero erucic acid, low glucosinolates since the 70s has left the modern day rapeseed cultivars susceptible to various fungal pathogens including *V. longisporum*. Diversity rich *Brassica* species can be a valuable resource to expand the narrow gene pool of oilseed rape. To date, only quantitative resistance against *V. longisporum* has been identified, mainly deriving from C-genome resistance sources but the selection of resistant varieties is challenging due to the late appearance of symptoms, low heritability and a lack of knowledge about the molecular mechanisms underlying the resistance response. In order to dissect the genetic control of *Verticillum* resistance in *B. napus*, we compared the transcriptomes of two doubled-haploid sister lines exhibiting strongly contrasting resistance reactions to *V. longisporum* infection in a similar genetic background. By localizing differentially expressed genes within key resistance QTL, we identified three genes potentially involved in the resistance reaction. Detailed analysis of these genes in the resistance response reveals novel insights that could be instrumental in the breeding of oilseed rape cultivars with effective resistance against *V. longisporum*.

**Keywords:** Fungal resistance, transcriptomics, *Verticillium longisporum*, *Brassica napus*
S6-O-07. Field identification of biochemical biomarkers for screening plant resistance to insects: an example from the pollen beetle – oilseed rape interaction

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Pollen beetle (Brassicog ethes aeneus syn. Meligethes aeneus) is one of the main insect pest affecting oilseed rape crops. Efficiency of insecticides used to control this pest is decreasing due to the development of resistance to compounds such as pyrethroids in many populations. Breeding oilseed rape for resistance to pollen beetle attacks could be an interesting strategy to find alternative control methods. However, screening plants for insect resistance remains complicated as it often involves field tests on large genotype collections which are complicated to carry out without biases. Current knowledge on the chemical ecology of interactions between oilseed rape and pollen beetles could help finding biochemical markers of this resistance and bypass this problematic field screening phase thus allowing an indirect breeding approach. Previous laboratory tests have shown that variations in attack levels among a small set of oilseed genotypes could be explained by the biochemistry of bud tissues. The present study aimed at validating this link under field conditions. For that purpose, we conducted a multi-site experiment in France with 19 genotypes exposed to pollen beetle attacks. We phenotyped pollen beetle damage and sampled buds in the field to assess their chemical composition. Large variability in pollen beetle attacks was observed over the genotypes. These attack levels were consistent between locations. Bud chemistry was highly variable but most compounds were well correlated between locations. Potential biomarkers previously identified in laboratory experiments were not confirmed but new compounds which may be considered interesting markers for resistance screening against the pollen beetle emerged.

Keywords: Phenotyping, Oilseed rape, Pollen beetle, Metabolite, Environmental variability
S6-O-08. Influence of belowground herbivory on dynamics of root and rhizosphere microbial communities

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Plant-herbivorous insect interactions play an important part in shaping the biochemical composition of plants. Reciprocally plant metabolites influence major life history traits in these insects and largely contribute to their fitness. Rhizospheric microorganisms are an important biotic factor modulating plant metabolites and adaptation to stress. Recent studies unraveled the impact of microorganisms from the root and rhizosphere on plant-herbivorous insect interactions and are gradually changing our perception but the reverse effect has seldom been investigated. We conducted our study on oilseed rape (Brassica napus) and its major belowground herbivore, the cabbage root fly (Delia radicum) in order to determine whether potential changes in root metabolites and elemental compounds produced during herbivory can be related to root and rhizosphere microbial community diversity. Different microbial diversities harboring a common soil matrix were obtained through a removal-recolonization method. Root and rhizosphere sampling targeted different stages of the herbivore development corresponding to different intensities of perturbation and were assessed through amplicon sequencing of 16S and 18S ribosomal RNA genes. Root bacterial communities were more affected by herbivory than rhizosphere and fungal communities. Root herbivory enhanced root and rhizosphere γ-Proteobacteria, as well as rhizosphere Firmicutes and it increased trehalose and indolyl glucosinolates. Three bacterial genera (Bacillus, Paenibacillus and Pseudomonas) were positively correlated following herbivory to some sulfur-containing compounds and trehalose. Further research would help to identify the biological function of the microbial genera impacted by plant infestation and their potential implications in the plant defense.

Keywords: Delia radicum, Brassica napus, soil microbial diversity, rhizosphere and root microbial communities, herbivory, metabolites, elemental compounds.

References
S6-F-01/P-626. Managing blackleg of canola in Canada – Pathogen race dynamics, cultivar resistance and fungicide control

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In western Canada, the incidence and severity of blackleg \textit{[Leptosphaeria maculans} (Desmaz.) Ces. & de Not.] of canola \textit{(Brassica napus} L.) increased noticeably in recent years, and there are several unique aspects on the disease in this region influenced possibly by cultivars used and environmental conditions. While most canola cultivars are labelled resistant to blackleg, \textit{Rlm1} and \textit{Rlm3} are the only specific resistance (R) genes found in commercial varieties and breeding lines. Field monitoring since 2007 has found that \textit{AvrLm1} and \textit{AvrLm3} were low or had declined to very low levels in most areas, while \textit{AvrLm2}, \textit{AvrLm4}, \textit{AvrLm6} and \textit{AvrLm7} were generally high (<50\%) in the pathogen population. When inoculated with \textit{L. maculans} isolates carrying no \textit{AvrLm1} and \textit{AvrLm3}, most cultivars were substantially more resistant to blackleg than “Westar” (susceptible), reducing pathogen hyphal spread from infected cotyledons into the stem (lower blackleg incidence), and/or restricting the infection in stem (lower severity). This indicates quantitative resistance (QR) exists in these cultivars. Using RNA-seq, we found that resistance conferred by \textit{Rlm1} seemed to be related to activated jasmonic-acid and salicylic-acid pathways, while QR in the cultivar 74-44BL appears to be triggered by the upregulation of genes involved in programmed cell death and generation of reactive oxygen species. The different resistance mechanisms associated with a specific R gene and QR seem to support the strategy of using R genes in a strong QR background. The QR performance also appeared stable under prevailing heat conditions (~32°C daytime high) in western Canada. Due to a much shorter crop season (75-100 days) than in many other canola-growing regions in the world, early infection is the key to blackleg impact on canola in western Canada. This raises the question about fungicide timing; seed dressing or foliar application? Spray or not to spray? The presentation will discuss these issues in light of recent research information.

**Keywords:** Stem canker, \textit{Leptosphaeria maculans}, avirulence-gene profile, specific R gene, quantitative resistance, race nonspecific resistance, molecular mechanisms, fungicide control

**References**

Liban SH et al. (2016) Plant Pathol. 65, 1161–9
Leptosphaeria maculans is a hemi-biotroph that causes blackleg disease and remains a significant threat to canola (Brassica napus) cultivation. Qualitative resistance has been utilized in many breeding programs around the world with some adult plant resistance in their background to mitigate this disease. Although Blackleg was well managed for over 3 decades with good genetics in the Canadian canola industry, there's been a steady increase of resistance being eroded due to the presence of new races of the pathogen in grower fields. Rlm3 is the predominant gene found in Canadian canola cultivars. AvrLm3 gene has been disappearing rapidly in many fields enabling the pathogen to cause moderate to severe disease in grower fields. Canada has also had a trade embargo with China due to blackleg presence in Canada. The successful implementation of research over 5 years by the government and grower groups has helped Canada to adopt new strategies in the mitigation of the blackleg disease. The understanding of the R-genes in Canadian canola germplasm and the blackleg-pathogen races across the canola growing regions has helped the industry to introduce a new R-gene rotation strategy. The development of a diagnostic tool, Kompetitive Allele Specific PCR (KASP) markers has helped the producers to identify the predominant races present in their fields. This would help them to strategically select the R-genes and their combinations to be selected in the variety they select to grow in the R-gene rotations. The seed companies are starting to label their varieties with the known R-genes and their combinations so growers can make a good selection of varieties that suits their needs. The adoption of major-gene resistance groups and the L. maculans race diagnostics test will provide producers with new tools to help manage and mitigate blackleg on their farms. The presentation will take you through the past, present and future of canola, and the mitigation of blackleg disease in Canada.

Keywords: canola; blackleg; R-gene rotation; KASP markers; R-gene labeling

References
S6-F-03. Oilseed rape crop debris and potential spread of *Leptosphaeria maculans* (phoma stem canker) into China

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Quarantine regulations are designed to prevent establishment of pathogens in new countries. Phoma stem canker (blackleg), which causes global losses in oilseed rape (canola) worth >£1000m per annum, is caused by *Leptosphaeria maculans* and the less damaging *L. biglobosa*. Chinese surveys provided evidence that only *L. biglobosa* is currently present in China but that *L. maculans* is present in crop debris associated with cargoes of oilseed rape seed imported into China (Zhang et al. 2014). Following work that applied models of the spread of *L. maculans* across Canada to assess potential spread across China, in November 2009 the Chinese quarantine agency imposed restrictions on the import of oilseed rape seed into China. Canadian exports to China decreased in 2010 but then returned to previous levels when it was agreed that they could enter China through ports in regions where oilseed rape is not grown. A memorandum of understanding signed between China and Canada during a visit by Chinese prime minister Keqiang Li to Canada in 2016, specifying the amount of crop debris (dockage) permitted in seed cargoes, is in place until 2020. Current modelling work will assess the risks of spread of *Leptosphaeria maculans* associated with different amounts of debris in cargoes. This work will establish principles that can be applied to help restrict the spread of pathogens to new countries in seed cargoes.

**Keywords:** *Leptosphaeria maculans*; oilseed rape; phoma stem canker; quarantine regulations

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S6-F-04. Molecular dissection of the *B. napus* A07 gene cluster conveying resistance against the blackleg pathogen *Leptosphaeria maculans*.

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Race specific resistance (R) genes have been widely deployed in breeding *Brassica napus* (canola, rapeseed) for resistance against the blackleg pathogen *Leptosphaeria maculans*. All of the genetically defined *B. napus* R genes against blackleg (named *Rlm* or *LepR*) reside on the A genome of *B. napus* and most of the matching *L. maculans* avirulence (*Avr*) genes have been cloned (1). Cloning the corresponding *Rlm* and *LepR* genes, however, has been hampered by the complexity of the genome and difficulties in genetic manipulation of *B. napus*. We reported cloning of *LepR3* and *Rlm2*, the first *B. napus* R genes against blackleg (2, 3). *LepR3* and *Rlm2* are allelic variants encoding membrane-localised receptor like proteins (RLPs).

The *Brassica-Leptosphaeria* pathosystem provides examples of deviation from the gene-for-gene concept for some of the *Rlm* and *LepR* recognition of their cognate *Avr* proteins (named *AvrLm* and *AvrLep* respectively). The best example is the perception of *AvrLm3, AvrLm4-7 AvrLm5-9* (4, 5, 6) by their corresponding R genes *Rlm3, Rlm4, Rlm7* and *Rlm9* respectively, that are clustered on the A07 chromosome of *B. napus*. We have produced a fine map for majority of the *Rlm* and *LepR* genes including the A07 gene cluster (7).

Here we report cloning of *Rlm9*, the first gene from the A07 cluster. *Rlm9* presents a new class of R proteins and differs significantly from *LepR3* and *Rlm2*. Recent work by our lab and others has shown that recognition of *AvrLm3* and *AvrLm5-9* is masked in the presence of *AvrLm4-7* and *AvrLm4* (5, 6). We will describe the role of *Rlm9* and the masking effect of *AvrLm4-7*, based on our cloning of *Rlm9* described here and also our recent work on the cloning and functional analysis of *AvrLm5-9*.

**Keywords:** *Brassica napus, Leptosphaeria maculans, Rlm9, AvrLm5-9*

**References**

S6-F-05/P-625. Genome-based identification of genes involved in pathogen interactions with Brassica crops

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A wealth of genomic information, deposited in public databases, is available to identify candidate genes that contribute to interactions between crops and their pathogens. Of interest to us are pathogens that pose global threats to production of Brassica oilseed and vegetable crops, including Brassica napus, Brassica juncea, Brassica rapa and Brassica oleracea. We have used published and in-house genome sequences to determine the distribution of genes encoding leucine-rich repeat (LRR) receptors in the genome of B. napus. Specific attention was given to a resistance (R) gene cluster on chromosome A7 that operates against the phoma stem canker pathogen Leptosphaeria maculans. Tests for positive selection were used to decrease the number of candidate LRR genes, which can be used as cloning and breeding targets. In addition, the genomic intervals of known major and quantitative trait loci for resistance against L. maculans, Sclerotinia sclerotiorum and the clubroot pathogen Plasmodiophora brassicae were used to determine the relative frequencies of genes encoding nucleotide-binding, secreted or transmembrane LRR receptors. Moreover, work is proceeding to better understand molecular components of compatibility of B. napus to L. maculans or its sister species Leptosphaeria biglobosa. This research will be of benefit to our academic and industry colleagues working on plant breeding.

Keywords: oilseed rape, stem rot

References
S6-F-06: Screening of rapeseed mustard germplasm and identification of new donors for biotic stress resistance

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Because of dynamic changes in race composition of the pathogen, identification and characterization of new sources of resistance is an important pre-requisite in managing biotic stress. About 3000 germplasm accessions have been evaluated under multiple environments at hot spots and under artificial epiphytophiic conditions against insect and diseases such as aphids, white rust and Alternaria blight [1, 2]. IC265495, IC313380, EC766091, EC766133, EC766134, EC766192, EC766230, EC766272 were identified as highly resistant to white rust (A. candida) with disease severity reaction (PDI = 0) across the locations and under artificial inoculation at both cotyledonary stage and true leaf stage. These resistant accessions coupled with agronomic superiority sources will be useful for selecting parents for white rust resistance breeding in Indian mustard. A new Leaf curl disease in rapeseed-mustard germplasm reported which is caused by a weed infecting begomovirus-betasatellite complex. Molecular characterization revealed the disease is associated with Croton yellow vein mosaic virus and its betasatellite. Results indicated that all the Brassica accessions differed in their susceptibility/tolerance to aphid infestation and it ranged from 11 to 173 mean no. of aphids/ top 10 cm inflorescence. IC262141, IC385673, IC331817 and IC423130 were found highly tolerant as the infestation level was < 10 mean no. of aphids/ top 10 cm inflorescence. Glucosinolates showed highly significant negative correlation (r = -0.88), palmitic and oleic acid showed negative correlation (r = -0.21 and -0.17) while, linoleic acid and erucic acid showed positive correlation (r = 0.20 and 0.23) with aphid infestation. We assume that several traits specific donors identified from cultivated and other related species will be useful for genetic amelioration of rapeseed and mustard.

Keywords: Rapeseed, Mustard, Genetic Diversity, Germplasm, White rust, Aphid

References
Understanding the mechanisms and generational durability of clubroot resistance

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Clubroot (Plasmodiophora brassicae Woronin) resistance relying on a single gene can be short lived and this has been observed in both crucifer vegetable and canola crops. It is not clear if multiple clubroot resistance (CR) genes can be deployed to improve the resistance versatility and durability, potentially due to different modes of action of CR genes. In this study, transcriptome, proteome, metabolome and synchrotron-based Fourier transform infrared (sFTIR) spectroscopy analyses were explored to characterize resistance mechanisms associated with specific CR gene or gene combinations. The generational durability of resistance was assessed by inoculating canola (Brassica napus L.) lines carrying single and double CR genes with P. brassicae resting spores from disease roots of previous planting cycles. In plants carrying the CR gene Rcr1 inoculated with P. brassicae pathotype 3, genes involved in jasmonic acid/ethylene pathways and callose deposition were strongly upregulated, whereas most genes involved in auxin biosynthesis and cell growth/development were downregulated. These defense responses appear to be triggered via a unique signaling pathway involving ubiquitin-26S, a proteasome frequently associated with plant cold tolerance. Metabolomic work identified increases in caulilexin C and jasmonic acid, while sFTIR spectroscopy found increased lignin and phenolic content in resistant root tissues. The latter was also supported by enhanced intensity of lignin autofluorescence in cell walls under confocal laser scanning microscopy (CLSM). The resistance to clubroot conferred by Rcr1 may involve several modes of action. Canola lines carrying two CR genes (on A3 and A8) consistently showed moderate resistance to a new P. brassicae pathotype (5x). These lines maintained the efficacy under controlled-environment conditions, despite repeated exposure (5 plantings) to the inoculum from root galls of prior plantings. Molecular resistance mechanisms associated with single and double CR genes against the pathotype 3 and 5x are being further investigated using RNA-seq and CLSM.

Keywords: Canola, clubroot, resistance mechanism, Gene Ontology, secondary metabolites, multi-geneic resistance, repeated exposure, resistance durability.

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Exploration of Brassica accessions for resistance to ‘old’ and ‘new’ isolates of Plasmodiophora brassicae in Alberta, Canada.

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Genetic resistance is the main tool used to manage clubroot of canola (Brassica napus) in Canada. However, the emergence of new virulent strains of the clubroot pathogen, Plasmodiophora brassicae, has complicated canola breeding efforts. In this study, 386 Brassica accessions were screened against five single-spore isolates and 17 field isolates of P. brassicae to identify resistance sources effective against these new strains. The results showed that one B. rapa accession [CDCNFG-046, mean index of disease (ID) = 3.3%] and two B. nigra accessions (CDCNFG-263, mean ID = 3.1%; and CDCNFG-262, mean ID = 4.7%) possessed excellent resistance to all 22 isolates evaluated. Fifty other accessions showed differential clubroot reactions (resistant, moderately resistant or susceptible), including 27 (one B. napus, two B. rapa, four B. oleracea and 20 B. nigra) accessions that were each resistant to 8 - 21 P. brassicae isolates, but developed mean IDS in the range of 5.3-29.6%. The remaining 23 accessions (two B. napus, one B. rapa, five B. oleracea and 15 B. nigra) were each resistant to 3 - 13 isolates, but developed mean IDS in the range of 30.3-47.0%. The three accessions which showed absolute resistance and the 50 accessions which showed differential clubroot reactions could be used to breed for resistance to the new P. brassicae strains.

Keywords: Screening, Brassica accessions, Clubroot tests, Plasmodiophora brassicae, pathotypes, resistance

References
Characterization of clubroot resistance in *Raphanus*

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*Raphanus* crops are important vegetables and play a major role as break crop or for biocontrol of nematode infestations in soil. Clubroot is a major disease of crucifers. *Plasmodiophora brassicae* infections are strongly increasing in all major crucifer crops. Despite a relatively high resistance level, clubroot affects also radish crops. Even minor infections in oil radish break crops receive public attention and cause farmers to avoid all crucifers as break crops. On the other hand, the use of break crops like oil radish is highly desirable due to their positive effects on soil fertility and reduction of N leakages. Due to this situation, clubroot resistance (CR) in oil radish needs strong improvement to support the use of this break crop without risking the increase of clubroot inoculum. The RAPHKORE project aims at characterization and improvement of CR in *Raphanus*. Different sources of CR are studied. The known *Raphanus* CR that is effective against the prevailing pathotypes, which originate from *Brassica* crops, needs to studies of its inheritance and broadness. The use of this CR type can be supported by an increased homogeneity. Newly collected *P. brassicae* isolates from oil radish crops were tested on different *Raphanus* and *B. napus* cultivars. So far, adaptation to this CR type is very seldom, and *Raphanus* cultivars usually show only few infections. One isolate has been identified showing strong virulence towards *Raphanus* CR. This isolate originates from a farm focusing on potato production with oil radish as a break crop to reduce nematodes. We used this isolate to screen *Raphanus* gene bank accessions and found a small number of resistant accessions. Resistant individuals were selected and selfed. The S1-progeny of CR plants was often significantly more resistant that the original population, indicating successful selection. Future studies on the inheritance of CR from *Raphanus* will further reveal the genetic potential to control clubroot in this crop.
Two Clubroot Resistance Genes Mapped into Chromosome A08 of *Brassica rapa* using Bulk Segregant RNA Sequencing

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Genetic resistance is widely used to manage clubroot (*Plasmodiophora brassicae*) in *Brassica* crops, but development of new pathotypes can result in rapid breakdown of resistance. In the current study, two new resistance loci (*Rcr3* and *Rcr10*) were mapped in *B. rapa* using bulk segregant RNA sequencing (BSR-Seq). *Rcr3* and *Rcr10* were effective against the most prevalent pathotype on the Canadian prairies (pathotype 3H) and a new pathotype (5X), respectively. BC₁ plants exhibited a 1:1 segregation ratio for resistance to both pathotypes, which indicated control by a single dominant resistance gene. Variants were identified by assembling reads against a reference genome of *B. rapa*. SNP variants were much more prevalent than InDels (89% vs. 10%). Chromosome A08 carried the highest number of polymorphic variants (38%), which were most abundant between positions 9–11 Mb. *Rcr3* was mapped using 23 polymorphic SNPs within a range of 38.3 cM, with flanking markers (A90_A08_SNP_M64 and M16) 417 Kb apart, between 9.8-10.2 Mb. Analysis of differentially expressed genes identified six genes in this flanked region that were highly expressed in the R bulk, and gene annotation indicated that four of these genes were associated with plant defence. *Bra020936* was highly expressed in the R bulk and annotated as a TIR-NBS-LRR class disease resistance protein, so it represents the most likely candidate gene for *Rcr3*. The other gene, *Rcr10*, was mapped with flanking markers (A90_A08_SNP_M28 and M65) between 10.8-12.0 Mb on A08 with the same set of SNP markers used for *Rcr3* mapping through BSR-Seq. The *Rcr10* interval region contains 14 genes related to plant immunity, but only the gene *Bra020814* was associated with a TIR-NBS-LRR protein in *A. thaliana*, which could be the candidate gene for *Rcr10*. *Bra020814* was 877.4 Kb away from *Rcr3* candidate gene *Bra020936* and 363.6 Kb away from *Crr1* gene *Bra020861*.

**Keywords:** *Brassica rapa*, *Plasmodiophora brassicae*, Bulk Segregant RNA Sequencing
Influence of nitrogen constraint on quantitative resistance to clubroot in *Brassica napus*

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Abiotic factors are known to influence quantitative resistance to plant pathogens, but underlying genetic and physiologic mechanisms are mostly unknown. In this work, we developed combined genetic and molecular physiology approaches to investigate the influence of nitrogen fertilization on quantitative resistance of *Brassica napus* to the clubroot causing agent *Plasmodiophora brassicae*. Disease response was studied in a panel of oilseed rape genotypes and *P. brassicae* isolates cultivated under low vs high nitrogen supplies. This work highlighted that lower nitrogen input can modulate disease symptoms (from strong symptom inhibition to no effect), depending on both plant genotype and *P. brassicae* isolate. QTL analysis conducted in a ‘Darmor-bzh’ x ‘Yudal’ doubled haploid progeny showed that nitrogen deficiency exerts a major switch between the effects of two QTL involved in resistance toward eH isolate. One low-nitrogen-dependent QTL identified on the chromosome C02 was found to exert a major effect on the resting spore content in infected roots, but moderately influencing club symptom development. By contrast, the effect of a major QTL involved in resistance toward K92-16 isolate was unaffected by nitrogen fertilization. Combination of metabolomics and transcriptomics highlighted the putative role of nitrate transporter encoding genes, which are specially induced under the double biotic-abiotic stresses in the genotype ‘Yudal’ expressing low nitrogen-triggered resistance. Altogether, our results indicated that nitrogen fertilization influence clubroot disease in a QTL x isolate dependent manner. A better understanding of QTL x pathogen isolate x fertilization crosstalk may help to rationalize the use of clubroot quantitative resistance in breeding.

**Key words:** *Plasmodiophora brassicae, Brassica napus*, nitrogen fertilization, abiotic/biotic stress crosstalk, disease resistance
Mimicking the host regulation of SA: a virulence strategy by the clubroot pathogen *Plasmodiophora brassicae*

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Salicylic acid plays a critical role in defense against biothrophic plant pathogens. *Plasmodiophora brassicae* (Pb) is an obligate biotrophic pathogen of Brassicaceae and the causal agent of clubroot disease on canola. Pb encodes a protein with very limited homology to benzoic acid (BA)/salicylic acid (SA)-methyltransferase (PbBSMT). PbBSMT has a BA/SA methyltransferase domain and an IAA-binding domain, which are also present in *Arabidopsis thaliana* BSMT1 (AtBSMT1). Here we show that PbBSMT suppresses local defense and provide evidence that PbBSMT is much more effective than AtBSMT1 at suppressing the levels of SA and its associated effects. PbBSMT-overexpressing plants were more susceptible to Pb than WT plants; they also were partially compromised in non-host resistance to *Albugo candida*. In contrast, AtBSMT1-overexpressing plants were not more susceptible than WT to either Pb or *A. candida*. Furthermore, transgenic Arabidopsis and tobacco plants expressing PbBSMT exhibited increased susceptibility to virulent *Pseudomonas syringae* pv. *tomato* (PstDC3000) and virulent *P. syringae* pv. *tabaci*, respectively. R gene-mediated resistance to PstDC3000/AvrRpt2 and TMV was also compromised in *Arabidopsis* and *Nicotiana tabacum* cv. Xanthi-nc plants expressing PbBSMT, respectively. Transient expression of PbBSMT or AtBSMT1 in lower leaves of NtXanthi-nc resulted in enhanced resistance to TMV in the distal systemic leaves, which phenotypically resembled systemic acquired resistance (SAR). The development of PbBSMT-mediated SAR was dependent on the MeSA esterase activity of NtSABP2 in the systemic leaves. Collectively, these results strongly suggest that PbBSMT is a novel effector, which is secreted by Pb into its host plant to deplete pathogen-induced SA accumulation.

**Keywords:** *Albugo candida*, *Arabidopsis*, AtBSMT1, SA/BA methyltransferase, PbBSMT, *Plasmodiophora brassicae*, Salicylic acid, tobacco
Workshop: Nitrogen Use Efficiency

W2-O-01. Breeding of oilseed rape for sustainable agriculture: Assessing 25 years of breeding progress

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The element nitrogen (N) is essential for plant growth and agricultural crop productivity and is the plant nutrient that has to be applied as fertilizer in the largest quantities. Unused nitrogen, however, can escape from the agricultural production system either by nitrate leaching into groundwater, runoff from the soil surface, or due to emissions of nitrous oxide or volatile ammonia. In the agro-ecological context these events can have potentially serious negative side effects on neighboring ecosystems. Furthermore, since mineral fertilizer production by the Haber-Bosch process is energy dependent, carbon dioxide emissions influence the greenhouse-gas balance in a negative way; simultaneously rising energy costs result in increasing fertilizer prices, thus diminishing the economic margin of farmers. In future the question will arise how a growing world population can match its demand without magnified impacts on the environment. Besides a more adjusted fertilizer management, a greater emphasis on breeding and cultivation of varieties with improved nitrogen use efficiency (NUE) is important for a more sustainable agriculture.

Oilseed rape (Brassica napus L.) is Europe’s most important oilseed crop and the second most important in the world. Its high quality plant oil for human nutrition purposes, the industrial use as substitute for fossil oil and not at least its high value protein as animal feed, has resulted in significant production increases: Today around 40 Mio tons of oilseed rape are produced per annum in the European Union on an average cultivation area of 8.6 Mio ha (FAOSTAT). As the dominating dicotyledonous crop, winter oilseed rape has assumed an integral role in cereal crop rotations. However, with its relatively high acquisition of nitrogen during vegetative growth stages but a comparatively low nitrogen seed yield, oilseed rape cultivation is often associated to an N-balance surplus.

The objective of this study was to assess the genetic improvement of winter oilseed rape varieties released to the market between 1989 and 2014. Therefore 30 elite varieties, clustered into older and modern hybrids as well as in older and modern open pollinated lines, were tested in a two-year experiment in 10 environments across Germany. The varieties were cultivated under two nitrogen fertilization levels (120 and 220 kg N/ha including N_{min}) during growing seasons 2014-2015 and 2015-2016. At two locations stems, leaves, flowers and pods of plants were harvested separately to determine the respective nitrogen contents in the particular plant segments at flowering (developmental stage BBCH 67-69). Later, at seed maturity, the nitrogen content of seeds and plant residues was analyzed alongside the primary yield components. The experiment delivered an up-to-date analysis of genetic improvement for both nitrogen uptake efficiency (NupE) and nitrogen utilization efficiency (NutE). Interestingly we observed a surprisingly low yield gap between both fertilization levels.

In future the cultivation of more efficient varieties might allow farmers to harvest more with less fertilizer. This will be beneficial for the environment and delivers economic advantages for the entire society.
W2-O-02. The key role of SAG12 cysteine protease in nitrogen remobilization associated with leaf senescence

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Oilseed rape (Brassica napus L.) is a crop requiring high inputs. In a context of sustainable agriculture especially by inputs reduction (including N), it becomes necessary to decipher the physiological mechanisms related to N management in plant. B. napus is characterized by a Low Nitrogen Use Efficiency due to a low Nitrogen Remobilization Efficiency associated with leaf senescence process. This developmental process involves numerous classes of proteases to ensure its good progression (1). Cysteine protease is the most overexpressed class during senescence in many plant species such as B. napus L. and A. thaliana (2). Among them, SAG12 protease is the most induced during leaf senescence of B. napus (3) and A. thaliana. However, the role of this cysteine protease in nitrogen remobilization and leaf senescence process remains unclear and controversial (4, 5). Therefore, the aims of this study is to clarify the role of the SAG12 protease during leaf senescence. To reach this, phenotypic characterization, SAG12 localization by GUS staining and proteomics approaches were realized on KO-sag12 and/or wild-type A. thaliana grown in optimal and low N conditions (HN and LN) until seed maturation. Main results have shown the existence of proteolytic compensation systems involving not only cysteine proteases but also aspartate proteases including a CND41-like aspartate protease. In optimal conditions, these compensation systems are sufficient to substitute SAG12 explaining the absence of phenotype demonstrated also by other studies (4, 6). Under LN condition, inducible proteolytic systems are not sufficient to compensate SAG12 depletion leading to a decrease of yield and N content. Finally, SAG12 was shown to be specifically located in vascular tissues of non-senescent organ and extended to the other cells during leaf senescence. Altogether, these results suggest a putative role of SAG12 in N export and a key role during grain filling associated with leaf senescence.

Keywords: Leaf senescence, nitrogen management, protease activity, SAG12 localization, seed yield

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W2-O-03. Genetic and molecular determinants of Nitrogen Use Efficiency in winter oilseed rape

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A major challenge in plant breeding is to propose cultivars adapted to low input cultural practices, especially low nitrogen (N) input, for environmental and economic issues. Winter oilseed rape (WOSR) is known for its low nitrogen use efficiency (NUE). Therefore improving NUE is a major breeding target. At the plot scale, NUE can be assessed by scoring seed yield (SY) variations between two contrasting N nutrition regimes, namely optimal vs. limiting N supplies (hereafter referred to as ∆SYN) (Bouchet et al., 2016). As a consequence, identification and characterization of QTL controlling specifically SY and SY components under low N input or ∆SYN will greatly improve our knowledge about genetic and molecular determinants controlling NUE in winter oilseed rape. To address this issue, we carried out GWAS on SY and ∆SYN related traits using a set of 174 WOSR inbred lines grown under two contrasting N regimes in 8 environments (location × year) and genotyped with 236k SNP well spread over the genome. For all traits analyzed, a high number of QTL was detected over the genome. In addition, most of the SY QTL was detected under both N conditions; and only few loci were specific to low N conditions. To confirm the N-specificity of these QTL, they were confronted to ∆SYN QTL. Afterwards, a QTL prioritization was carried out based on homoeologous relationships between the two rapeseed subgenomes. Four pairs of homoeologous QTL were identified that allowed reducing the candidate gene list underlying the corresponding loci. To conclude our analysis, we suggested that the small number of SY QTL that were specific to low N supply may result from an interaction with the environment. This hypothesis was supported by the identification of many environment-specific QTL. This prompted us to further get insights into the G×N and G×N×E interactions.

Keywords: Brassica napus L., seed yield, NUE, genome wide association study, quantitative trait loci

Reference