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ORAL PRESENTATION THEME A

New population genomics re-capture lost genetic diversity in rapeseed

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

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

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

Conclusion: We established a highly interesting genomic platform for studying and using genetic diversity in an important global crop species and for dissecting and predicting complex traits.

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ORAL PRESENTATION THEME A

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Increasing the market value of canola through improved oil and meal quality traits

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

Genetic diversity increases heterosis – The Sprinter *Brassica napus* Project

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

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

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

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

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

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ORAL PRESENTATION THEME A

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Genomic insights into seed development in *Brassicas*

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

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

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

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

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

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044
ORAL PRESENTATION THEME A

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

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

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

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

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

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

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WCC/RRC data from Canola Council of Canada, CPT data from Canola Council of Canada.

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ORAL PRESENTATION THEME A

Heterotic gene pool development in *Brassica napus*

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

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

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

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

Conclusions: Phenotypic and genotypic heterotic clustering was similar despite different methods and agreed with pedigree information. Genetic distance was positively correlated with hybrid heterosis (R2 = 0.18); however, it lacks predictive power for high heterotic crosses using these specific distance/clustering techniques.

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

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

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

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

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

Synthesis of a stable allohexaploid *Brassica*

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

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

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

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

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

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049
ORAL PRESENTATION THEME A

Homologous pairing control in *Brassica napus*

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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051
ORAL PRESENTATION THEME A

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

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

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

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

Results: The dominant effects of all yield-related traits were obviously greater than their additive effects and epistatic effects, suggesting that heterosis is important to improving the yield of rapeseed. Among three yield components, Siliques per plant (SPP) and seed per silique (SPS) both showed a significant negative correlation with thousand-seed weight (TSW), but silique density (SD) was genetically correlated with all three components of yield to a certain extent. GDs of the 14 parental lines ranged from 0.1883 to 0.8811, with an average of 0.5217. At genetic similarity of 0.65, the parental lines were divided into four groups. Except for number of effective primary branches, all other nine yield-related traits showed significant heterosis in F1 hybrids. Especially for plant height (PH), SPS, branch height (BH) and yield per plant (YPP), the average mid-parent heterosis were 6.83%, 15.31%, 16.13% and 38.78%, respectively, and the average high-parent heterosis reached 3.18%, 5.19%, 7.85% and 20.78%, respectively. Significant positive correlation between heterosis and SNP estimated GD was detected for PH, BH and YPP.

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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053
ORAL PRESENTATION THEME A

Genomic prediction of hybrid performance in canola *(Brassica napus)*

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

Methods: We constructed and sequenced RNAseq libraries made from 12 different tissue samples collected in triplicates at four major developmental stages, including germination, vegetative growth, flower development and seed development. Using the Illumina HiSeq2000 platform, ~ 727 million 100 bp paired-end reads (73 GB) were generated, corresponding to an average of 61 million reads per tissue sample. After filtering low-quality, adapter contaminated, and short reads, high quality reads from each sample were aligned to the *C. sativa* reference genome using Tophat2, and expression levels were calculated using Cufflinks.

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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058
ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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ORAL PRESENTATION THEME A

Using the Illumina Infinium 60K array to identify species and genetic diversity in *Brassica* germplasm

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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

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066
ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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High-throughput multiplex cpDNA re-sequencing clarifies the genetic diversity and genetic relationships among *Brassica napus*, *Brassica rapa* and *Brassica oleracea*

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

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

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

Results: The cpDNA of the current worldwide *B. napus* population comprises more than 400 variants (SNPs and short InDels) and maintains one predominant haplotype (Bncp1). Whole-genome re-sequencing of the cpDNA of Bncp1 haplotype eliminated its direct inheritance from any of the *B. rapa* or *B. oleracea* species. The distribution of the polymorphism information content (PIC) values for each variant demonstrated that *B. napus* has a much lower cpDNA diversity than *B. rapa*. However, a vast majority of the wild and cultivated *B. oleracea* appeared to share one same distinct cpDNA haplotype, definitely contrasted to its wild relatives.

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

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068
ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

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

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ORAL PRESENTATION THEME A

Brassica oleracea as a model for epigenome analysis

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

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

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

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

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

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071
ORAL PRESENTATION THEME A

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

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

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

Breeding strategies for oilseed *Brassica* improvement in India

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

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

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073
ORAL PRESENTATION THEME A

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

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

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

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

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

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

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074
ORAL PRESENTATION THEME A

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

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

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

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

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

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ORAL PRESENTATION THEME A

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Development of *Alternaria* blight resistant Indian mustard (*Brassica juncea L.* Czern & Coss.) lines derived from *Brassica juncea* x *Brassica alba* through conventional and embryo rescue techniques

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

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

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

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

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

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ORAL PRESENTATION THEME A

Proteomic analysis of temperature sensitive male sterility SP2S in rapeseed

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

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

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

Conclusions: Disturbance on the expression of these proteins may disrupt the coordination of developmental and metabolic processes, resulting in defective tapetum and unviable microspores. This is the first proteomic investigation on temperature-sensitive male sterility in rapeseed, and the results provide new insights into molecular events associated with the male sterility.

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ORAL PRESENTATION THEME A

Identification and analysis of BnaA.tsMs:A novel gene required for meiotic chromosomal organization in *Brassica napus*

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Background: We discovered a newly bred thermo-sensitive dominant genic male sterility (TSDGMS) line TE5A which originated from a spontaneous mutant of the inbred line TE5 in *Brassica napus*. The TE5A exhibits ecotypic sensitivity, the fertility of TE5A is normal at low temperature, and it will transform to completely sterility when temperature is higher than 20°C during florescence (Zeng et al., 2014). Based on the observation of morphological, the TE5A mutant phenotype was observed at the PMC stage, in which the PMCs did not progress to tetrad production. We have cloned the gene from TE5A, *BnaA.tsMs,* that is involved in sister chromatid cohesion and chromosome segregationthe during male meiosis.

Objectives: To date, many dominant genic male sterility (DGMS) lines have been studied in *Brassica napus*. However, the abortion mechanism and gene functions of DGMS have been unclear. This research of abortion mechanism of TE5A and function of *BnaA.tsMs* gene will greatly accelerate construction of new male sterility line in *Brassica napus*.

Methods: The *BnaA.tsMs* gene was cloned by map-based cloning from TE5A. To perform observations of flower development, standard paraffin and plastic sections were generated. To obtain chromosome spreads, inflorescences were harvested and fixed in Carnoy's solution (ethanol:glacial acetic, 3:1, v/v). Anthers containing PMCs undergoing meiosis were incubated with 3% cytohelicase, 3% pectolyase, and 3% cellulase in citric acid buffer for 90 min at 37°C and then counterstained with DAPI. FISH was performed in meiotic chromosome spreads by 45S rDNA from clone pTa71.

Results: In the *BnaA.tsMs* mutant allele, an L-to-F transition converts a Leu at position 281 to a Phe (L281F), causing thermo-sensitive dominant genic male sterility (TSDGMS). In TE5A male meiosis, the classical steps of prophase were not observed; chromosomes did not undergo synapsis, and they formed 38 univalents instead of 19 bivalents. The 38 univalents generated an ordered metaphase plate and underwent an equational division. Then, the chromatids formed chromatin again, in the same manner as in S-phase, and stopped progression at telophase I.

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

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ORAL PRESENTATION THEME A

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

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

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

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

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

Conclusions: A novel recurrent selection population of new-type *B. napus* diversed on Ar/Cc genome was developed, and extensive recombination among Ar/Aj/An/ and Cc/Cn subgenomes has been achieved in the population. An intensive phenotypic and genomic selection will be recurrently carried out with the population and unique lines are expected developed from a novel pre-breeding program.