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# Evaluation of rapeseed- mustard genotypes from Australia, India and China for agronomic and biochemical traits

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**Background:** The rapeseed-mustard varieties being grown in Australia, India and China do not conform to 'canola' quality and/or is susceptible to fungal diseases

**Objectives:** Breeding good agronomy canola quality *B. juncea/ B.napus* free of fungal diseases by combining selected genotypes through ACIAR support.

**Methods:** Australian, Indian and Chinese cultivars/breeding lines of *B.juncea* (95) and *B.napus* (155) were evaluated at TERI research field (NCR, India). DI was evaluated (three leaves/plant) on a 0-5 scale (0–no symptoms, 0.5-£5%, 1.0-£10%, 1.5-£15%, 2.0£20%, 3.0-£30%, 4.0-£40%, 5.0-£50%). Fatty acids, glucosinolates and oil content were estimated by GC, HPLC/Elisa, and NMR/NIR respectively.

**Results:** All *B. napus* accessions had low incidence (5-10%) of white rust (WR)/Alternaria blight (AB). Indian *B. juncea* recorded 5-50% WR/AB, while exotic accessions had 0-10% WR/ 5-10% AB. This could be due to different host pathogen susceptibility of exotic accessions to Indian isolates. Exotic *B. juncea* showed high incidence of bacterial wilt (10-40 plants/genotype) and *Sclerotinia* stem rot (5-20 plants/genotype), except JN028 and JN033 that were free from both. Indian cultivars had high erucic/high glucosinolate. Promising *B. napus* accessions with 68-71% oleic acid are OscarAG Outback, ZY007, ZY009, Rivette and TERI(OO)R9903. *B. juncea* genotypes CBJ002, BJ3, JM06006, JR042 and JN031 had 50-63% oleic acid, and JN004, JN010, JR042 and Rohini had 39-40% oil content. Promising accessions- Pusa Bold (PB)/PCR-7 (good agronomy), GZ5 (double low, yellow seed), GPZ (double low, good agronomy) from India; JR049 (double low), JR042 (oil content 40%), JN028, JN033 (free from Sclerotinia/bacterial wilt) from Australia; and CBJ003 (long pods) from China were used for intra-specific hybridization.

**Conclusions:** The useful variability in BC2/BC3 generations derived from intra-specific hybridization are: palmitic acid 2.46 (GZ5/PB) to 5.13 (GPZ/JR042)%; oleic acid 13.29 (GZ5/PB) to 46.81 (GPZ/CBJ003)%; linoleic acid 17.9 (GZ5/PB) to 37.75 (PB/JR042)%; linolenic acid 7.01(GZ5/PB) to 18.75 (GZ5/JO009)%; erucic acid <2 to 34.04(PCR7/JR049)%. Four populations derived from hybridization of GZ5 and three from GPZ with exotic genotypes have double low traits needing further agronomic improvement. The near double low populations from Pusa Bold/JR042 are promising with good agronomy.

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# Characterization of a *Brassica napus* doubled haploid population derived from a winter by spring cross

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A doubled-haploid (DH) population containing 115 lines was derived from a cross between a European winter growth habit *Brassica napus* cultivar and an Australian spring growth habit *Brassica napus* cultivar. Important traits in this population that are segregating include the vernalization requirement, as well as flowering and maturity. Even spring-like lines in this population, where vernalization is not required, tend to show later maturity than that of the spring checks. The current study aims to understand whether the traits for early flowering and maturity, as well as enhanced cold tolerance can be selected for together. It is hypothesized that individuals will be found that have cold tolerance as well as early flowering and maturity. Three major areas of research have been undertaken: 1) evaluation of cold tolerance in a field setting, 2) evaluation of cold germination in a laboratory setting, and 3) characterization of the agronomic traits of this population including flowering times and maturity. Analysis of the agronomic and cold tolerance data together suggests that while there are few examples in this particular population, it is possible to combine enhanced cold tolerance derived from winter germplasm with early maturity in a spring growth habit. Early maturity in a winter background is desired due to improved agronomic performance in hybrid combinations. The combination of enhanced cold tolerance, lack of vernalization requirement and early maturity in an otherwise winter-type background represents a step forward in germplasm development within *Brassica napus*.

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# Extraction of *Brassica* monogenomic from digenomic species: Reversing evolutionary pathway

**Background:** Resynthesis of natural allotetraploids is a common procedure to create novel variation as well as to infer structural and/or functional modifications in the participating genomes as a consequence of the process of polyploidizationfollowing interspecific hybridization and subsequent chromosome doubling. A large number of such examples are available in crop *Brassicas*. Uncoupling of the diploid components of a polyploidy is another avenue which have been sought but never adequately investigated. We report successful extraction of *B.rapadiploid* from *Brassica juncea*, a natural hybrid between *B.rapa* and *B.nigra*.

**Objectives:** To extract *B.rapafrom* a natural *B junceaallotetraploid*.

**Methods:** An interspecific hybrid was first produced by hybridizing *B.juncea* with *B.nigra* followed by cochiploidy to produce an allohexaploid. This was followed by three cycles of selfing and selection for reduced genome size using flow cytometery(Dolezelet al.2007).In every generation plants resembling *B rapa*, with lower chromosome number were retained for next cycle of selfing. GISH was performed as per (Schwarzacher& Heslop-Harrison,2000) to demonstrate the genomic constitution of the extracted *B rapa*.

**Results:** Crossing *B. juncea* with *B. nigra* followed by chromosome doubling produced an allohexaploid (AABBBB;2n=52) combination with B genome in tetrasomic dose. The allohexaploid plant was partially fertile but some seeds were produced following selfing. Plants in S1 progeny were queried for the genome size based on flow cytometeric analysis. S1 selects wereselfed to obtain progeny for the next cycle of selection. After three cycles of selfing and selection, plants with genome size similar to *B.rapa* were retained for detailed meiotic analysis. We could identify one plant with 2n=22 ( 11II). Genomic in situ hybridization using *B.nigra* probe confirmed disomic addition of one B genome chromosome pair. Remaining 10II were confirmed to be from complete set of A genome. This plants was selfed to raise S4 generation. Plants with *B. rapa* phenotype and euploid chromosome number of 2n=20 could be identified. Molecular characterization of these plants using a set of chloroplast, mitochondrial andnuclear SSRs confirmed their distinctness from natural forms of *B. rapa* . Interestingly the extracted *B. rapa* plants seemed phenologically closer to winter forms of *B. rapa* as compared to spring types.

**Conclusions:** The novel crossing scheme coupled with molecular cytogenetic techniques helped to derive *B. rapa* (10II) from alloteraploid *B. juncea* (18II), a valuable resource for future genome analysis studies.

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# A novel procedure to resynthesize *Brassica napus* from related allotetraploids

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**Background:** Rapeseed (*Brassica napus L.*; AACC, 2n = 38) is an allopolyploid that arose through natural hybridization between *B. rapa* (AA, 2n = 20) and *B. oleracea* (CC, 2n= 18). Its narrow genetic base is implicit in dual bottlenecks of polyploidy and domestication. Intensive plant breeding activities, especially for canola quality may have further eroded the variation for traits of high breeding interest. We have developed a new method of derived amphiploidy. This procedure envisaged an alternate pathway to polyploidy by sourcing desired diploid genomes from related and intensively bred *Brassica* digenomics (Banga and Kaur 2009). Efficacy of this method was demonstrated through recreation of *B. juncea* by combining 'A' genome from *B. napus* and 'B' genome from *B. carinata* (Gupta et al. 2015). We report an extension of this procedure for resynthesis of *B. napus* through hybridization between *B. juncea* (2n=36; AABB) and *B. carinata* (2n=34; BBCC).

**Objectives:** To explore an alternate pathway for *B. napus* resynthesis by sourcing desired diploid genomes from related and intensively bred *Brassica* digenomics.

**Methods:** *B. napus* (AACC) was resynthesized by hybridization between two related digenomic species *B. juncea* (AABB) and *B. carinata* (BBCC). This was facilitated by spontaneous chromosome doubling in two hybrid (ABBC) plants and elimination of extra 'B' genome chromosomes following several cycles of selfing and selection for fertile plants with *B. napus* morphology in subsequent generations of selfed selects.

**Results:** Twenty-five progenies with varying degree of resemblance to natural *B. napus* were assayed for cytogenetic stability and genetic diversity. Plants of only six progenies showed predominant occurrence of 19 bivalents (2n=38) and equal distribution of chromosomes at anaphase as in natural *B. napus*. Genotyping with 'B' genome specific primers and genomic in situ hybridization (GISH) indicated introgression of 'B' genome segments. Genotyping with 'A' and 'C' genome specific primers confirmed genetic identity of six derived *B. napus* plants with natural *B. napus*.

**Conclusions:** We report resynthesis of *Brassica napus* by combining 'A' and 'C' genome from *B. juncea* and *B. carinata*, respectively. Study also documents 'B' genome introgression into resynthesized *B. napus*.

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# Molecular analysis of yellow-seeded winter rapeseed *(B. napus L.* var. *oleifera)*

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**Background:** Rapeseed meal is a very important by-product remaining after oil extraction from seeds and containing over 40% of protein in a dry matter. However, the utilization of rapeseed meal in feeding of non-ruminants, especially of poultry, is limited by the antinutritive products of glucosinolate breakdown, also in seeds of canola quality, as well as by high fibre content. The reduction of fibre content is possible by development of yellow-seeded genotypes of rapeseed, as the trait is associated with reduced seed coat thickness.

**Objectives:** Identification of quantitative trait loci (QTL) for seed colour and fibre content in *Brassica napus*.

**Methods:** In order to identify major QTLs contributing to reduced seed coat and yellow colour of seeds as well as fibre content two mapping populations were developed. They consisted of the offspring of reciprocal crosses between two black-seeded and two yellow-seeded doubled haploid lines (DH) of winter oilseed rape. The first population included 91 and the second one 103 DH lines. The phenotype traits of plants from both populations were analyzed during five vegetation periods in two environments. Molecular characteristics was conducted using RAPD, AFLP and STR primers. Some of them were described in literature as generating markers linked to the seed colour (Somers et al. 2001; Sabharwal et al. 2004, Yan et al. 2007).

**Results:** For both populations, 1061 products of amplification were obtained, whereas 448 of them were polymorphic. For the first mapping population 22 linkage groups were obtained and for the second one 26 groups. 163 polymorphic amplification products were included into the linkage groups; 28 of them were linked to different phenotypic traits and 6 were linked to seed colour.

**Conclusion:** From the obtained 22 and 26 linkage groups 19 will be located on the *B. napus* chromosomes and the residual small groups will be properly linked when the constructed genetic map will be supplemented with larger number of markers.

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# Identification of QTL controlling glucosinolate contents in leaf, stem and seed in a *B. napus* DH-population

**Background:** The development of low erucic acid varieties and subsequently the introduction of low seed glucosinolate (GSL) content through the polish cultivar 'Bronowski' were two milestones in the breeding of *B. napus* as oil crop. While the inheritance of seed GSL content has been studied well due to its importance, only a few quantitative trait loci (QTL) mapping studies with the focus on GSL contents in vegetative tissues exist (Feng et al 2012).

**Objectives:** We used a *B. napus* DH-Population derived from the cross of the resynthesized line 'L16' and the cultivar 'Express' to identify regions in the genome which are possibly involved in GSL metabolism.

**Results:** In a field trial we measured individual GSLs in leaf, stems and seeds in order to investigate the tissue-specificity of GSL-QTL. Applying Composite interval mapping we found a total of 115 QTL related to individual or groups of GSL. Of these 49 QTL influenced GSL traits of seeds, 31 QTL leaf GSL traits and 35 QTL stem GSL. Most of the seed QTL were mapped in linkage groups (number of QTL) A03 (13), C02 (11) and C09 (12). Linkage groups A03 and C09 also had the highest number of QTL in vegetative tissue (A03 15 and C09 14 QTL). Comparison of the QTL positions by trait between tissues generally showed more overlapping regions between leaf and stem than between seed and leaf/stem. Nevertheless, some of the mapped QTL were trait specific and detected only in leaf, stem or seed. The phenotypic correlation for the sum of GSL contents was high (> 0.94) between stem and leaf, but lower (about 0.5) between vegetative tissues and seeds.

**Conclusions:** The GSL content and composition of leaves and stem is mainly controlled by the same QTL. The QTL for seed GSL are partly the same, but many seed QTL are tissue specific.

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# Genetic and omics resources for integrated *Brassica* researches at the Institute for Genetics Environment and Plant Protection (IGEPP), Rennes France

*Brassica* research programs at IGEPP aim at i) studying the dynamics of polyploid genomes and the regulation of homologous and homoeologous recombination ii) identifying resistance genetic factors and deciphering the plant response to different bioagressor infections and iii) studying the determinants that guarantee the stability of grain yield (quantity and quality) under low nitrogen input and water shortage conditions. To address these different questions, integrated approaches are developed based on the exploitation of the genetic diversity of *B. napus*, *B. oleracea* and *B. rapa* species through the development of specific genetic and genomic resources and on the use of core facilities of different platforms.

**Genetic resources:** IGEPP hosts the French Biological Resource Center "BraCySol" (site web). "BraCySol" collections gathered 1000 *B. napus* accessions (oilseed rape, fodder rape,...) and 1200 *B. oleracea* accessions (mainly French landraces): cauliflower, kale, cabbages... "BraCySol" seeds are available on request. Specific research material was developed and can be shared through collaborations:

- diversity sets (150 to 200 inbred lines) dedicated to the analysis of disease resistance and abiotic stress response by genome-wide association studies,
- seven doubled-haploid segregating populations for linkage analysis,
- a nested-Association Mapping populations constituted of 15 crosses of 200 RILs each (out of which 8 are public)
- synthetic and semi-synthetic forms derived from different *B.rapa* and *B.oleracea* accessions as well as monosomic addition lines (AA+C chromosomes)
- one tilling population

**Genomic resources:** Our main genomic resources consisted in i) the seven genetic maps (96 to 356 individuals per DH segregating population) and an integrated map with 47000 markers anchored to the sequences of Darmor-bzh, Chiifu-401, TO1000 and *Arabidopsis thaliana* including the *Brassica* subgenomes and the ancestral *Brassicaceae* blocks), ii) the characterization of the diversity sets using the 60K Infinium array, as well as iii) the resequencing of various oilseed rape accessions.

**Plant cytogenetic platform:** Our group has an expertise on cytogenetic analysis in mitosis and meiosis. The establishment of the meiotic behaviour allows determination of the pairing frequency in metaphase 1 between homologous and homoeologous chromosomes in *B. napus* haploid or diploid and in interspecific hybrids. The analysis can be completed by observation either at the genome scale through Genomic In Situ Hybridization with DNA of related species or with BAC specific of C genome or at the chromosome level through Fluorescent In Situ Hybridization with BAC specific of different chromosomes.

**Metabolomic platform:** Our metabolomics platform is dedicated to plant metabolomics, with a special expertise on *Brassica* species. Our set of chromatographic tools (LC-MS/MS, LC-UV, GC-FID and GC-MS) gives access to a wide set of profiling analyses, including primary and secondary metabolism, phytohormones, and untargeted metabolomics.

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# Identification of ideal tester genotype(s) in Indian mustard for screening of aphid infestation tolerant germplasm adapted to south-western region of Punjab province in India

**Background:** Indian mustard {*Brassica juncea (L.)* Czern & Coss} commonly known as raya, is confined to south-western region of province having about 30, 000 ha under cultivation in Punjab, a north-western province of India. This crop gave maximum yield potential when planted from 10-25th October, but sowing of crop is delayed about one month due to prevailing cropping system of this region, which imposes serious impact of both biotic stresses as well as abiotic stresses. Among the biotic stresses, Aphid *(Lipahis eyrsimi Kalt.)* causes about 10-90% losses in yield in India to *Brassica* crops depending upon severity of damage and crop stage (Singh, 2005). Cultivation of raya in Punjab particularly under delayed sowing is facing above biotic stresses thus emphasizing the need to develop resistant/ tolerant germplasm for sustainable productivity.

**Objectives:** The objective of the present study were to use the GGE and AMMI biplot approaches to determine the performance of lines and their crosses under diverse environments to examine the combining abilities (GCA) and heterotic pattern (SCA) of the lines and identify the best tester(s) having acumen to select lines with good GCA for aphid infestation on raya under diverse growing conditions for sustainable productivity.

**Methods:** PBR 91, PBR 97, PBR 210, PBR 357, RL 1359, RLC 1, IC 255435, IC 255439, IC 266917 and IC 266933 were crossed in diallel fashion to generate 45 F1 crosses. These F1 crosses including parents were grown under optimum, delayed sowing and brackish water conditions for two years 2011-12 and 2012-13. The aphid population counts were made at weekly intervals from 10 cm terminal portion of central shoot and 3 leaves (one each from top, center and bottom) on each of 10 plants every F1 population.

**Results:** Significant differences among hybrids across environments were observed for aphid infestation. The GGE biplot explained 88.8% of the total variability GCA effects across the environment. Similarly, AMMI biplot captured 71.5 % of the non-additive portion of the variability for this trait. Based on the visualization of AMMI biplot three F1 hybrids viz., PBR 91 x IC266917, RL 1359 x IC 266933 and PBR 357 x PBR 97 showed heterotic (non-additive) effects for tolerance to aphid infestation. While GGE biplot showed that PBR 91 and PBR 357 had high GCA effects for aphid infestation tolerance can be utilized to generate genotypes having tolerance to aphid infestation.

**Conclusion:** Biplots visualization revealed that IC 266917 and PBR 91 were good tester for wide adaptability and hence are also ideal tester(s) as they have power to discriminate GCA of germplasm possessing tolerance to aphid infestation under diverse agro-climatic condition in south-western region of Punjab province for sustainable productivity of this crop.

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# Association of SNP markers and chlorophyll fluorescence parameters with low temperature tolerance in multiple *Brassica* species

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**Background:** The Canadian Prairies is the major growing region for canola in North America with an average annual production of 12 million tonnes (Council of Canada, 2008). Although Canadian canola production has increased in recent years, it has largely been due to increased acreage. A new goal for the industry is to improve yield of the Canadian crop by 50% to continue to increase total production. A major factor affecting spring canola production in Canada is frost during seedling development in the late spring, resulting in significant yield reduction (Canola Council of Canada, 2008). Efficient tools to screen canola lines would enable breeders and producers to develop and select canola varieties that would perform better under low soil temperature and spring frost conditions.

**Objectives:** The objectives were to 1) determine the effect of low temperature on seed germination and seedling performance of a diverse set of *Brassica napus* and *B. rapa* lines, and 2) conduct an association study to identify SNP markers linked to alleles for improved cold tolerance.

**Methods:** Untreated field-grown seeds of 169 genotypes, including *B. napus, B. rapa, B. juncea,* and *B. oleracea* were sown in pots containing field soil and placed in Conviron TC80 germination chambers (Conviron, Winnipeg, Canada). Seed quality was visually assessed prior to planting. In each pot, 50 seeds of each genotype were sown at a seeding depth of 1 cm. Emergence was assessed at 5, 10 and 15°C. In a seedling performance experiment, seedlings at the cotyledon stage were freeze-shocked at -5°C for 75 minutes. Frost injury was visually assessed as well as being inferred from the measurement of the chlorophyll fluorescence parameter [FV/FM = (FM – FO)/ FM]. DNA of all genotypes were hybridized and scanned using the 60K *Brassica* Illumina® Infinium array according to the manufacturer's instructions. We used the software STRUCTURE (Pritchard et al. 2000) to identify underlying populations among the lines and this was confirmed through PCA analysis. Genome-wide association analyses is being carried out with TASSEL (Bradbury et al, 2007).

**Results:** The time to onset of emergence, 50% (T50) emergence, and maximum (T≥90%) emergence was positively correlated with temperature. Onset of emergence began within 2 to 4 days of seeding for all genotypes at 15°C. At 5°C there was significant variability in seedling emergence among genotypes. As temperature decreased, smaller-seeded genotypes tended to emerge slightly faster than larger-seeded genotypes. However, a few larger-seeded genotypes emerged slightly faster than smaller-seeded genotypes. Chlorophyll fluorescence was highly correlated with the cold tolerance of the genotypes. The FV/FM parameter showed higher correlation with plant survival than any other parameters such as FV/FO, FM, and FV, suggesting that FV/FM could be used to assess cold hardiness in spring canola varieties. Out of the 47,304 SNPs arrayed, a total of 36,079 SNPs were retained for the purposes of association analysis. Of these, 6, 416 high quality SNPs evenly distributed across the genome have been selected for characterizing the population structure, relationship among the lines and for potential association with FV/FM.

**Conclusions:** The study demonstrated that the chlorophyll fluorescence method can be used in combination with molecular markers to achieve efficient gains for low temperature tolerance. With the availability of tools to screen large breeding populations for low temperature tolerance, it may be feasible for deployment in the canola breeding programs for selection.

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# In vitro culture of isolated microspores and plant regeneration of white mustard syn. yellow mustard *(Sinapis alba* syn. *Brassica hirta)*

**Background:** White mustard syn. yellow mustard (*Sinapis alba L.* syn. *Brassica hirta*) is open-pollinated, an annual plant of the family *Brassicaceae*. *Sinapis alba* as an oil plant is of growing economic importance due to the multilateral uses for example: seeds for food production, as green manure crops, phytosanitary crops and valuable honey plant and other. White mustard among *Brassicaceae* is the most resistant to drought occurring in Poland late spring and summer. The seeds of traditional varieties of white mustard, including var. Nakielska, is characterized by high erucic acid content in oil (35-45%) and high content of glucosinolates especially sinalbine (approx. 160 µmol/g-1), which remain in the meal extracted. In contrast, white mustard var. Bamberka, has lower than 1,2% erucic acid, but a similar content of glucosinolates as the traditional varieties. But the, first in Poland, double-low white mustard var. Warta is characterized by lower than 1,2 % of erucic acid in oil and 15 µmol g-1glucosinolates content and without sinalbine (Krzymański, 2014).

**Objectives:** We report preliminary results concerning the develop of efficient and reliable isolated microspore culture methodology for three different types of white mustard: traditional, with low erucic acid content and double low quality.

**Methods:** While in our previous studies observed a negative impact on *Sinapis alba* microspores rich in mineral and organic compounds NLN medium (Lichter, 1982) we have used KB5 medium (Leelavathi et.al 1984). Microspores of white mustard were isolated from anthers which were cultured on solid medium KB5 after exposure to low temperatures 4°C. The first divisions of microspores were observed after a few days of isolation from anthers. Over the next two weeks were recorded multicellular divisions and then forming the embryos. Visible to the naked eyes embryos were transferred to fresh KB5 liquid medium and cultured at light. Green embryos were planted on stable MS medium. After a few weeks, were observed the formation of shoot primordia. Developed shoots on MS medium with kinetin were transferred to rooting MS medium with IBA (Klóska et al. 2012). Androgenic plants of white mustard were planted to the soil and their further development took place in green house till the seed were collected.

**Conclusions:** The method of obtaining of white mustard androgenic plants from isolated microspore culture has been developed.

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**POSTER THEME A**

# Development of an engineered male-sterile line 15A in *Brassica napus*

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**Background:** High heterosis has been found in rapeseed (*Brassica .napus*). Many approaches to utilizing heterosis including cytoplasmic male sterility and chemically induced male sterility have been established. However, these accepted approaches each have drawbacks. It is worth developing a new way to utilization of heterosis in rapeseed.

**Objectives:** This study is to develop a new rapeseed male sterile line by genetic transformation and evaluate utility of the resultant male sterile line.

**Results:** The gene barnase causing male sterility was introduced into the double-low rapeseed cultivar Xiangyou 15 by genetic transformation. The resultant male-sterile tranformant was backcrossed to Xiangyou 15 for successive generations and a genetically stable male sterile line 15A has been bred. This male sterile line 15A is completely male sterility and its sterility is not influenced by air temperature. Observations showed that male fertility segregation fitted to the expected 1 fertile: 1 sterile in field. Fifty percent plants were killed after spray of 1% PPT at the seedling stage. The surviving plants were observed at the flowering stage and about 2% surviving individuals male-fertile. This implies that the surviving plants contain a single linked barnase-bar copy. There are no differences in characters including the number of flower buds, siliques per plant between 15A and its original parent Xiangyou 15 except male sterility. The male sterile line 15A has smaller flowers and completely deteriorated stamens at the beginning of flowering stage. However, its stamens are not completely deteriorated and aborted pollen grains can stain blue by methylene blue squash from the middle of flowering stage forward on. The male sterile line 15A, whether open pollinated or hand pollinated, sets seeds normally.

**Conclusions:** A engineered male sterile line has been bred in rapeseed successfully. This new male sterile line is better than traditional cytoplasmic or chemically induced male sterile lines because of its stability in male sterility and its linkage to herbicide resistance.

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**POSTER THEME A**

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# Application of In vitro vernalization in winter canola doubled haploid production

**Background:** In vitro vernalization has been studied in several plant species including oilseed rape or canola (*Brassica napus L*). Shi et al. (2004) reported that in vitro vernalized doubled haploid plants of semi-winter oilseed rape had plant flowering frequencies comparable to that observed with the normal vernalization protocol.

**Objectives:** We studied in vitro vernalization with nine winter-type European canola doubled haploid populations in comparison to regular artificial vernalization using a cold room.

**Methods:** Regenerated plants from microspore culture were vernalized in solid culture medium.

**Results:** In vitro vernalization gave flowering frequencies and seed production similar to regular artificial vernalization. The application of in vitro vernalization shortened the doubled haploid production cycle by six weeks, potentially increasing flexibility and efficiency in breeding applications.

**Conclusions:** We developed an effective in vitro vernalization procedure in winter canola doubled haploid production.

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# Extra-large seed germplasms of *Brassica napus* created through microspore culture

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**Background:** Rapeseed yield per unit area is determined by two main components, the number of seeds per unit area and the seed weight (Berry et al. 2006; Shi et al. 2009). Thousand seed weight (TSW) is one of major yield components of rapeseed (*Brassica napus L.*). Thus, creation of germplasm with high TSW of rapeseed make a great significance for promoting the yield per unit area.

**Objectives:** Here, an extra-large seed germplasm GM01, with the biggest TSW 8.68g, was obtained through isolated microspore culture. Furthermore, we investigated the stability of TSW, observed and evaluated the morphological traits especially flora organ and agricultural characteristics of GM01.

**Methods:** The three-way cross was made from H8 (TSW of 3.6g), a Yunnan spring early-maturing rapeseed variety, "Legency" (TSW of 3.8g), a Canadian canola variety, and "020010" (TSW of 4.6g), a semi-winter late-maturing rapeseed variety. After that, microspores of F1 of the three-way cross was used to culture to obtain doubled hyploid lines (DH). The TSWs of DH were tested in Kunming, Yunan. The DH with the highest TSW, GM01, was selected out to conduct the performance of the material in multiple sites for years. In detail, the fresh weight and diameter of flower bud, the diameter of pedicel, the length and width of petal, the fresh weight and length of ovary, and the morphological traits of stigma were investigated.

**Results:** One hundred and forty eight doubled hyploid lines(DH) were obtained from plantlets regenerating from microspore culture. For those DH, the TSWs of 53 lines were above 5.0g, among which the TSW of GM01 amounted to 8.68g. Furthermore, the TSW of GM01 was relatively stable with the variation only 10-15% among the multi-location field trials from 2007 to 2013. Compared with H8 and Legency, GM01 had larger flower organs, which were with the diameter (4.07±0.17cm), fresh weight (7.73±0.59g), larger flowers whose petal width was 0.96±0.02cm, thicker pedicel (0.82±0.08mm), bigger siliques whose length were 12.11±0.86cm and width (5.98±0.49cm), and the biggest seeds with the diameter(2.62±0.74mm). The DH lines of GM01 had less branches, siliques per plant, and seeds per silique on quality respect, howere, the glucosinolate and erucic acid contents were critically high, up to 130-140_mol/g and 30-40%, respectively.

**Conclusions:** One new higher TSW germplasm was obtained from the F1 DH of three-way cross by microspore culture. The material could blossom and bear fruits normally, to the most important, it showed considerable stability in multiple sites for years. However, the glucosinolate and erucic acid contents were much higher than that of their parents.

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# Variation and inheritance of mucilage content in yellow mustard *(Sinapis alba L.)*

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**Background:** Mucilage in yellow mustard seed is an important quality trait and functional food ingredient. Mucilage contributes to the consistency of prepared mustard products and has potential as a food gum additive (Cui et al., 1994). Therefore, development of yellow mustard cultivars with different mucilage contents is desired by the food industry. However, limited information is available on mucilage variation and inheritance in this crop.

**Objectives:** The objectives of the present study were to study the variation and inheritance of mucilage amount and viscosity in yellow mustard.

**Methods:** Mucilage was extracted from whole seeds and the amount was determined as a percentage of seed weight (Cui and Mazza, 1996). The mucilage viscosity of the samples was calculated as the viscosity of extract minus the viscosity of water and then multiplied by the volume of water and finally divided by the weight of the seed (cS*mL/g seed) (Raney and Rakow, 1999). The inbred line Y1494 has a high mucilage amount of 6.9 g/100g seeds and high viscosity of 139 cS*ml/g seed, while Y1497 has a low mucilage amount of 4.1g/100g seeds and low viscosity of 4.1 cS*ml/g seed. To study the inheritance of mucilage amount and viscosity, Y1494 was crossed with Y1497 to produce $F_1$ seeds. The $F_3$ seeds borne on the $F_2$ plants were used to study the inheritance of mucilage in yellow mustard.

**Results:** The yellow mustard inbred lines exhibited great variation in mucilage amount and viscosity ranging from 2.3 to 6.5 g/100g seeds and from 12 to 184 cS*mL/g seed, respectively. The mucilage viscosity was positively correlated with the mucilage amount in yellow mustard ($r = 0.90$). Mucilage was controlled by the maternal genotype. The $F_2$ seeds borne on the $F_1$ plants averaged 5.8g/100g seeds in mucilage amount and 84.8cS*mL/g seed in viscosity, suggesting the high mucilage amount and viscosity were partially dominant over the low. The mucilage amount and viscosity of the $F_3$ seeds borne on the $F_2$ plants showed continuous distribution ranging from 1.3 to 6.2 g/100 g- seed and 12 to 242 cS*mL/g seed, respectively. QTL mapping on mucilage amount and viscosity is underway.

**Conclusions:** Yellow mustard comprises extensive variation in mucilage. Preliminary genetic studies suggest that mucilage seems like a multigenic quantitative trait.

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**POSTER THEME A**

# Hybrid breeding using the improved Ogura cms system in *Brassica juncea*

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**Background:** Hybrid breeding strategy has been used to enhance yield in *Brassica juncea* (AABB, 2n=36). Hybrid varieties based on the Ogura cms, Moricandia cms and 126-1 cms systems, respectively, have been reported in this species (Archana et al. 2012; Sodhi et al. 200; Yao et al. 2012 and Canadian Food Inspection Agency, plant variety database). However, the hybrid variety 45J10 developed using the Ogura cms system had problems such as enlarged roots due to linkage drag. Agriculture and Agri-Food Canada obtained the *B. juncea* Ogura cms A line and restorer (R) line (RfoRfo) from INRA in 2003. Our molecular analysis indicated that the *B. juncea* R line (*RfoRfo*) carried a large radish introgression segment with the *Rfo* gene and the radish markers ScH03, ScA14, SG34, BolJon and PGlint as observed in the unimproved *B. napus* R line RRH1 (Primard-Brisset et al. 2005). In consequence, the R line has reduced male and female fertility as well as poor agronomic performance due to linkage drag. Improved *B. juncea* Ogura cms restorer (R) line VR441 *(RfoRfo*) with only two radish markers ScH03 and BolJon has been successfully developed via marker-assisted selection in combination with the increased recombination frequencies involving the Rfo gene in resynthesized *B. juncea* germplasm (Tian et al. 2014).

**Objectives:** 1) Development of improved condiment and canola *B. juncea* Ogura cms R lines (*RfoRfo*) using the *B. juncea* R line VR441 (*RfoRfo*) as restorer gene (*Rfo*) source; 2) Production and agronomic evaluation of test-cross hybrid based on the improved *B. juncea* Ogura cms hybrid system.

**Results:** Improved condiment and canola *B. juncea* Ogura cms R lines (*RfoRfo*) were developed using VR441 as the *Rfo* gene source via pedigree breeding. The improved R lines (*RfoRfo*) had strong growth vigour compared with the checks (the elite canola *B. juncea* line 5607 and condiment *B. juncea* variety Cutlass) in the two-replicated field nursery in 2014. Test-cross hybrid O2152 (*Rforf*) between the oriental mustard variety "Cutlass" Ogura cms line (rfrf) and the improved R line VR445 (*RfoRfo*) was produced in an isolation tent in 2013. The agronomic performance of the hybrid O2152 was evaluated in four-replicated yield trials in four locations (Saskatoon, Scott, Swift Current and Lethbridge) in western Canada in 2014. On average, O2152 yielded statistically significant higher (17.4%) than the check variety Cutlass.

Currently, heterozygous restorer lines (*Rforf*) are used as donor genotypes to produce doubled haploid (DH) Ogura cms R lines (*RfoRfo*) in condiment and canola *B. juncea*. Preliminary result indicates that the microspores carrying the *Rfo* gene is more embryogenic than those *Rfo*-negative ones in *B. juncea*. For instance, 150 plants were obtained using the canola *B. juncea* heterozygous restorer line C2532 (*Rforf*) as donor genotype, of which 122 (78.5%) plants were *Rfo*-positive.

**Conclusion:** The development of improved R line VR441 (*RfoRfo*) has made the Ogura cms hybrid system fully functional in *B. juncea*. We can use the improved Ogura cms system to develop high yielding hybrid varieties in both condiment and canola *B. juncea*.

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# Development and genetic studies of inbred lines with different linoleic acid contents in yellow mustard *(Sinapis alba L.)*

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**Background:** Yellow mustard (*Sinapis alba L.*) is cultivated as an important condiment crop in the world. It is an obligate outcrossing species due to its sporophytic self-incompatibility. Open-pollinated population varieties of yellow mustard comprise great genetic variation. Different erucic and linolenic acid variants have been identified among the inbred lines developed via pedigree breeding in yellow mustard (Cheng et al., 2012). Genetic studies indicated that the erucic acid content is conditioned by multiple alleles of the *FAE1* locus, while two gene loci are responsible for the linolenic acid variation in this crop (Javidfar and Cheng, 2013; Zeng and Cheng, 2014; Tian et al., 2014).

**Objectives:** 1) Development of inbred lines with different linoleic acid (C18:2) contents; 2) Genetics studies and QTL mapping of C18:2 content in yellow mustard.

**Methods:** Inbred lines with different C18:2 contents were developed via pedigree breeding. The intron length polymorphism markers were used for construction of the regional linkage map of linoleic acid gene. QTL analysis of the C18:2 content was performed using the interval mapping method of MapQTL 6.0 (Van Ooijen, 2009).

**Results:** The inbred lineY1798 has a low C18:2 content (average: 4.7%), while line Y1801 contains a high C18:2 (average: 27.5%). Inheritance studies indicate a continuous distribution of the C18:2 content in the F2 population of Y1798 × Y1801. Two QTLs for C18:2 content were identified and mapped, respectively, to the linkage groups Sal01 and Sal02 in the reference map (Javidfar and Cheng, 2013). The first QTL explaining 39.0% of the C18:2 variation, was mapped to the linkage group Sal01. The second QTL was located in the linkage group Sal02 and responsible for 37.0% of the C18:2 variation. Together, the two QTLS accounted for 76.0% of the total C18:2 phenotypic variation.

**Conclusions:** Two linoleic acid variants have been developed in yellow mustard. QTL mapping indicated that the two QTLs located on the linkage groups Sal01 and Sal02, respectively, are largely responsible for the phenotypic variation of C18:2 content in this species.

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**POSTER THEME A**

# Studies on CMS line 1193A and its hyrid of *Sinapis arvensis L.*

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**Background:** Chinese hybrid rapeseed acreage is the largest country in the world, and most of the cytoplasmic male sterile hybrid comes from Pol (Pol CMS).

**Objectives:** Due to the cytoplasm relatively simple, it is urgent to creat a new type of canola cytoplasmic male sterility. This study is by using unique Chinese Xinjiang wild rape (*Sinapis arvensis L.*) and Xiangyou15 (*Brassica napus L.*) to make the new cytoplasmic male sterile line.

**Methods:** By using bud pollination between Xinjiang wild rape (*Sinapis arvensis L.*) as female and *B. napus* varieties Xiangyou 15 as male parent. The hybrids obtained. F1 is highly sterile. The Xiangyou 15 self line 02-902 get backcross, The sterile plant rate of descendant is 43.33%. Since then, stirile plant and Xiang you 15 self line repeated backcrossing, The sterile plant rate in. 2007 is 53% -67%, in 2010 sterile plant rate remained at 100%. Then by two backcross eventually bred sterile high fertility stable CMS 1193A and corresponding maintainer 1193B.

**Results:** 1.CMS1193A basic characteristics:CMS1193A belong to *Brassica napus* weak-winterness cytoplasmic male sterile line, CMS and maintainer line hybrid offspring complete sterility and stability. Bagging has strong self-incompatibility. After bagging and artificial pollination, seed normal, outcrossing normal seed rate of about 85%. Petals large, overlapping, stamens degradation, anthers triangular, pistil normal; petal length 13.2mm, petal width 9.3mm, an area of about 122.8mm2, pistil length 8.5mm, long stamens long 5.2mm, short stamens long 4.3mm. Height 172 cm or so, once effective branches 8, 9 or so secondary branches, number of pods per plant is about 340, 18 grains per pod, grain weight 3.6 grams. Double low quality. Resistance to stem rot. 2. Restorers found: 2012 summer lines 12 (91) are all normal fertile plants, and sterile hybrids fertile full recovery rate of 100%. And the heterosis is significant. Test cross found its extensive restoration sources.

**Conclusions:** This study is by using unique Chinese Xinjiang wild rape (*Sinapis arvensis L.*) and cultivars Xiangyou 15 (*Brassica napus L.*) to make the new CMS line.

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**POSTER THEME A**

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# Identification of *Brassica napus* sources of resistance against pathogenicity groups 3 and 4 of *Leptosphaeria maculans*

**Background:** Blackleg caused by the fungus *Leptosphaeria maculans* is a serious disease of canola (*Brassica napus L.*) worldwide. In North Dakota, *L. maculans* populations have recently shifted their virulence profile from one dominated by strains of pathogenicity group (PG) 2 to one where the dominant group is PG-4. PG-2 strains cannot infect cultivars that have resistance genes *Rlm2* and/or *Rlm3* while the latter infects plants carrying *Rlm1, Rlm2* and/or *Rlm3* (del Río Mendoza et al., 2012; Marino, 2011; Nepal et al., 2014). Strains of PG-3, which are capable of infecting cultivars with resistance genes *Rlm2* and/or *Rlm3*, but are not that effective against *Rlm1*, constitute the second most prevalent group (Nepal et al., 2014). The recent occurrence of blackleg outbreaks that were caused by strains of these new groups (del Río Mendoza et al., 2012) adds a sense of urgency to the identification of effective sources of resistance against them.

**Objectives:** Identify *B. napus* plant introductions with effective levels of resistance against *L. maculans* strains of pathogenicity groups 3 and 4.

**Methods:** The screening approach consisted of two stages; in the first stage seedlings were evaluated in greenhouse conditions using the cotyledon inoculation method described by Nepal et al. (2014). Materials that advanced to the second stage were evaluated at the adult stage under field conditions. In the first stage, 579 *B. napus* plant introduction materials were evaluated using a mixture of five strains of *L. maculans* belonging to PG-4. All accessions were evaluated in replicated trials conducted at least two times. The materials that entered the second stage were inoculated with laboratory and field produced inoculum in 2014 and will be evaluated again in 2015. Disease severity at the adult plant stage was conducted before harvest by estimating the proportion of internal crown tissues visibly affected by the disease.

**Results:** Sixty plant introductions were considered resistant or moderately resistant to PG-4 isolates in greenhouse trials. Of the 21 accessions evaluated in field conditions, nine of them had significantly less disease ($P < 0.01$) than cultivar Westar and the two commercial controls used. The average severity for these accessions was < 10% compared to 81% of cultivar Westar and 26% of the commercial controls. The first stage of the screening of materials for reaction to PG-3 is under way.

**Conclusions:** *B. napus* plant introductions with high levels of resistance against *L. maculans* strains belonging to PG-4 have been identified and are currently being evaluated for their reaction to strains of PG-3. Doubled haploids will be developed from crosses involving some of these materials.

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**POSTER THEME A**

# Marker assisted selection of Ogu-INRA cms system in NS rapeseed

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**Background:** At the end of the 20th century, trends in rapeseed breeding changed direction from developing and using varieties to developing hybrids. This change in breeding programs was enabled by introduction of male sterility (cms) systems in rapeseed. At the Institute of Field and Vegetable Crops there is an ongoing breeding program with the goal of developing NS cms and fertility restorer (Rf) lines and hybrids (Marjanović-Jeromela et al. 2014). Ogu-INRA was chosen as a source of cms gene and R-2000 as a source of Rfo gene. It takes 6 years to develop rapeseed inbred lines; however, use of molecular markers can facilitate and accelerate this process.

**Objectives:** The aim of this study was to test the efficiency of markers linked to Ogu-INRA cms and Rfo genes (Sigareva and Earle 1997; Primard-Brisset et al. 2005) in NS experimental breeding material. This is especially important in testing experimental hybrids since some deviations in F1expected segregation ratios were observed in the past.

**Methods:** Analyzed plant material included different experimental Rf and cms lines, and F1. DNA was extracted by use of modified CTAB method (Permingeat et al. 1998). Extracted DNA was used for amplification with BolJon and CMS primers in reaction (Dimitrijević et al. 2010). Obtained profiles were analyzed by BIO-CAPT V.97 program (Vilber Lourmat, France).

Results: Most of the obtained profiles corresponded to the ones amplified by Sigareva and Earle (1997) and Primard-Brisset et al. (2005). However, some deviations from expected profiles were observed. These will further be examined.

**Conclusions:** Preliminary results of this study showed that tested markers could be prosperous in facilitating NS rapeseed breeding programs and thus accelerating the process of developing not only, cms and Rf inbred lines, but also hybrids.

**Acknowledgment:** This work is a part of the project TR31025, supported by Ministry of Education, Science and Technological Development, Republic of Serbia.

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**POSTER THEME A**

# Fine mapping a major early flowering QTL QFTC2-1 by high through sequencing combine with genetic mapping in rapeseed *(Brassica napus L.)*

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**Background:** In rapeseed, proper timing of flowering is a crucial determinant for adaption to different cultivation area and cropping seasons, also is an immediate indicator of the productivity. In South multiple cropping area of China, the rapeseed - rice -rice or rapeseed - rice or rapeseed -cotton rotation systems are rather pervasively. In those rotation systems, rapeseed with early maturity is one of the important limiting factor. So it is necessary to find and use key flowering time genes to breed early maturity rapeseed. Two rapeseed NIL lines R11 and R15 performed different flowering time were selected in pedigree breeding program. R11 usually blossomed 7-15 days earlier than R15 in winter rapeseed area while 6-12 days earlier in spring rapeseed area.

**Objectives:** Preliminary genetic analysis suggested that different flowering in the NILs was controlled by a major QTL. The objective of this study was to fine map and clone it, while using it in early maturity breeding was our final goal.

**Methods:** A F2 population was generated after self-pollinating F1 from R11 and R15. Flowering time data was recorded as the first flower blossomed and two extreme DNA pools, early flowering pool and late pool were constructed, respectively. Libraries of two pools and parents were prepared and then sequenced by an Illumina Solexa Hiseq2000 machine. Sequences analysis by BWA(Li and Durbin 2009) and GATK(McKenna et al., 2010) software to identify early flowering QTL. SSR markers were developed in QTL region by referring to the Darmor-bzh reference genome and an extreme early flowering population from F2 were scanned to confirm and fine map this QTL.

**Results:** A major early flowering QTL, QFTC2-1 was identified on the end of chromosome C2 after analyzing the sequences of two pools combine with parents. To confirm the early flowering QTL detected by high through sequencing, 40 linked markers were developed and a population containing 323 extreme early flowering plants was scanned. Finally, QFTC2-1 was fine mapped to a DNA region of approximate 95 kb. Now, we preliminary identified a gene that is homologous Arabidopsis genes involved in the flowering time pathway as candidate gene.

**Conclusions:** In this study, we fine mapped QFTC2-1 to a 95 kb DNA region and identified a candidate gene by high through sequencing combine with genetic mapping. This method took advantage of the high-through whole genome re-sequencing and bulked-segregant analysis (BSA) and genetic mapping. The results might be useful for cloning QFTC2-1 and using it in early maturity breeding by MAS.

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**POSTER THEME A**

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# *AP2/EREBP* transcriptional factors responsible for cold stress in rapeseed *(Brassica napus L.)* revealed by dynamic transcriptome analysis

**Background:** Changes in temperature significantly affect the development of plants. *Brassica napus L.* is one of the ideal crops for studying cold tolerance capacity. Although certain progress has been made in studies of cold-tolerant genes and related transcriptional factors, a comprehensive and dynamic research on the cold-tolerant network and AP2 transcriptional factors regulation from the perspective of the has not yet been reported.

**Objective:** A complete transcriptome data responsible for cold stress was obtained for comprehensive understanding of the regulatory mechanism of AP2-EREBP transcription factors, and providing an effective reference for further development of cold-tolerant genes.

**Methods:** Transcriptome high-throughput sequencing method was applied in this paper to conduct temporal and spatial dynamic analyses for the entire transcript of rapeseed subjected to 4 °C low temperature stress for 2, 6, 12, and 24 h. All the *Arabidopsis* AP2 domain of each treatment was used as a query to search the *AP2/ERF* gene domains in *B. napus* in the genome database using BLAST. We subsequently obtained the *AP2/ERF* genes for each species. The physical distribution of *AP2/ERF* genes on chromosomes was drawn by PERL scripts based on gene position in the genome. Phylogenetic and molecular evolutionary analyses were conducted using MEGA5. The dynamic and reliability of those transcription factors were further validated by RT-PCR.

**Result:** A total of 15,316 differentially expressed genes were identified, among which 451 *AP2-EREBP* transcription factors were discovered after comparing with the complete genome of rapeseed that was located in different chromosomes. After cluster analysis, these transcription factors were found to belong to five subfamilies, namely, DREB, ERF, AP2, RAV, and soloist.

**Conclusion:** A complete genome dynamic analysis was conducted on the stress-response gene and transcription factor regulation of rapeseed under cold stress. We obtained favorable transcriptome data for the comprehensive understanding of the regulatory mechanism of *AP2-EREBP* transcription factors, thereby providing an effective reference for further development of cold-tolerant genes and promoting the cold-tolerant capacity of plants.

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**POSTER THEME A**

# Cloning, sequence analysis of *EsPL* gene from *Eruca sative* Mill and its plant expression vector construction

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**Background:** *Eruca sative* Mill has a sporophytic self-incompatibility reproduction system. Genetically stable self-incompatible (SI) and some self-compatible(SC) mutant species have also been identified in this crop. Cloning of SC genes has important significance in biology and breeding research. To isolate the genes associated with the self-compatibility of *Eruca sative* Mill, we screened differential gene expression in SC and SI of *Eruca sative* Mill by differential display reverse transcriptase polymerase chain reaction (DDRT-PCR) in previous study, The pectate lyase gene EST was obtained.

**Objectives:** Experiments were performed to clone the full-length cDNA of *Eruca sative* Mill pectate lyase gene *(EsPL)* .

**Methods:** The full-length cDNA of *EsPL* was obtained by the technology of rapid amplification of cDNA ends (RACE). The cDNA sequence and the deduced amino acid sequence were analyzed by bioinformatics method. The expression vector of *Eruca sative* Mill *EsPL* gene were constructed successful. The expression of *EsPL* before and after flowering in SI and SC were determined by real-time PCR.

**Results:** In this study, a novel gene, *EsPL*, from the anther of *Eruca sative* Mill was isolated and characterized. The full-length cDNA was 1657bp, containing a 1371bp opening reading frame(ORF). The deduced protein was 456 amino acids with molecular weight 51.179kD and isoelectric point 9.42. The sequence aligment demonstrated that *EsPL* was high identity with other PL proteins from other species. So it was named the *EsPL*. Phylogram tree showes that there were closest evolutionary relationship of *Brassica napus*, *Brassica* rape and *Eruca sative* Mill gene. Analysis by real-time PCR indicated that *EsPL* gene expression levels in SC anther is significant higher than SI anther after flowering. The expression vector of *Eruca sative* Mill EsPL gene were constructed successful, which laid the foundation for further study the function of this gene.

**Conclusions:** The *EsPL* gene was cloned from the anther of *Eruca sative* Mill and the analysis by real-time PCR suggested that *EsPL* played an important role in SI and SC Characteristics of regulation of *Eruca sative* Mill.

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**POSTER THEME A**

# The BRASSINAM population, a new tool for investigating agronomic traits in *Brassica napus L.*

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**Background:** A key component in the identification of genetic factors underlying agronomic traits relies on the availability of genetic material, such as mapping populations or panels of lines segregating for the desired trait. In winter oilseed rape (*Brassica napus L.*), several bi-parental populations have been developed for linkage analyses and at least one association panel is publicly available (ASSYST project, Bus et al, 2011). However, these resources have their drawbacks, notably in terms of power, resolution or allele frequency. Recently, multi-parental population schemes combining benefits from both linkage and association mapping approaches have been developed in crop plants, such as the maize NAM population (Yu et al 2008) or the wheat MAGIC population (Huang et al 2012).

**Objectives:** The BRASSINAM project aimed at creating a new genetic resource for rapeseed that will allow powerful analysis of genetic determinism of quantitative agronomic traits. This population should be of particular interest for both breeders and scientists. It will combine wide genetic diversity with adaptation to high-throughput and large scale phenotyping under European conditions.

**Methods:** The parents of the population were selected among a *B. napus* diversity set of 280 accessions that represented the main genetic pools. The diversity set was screened using 76 SSRs. Parents were selected and crossed with the french-adapted line AVISO. RILs were produced for each cross up to the F6 stage, were genotyped with 9,500 SNP and observed in nursery trials in 2 sites in 2014-15 to assess adaptation to french growing conditions.

**Results:** A set of 15 parents (12 WOSR, 3SOSR) was selected based on both genetic diversity as well as agronomic knowledge acquired by breeders. The population has been advanced to the F6 stage with around 200 individuals per cross. Field observations at F3 stage have allowed to assess winter hardiness of the SOSRxAVISO material in order to keep 200 adapted RIL for each population. Field observation at F6 stages such as flowering time allowed to identify potential subsets within the population to be used or avoided for particular traits. The genotyping data allowed a first run at association analyses with the traits observed, giving preliminary insights into the power and resolution that could be expected from this population. Finally, seeds are now available to potential collaboration.

**Conclusions:** This population represents a tremendous resource for further genetic studies and now needs to be exploited. Seeds are now available to potential collaborations. Within the frame of the Rapsodyn project, the parental lines and Aviso were re-sequenced in order to provide a high resolution allele scan of the population and a genetic map will be produced for the population. Imputation strategies will be investigated to further strengthen the resolution of the association analyses that could be performed with this material. Moreover, agronomic data will be obtained to assess performance under normal or N stressed conditions.

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**POSTER THEME A**

# Use of rutabaga *(Brassica napus* var. *napobrassica)* for the improvement of Canadian spring canola *(Brassica napus)*

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Spring-type oilseed *Brassica napus L.*, commonly known as canola, has become the cornerstone of agricultural production in western Canada with total acreage seeded increasing in each production year over the past two decades. However, the narrow genetic base of spring *B. napus* canola coupled with ever-increasing acres has led to emergence of clubroot disease, caused by *Plasmodiophora brassicae*, on the canola production areas. *Brassica napus* var. *napobrassica*, or Rutabaga, is a biennial fodder-type *Brassica* species that has potential to serve as not only a source of genetic diversity for *B. napus*, but provide strong resistance to *P. brassicae* pathotypes prevalent in the canola fields in western Canada. A F2 derived population of Rutabaga-BF × A07-26NR and a 3-way cross derived population of (A07-45NR ×Rutabaga-BF) × A07-26NR were evaluated for different agronomic and seed quality traits including resistance to *P. brassicae* pathotypes prevalent in western Canada. Results demonstrated that Rutabaga has the potential to broaden genetic diversity in spring type *B. napus*, as well as provide resistance to *P. brassicae* pathotypes.

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# Novel interspecific hybrid populations to investigate the problem of meiotic stability in *Brassica* synthetics

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**Background:** *Brassica napus* (oilseed rape, 2n = AACC), *B. carinata* (Ethiopian mustard, 2n = BBCC) and *B. juncea* (Indian mustard, 2n =AABB) are all allopolyploid, with two genomes each resulting from ancestral hybridisation events between diploid species *B. rapa* (Chinese cabbage, turnip; 2n =AA), *B. nigra* (black mustard; 2n = BB) and *B. oleracea* (cabbage, cauliflower, broccoli; 2n = CC). However, attempts to recreate these ancestral hybridization events to produce novel allopolyploid genotypes from the current-day diploid progenitor species have been mostly unsuccessful, particularly in the case of synthetic oilseed rape. Resynthesised *B. napus* "falls apart" due to meiotic instability, which causes loss of chromosomes and chromosome segments and hence loss of critical genetic information and fertility over generations.

**Objectives:** We aim to isolate genetic and genomic factors contributing to fertility and subsequently meiotic stability in the three *Brassica* allopolyploid crop species.

**Methods:** Novel interspecific hybrid populations were generated by pairwise crossing between different genotypes of *B. juncea*, *B. napus* and *B. carinata* in order to produce populations segregating for A, B and C genome alleles and whole chromosomes. First generation hybrids had genome compositions AABC, BBAC, CCAB, AABCC and AABBCC. Self-pollination and selection over generations for increased fertility as measured by self-pollinated seed set was undertaken. Molecular genotyping and cytogenetics approaches were used to track A, B and C genome allele inheritance, and self-pollinated seed set and pollen viability data were collected.

**Results:** Many hybrid genotypes were sterile, producing no self-pollinated seeds or even viable pollen, particularly in the first generation. However, several different genetic and genomic hybrid combinations also showed greatly increased fertility over subsequent self-pollinated generations. Involvement of unreduced gametes in early generations of *B. napus* x *B. juncea* and (*B. napus* x *B. carinata*) x *B. juncea* hybrid crosses appeared to greatly increase the chance of restoring fertility in later hybrid generations. Assessment of meiotic stability through allelic inheritance and cytogenetics and attribution to specific genetic and genomic factors is underway.

**Conclusions:** These interspecific hybrid populations will provide a valuable tool with which to identify key genetic and genomic factors responsible for meiotic stability in *Brassica* allopolyploids. Understanding the mechanisms underlying the process of hybrid species formation in *Brassica* will facilitate resynthesis of oilseed rape and the development of novel crop types in this genus.

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# Genetic variation and inheritance of epicotyl elongation before winter and its correlation with winter hardiness and vernalization requirement

**Background:** Epicotyl elongation before winter is considered as one critical component of the complex trait winter hardiness in winter oilseed rape (Kole et al. 2002). Thereby, genotypes with an enhanced epicotyl elongation before winter are most likely affected by strong frost. At present, little is known about the genetic variation and inheritance of this trait in winter oilseed rape and its correlation to other traits, e.g. vernalization requirement and flowering time.

**Objective:** The present work has been conducted to study the variation and inheritance of epicotyl elongation in a DH population derived from the cross of the two winter oilseed rape cultivars Sansibar x Oase in three different environments.

**Methods:** The DH population consisting of n=224 lines of the cross Sansibar x Oase were tested in three different environments in Germany: (a) in field experiments sown in August, (b) in field experiments sown in spring and (c) in greenhouse experiments without vernalization. Epicotyl length was measured before winter (a) and three months after sowing (b, c). Results obtained from replicated experiments were used for ANOVA and means over replicated experiments were used for correlation and QTL analysis.

**Results:** In all three experiments large and significant genetic variation was found for epicotyl length. Heritability for epicotyl length was high in all environments (91% to 94%). In the spring sown experiments 54% of the DH lines had a shoot length longer than 70 cm and produced flower buds, indicating a low vernalization requirement. Spearman rank correlation between the phenotypic mean values of the different environments were low (winter: spring rS=0.14*; spring: greenhouse rS=0.39* and winter: greenhouse rS=0.12*), indicating that in different environments different genetic loci are involved in the expression of the trait "epicotyl length".

**Conclusion:** The majority of the DH lines appear to have a low vernalization requirement. This seems not to be related to frost tolerance, because frost damage was neither observed during the present field experiments nor during earlier field testing. Low correlation between phenotypic mean values of the different test environments excludes the possibility for early and simplified selection e.g. in greenhouse experiments.

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**POSTER THEME A**

# Expression of oleic acid character in different generations of rapeseed

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**Background:** During the course of present investigation law of inheritance for oleic acid character in rapeseed was studied. One high oleic acid line- 04-863(80.5%) and a low oleic acid line-04-1020 (developed by self-pollination of Xiangyou No.15 with 62.7% oleic acid) were used.

**Objectives:** The character of oleic acid is quantitative character and the expression of oleic acid character is related to the environment.

**Methods:** Measuring continuous variation of oleic acid character in rapeseed; Measuring broad sense heritability of oleic acid character in rapeseed; goodness of fit test for separate proportion of oleic acid character in rapeseed.

**Results:** The results are as follows: (1) Oleic acid character in rapeseed is an quantitative character, which can not be distinguished distinctly; (2) Oleic acid content in F1 is in between both the parents but female parent has more influence on it. If high oleic acid line-04-863 is used as female parent, the distribution of oleic- acid content ( =73.41) is reflected to female. If low oleic acid content line-04-1020 is used as female parent, the F1 oleic acid content ( =67.75) is also reflected to female parent; (3) The oleic acid contents in both the parents and F1 are susceptible to environmental changes, as they show phenotypic differences and in one of their genotypes respectively; (4)F2 generation shows wide-ranging separation which is due to both genotypic separation and environmental Change. The measurement of phenotypic difference is studied by variance (V) and standard deviation (S). It has been recorded that the degree of variation departs from mean and standard deviation in F2 generation is larger than other generations, P1, P2 and F1, that is V=52.81 and S=7.28 in F2; (5) The distribution of variance in P1, P2, F1 and F2 shows nearly normal distribution. It is extremity type in a few and middle type in great many; (6) One major pair of gene is necessary to control rapeseed oleic acid character. This is due to separation of oleic acid character which shows a ratio of 3:1 i.e. law of segregation of Mendel, and by backcross ratio 1:1. The heritability of oleic acid is 69-71%, which is 69-71% due to the influence of heredity difference and 31-29% by environmental influence.

**Conclusions:** The above results show that the difference between quantitative and qualitative characters is not absolutely relative.

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**POSTER THEME A**

# Identification of QTLs for antioxidants in relation to Alternaria Blight in *Brassica juncea L.* Czern & Coss.

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**Background:** Plants possess a series of enzymatic and non-enzymatic detoxification systems to prevent damage caused by accumulation of reactive oxygen species (ROS) in plant cells due to adverse environmental conditions and pathogen attack. These include: superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), Phenylalanine ammonia lyase (PAL), carotenoids (CAR), phenols, proline (PRO) and glutathione (GSH). Little information is available on the genetic and molecular aspects of these enzymatic and non-enzymatic antioxidants.

**Objectives:** To develop a QTL map for various antioxidants in relation to alternaria blight and high temperature stress.

**Methods:** A population comprising 53 F6 RILs, developed from a cross between an Indian variety, RL-1359, and European mustard line, NUDH-YJ-04 was used for the study (Gupta et al. 2014). POD, PAL, total phenols, O-hydroxy phenols (o-OHPHENOLS), PRO, CAR, GSH, alternaria blight severity and seedling survival were estimated by using their standardized methods. QTL mapping was performed by regression based composite interval mapping using WinQTL Cartographer v2.5_011 (Wang et al. 2012). It was graphically displayed using MapChart Version 2.1 (Voorips 2002).

**Results:** Extensive phenotypic variation was recorded for different biochemicals under question. Alternaria blight severity varied from 33.24% to 76.67% with mean disease severity of 51.26%. The per cent seedling survival under high temperature stress depicted a range of 0 to 100% with mean value of 67.58%. Alternaria blight had significant positive correlation with POD and proline; negative with PAL, o-OHPHENOLS and GSH whereas no significant correlation was observed between total phenols and severity of alternaria blight. A significant positive correlation has also been registered for survival percentage of seedlings under high temperature stress with proline and negative with GSH. A total of 13 quantitative trait loci (QTL) (ALTBLT-2; PAL-2; T-PHENOL-2; GSH-3; PRO-1; CAR-1; POD-1 and O-O HPHENOL-1) were detected and assigned to different loci in linkage groups with LOD score values ranging from 3.1-10.8 and R2 value of 16-48%.

**Conclusions:** Significant QTLs impacting variation for T-phenol (A1, A9), PAL(A1, B2), GSH (A2, A5, A6), PRO(A3), POD(A9), o-OHPHENOL(A10), CAR(A7) and ALTBLT(B7,B8) could be located respectively. As limited information is available for tagging of various biochemicals studied, our study can act as skeleton for detailed analysis of genetic control of these biochemicals.

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**POSTER THEME A**

# Map-based cloning of a major quantitative trait locus (QTL), qSS.C9, for the number of seeds per silique (NSS) in oilseed rapa *(Brassica napus L.)*

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**Background:** As a complex agronomic trait, seed yield of a rapeseed plant is multiplicatively determined by the number of seeds per silique (NSS), the number of siliques per plant and seed weight. It is well known that NSS is a typical quantitative trait like the other two yield components; however, the genes and the putative molecular mechanism underlying the natural variation of NSS remain unknown thus far.

**Objectives:** Our previous study mapped a major QTL (qSS.C9) controlling NSS on the *B. napus* C9 chromosome (Zhang et al. 2012). Comparative genomics showed that the closest flanking markers delimited qSS.C9 to a syntenic region of 911 kb on the C9 chromosome of *Brassica oleracea* genome. We expected to isolate qSS.C9 and primarily reveal the underlying molecular mechanism of how it controls the natural variation of NSS.

**Methods:** Near-isogenic lines (NILs) segregating at qSS.C9 were prepared by backcrossing a low-NSS parent HZ396 (SS, 11.0 ± 2.6) to a high-NSS parent Y106 (SS, 27.3 ± 3.7) for three times. We also constructed a new bacterial artificial chromosome (BAC) clone library from *B. napus* lines with high NSS and explored a map-based cloning strategy to identify the target BAC clone covering the candidate gene. Transgenic complementation and RNAi experiments were adopted to confirm the target gene of qSS.C9. The ovule development and cell plate formation in female meiosis were respectively observed by CLSM and callose staining.

**Results:** We identified a BAC clone covering part of the candidate region and got partial sequences of it which shows obviously sequence variation with the *B. napus* reference genome. Based on the sequence of this BAC clone, we analyzed the target sequences between NIL(Y106) and NIL(HZ396), and found obvious variations in two candidate genes (qSS.C9a and qSS.C9b). Genetic transformation of a 5.3-kb genomic fragment covering the predicted qSS.C9a from NIL(Y106) into NIL(HZ396) could significantly increase NSS in T0 and T1 generations, while knockdown the expression of qSS.C9a in a line with high NSS resulted in extremely low NSS, proving that qSS.C9a is the target gene of qSS.C9. Expressional and cytological analyses showed that qSS.C9a plays an exclusive role in regulation of cell cycle progression during the megaspore meiosis stage.

**Conclusions:** qSS.C9a functions as a positive regulator of NSS by promoting the normal progression of female meiosis in rapeseed. The functional characterization of qSS.C9 represents the first step toward unraveling the molecular mechanism controlling natural variation of NSS in rice and provides a potential locus for yield improvement in rapeseed.

**References:**

Zhang, L., S. Li, L. Chen, G. Yang, 2012. Identification and mapping of a major dominant quantitative trait locus controlling seeds per silique as a single Mendelian factor in *Brassica napus L.* Theor Appl Genet 125:695-705.

# The male-sterile gene *BnRfb* from 9012AB disrupts the tapetum degeneration and tetrad release both in *Brassica napus* and *Arabidopsis*

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**Background:** A genic male sterility (GMS) line, 9012AB, has been used as an efficient pollination control system in China for hybrid production. Genetic analysis showed that *BnRfb* alone can cause the male-sterile phenotype in 9012A which can be temporarily maintained by *BnRfc* while recovered by *BnRfa* or *BnMs3* (Dong et al. 2012). Though we previously mapped the *BnRf* locus to a 13.8-kb DNA fragment of a *BnRfc*-containing bacterial artificial chromosome (BAC) clone on the *B. napus* A7 chromosome (Xie et al. 2012), marker assay showed that great sequence variations may exist in the flanking regions of the three alleles.

**Objective:** An efficient approach should be adopted to obtain the sequences around the *BnRf* locus, and the target gene of *BnRf* should be narrowed down to a reasonable region. We expected to reveal the allelic variations between different alleles of *BnRf* and how *BnRfb* cause male-sterility fertility in this study.

**Methods:** A bulked *B. napus* bacterial artificial chromosome (BAC) clone library containing both the *BnRfa* and *BnRfb* locus were prepared. A map-based cloning strategy was adopted for the identification of *BnRf* by integrating the genetic maps from different mapping populations. The target BAC clones covering the candidate genes were screened by PCR method. The wide-type *Arabidopsis* plants was used for transgenic complementation assay. The cytological observation of developmental anthers was comparatively observed between the male-fertile and male-sterile *Arabidopsis* and *B. napus* plants.

**Results:** Sequence comparison found around a 61-kb DNA fragment insertion in *BnRfa* or *BnRfb* candidate region relative to *BnRfc*. *BnRfa* and *BnRfb* were further mapped to a 40.9-kb region of the insertion fragment including eight predicted open reading frames (ORFs). Genetic transformation showed that G14 both from the *BnRfa*- and *BnRfb*- containing BAC clones can cause the similar male-sterility in *Arabidopsis* as in 9012A, and the resulting male-sterility can be reversed by introduction of the restorer gene *BnMs3*, demonstrating G14 as the target of *BnRfb*.

**Conclusion:** We cloned the male-sterile gene *BnRfb* from 9012AB. The expression of *BnRfb* in *Arabidopsis* negatively regulated some vital genes responsible for tapetum degeneration and resulted in the delay of tapetum PCD and developmental arrest of tetrads. The true *BnRfa* is actually not allelic to *BnRfb* but corresponds to another tightly associated gene in the 40.9-kb candidate region, while *BnRfc* is indeed a deleted nonfunctional allele. These findings laid a solid foundation for elucidating the molecular mechanism underlying the sterility maintenance and restoration in 9012AB.

**Reference:**

Dong, F., D. Hong, Y. Xie, Y. Wen, L. Dong, P. Liu, Q. He, G. Yang, 2012. Molecular validation of a multiple-allele recessive genic male sterility locus (*BnRf*) in *Brassica napus L*. Molecular Breeding 30: 1193–1205.

Xie, Y., F. Dong, D. Hong, L. Wan, P. Liu, G. Yang, 2012). Exploiting comparative mapping among *Brassica* species to accelerate the physical delimitation of a genic male-sterile locus (*BnRf*) in *Brassica napus*. Theor Appl Genet:125, 211–222.

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**POSTER THEME A**

# *BnMs5* causes the male-sterility phenotype in rapeseed *(Brassica napus L.)* by suppressing the early event of meiosis

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**Background:** Male sterility has been used as one of the most important pollination control systems in rapeseed. A spontaneous mutation in Rs1046AB which was transferred from Yi3A could be used as a possible alternative to the extensively used Polima cytoplasmic male sterility (Pol CMS) in China. Previously genetic and molecular marker assays have showed that the male fertility of this genetic male sterility (GMS) line is controlled by a locus assigned as *BnMs5* with three different alleles: *BnMs5a* (restorer type), *BnMs5b* (male-sterile type) and *BnMs5c* (normal male-fertile type) (Lu et al. 2013).

**Objectives:** Previous study has delimited *BnMs5* to a 21kb region on a *Brassica napus* BAC clone JBnB034L06 which contains six predicted functional genes (Lu et al., 2013). The aim of this study was to clone *BnMs5* and identify the mechanism of BnMs5 in fertility regulation in *Brassica napus*.

**Methods:** A bacterial artificial chromosome (BAC) library with in the *BnMs5* locus was constructed and screened to identify BACs with *BnMs5a* and *BnMs5b*. A genetic complementation experiment was performed to confirm the candidates for *BnMs5a*. The sequences of the three *BnMs5* alleles were compared and the functions of BnMs5 were verified by qRT-PCR, GUS assays, RNA in situ hybridization and protein localization.

**Results:** We identified the target BAC clones covering the candidate region of *BnMs5a* and *BnMs5b*, respectively. Sequence comparison revealed that obvious variations exist between the candidate regions of the three alleles. From the six candidate genes, we found the introduction of one gene from the restorer line DH195 into a male-sterile line could completely restore the male-sterility phenotype in T0 and T1 generations, and the fertility conversion was cosegregated with the transgenic events. These results proved that this gene is the target gene of *BnMs5*.

**Conclusion:** We characterized a novel nucleus-localized gene *BnMs5* by a map-based cloning strategy. The results here showed further insights into the molecular control of *BnMs5* in meiosis and anther development. This work represents major progresses towards final understanding of the mechanism of *BnMs5* and how three alleles acts in a dominance or recessiveness manner to condition the fertility conversion in Rs1046A.

**Reference:**

Lu, W., J. Liu, Q. Xin, L. Wan, D. Hong, G. YANG, 2013. A triallelic genetic male sterility locus in *Brassica napus*: an integrative strategy for its physical mapping and possible local chromosome evolution around it. Annals of botany 111: 305-315.

# Comparative transcriptome analysis reveals carbohydrate and lipid metabolism blocks in *Brassica napus L.* male sterility induced by the chemical hybridization agent monosulfuron ester sodium

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**Background:** Chemical hybridization agent (CHA) inducing male sterility is a widely used tool for seed production in crop heterosis utilization. We previously discovered that monosulfuron ester sodium (MES), an acetolactate synthase (ALS) inhibitor of the herbicide sulfonylurea family, can induce rapeseed (*Brassica napus L.*) male sterility at approximately 1% concentration required for its herbicidal activity.

**Objectives:** To find some clues to the mechanism of MES inducing male sterility.

**Methods:** The ultrastructural cytology observations, comparative transcriptome analysis, and physiological analysis on carbohydrate content were carried out in leaves and anthers at different developmental stages between MES-treated and mock-treated rapeseed plants.

**Results:** Cytological analysis revealed that the plastid ultrastructure was abnormal in pollen mother cells and tapetal cells in male sterility anthers induced by MES treatment, with less material accumulation in it. However, starch granules were observed in chloroplastids of the epidermis cells in male sterility anthers. Comparative transcriptome analysis identified 1501 differentially expressed transcripts (DETs) in leaves and anthers at different developmental stages, most of these DETs being localized in plastid and mitochondrion. Transcripts involved in metabolism, especially in carbohydrate and lipid metabolism, and cellular transport were differentially expressed. Pathway visualization showed that the tightly regulated gene network for metabolism was reprogrammed to response to MES treatment. The results of cytological observation and transcriptome analysis in MES-treated rapeseed plants were mirrored by carbohydrate content analysis. MES treatment led to decrease in soluble sugars content in leaves and early stage buds, but increase in soluble sugars content and decrease in starch content in middle stage buds.

**Conclusions:** Our integrative results suggested that carbohydrate and lipid metabolism was influenced by CHA-MES treatment during rapeseed anther development, which might responsible for low concentration MES specifically inducing male sterility. A simple action model of CHA-MES inducing male sterility in *B. napus* was proposed. These results will help us to understand the mechanism of MES inducing male sterility at low concentration, and might provide some potential targets for developing new male sterility inducing CHAs and for genetic manipulation in rapeseed breeding.

**References:**

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**POSTER THEME A**

# Heterotic grouping and the heterotic pattern among Chinese rapeseed accessions *(Brassica napus L.)*

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**Background:** Heterotic groups and patterns are extremely important in hybrid breeding. However, to date, there have been limited reports on the heterotic patterns within Chinese semi-winter rapeseed, which is extremely important for Chinese rapeseed hybrid breeding.

**Methods:** Nine elite inbreds widely used in Chinese rapeseed hybrid breeding programs were crossed in a diallel mating design to develop 36 hybrids. These hybrids and their parents were evaluated for two successive years in Northern China. Five different methods, which were based on specific combining ability (SCA) effects, SCA-Yang's effects, molecular markers, heterotic group specific and general combining ability (HSGCA), and heterotic grouping based on GCA of multiple traits (HGCAMT), were compared for their ability to classify the tested inbreds into heterotic groups.

**Results:** With regard to grouping of inbreds, breeding efficiency, and cross-mean yield variation explained by the cross types, the SCA method was the most promising one, followed by the SCA-Yang's and molecular marker methods. Using the SCA method, three testers (8D129, 8C343, and 8D153) and opposing heterotic groups[(8C108, 8C189, and 8D129), (8C343, 8C360, and 8E001), and (8C272, 8D153, and 8E019)] were identified across environments. Chinese southern and northern rapeseed lines formed a different heterotic group. Four out-yielding crosses, 8D129 × 8E001 (high-check heterosis, 29.52%), 8C343 × 8D129 (24.76%), 8C189 × 8C272 (23.98%), and 8C272 × 8C343 (22.95%), were identified as ideal hybrids for further extensive testing in multi-location trials and are promoted for adoption and commercialisation in China.

**Objectives:** To assess the genetic diversity and the combining ability of the nine rapeseed breeding lines in China; to compare the five different methods, SCA method, SCA-Yang's method, molecular markers method, HSGCA, and HGCAMT methods, for their ability to classify the tested inbreds into heterotic groups; to identify inbred testers for heterotic groups and heterotic patterns among elite Chinese breeding lines.

**Conclusions:** Among the five different methods of classifying heterotic groups in Chinese *B. napus,* SCA method was the most promising one, followed by SCA-Yang's and molecular marker methods. Chinese southern and northern rapeseed lines formed a different heterotic group.

**References:**

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**POSTER THEME A**

# QTL analysis for pod shatter resistance in Chinese breeding populations of rapeseed

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**Background:** Rapeseed shatter seeds upon maturity, making combine harvesting difficult. This becomes more serious under hot dry conditions at harvest time in the regions such as the Yangtze river valley in China. Development of varieties with pod shatter resistance suitable for mechanized harvesting has become the main breeding objective. Studies have shown that genetic variation exits in Chinese breeding populations and lines with both high and moderate resistance to pod shattering have high general combine ability (GCA) (Liu et al., 2013). SNPs on A9 were identified to be associated with pod shatter resistance in *Brassica napus* (Hu et al., 2012).

**Objectives:** One DH population and one natural population comprised mainly Chinese breeding lines with various resistance levels to pod shatter were used to analysis the genetic basis of pod shatter in *Brassica napus* combined with genome-wide SNP assay. By linkage and association analysis, QTL mapping and development of markers associated with pod shatter resistance are of great potential for accelerating variety development for pod shatter resistant and understanding the genetic mechanism of pod shatter.

**Methods:** One DH population comprising 96 lines was generated using a high resistant line × a susceptible line. Pod shatter resistance index (PSRI) were measured by random impact method in three environments. High density genetic linkage map was constructed using genome-wide single nucleotide polymorphism (SNP) markers assayed by the *Brassica* 60K Infinium BeadChip Array. Linkage analysis and SNP map construction were performed using QTL IciMapping V4.0 and JoinMap 4.0. WinQTLCart2.5 was used to detect QTLs. TASSEL was used for association analysis.

**Results:** Association analysis resulted in six QTLs located on A1, A6, A7, A9, C2 and C5, respectively, with the one on A9 explaining the largest phenotypic variation. Five, two and two QTLs were detected from DH population in three environments respectively. Two of the QTLs could be repeated detected across environments indicating that there are at least two additive genes involved in resistance to pod shattering and that the A9 and A6 loci were the main contributor to this resistance. The locus on A6 (BnSRI.A6) is a new one identified for pod shattering and fine mapping with near isogenic lines (NILs) is ongoing.

**Conclusions:** QTLs for pod shatter resistance were detected from Chinese breeding populations. Two major QTLs for pod shatter resistance are located on A9 and A6, with the one on A6 is newly identified and will be further investigated for pyrimiding pod shatter resistant loci in rapeseed varieties.

**Reference:**

Liu, J., D. Mei, Y. Li, J. Cui, H. Wang, P. Peng, L. Fu, Q. Hu, 2013. Combining ability and breeding potential of rapeseed elite lines for pod shatter resistance. Journal of Integrative Agriculture, 12: 101-104.

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**POSTER THEME A**

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# Single-point mutation in the rapeseed acetohydroxy acid synthase (AHAS) gene confers resistance to imidazolinone herbicides and its application in hybrid rapeseed production

**Background:** Imidazolinone herbicides (IMIs)-resistant varieties, induced by mutations to acetohydroxyacid synthase genes (AHAS), are planted worldwide with many crops (Thompson and Tar'an B, 2014). However, in the case of rapeseed, which is a common source of edible oil for nearly one-half of China's population, no IMI resistance has been reported for any of the varieties currently cultivated.

**Objectives:** We have developed an imazethapyr-resistant rapeseed (M9) derived from a naturally occurring mutant plant. The goals of this study were to determine the biochemical and molecular bases of herbicide resistance in M9, to develop molecular markers for the detection of herbicide-resistant genes, and to utilize herbicide-resistant traits to enhance seed purity in hybrid rapeseed production.

**Methods:** AHAS extraction and inhibition measurements of AHAS activity were performed. Three genes *BnALS1-BnALS3* encoding ALS were isolated from the mutant and wild type by using PCR method. The RT-PCR analysis of AHAS transcripts was performed using TaqMan probe detection. An allele-specific PCR (AS-PCR) marker for *BnAHAS1R* was designed and detected in the F2, BC1, and BC2 populations. The resistant trait of M9 was introgressed into a susceptible restorer line of a CMS system using a marker-assisted backcrossing selection procedure.

**Results:** An in vitro AHAS activity assay indicated that the AHAS enzyme from M9 conferred a specific resistance to IMIs. Molecular analysis identified a single-point mutation leading to an amino acid substitution from serine 653 (AGT) to asparagine (AAT) at the herbicide-binding site of the rapeseed *BnAHAS1* gene. This substitution mutation (Ser653Asp) did not change the transcription levels of *BnAHAS1* in M9 compared with the wild type. An AS-PCR marker for the *BnAHAS1R* was cosegregated with IMI resistance in three populations. Finally, the CMS restorer line 10M169 was developed to show the resistance of M9 and the different purity of F1 seeds were generated from 10M169 and Ning A7 under different pollination conditions. The increases in seed purity under natural hybridization and hybridization in tents reached 13.41% and 16.41% after IMI treatment, suggesting that the herbicide-resistant trait can be utilized for the efficient elimination of false hybrids in hybrid rapeseed production, and leading to yield increases of up to 322 kg/ha and 394 kg/ha, respectively.

**Conclusions:** The molecular mechanism and molecular marker of herbicide resistance described herein provide the basis for the release of IMI-resistant rapeseed cultivars.

**References:**

Thompson C, Tar'an B, 2014. Genetic characterization of the acetohydroxyacid synthase (AHAS) gene responsible for resistance to imidazolinone in chickpea (*Cicer arietinum L.*). Theor Appl Genet 127: 1583-1591

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**POSTER THEME A**

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# Natural variation at *BnRSW* gene affects seed weight by regulating development of silique wall in polyploidy rapeseed

**Background:** Seed weight has been widely accepted as a complex trait controlled by polygenes in crops. Although some genes have been identified to regulate seed weight, mechanisms for seed weight regulation are still not well understood. Especially in polyploidy crops, no gene regulating seed weight has been cloned in polyploidy so far. *Brassica napus L.*, as the world's second leading crop source of vegetable oil, is a tetraploid (4x) species.

**Objectives:** We identified a major QTL on A9 chromosome that controls rapeseed seed weight (explaining approximately 30% of the phenotypic variation) and silique length. To gain a better understanding of how this QTL controls seed weight, we cloned and analyzed the targeted gene by fine mapping and association analysis.

**Methods and results:** Firstly, we used map based cloning and target-regional association to clone and characterize the genetic basis underlying the major QTL, *BnRSW* for seed weight. We uncovered a 165-bp deletion in *BnRSW* associated with the increased seed weight. *BnRSW* encodes an auxin response factor and shows inhibition activity on the auxin downstream genes. The deletion in *BnRSW* increases silique length and seed weight by decreasing the inhibition activity. Furthermore, reciprocal crossing result shows that this QTL affects seed weight by maternal effects. Based on the transcription analysis, we further proved that *BnRSW* regulates cell growth in silique wall by acting in the auxin response pathway.

**Conclusions:** Together, our results suggest that *BnRSW* regulates silique wall development and then finally determines the seed weight by controlling the amount of photosynthates as a new way of maternal regulation. Also, our study revealed one QTL gene in tetraploid and will provide the insights for QTL genes cloning in polyploidy crops.

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**POSTER THEME A**

# Resynthesizing *Brassica amphidiploids* for resistance to clubroot

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**Background:** Clubroot is an economically important disease affecting plants in the family *Brassicaceae* worldwide. In amphidiploid *Brassica* species, there are limited resistant sources available for the economically important canola crop *B. napus* and no resistant genotypes available for the mustard species *B. juncea* and *B. carinata*. Resistant germplasms resistant to a broad range of pathotypes of *P. brassicae* were identified in the progenitor diploid *Brassica* species *B. rapa, B. nigra* and *B. oleracea* (Peng et al. 2014), which could be used for developing canola and mustard crops for resistance to clubroot by re-synthesizing the *Brassica amphidiploids*.

**Objectives:** To introduce new sources of resistance to clubroot into the amphidiploid crops, *B. napus*, *B. juncea* and *B. carinata* lines were resynthesized through inter-specific hybridization using diploid progenitor species identified at Saskatoon Research Centre, Agriculture and Agri-Food Canada.

**Methods:** Four resistant *B. rapa* lines (Jazz, Milan, 96-6992 and T19), two R *B. oleracea* (Tekila and Kilaherb), one *B. nigra* R line (CR2716), and two susceptible lines (ACDC, *B. rapa*; T010000DH3, *B. oleracea*) were used as the parents for interspecific crosses. Embryos at 15 days after pollination were cultured in the MS liquid medium with 2% sucrose. When cotyledons appeared, they were transferred into B5 solid medium supplemented with 2% sucrose. Plantlet roots were submerged in 0.2% colchicine solution for 2 hours, then washed for three times and transferred them to soil directly. *Amphidiploids* were identified by Ploidy Analyzer and then confirmed by the development of fully formed stamens.

**Results:** Three *B. napus* lines (Tekila × 96-6992, Kilaherb × Milan and T010000DH3 × Jazz) and two *B. juncea* lines (T19 × CR2716 and ACDC × CR2716) and one *B. carinata* (T010000DH3 × CR2716) were successfully re-synthesized. The presence of clubroot resistance genes derived from the *B. rapa* R lines in the *amphidiploids* were confirmed using SNP markers. Seeds were obtained by self-pollinating the re-synthesized *B. juncea* and *B. carinata* lines as well as the *B. napus* line derived from T010000DH3 × Jazz. Due to male-sterility in Tekila and Kilaherb, the re-synthesized *B. napus* from the cultivars were not able to produce pollen therefore they were crossed to a DH *B. napus* line DH16516.

**Conclusions:** Clubroot resistant germplasms in each of the three amphidiploid species were developed. Each will serve as new resistant sources for developing canola and mustard crops with resistance to clubroot thereby broadening genetic diversity for the crop improvement.

**References:**

Peng, G., K.C. Falk, R.K. Gugel, C. Franke, F. Yu, B. James, S.E. Strelkov, S.F. Hwang, L. McGregor, 2014. Sources of resistance to *Plasmodiophora brassicae* (clubroot) pathotypes virulent on canola. Can J Plant Pathol, 36:

# Identification of QTL involved in resistance to clubroot in canola *(Brassica napus)*

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**Background:** Clubroot, caused by *Plasmodiophora brassicae* Woronin, is an important disease of canola (*Brassica napus L.*). Over the past decade, clubroot has spread throughout central Alberta and also has been confirmed in a few fields in Saskatchewan and Manitoba. Breeding for resistance is the most effective management approach (Strelkov and Hwang 2014). European fodder turnips (*B. rapa* ssp. *rapifera*) were identified as the main source of clubroot resistance and have been extensively used in breeding (Diederichsen et al. 2009). Several resistance genes and QTL were identified in *B. napus* on chromosomes N02, N03, N08, N09, N13, N15, N16 and N19.

**Objective:** The objective of this study was to identify QTL conferring clubroot resistance in canola.

**Methods:** A doubled haploid (DH) population (N=133) of canola was developed from a cross between genotype 11-99 and genotype 12-1. The resistant parent 11-99 is a spring-type inbred line, and its resistance originated from 'TOSCA' (*B. napus*). Three *P. brassicae* single-spore isolates (SSI) classified as pathotype 2, 3 or 5 on the differentials of Williams (1966) were used as inoculum. Seedlings were inoculated with a resting spore suspension (1×107 spores/ml) and evaluated for clubroot reactions after six weeks with a 0-9 scale. A disease index (DI) was calculated according to the formula DI= [(0n0+1n1+…+9n9) ×100]/ (9×NT) ×100%. One hundred and seventy five anchor simple sequence repeat (SSR) markers were used to generate a genetic map. JoinMap v. 4 was used to construct the map. The QTL for clubroot resistance were analyzed by composite interval mapping (CIM) with Map QTL v. 3.

**Results:** The resistant parent was resistant, while the susceptible parent was susceptible, to all SSI. The frequency distribution for resistance to the SSI in the DH population showed continuous segregation patterns. A genetic map was constructed with 175 SSR which included 18 linkage groups and covered 18 chromosomes (except N16). A total of six QTL were detected on two chromosomes that confer resistance to the three *P. brassicae* SSI, which explain 62.2% to 65.8% of the total phenotypic variance. One QTL located on N03 confers resistance to all isolates and explained 57.5% to 61.4% of the phenotypic variation. A QTL on N06 with a minor effect also was identified that contributed to resistance to all three isolates.

**Conclusion:** Two QTL were identified on a draft genetic map of canola consisting of 175 SSR markers. One QTL was located on N03 and confers resistance to all isolates, explaining 57.5% to 61.4% of the phenotypic variation. The other QTL was on N06 and had a minor effect on resistance.

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**POSTER THEME A**

# Transcriptome analysis of interspecific hybrid between *Brassica napus* and *B. rapa* reveals heterosis for oil rape improvement

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**Background:** Heterosis is a prevalent phenomenon in evolution and breeding process of plants. Development of interspecific hybrids has been widely exploited for the heterosis breeding of *Brassica* crops. The hybrid between *Brassica napus* and *B. rapa* displays obvious heterosis in both growth performance and stress tolerance. The hybrid offspring obtains the advantage in many agricultural and developmental traits including biomass yield, plant height, vigor, stress tolerance from their parents, so as to improve the adaptation and enlarge the planting area in the world.

**Objectives:** In this study, the transcriptomes of *B. rapa* ssp. chinensis Makino and *B. napus* and their interspecific hybrid were sequenced to investigate the molecular basis of heterosis. Gene expression profiles of Ar and An genomes in two parents and interspecies hybrid were analyzed.

**Methods:** In the present study, *B. napus* (AnAnCC genome), *B. rapa* (ArAr genome), and its hybrid F1 (AnArC genome) were used to identify possible molecular mechanisms of heterosis at gene expression level. Total RNA of each sample was isolated using RNAprep pure Plant Kit. cDNA library was constructed following the manufacturer's instructions of mRNA-Seq Sample Preparation Kit. Whole transcriptomes were sequenced using the platform of Illumina/Solexa. The uniquely mapped reads for a specific gene were counted by mapping reads to de novo assembled distinct sequences using SOAP2 software, and the RPKM (Reads Per Kb per Million reads) values were computed as proposed by Mortazavi et al. RNAseq data were further validated using qRT-PCR for a selected number of genes using gene-specific primer sets.

**Results:** A total of 40,320 nonredundant unigenes were identified using *B. rapa* (AA genome) and *B. oleracea* (CC genome) as reference genomes. A total of 6,816 differentially expressed genes (DEGs) were mapped in A and C genomes, and 4,946 DEGs displayed nonadditively by comparing the gene expression patterns among the three samples. The coexistence of nonadditive DEGs including high-parent dominance, low-parent dominance, over-dominance, and under-dominance was observed in the gene action modes of F1 hybrid, which were potentially related to the heterosis. The nonadditive DEGs in hybrids from A genome mainly participated in metabolism and development, while those from C genome largely involved in stress resistances. The coexistence of multiple gene actions in the hybrid, and provided a list of candidate genes and pathways for heterosis. Furthermore, the expression bias of transposable element-associated genes from A and C genome was observed in the hybrid compared to their parents.

**Conclusions:** The present study could be helpful for the better understanding of the determination and regulation mechanisms of heterosis with purpose to *Brassica* improvement.

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# QTL mapping of traits associated with plant architecture and yield in *B. napus*

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**Backgound:** Plant architecture directly affects the adaptability to cultivation, harvest index and potential yield of crops. Rapeseed plant architecture is a collection of many important agronomic traits including plant height, branch angle, number of branches, length of branches and silique density. In last decades, significant progress has been made in elucidating the molecular mechanism of plant architecture in rice, barley and maize. However, the molecular factors that shape plant architecture of *Brassica* crops are still largely unknown partly due to lack of mutants. Recently, we found a double haploid (DH) *B. napus* line (4942) exhibiting dwarf and compact phenotype. It has significantly fewer primary branches, shorter branch length and flowering period, higher silique density and no secondary branches.

**Objectives:** The aim of this study was to construct a linkage map using two *B. napus* genotypes with contrasting plant architecture for mapping of major quantitative trait loci (QTLs) for key agronomic traits.

**Methods:** A population of 181 DH lines derived from a cross between 4942 and 8008 (a *B. napus* inbred line with normal plant type) was used to construct a molecular linkage map using a 6K *Brassica* Infinium® SNP array and molecular maker techniques. Phenotypic data was collected from 16 traits associated with plant architecture and yield including two new indexes such as the length of silique layer and the width of silique layer under three environmental conditions.

**Results:** The map is comprised of three SCAR, 80IP, 160 AFLP, 253 SSR, and 1406 SNP markers distributed across 20 linkage groups and has a total length of 2,328.97 cM. A total of 337 quantitative trait loci (QTL) for 16 plant architecture and yield-associated traits were identified in three natural environments in the DH population. The proportion of phenotypic variance explained by the individual QTLs ranged from 2.6% to 60.0%. Maximum number of major QTLs were present on A01 followed by A09 linkage group. A trait-by-trait meta-analysis revealed 234 consensus QTLs, of which 70.5% were clustered and integrated into 59 pleiotropic unique QTLs by meta analysis. Two pleiotropic QTLs (mqA1.13 and mqA1.14) for length related traits such as plant height, the total and average length of primary branches, the length of silique layer and the width of silique layer were identified.

**Conclusions:** *B. napus* 4942 has promising plant architecture for mechanical harvesting. Our study is the first report on identification of QTLs for plant architecture traits in *Brassica*. The QTLs identified here provide a strong foundation for fine mapping and further cloning of the major QTLs shaping plant architecture, which would help in breeding new rapeseed varieties suitable for mechanization and unveiling the mechanisms that control the rapeseed plant architecture.

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# Single cell genomic sequencing in *Brassica napus* and wheat: Applications in monitoring recombination frequency

**Background:** Transfer of high value target loci from genetically diverse wild relatives to adapted elite varieties is seen as a key to the future of wheat breeding. Efforts to modulate homoeologous recombination between the chromosomes of alien donor and those of cultivated wheat via mutating the Ph1 (Pairing homoeologous 1) locus, chemical treatment or changes in environmental conditions are currently underway. However, the fast progress of such projects is hindered by the lack of a rapid screening method for monitoring the impact of modulation of homoeologous recombination in polyploid crops, such as *Brassica napus* and wheat. Currently used cytogenetic methods for assessing recombination frequency are cumbersome and time consuming.

**Objectives:** The main objective of this study is to establish an easy and efficient method for monitoring the impact of modulation of recombination in plants. We have devised a strategy to assess homoeologous recombination frequency in an F1 plant which leverages the combined advantages of single cell whole genome sequencing technology and the relative ease of enrichment of single microspores.

**Methods:** Single cell haploid microspores from an F1 plant is ideal material to quickly assess homoeologous recombination frequencies as it is relatively easy to isolate thousands of microspores carrying segregating genotypes. Our method involves DNA isolation from single microspores derived from F1 progenies using the Fluidigm C1 single cell auto prep system which offers a simplified single cell isolation and DNA extraction workflow. Subsequent sequencing of DNA and genotyping of multiple segregating microspores facilitates assessment of the frequency of homoeologous recombination.

**Results:** Based on our results, the Fluidigm's C1 based single cell sequencing method works well for isolation of DNA from *B. napus* microspores. *B. napus* microspores were sorted successfully in individual Integrated Fluidic Circuit (IFC) wells. The cell capture frequency ranged from 40 to 55%. The capture frequency can be further improved by optimizing the concentration of cell suspension and elimination of clumps formed by dead cells. Bioanalyzer traces showed an enrichment of amplified DNA fragments at ~10 kb from all the IFC wells in which a microspore was captured. Empty wells did not have any DNA fragments which potentially ruled out the possibility of DNA amplification from contaminants. Successful PCR amplification of two well characterized *B. napus* genes further confirmed that the DNA isolated was derived from microspores. In the case of wheat, the bigger size of its microspores (40-60 µM) prohibits their flow and capture in the narrower IFC module/wells (maximum 25 µM). The use of smaller microspores (<25 µM) from very early stages of spike development is being tested to help resolve this issue. Alternatively, FACS based cell sorting system can be used for sorting larger wheat microspores.

**Conclusions:** Single microspores can be successfully captured, lysed and their DNA amplified using the Fluidigm C1 single cell module. Future work will consist of the optimization of microspore capture frequency and confirming uniform coverage of whole genome amplification.

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# Identification of genomic regions that control the diploidization rate of microspores in intervarietal substitution lines of rapeseed *(Brassica napus L.)*

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**Background:** Isolated microspore culture (IMC) of *Brassica napus* treated with colchicine is an important technique that efficiently produces homozygous doubled haploid lines in one generation, thus accelerating crop improvement and breeding programs. A large variation in diploidization rates among genotypes can be detected even after identical treatments of microspores. Little is known about the genetic control of these differences. In segregating DH populations, genes that have an effect on the diploidization rate of microspores should lead to skewed segregation at markers linked to loci where the different alleles are segregating.

**Objectives:** Localize genomic regions that control the diploidization rate of microspores by evaluating the diploidization rates of intervarietal substitution lines (ISLs) of rapeseed (*Brassica napus L.*).

**Methods:** A mapping population of 197 diploid microspore-derived embryos (MDEs) was developed from isolated, colchicine treated microspores of one F1 plant of the cross 'Express 617' x 'RS239'. Similar to the approach of Ecke et al. (2015) regions with skewed segregations that may carry genetic factors controlling diploidization rates were identified by genetic mapping in the MDE population and ISLs from the same cross as the MDE population with donor segments covering a number of these regions were selected. Diploidization rates of microspores from the selected ISLs were evaluated by measuring ploidy levels of MDEs with flow cytometry. The significance of deviations of the diploidization rates of the ISLs from the diploidization rate of 'Express 617', which is the recurrent parent of the ISLs, were tested by $\chi 2$-tests using the ratio of diploid and non-diploid MDEs of 'Express 617' as the expected ratio.

**Results:** A genetic map of 483 AFLP markers was constructed in the MDE population. A total of 75 markers in 13 regions showed significant deviations ($P<0.05$) from the expected 1:1 segregation ratio. Five regions and 10 ISLs were chosen for further studies. 'Express 617' showed a diploidization rate (DR%) of 51.8%. The DR% of the ISLs ranged from 23.9% - 58.7%. Two ISLs showed higher DR% and eight ISLs showed lower DR% compared to 'Express 617'. The statistical analysis showed three ISLs, ER281, ER984 and ER964, to have significantly lower DR% of 23.9% ($\chi 2=50.77$), 25.6% ($\chi 2=50.77$) and 27.1% ($\chi 2=44.13$), respectively, than 'Express 617'. No ISLs with significantly higher DR% were detected. The three ISLs were carrying two to four donor segments. After comparing the donor segments between significant and non-significant lines 6 genomic regions were identified distributed in 6 linkage groups that possibly carry genetic factors affecting the diploidization rates of microspores.

**Conclusions:** Three ISLs, ER281, ER984 and ER964, with significantly reduced diploidization rates compared to the recurrent parent 'Express 617' were identified. A total of 6 genomic regions on 6 linkage groups may carry genetic factors controlling diploidization rates of microspores.

**References:**

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# Introgression of resistance to blackleg from *Brassica juncea* into *B. napus* and analysis of blackleg resistance in synthetic hexaploid *Brassica* species

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**Background:** Canola (*B. napus*), one of the valuable oilseed crops in the world, has been reported with significant yield losses due to blackleg disease caused by *Leptosphaeria maculans.* The primary method to control blackleg is to develop resistant cultivars. Blackleg resistance can be introduced from the B-genome containing *Brassicas* into canola. The synthetic hexaploid *Brassica* species (AABBCC) created with the genomes of *B. rapa* (AA) and *B. carinata* (BBCC) (Chen et al. 2011) have high levels of blackleg resistance that can be introduced into canola.

Objectives: To introduce blackleg resistance from *B. juncea* to *B. napus* and to analyze the high level of blackleg resistance in synthetic hexaploid *Brassica* species (AABBCC).

**Methods:** Fifty *B. juncea* accessions from the collection of University of Manitoba and eight lines of synthetic hexaploid *Brassica* developed by Dr. Meng were tested with cotyledon inoculation. All 'Meng' accessions were completely resistant to blackleg while most resistant and susceptible *B. juncea* accessions to blackleg were identified. Most resistant *B. juncea* were crossed with susceptible canola 'Westar' and all F1 plants were backcrossed to 'Westar' to produce BC1 generation. The BC2 generations are produced and being tested to find resistant progeny and eventually the blackleg resistance will be introduced into canola. To test blackleg resistance in synthetic hexaploid *Brassica* 'Meng', the interspecific hybridization between susceptible *B. juncea* and 'Meng' were made and embryo culture was used to obtain F1 plants. These F1 were backcrossed to the susceptible *B. juncea* to produce BC1 progenies.

**Results:** Hybrid seeds between *B. juncea* and canola were produced and backcrossed to canola. The seed setting rates of the F1 hybrids were low and a few to a dozen of seeds were obtained from different crosses. The lower survival rate was observed in BC1 than F1 where there was about 10 times of difference. In the crosses of *B. juncea* and 'Meng', the seed setting rates of the backcrosses to the susceptible *B. juncea* were very high and hundreds of BC1 seeds were harvested. These BC1 seeds are being tested and the resistance will be analyzed through genetic analysis of the BC1 and following generations.

**Conclusions:** Although the high level of blackleg resistance exists in the B-genome containing brassica species, it is difficult to introduce the blackleg resistance to canola. Understanding the mechanism of blackleg resistance in hexaploid 'Meng' will lay a foundation to introduce the blackleg resistance in the hexaploids to canola. The good seed setting indicated that genetic analysis of blackleg resistance by crossing synthetic hexaploid *Brassica* species to susceptible *B. juncea* is feasible since all hexaploid 'Meng' accessions are completely resistant to blackleg.

**References:**

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**POSTER THEME A**

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# Investigation of genetic resistance operating in oilseed rape against the light leaf spot pathogen, *Pyrenopeziza brassicae*

**Background:** Light leaf spot disease, caused by *Pyrenopeziza brassicae*, is currently the most damaging foliar disease on winter oilseed rape in the UK. *P. brassicae* is also able to infect and cause the disease on vegetable brassicas, including Brussels sprouts. Previously, severe disease epidemics have been recorded in Scotland and northern England, where the climatic conditions are favourable for disease development. However, according to recent disease survey data, the severity of epidemics has increased progressively across the UK since 2006 (http://www.cropmonitor.co.uk/). This frequent, widespread occurrence of light leaf spot has made it a high priority for many oilseed rape growing areas in the UK. Severe epidemics have also been reported in some parts of the continental Europe, including France and Germany. Breeding for host resistance can be used as an effective control strategy against this economically damaging pathogen. According to HGCA recommended list trials, there are commercial oilseed rape cultivars with good to moderate resistance against the pathogen. There is little information available about the genetic resistance operating in these cultivars.

**Objective:** To investigate genetic resistance in oilseed rape against *P. brassicae* to identify new sources of resistance, which can be exploited in oilseed rape breeding programmes.

**Methods:** Recent identification of a major gene locus for resistance against *P. brassicae* that has been mapped to the bottom end of chromosome A1 (Boys et al. 2012) and the genome sequencing data for *B. rapa*, *B. oleracea* and *B. napus* will be used for fine mapping and sequencing of this major gene. A DNA fragment of c. 260 bp was amplified from parental lines of a doubled haploid (DH) population segregating for resistance, using primers of a flanking marker closest to the resistance gene on chromosome A1. The PCR products will be sequenced and aligned to the *B. napus* genome sequence to locate the closest marker on the chromosome A1. SNP markers between this region and the telomere will be tested using DNA samples of the DH population to fine map disease resistance. In addition, QTL analysis will also be done on a population segregating for quantitative resistance against *P. brassicae* to map QTL and to identify candidate resistance genes.

**Results:** Physical locations of markers flanking the corresponding R gene on the chromosome A1 have been identified.

**Conclusion:** Fine mapping of the R gene at the bottom of chromosome A1 will become available in the future and will be used to sequence a resistance gene operating against *P. brassicae.*

**References:**

Boys, E. F., Roques, S. E., West, J. S., Werner, C. P., King, G. J., Dyer, P. S., & Fitt, B. D. L. (2012). Effects of R gene-mediated resistance in *Brassica napus* (oilseed rape) on asexual and sexual sporulation of *Pyrenopeziza brassicae* (light leaf spot). Plant Pathology, 61(3), 543-554.

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**POSTER THEME A**

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# Synthesis and characterization of a wide hybrid between *Brassica napus* and *Raphanus raphanistrum*

**Background:** The *Brassicaceae* family includes an array of wild and weedy species which represent a massive reservoir of genetic and agronomic variation. The bringing of this variation into cultivated germplasm through interspecific/ intergeneric hybridization is expected to further enhance the scope and pace of *Brassica* breeding efforts by broadening the genetic base of crop *Brassicas* (Garg et al. 2007, Nicolas et al. 2008). Wild radish (*Raphanus raphanistrum*) is an important source of resistance to blackleg, diamond back moth, pod shattering, acetolactate synthase herbicide, tolerance to salinity and downy mildew. Genetic relatedness between *Raphanus raphanistrum* and cultivtated *Brassicas* will provide possibility to exploit desirable genes.

**Objectives:** To develop the Intergeneric hybrid between *B. napus* and *R. raphanistrum* and to study the genetic relatedness between genomes of *B. napus* and *R. raphanistrum*.

**Methods:** Sequential ovary-ovule culture was used to develop the intergeneric hybrid between *B. napus* and *R. raphanistrum*. Morphological, molecular, cytological studies and fluorescent genomic in situ hybridization were conducted to establish the hybridity and genomic relatedness. For cytological analysis young flower buds were fixed in Carnoy's solution II (Ethanol: Chloroform: Acetic acid in a ratio of 6:3:1) and squash preparations were made in 2% acetocarmine. Gish analysis was carried on air dried metaphase spreads as per Heselop-Harrison protocol.

**Results:** F1 hybrid plants were morphologically intermediate between the two parents. Cytological investigations of the intergeneric hybrid (F1) was carried out to establish the extent of genomic relatedness between AC and Rr genomes. The F1 hybrid plant had 2n=28 chromosome number. The cytogenetic analysis of the pollen mother cells of the F1 hybrids (2n=28) revealed the occurrence of varied chromosome configuration during diakinensis/metaphase-I with 10II+8I as the predominant configuration. The number of bivalents ranged between 7-13II. Maximum of 13II were observed in 8.24 per cent of the PMC's. The mean bivalent frequency was 8.83. The quadrivalent and trivalent were also observed in 3.09 and 23.7 per cent of PMC's respectively. Chromosome separation during anaphase was irregular with frequent chromosome laggards. Occurrence of more number of bivalents (13II) than expected along with a quadrivalent and a trivalent in few cells was indicative of allosyndetic pairing between the genomes under consideration. GISH studies also confirmed the hybridity and allosyndetic pairing between AC and Rr genomes.

**Conclusions:** Presence of trivalent/quardrivalent indicated the allosyndetic pairing between the AC and Rr genomes. This suggested the possible recombination between A, C and Rr genomes. Therefore intergeneric hybrid, *B. napus* × *R. raphanistrum* has the potential to be used as a bridging species for transfer of desirable genes from the wild to the cultivated *Brassica* species.

**References:**

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**POSTER THEME A**

# Development of nutritionally improved Indian Mustard *(Brassica juncea)* varieties having low erucic acid and low glucosinolate content using marker assisted selection

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**Background:** India is one of the largest rapeseed-mustard growing countries in the world, occupying the third position in production after China and Canada with 12% world total production. Due to high content of glucosinolate, the Indian cultivars have limited preference in Southern and Central Zone of India and in International market as glucosinolate is undesirable to animal feed. On one hand erucic acid is an anti-nutrient while on the other presence of Glucosinolate in the oil cake makes it unfit for cattle feed. TERI Developed double low strains of *Brassica juncea* however these lines did not had good seed yield. In order to develop high yielding double low variety of Indian mustard, TERI and Nirmal Seeds joined hands under the aegis of BIPP scheme of Department of Biotechnology, Government of India.

**Objectives:** The main objective of this project was to transfer low erucic (<2%) and low glucosinolate ( <30 micro mole/g defatted meal) content in high yielding line Nirmal 100 using marker assisted selection.

**Methods:** The present investigation work underlying this paper, conducted during Rabi & Kharif season (field and polyhouse conditions) of 2011-2015 at Research farm of Nirmal Seeds Pvt. Ltd. Pachora, Dist- Jalgaon. Based on phynotyping (i.e. HPLC and GC analysis) and genotyping (molecular marker analysis) results, TERI N-4 was selected as a donor parental line and agronomically superior high yielding NML- 100 *Brassica juncea* line was selected as a recipient parent . Sowing of the recipient line NML-100, donor line Teri-4, backcross seeds (BC1F1, BC2F1, BC3F1 and BC4F1) and self seeds (BC4F2) were conducted in their respective period in polyhouse and field condition. Backcrossed progenies were was validated for presence of glucosinolate and Euricic acid loci and AFLP markers was used for selection of recurrent parent genome (NML-100) recovery and reconfirmed with GC and HPLC analysis.

**Results:** Individual plants of donor line T-4 is used for crossing with recipient line NML-100. These plants have all low alleles in homozygous conditions for glucosinolate and euricic trait. Once the donor and recipient lines are selected, the erucic acid controlling genes were cloned and sequenced from these lines to use them for marker-assisted breeding in later generations. Plants which were close to recurrent parent genome were used for backcrossing programme. A total of 1153 BC4F2 plants were screened for GSL and Erucic acid loci. Finally 42 DL plants were recognized at BC4F1 containing 96% of recurrent parent genome. These BC4F1 population was selfed to fix the homozygosity and harvest BC4F2 .

**Conclusions:** The 42 homozygous BC4F2 lines will be developed as double low variety reday for cultivation in India.

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# Quantitative trait loci (QTL) mapping in recombinant inbreed lines of *B. rapa* for agronomic and seed quality traits

**Background:** *Brassica rapa L.* (AA, 2n = 20), an important vegetable and oilseed crop, is one of the parental species of *Brassica napus L.* (AACC, 2n = 38). Total land area coverage of *B. rapa* species reduced to <2% in Western Canada in the last decades due to the release of early-maturing *B. napus* cultivars with high seed yield. However, earliness in current early-maturing *B. napus* cultivars is not still adequate especially in the short-growing season areas. *B. rapa* L is attractive for traits, such as, early-maturity, yellow seed associated with lower fibre content in meal, and reduced silique shattering. QTL mapping of these traits in *B. rapa* can facilitate understanding of the genetic control of the traits in the amphidiploid species, such as *B. napus* (AACC, 2n = 38) and *B. juncea* (AABB, 2n = 36), which shares the A genome of *B. rapa*.

**Objectives:** To understand genetic controls in recombinant inbred lines (RILs) from *B. rapa* cross for agronomic and seed quality traits and to map QTL for seed color, glucosinolate (GLS) content and silique length.

**Methods:** The F1 plants (Sampad × 3-0026.027) were self-pollinated for F2 seed. A total of 96 F2 plants segregating for different agronomic traits were subjected to a single seed descent for the development of RILs. The RILs were grown in a greenhouse, growth chambers and field trial. Seed color was visually assessed in individual plants, GLS content was estimated in µmol g–1 seed by near-infrared reflectance spectroscopy and silique length was measured for five pods per plant in mm. QTL mapping was conducted using composite interval mapping (CIM).

**Results:** One major and a minor QTL on linkage group (LG) A9 and two minor QTL on LG A3 and LG A5 were detected for seed color, explaining ~2% to 64% of the phenotypic variance. QTL mapping detected three loci on linkage groups A2, A7 and A9 for GLS content. These QTL individually explained 5 to 22% of total phenotypic variation. For silique length, three QTL were detected on the linkage groups A3, A5 and A7, explaining 36.0–42.3% of the total phenotypic variance.

**Conclusions:** The two QTL for seed color on LG A9 are apart by ~70 cM in *B. rapa* map (Kebede et al. 2012). A single major QTL was detected on LG N9 _ A9 in *B. napus* (Fu et al. 2007). The alignment of common SSRs indicated that the SSR markers were spread more widely across LG A9 as compared to LG N9 of *B. napus* developed by Piquemal et al. (2005), which can explain for the presence of another QTL region on LG A9 in *B. rapa*. LG A9 carries a major QTL while A7 carries a minor QTL for total seed GLS content (Rahman et al. 2014). For silique length, a QTL on LG A5 was detected in all environments and explained about 9% of the total phenotypic variance (Kebede et al. 2014). However, no QTL was detected for silique length on LG A5 in *B. napus* (Chen et al. 2007, Qi et al. 2014). QTL mapping in *B. napus* indicated that the chromosome C5, which is homologous to LG A5, carries a locus controlling silique length (Chen et al. 2007, Qi et al. 2014). Thus, understanding the genetic composition of diploid parental species can give a better insight of the genetic architecture of the amphidiploid species.

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**POSTER THEME A**

# Transcriptome atlas of the *Arabidopsis* funiculus – a study of maternal seed regions

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**Background:** The funiculus is the structure that connects the developing seed to the maternal plant, and is the only direct conduit for the transport of nutrients from the plant to the seed. While the use of genetic and molecular screens has contributed greatly to our understanding of seed development in recent years, our understanding of the funiculus development remains limited.

**Objectives:** Understanding the molecular genetics of funiculus development could contribute greatly to oilseed improvement research, but the accessibility of this structure and the analysis of large-scale datasets remains a challenge in the growing field of transcriptomics. We therefore studied the funiculus of the model plant, *Arabidopsis thaliana*, in order to provide insight into the biological processes and regulatory molecules that control these processes in space and time.

**Methods:** Using laser micro-dissection coupled with global mRNA profiling experiments of the seed transcriptome throughout development, we compared the funiculus with all subregions of the maternal (funiculus, seed coat) and zygotic (embryo, suspensor, endosperm) regions of the *Arabidopsis* seed. Using fuzzy-K means clustering, we generated dominant patterns of gene activity and performed Gene Ontology term enrichment analysis to uncover biological processes in the funiculus and other seed regions. These data are supported by our histological and anatomical analyses of the funiculus. Enrichment of sequence motifs, transcriptional regulators, and GO terms in funiculus mRNA populations was used to predict putative regulatory networks underlying funiculus development and function throughout seed development.

**Results:** The funiculus is a transcriptomically distinct region of the seed. Our data indicate that the funiculus is an energetically active region that is enriched for fatty acid metabolism, auxin response, and vascular development. The funiculus was also found to be enriched for glucosinolate biosynthesis, and many transcripts involved in glucosinolate biosynthesis and regulation were found to accumulate almost exclusively in the funiculus. Our analysis predicts MYC4 and AT4G00870 as regulators of glucosinolate biosynthesis and auxin response in the funiculus via interaction with the MYC2 binding motif. We also identify BEE2 and AT1G22490 as potential regulators of auxin response in the funiculus. We also identified several other transcripts that are specific to the funiculus in seed development, and unique to the funiculus in plant development overall.

**Conclusions:** Our study provides the first comprehensive analysis of funiculus development, setting a new foundation in the field of seed improvement research in *Arabidopsis* that can be extended to other members of the *Brassicaceae*. Understanding regulators of funiculus development and function in *Arabidopsis* may have great implications for our ability to enhance seed development via the manipulation of maternal tissues.

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# Gene-expression networks controlling seedling development and vigour in *Brassica napus*

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**Background:** Different *Brassica napus* genotypes show considerable variation for germination, seedling vigour and emergence when seeds are produced or sown in different environments. The mechanisms underlying this variation are unclear. Systems biological approaches, involving different levels of information from the genome, transcriptome and metabolome, can give deep insight into the regulation of poorly understood, environmentally sensitive traits like emergence and vigour in crops with complex genomes such as *B. napus*.

**Objectives:** The main goals of this study were to correlate seedling gene expression networks to emergence-related traits in diverse winter-type *B. napus* accessions, identify genes with high connectivity within trait-associated expression clusters and investigate interconnection of potential regulatory genes to quantitative trait loci (QTL) for germination and vigour traits.

**Methods:** Shoot and root transcriptomes from 4 week old seedlings of 42 *B. napus* genotypes with high, low and intermediate germination rate were assayed by Illumina sequencing of 100bp 3'-EST sequences. Sequence reads were mapped to *Brassica* unigenes and quantified. Weighted gene co-expression network analysis (WGCNA) was performed to cluster co-expressed genes into shoot and root gene co-expression networks and correlate gene expression modules (summarizing seedling expression clusters) to in vitro germination (early seedling development) and vigor traits (2 and 4 week old seedlings from multi-environment field trials). Trait-associated gene expression networks were investigated by functional annotation and co-localization of hub genes to QTL for germination and seedling development traits.

**Results:** Gene expression modules showing high eigengene correlations with field emergence and vigour traits were identified for both shoot and root networks, and some modules showed correlations to both germination and field emergence traits. Modules of interest exhibited hub genes related to growth and development. Of particular interest were hub genes, or genes with high interconnectivity to hub genes, that were located within QTL for early seedling development traits

**Conclusions:** Co-localization of potential regulatory genes for vigour-associated expression networks to QTL regions identified promising candidates in seedling growth and vigor.

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# Investigating the influence of genome structure on QTL for disease resistance in *Brassica napus*

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**Background:** Genome rearrangemments in the allopolyloid *Brassica napus* genome have been shown to generate selectable genetic variation that can contribute to advantageous phenotypes, potentially including disease resistance.

**Objectives:** Within the French-German consortium GeWiDis ("Exploiting genome wide diversity for disease resistance improvement in oilseed rape") we are performing comparative analysis of structural organization and allelic diversity associated with resistance factors to important oilseed rape diseases. In particular we aim to determine how structural chromosome rearrangements affect quantitative trait loci (QTL) contributing to pathogen resistances and how these can be used to improve resistance in breeding.

**Methods:** Parental lines from doubled-haploid (DH) mapping populations, including donors of quantitative disease resistance factors, were resequenced to determine subgenomic structural variants including homoeologous and non-homoeologous chromosome exchanges. Sequencing and genome-wide SNP-based genotype data from DH lines were then utilised to trace structural variants in the segregating population and compare their positions to those of QTL for disease resistance.

**Results:** Preliminary results suggest that resistance loci can be influenced by homoeologous exchanges. These can cause gene dosage changes that may confer a selective advantage.

**Conclusions:** Analyses of genes in exchanged segments associated with resistance QTL is a promising new approach to deciphering the genetic basis of quantitative resistances in oilseed rape.

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# A review of the rise in volume and complexity of winter oilseed rape variety testing and the challenges ahead

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**Background:** The size and complexity of variety trials has increased steadily over the last 30 years, requiring constant review of methodology to accurately demonstrate a yield improvement. However, the crop remains relatively poorly domesticated, with establishment problems associated with the small seed , as well as indeterminate maturity, pod shatter, lodging and inter-plot competition continuing to present difficulties.

**Objectives:** This paper reviews evolving trials methodology and identifies emerging challenges to provide a forum for exchange of new ideas.

**Methods:** NIAB has been conducting oilseed rape (OSR) trials since 1966 and set up the UK National and Recommended List (NL and RL) systems and has had involvement will all subsequent phases of trial development. The author draws on 25 years of direct experience of OSR variety trials and has accessed official published protocols and variety reports to produce this review.

**Review findings:** UK variety testing is similar in structure to that described by Kightley, (1993) with a two-year statutory NL programme which feeds the most promising varieties into a levy-funded (RL) system. Numbers peaked in 2014 with 107 NL1 entries and 71 varieties continuing into NL2 from the previous year. From 1992 onwards trials have become increasingly complex because of the entry of hybrids into trials, further increasing the already considerable inter-plot competition effects associated with different varieties (Talbot, 1993; NIAB, 1999).

Incomplete Block Design (Patterson, 1978) randomisations are used routinely to address field effects in large trials. These layouts are further adapted by blocking variety types to minimise inter-plot competition and, where necessary, using restricted neighbour design for height. At the plot level, drills modified to sow border plots between harvested areas have also contributed to reducing plot interference. Fitted Constant and REML Analyses (Kempton, 1997) have been used for linkage of data from different variety sets, sites and years. The large size of individual trials makes sowing and harvest operations increasingly lengthy and prone to interruption by rain but increasingly sophisticated machinery and access to Global Positioning Satellite (GPS) technology have introduced great efficiencies and improved operational accuracy. Modern plot combines have greatly reduced seed carry-over, allowing a move to shorter plots. On-board weighing, sampling and analysis systems have increased overall efficiency of harvesting and quality analysis but the precision of analytical methodology (NIRS) is not yet universally accepted.

On-going challenges include management of seed shedding risks in trials with diverse maturity types, and consideration of appropriate fungicide and nitrogen regimes. There are difficulties of finding long-rotation trial sites without volunteer problems and, increasingly, a need to adapt trial drills to min- or zero-till field conditions. New challenges include emergence of *Verticillium brassicae* as a widespread disease threat and the loss of control of cabbage stem flea beetles (*Psylliodes chrysocephela*) as a result of the EU ban on the use of neonicotinoid seed dressings.

**Conclusions:** Constantly evolving, robust trials programs are required to that ensure that the yield potential and other characteristics of new varieties are correctly assessed.

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# Association mapping pilot study for the investigation of complex traits in canola

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Association mapping has the potential to map complex traits at high resolution, allows for the identification of the best allele from a diverse panel of lines, and can make recommendations regarding the use of new germplasm containing these alleles in the breeding program. For this purpose a diverse panel of spring canola lines was selected consisting of lines from the Dow AgroSciences breeding program and exotic lines from around the world. All lines were genotyped through GBS generating over 70,000 markers. Field trials were conducted at two locations for two years and phenotypic data for a range of traits have been collected. Substantial phenotypic variation was observed for all traits, together with the observed medium to high heritability, should allow for genome wide association of these traits.

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# Performance of MAXIMUS® semi-dwarf hybrids

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**Background:** On-farm, high grain yield in winter canola is only realized in a crop not damaged by winter and plants not lodging until harvest. Pioneer HI-Bred is using a dwarf gene (bzh) identified by INRA exhibiting additive inheritance (Barret et al. 1998). Using the OGU INRA hybridization system, female inbreds homzygous for bzh are crossed to tall restorers resulting in semi-dwarf MAXIMUS® hybrids.

**Objectives:** To demonstrate high performance of MAXIMUS®hybrids based on evaluations in large and small plot trials. In regions where rapeseed develops fast before winter and tall plant height is expected growers apply growth regulators in the fall and during elongation in the spring to reduce winter losses and to avoid lodging. Pioneer's MAXIMUS®hybrids offer genetic improvements in both traits, namely winterhardiness and standability as well as high oil yields and additional agronomic benefits.

**Results:** In the period from 2006/7 to 2013/14 Pioneer Hi-Bred conducted more than 800 large plot trials in the PACT (Pioneer Accurate Crop Testing) system across Germany and Eastern Europe. The on-farm evaluation demonstrates the high yield performance of MAXIMUS® hybrids. In small plot trials the performance of the semi-dwarf hybrids is often underestimated. Moreover other important agronomic advantages of MAXIMUS® hybrids are only appreciated in large plots on farm (Feiffer and Koch. 2007).

**Conclusion:** Over the past 7 years hybrids like PR44D06, PX104, PX109 and PX113 have delivered step-wise improvements in yield and oil content. Additionally, MAXIMUS® hybrids provide significant agronomic benefits to European rapeseed growers. Despite a longer history and more intensive breeding efforts in tall hybrids, PACT results show that both growth types are at the same level in grain yield. Growers confirm that besides strong winter hardiness and standability, MAXIMUS® hybrids show less damage during spraying (Sauermann 2010), more synchronous maturation, very easy and economically beneficial harvest in comparison to Tall hybrids. Most farmers who have grown MAXIMUS® hybrids repurchase them underlining the high value of this innovative variety concept.

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# Global gene expression responses to waterlogging in roots and leaves of rapeseed seedlings *(Brassica napus L.)*

**Background:** Rapeseed (*Brassica napus L.*) has the potential to follow rice as a rotation crop in paddy fields in Korea. Waterlogging is often a problem in paddy fields during the winter season (Ku et al. 2009). The effects of waterlogging stress include reduction in stomatal conductance, photosynthetic rate, and plant height, as well as premature senescence and disturbances to yield components (Ku et al. 2009; Zhou et al. 1997).

**Objectives:** To investigate the molecular responses of rape seedlings to waterlogging, we assayed global gene transcription in the aerial leaves and roots of waterlogged rape seedlings, as well as the physiological responses of seedlings to waterlogging.

**Methods:** Seedlings of 'Tammi' and 'Youngsan' cultivars were subjected to waterlogging for 3 and 6 days and recovery for 5 days. Physiological responses of seedling leaves to waterlogging were analyzed. We also examined NO production in roots of 'Tammi' cultivar during a 72-h waterlogging period. To examine changes in gene transcription in aerial leaves and roots of waterlogged seedlings and to investigate whether the observed physiological responses were correlated with the regulation of waterlogging-responsive genes, we analyzed the global transcriptional profile of leaves and roots of 'Tammi' cultivar seedlings exposed to waterlogging stress for a short period (36 and 72 h).

**Results:** Waterlogging stress caused a significant decrease in leaf chlorophyll content and premature senescence of the leaves. In addition, waterlogging stress repressed many genes that encode photosynthetic reactions, including the light reactions and carbon-fixing reactions. On the other hand, a majority of the genes that function in ROS scavenging, degradation (proteins, starch, and lipids), premature senescence, and abiotic stress tolerance were upregulated. In roots waterlogging for up to 72 h enhanced NO production rapidly in the roots. Maximum NO generation (sixfold higher than the control) occurred at hour 18, after which it decreased gradually until hour 72. Of 53,107 root genes assayed, 9,692 showed a twofold change in expression within 36 h of waterlogging. Two nitrate reductase (NaR) genes (TC201891, TC161540) and four nitrite reductase (NiR) genes (TC168889, TC164215, TC163914, TC185634) were potentially involved in NO production in response to waterlogging stress. Strong hypoxic induction of nonsymbiotic hemoglobin (Hb) gene (TC165566), which increased 656- and 645-fold at 36 and 72 h of waterlogging, respectively, could oxidize the NO overproduced in the roots. The up-regulation of many additional waterlogging-responsive genes with potential roles in the anaerobic respiration, sucrose and starch degradation, glycolysis, and pyruvate metabolism, may acclimate the plant to waterlogging-induced hypoxic condition.

**Conclusions:** Waterlogging stress caused a decrease of leaf chlorophyll content and premature leaf senescence. Waterlogging for up to 72 h enhanced NO production rapidly in the roots. Thesis physiological responses were associated with dramatic changes in gene expression profiles in the aerial leaves and roots of waterlogged rape seedlings.

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**POSTER THEME A**

# Genetic diversity of germplasm resources on winter rape *(Brassica compestris L.)*

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**Background:** Winter turnip rape (*Brassica campestris L.*) began to be planted in spring rape production area of northwest China, with global climate warming and ultra cold-tolerance winter rape cultivar breeding. High resistance winter rape cultivar breeding provided with cultivar guarantee for development of new winter rape area. Winter rape production played important roles in Agricultural Production in China. But there was little research on genetic diversity of winter rape.

**Objective:** Genetic diversity of 51 winter rape cultivars in Gansu province were studied to provide the theory basis for collection, conservation exploitation, and utilization of germplasm resources and cold-tolerance breeding of winter rape.

**Method:** Genetic diversity of analysis was studied by SSR molecular marker and UPGMA clustering analysis.

**Results:** 15 SSR markers were screened for polymorphism among 51 winter rape cultivars. 96 polymorphic bands were detected.11.45 the polymorphic rate of SSR markers was 100%, and the average allelic variants per SSR primer pair being 6.4.The range of PIC was 0.5834 - 0.8740, The mean value of PIC was 0.7794. The genetic similarity coefficient of 51 winter rape cultivars varied from 0.5000 to 0.969. The results of clustering analysis showed that 51 winter rape cultivars were devided two groups. The first group and the second group had 18 cultivars, which could be devided many sub-clusters.

**Conclusion:** winter rape cultivars in Gansu province showed abundant genetic diversity, which also showd the genetic diversity of winter rape mainly caused by geographical and ecological environment.

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# Genetic variation of new-type rapeseed derived from interspecific hybridization between *B. napus* and its parental species

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**Background:** *Brassica napus* is originated from natural hybridization between *B. rapa* and *B. oleracea*. The narrow genetic basis of *B. napus* limited its improvement due to its intensive modern breeding and short history of origination and domestication. However, its parental species possess more diverse genetic basis than it. It suggests that utilization of *B. rapa* and *B. oleracea* offers an approach to improving *B. napus*.

**Objectives:** Two strategies introgressing genomic components of parental species into *B. napus* were reported, one was using hexaploid (AACCCC) crossing with *B. rapa*, the other was crossing *B. napus* with *B. oleracea* (Li et al. 2013, 2014). New-type *B. napus* from each of the crosses could have the potential to widen genetic basis of current *B. napus*.

**Methods:** 76 new-type *B. napus* (Na) derived from hexaploid (AACCCC) crossing with *B. rapa*, 51 new-type *B. napus* (Nc) derived from interspecific hybrid between *B. napus* and *B. oleracea*, are compared with 3 *B. oleracea*, 6 *B. rapa*, 52 current *B. napus* (16 spring, 16 winter an 20 semi-winter) via 155 SSR markers to clarify genetic variation.

**Results:** Diverse of *B. rapa* and *B. oleracea* are used to broaden genetic basis of *B. napus* via interspecific hybridization. Here, 486 polymorphic loci are amplified by 155 SSR markers in two kinds of new-type *B. napus*, comparing with *B. oleracea, B. rapa* and current *B. napus*. All the genotypes are separated into 3 groups: *B. rapa, B. oleracea* and *B. napus*, by principal components analysis. In which, the total variation explained by the first, second and third principal components are 20.33, 14.02 and 8.93%, respectively. Whereas all the *B. napus* are clustered into three groups: new-type *B. napus* (Na) introgressing A genome of *B. rapa*, new-type *B. napus* (Nc) having genomic components of *B. oleracea* and current *B. napus*. Genetic distance among populations of *B. napus* is further than within populations, except that between Nc and current *B. napus*. The genetic distance between Na and current *B. napus* (0.53 ± 0.11) is biggest than others, followed by the genetic distance between Na and Nc (0.34 ± 0.09). It indicates that the genetic variation of new-type *B. napus* introgressing A genomic components of *B. rapa* is different from the new-type *B. napus* having C genomic components of *B. oleracea*.

**Conclusions:** The genetic variation between two kinds of new-type *B. napus* are different. Both of them are different from parantal species, and have the potential to broaden genetic basis of current *B. napus*.

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**POSTER THEME A**

# The efficient breeding of rapeseed male sterility hybrid induced by chemical hybridization agent and its production technology

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**Background:** Hybrid rapeseed was focused because of its heterosis in yield and quality by breeders and producers generally, but how to raise the breeding efficiency continually, and to get high quality and enough quantity parent seeds for hybrids, it will be an eternal task of breeders. Though breeders have made new progresses in efficient breeding and parents reproduction, but these can not still meet the progress of rapeseed hybrid breeding.

**Objective:** Adopting simple and practical breeding method and mechanized reproduction technique make rapeseed breeding and parent seeds reproduction efficient, saving labours, high quality and yield.

**Method:** 1. The chemical hybridization agent (CHA) SX-1 was used to induce male sterility of hybrids for improving rapeseed varieties ; and for artificial hybridization through smearing stem with CHA to induce sterility of rapeseed in the bolting stage; for creating more combinations for test through one male parent and more female parents (induce sterility of female parents) in net house; At the same time, DH was cultured to good F1 hybrids for creating new germplasms. 2. Reproduction more parents seeds of hybrids through mechanized pollination in big isolation net house; spraying CHA SX-1 and pesticides and fertilizers as well as harvesting through machineries for realizing entire mechanization of hybrid seeds production using CHA SX-1 to induce sterility in fields.

**Result:** CHA SX-1 inducing male sterility and microspore culture DH were used successfully in hybrid breeding, these improved efficiency of breeding greatly. In addition, pollination using machinery may get parent seeds with high yield and good quality in net house. In hybrids production field of rapeseed, mechanized spraying CHA SX-1 and pesticides and fertilizers can save a lot of labors and expenses, also assure quality of hybrids.

**Conclusions:** the results showed that the above-mentioned four methods were utilized in cross breeding of rapeseed successfully , breeding efficiency has been improved greatly. Meanwhile, entire mechanization of parents reproduction of hybrids and hybrids production will promote cross breeding of rapeseed to step into a new stage with high production, good quality and efficient .

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# Male sterility induction mechanism study of the SX-1 in cytology and comparative proteomics

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**Background:** Rapeseed (*Brassica napus L*) is one of the main oilseed crops in the world. The major task for breeders is to increase the seed production at present. Hybrid cultivars have been used to increase the production of rapeseed worldwide successfully. Male sterility of rapeseed induced by chemical hybridization agents (CHA) is one of the main ways to produce hybrid rapeseed. SX-1 as a new-typed CHA had been widely applied on hybrid seed production with high efficiency in China. A series of sterile lines induced by SX-1, such as Y133, YD61A, had been selected out, and Qinyou33, Qinzayou4 and Qinzayou19 new hybrids cultivars were also cultivated successfully.

**Objectives:** Observing the anthers changes after SX-1 treatment and ultrapure water treatment in *B. napus*.

**Methods:** Firstly, the development of microspore and tapetum were observed using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Proteomic analysis was conducted in different development stages of anthers after *B. napus* treated with 6mg/L SX-1.These changes were analyzed using two-dimensional electrophoresis (2-DE).

**Results:** The results showed that, few obvious morphological changes were observed between microspore of control group and SX-1 treatment group in the beginning, all of them seemed to be full and round. However, nearly 100% of treated pollen grains were crimpled and these pollen coats lacked in the late stage of pollen grains in maturation process. Plasmolysis occurred in the crimpy pollen grains, the organelles were not evident and the microspores were almost empty of contents at last. Meanwhile, in the early stage of anthers development, developing tapetosomes and elaioplasts could be observed both in SX-1 treatment group and control group. However, tapetosomes and elaioplasts in tapetum treated with SX-1 became disordered and broken in advance in the late stage of pollen grains maturation process, resulting in tapetal extrusions and no oil body formation in treated pollen grains. These extruded regions appeared to have an extremely high density of organelles and electron-dense materials. These results suggested that SX-1 ruined the proper development of oil body in anthers. We also found that the filaments became shorter and thinner, the petal became smaller in the treated group than in the control group.

About 1000 protein spots were detected on each gel, a total of 130,220, 329, 366 protein spots were down-regulated and 87, 25,74, 60 protein spots were up-regulated, at four different developmental stages, in response to SX-1 treatment. Protein identity is in progress through liquid-chromatography–tandem mass spectrometry. By the available databases for rapeseed and other species, a comprehensive analysis of the *B. napus* anthers proteome could be performed.

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**POSTER THEME A**

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# *ocsESTdb:a* database of oil crop seed EST sequences for comparative analysis and investigation of a global metabolic network and oil accumulation metabolism

**Background:** Oil crop seeds are important sources of fatty acids (FAs) for human and animal nutrition. Despite their importance, there is a lack of an essential bioinformatics resource on gene transcription in oil crops for a comparative perspective.

**Objectives:** In this study, we developed *ocsESTdb*, the first database of expressed sequence tag (EST) information from seeds of four oil crops with an emphasis on global metabolic networks and oil accumulation metabolism that target the involved unigenes.

**Methods:** Developed seeds were sequenced from cDNA libraries or directly from RNA and sequences were analyzed using multiple tools.

**Results:** A total of 248,522 ESTs and 106,835 unigenes were collected from the cDNA libraries of rapeseed (*Brassica napus*), soybean (*Glycine max*), sesame (*Sesamum indicum*) and peanut (*Arachis hypogaea*). These unigenes were annotated by a sequence similarity search against databases including TAIR, NR protein database, Gene Ontology, COG, Swiss-Prot, TrEMBL and Kyoto Encyclopedia of Genes and Genomes (KEGG). Five genome-scale metabolic networks that contain different numbers of metabolites and gene–enzyme reaction–association entries were analyzed and constructed using Cytoscape and yEd programs. Details of unigene entries, deduced amino acid sequences and putative annotation are available from our database to browse, search and download. Intuitive and graphical representations of EST/unigene sequences, functional annotations, metabolic pathways and metabolic networks are also available. *ocsESTdb* will be updated regularly and can be freely accessed at http://ocri-genomics.org/*ocsESTdb*/.

**Conclusions:** ocsESTdb may serve as a valuable and unique resource for comparative analysis of acyl lipid synthesis and metabolism in oilseed plants. It also may provide vital insights into improving oil content in seeds of oil crop species by transcriptional reconstruction of the metabolic network.

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# Phenotypic and molecular characterization of winter oilseed rape germplasms collected at the IHAR-NRI, Poznan, Poland

**Background:** Different methods are available to investigate the genetic diversity in winter oilseed rape breeding materials. Biochemical, phenological, agronomical traits and in recent years also DNA polymorphism analyses have been used to characterize and identify germplasms for use in winter oilseed rape cultivars breeding.

**Objectives:** The aim of this work was molecular characteristics and assessment of genetic diversity among oilseed rape cultivars and breeding lines of agronomic value, collected at the Plant Breeding and Acclimatization Institute-NRI, Research Division in Poznan, Poland.

**Methods:** The plant material comprised winter oilseed rape genotypes including Polish and foreign double-low and traditional (++ and +) cultivars, F1 ogura CMS hybrid and its parental components, in addition to domestic doubled haploid (DH) and recombinant lines developed from mutants with changed composition of fatty acids in seed oil, as well as selected high oleic genotypes, yellow-seeded and resynthesized lines.

Genomic DNA was isolated and degree of similarity among the studied genotypes was assessed using fluorescently labeled AFLP and STR markers separated by capillary electrophoresis. The UPGMA dendrogram was constructed based on the estimated Nei and Li genetic similarity (GS) coefficients. The presence of the ogura male-sterile cytoplasm (CMS) and the Rfo restorer gene was monitored with the multiplex PCR assay (Mikolajczyk et al., 2011), allelic forms of the FAD2 and FAD3 desaturase genes were identified by specific CAPS markers (Falentin et al., 2007) and SNaPshot analysis (Mikolajczyk et al., 2010), respectively.

First round of field trials has been conducted in the 2014-2015 growing season in two environments, in completely randomized block design and in four repetitions.

**Results:** The degree of similarity among studied genotypes was assessed with molecular markers using 10 AFLP primer combinations, and by PCR amplification with primer pairs specific for 48 STR loci. The constructed UPGMA dendrogram revealed pedigree chart of the genotypes. The ogura CMS was detected in 11 genotypes, the Rfo restorer gene – in 7, the fad2 mutant homozygous and heterozygous alleles – in 3 and 2 genotypes, respectively, whereas the fad3 mutant homozygous alleles were identified in 2 of the analyzed genotypes.

The evaluation of agronomical traits before winter dormancy revealed statistically significant differences among the genotypes.

**Conclusions:** This work will be continued on extended population of oilseed rape genotypes and the field trials will be continued in the 2015-2016 growing season, to establish association between the phenotype traits and molecular characteristics of the analyzed plant material of agronomical value.

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**POSTER THEME A**

# Organelle genome sequencing of Nsa cytoplasmic male sterility for identification of CMS gene in *Brassica napus*

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**Background:** A novel cytoplasmic male sterility (Nsa CMS) was estalished by somatic hybridization between *Brassica napus* and its wild relative Sinapis arvensis. The sterility of Nsa CMS is more stable than the widely used Polima CMS and is of potential for safe hybrid production of oilseed rape. As a maternally inherited trait, CMS was often observed when an alien cytoplasm is transferred into a cultivated species (Igarashi et al. 2013). Genes responsible for CMS are usually located in new chimaric ORFs which may be caused by mutations, rearrangement or recombination in mitochondrial genome.

**Objectives:** In order to understand the mitochondrial genomic composition of the novel CMS and to identify genes responsible for the male sterility in Nsa CMS, comparative analysis of mitochondrial genomes of Nsa CMS line and its two original parental lines, *S. arvensis* var.Yeyou 18 and *B. napus* var. Zhongshuang 4 were carried out and structural differences among mitochondrial genomes were uncovered.

**Methods:** The etiolated one-week-old *S. arvensis* seedlings were used for mtDNA extraction. mtDNA sequencing was performed using the Roche 454 FLX system (Roche Applied Science, Indianapolis, USA) and the clean read sequences were assembled by Newbler Assembler Software Version 2.8. The contigs were joined by PCR sanger sequencing. ORF Finder, BLASTX, BLASTN, and tRNA-SE were used to identify mitochondrial ORFs, genes, rRNA, and tRNA. Circularized RNA (CR)-RT-PCR(Kuhn and Binder 2002), was performed to determine *orf347* and *nad3* co-transcripts.

**Results:** We obtained the complete mitochondrial genome sequences of the *S. arvensis* (240,024bp), Nsa CMS line (269,973 bp) and Zhongshuang 4 (221,862 bp). Nsa CMS mitochondrial genome showed a fusion type with 64.7% identity to *B. napus* and 92.5% identity to *S. arvensis,* indicating mitochondrial genome recombination mediated by protoplast fusion. Different mitotypes are co-exist substoichiometrically in Nsa CMS lines. Comparative analysis the mitochondrial genomes of Nsa CMS line and its maintainer line Zhongshuang 4 resulted in three candidate ORFs which might be CMS-associated genes based on their chimeric nature or they encode peptides with transmembrane domains. These genes are usually located on the edges of highly-rearranged CMS-specific DNA regions and from *S. arvensis.* One of the candidates, *orf347*, was co-transcribed with i encoding NADH dehydrogenase subunit 3, and thus was likely to be the CMS gene. Functional analysis of the candidate gene is in progress.

**Conclusions:** To our knowledge, this is the first time that organelle genome derived from a hybrid was sequenced and analyzed. The Nsa CMS mitochondrial genome was highly rearranged compared with its parental lines. Although large portion of sequence context was shared by mitochondrial genomes of CMS and *B. napus*, extensive genomic rearrangements were detected. orf347, which is from *S. arvensis*, was selected as a candidate CMS gene for further investigation.

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**POSTER THEME A**

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# Effects of low nocturnal temperature on photosynthetic characteristics and chloroplast ultrastructure of winter rapeseed *(Brassica napus L.)*

**Objective:** To investigate the effects of low nocturnal temperature on photosynthetic apparatus of winter rapeseed (*Brassica napus L.*).

**Method:** An artificial climate chamber was used to simulate the effects of low nocturnal temperature on seedling and stomatal morphologies, chloroplast ultrastructure, photosynthetic parameters, and dry matter distribution and accumulation in two winter rapeseed cultivars, Longyou-7 with ultra cold resistance and Tianyou-2 with weak cold resistance.

**Results:** Compared with those at diurnal/nocturnal temperatures of 20°/10°C (control), rapeseed seedlings at 20°/5°C had increased leaf chlorophyll content, deepened green leaf color, decreased stomatal conductance (Gs), intercellular CO2 concentration (Ci), and photosynthetic rate (Pn), and improved root/shoot ratio; the majority of stomata remained open in Longyou-7 while those in Tianyou-2 were mostly closed or semi-closed. At diurnal/nocturnal temperatures of 20°/-5°C, rapeseed seedlings had decreased leaf chlorophyll content with increased Ci but decreased Gs and Pn; Tianyou-2 exhibited ruptured chloroplast membrane, dissolved grana, broken stroma lamella, and decreased root/shoot ratio, whereas Longyou-7 had chloroplasts retaining partial structure of grana with a small amount of starch granules in guard cells.

**Conclusions:** Low nocturnal temperature caused damage to the structure of photosynthetic membrane of chloroplasts and reduction of Pn in leaves of winter rapeseed, thus influencing photosynthetic processes in this crop. The reduction of Pn was mainly related to stomatal limitation at diurnal/nocturnal temperatures of 20°/5°C and non-stomatal limitation at diurnal/nocturnal temperatures of 20°/-5°C.

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# Disruption of a CAROTENOID CLEAVAGE DIOXYGENASE 4 gene converts flower color from white to yellow in *Brassica* species

**Background:** The distinctive yellow flower color of *Brassica* makes a striking contribution to the visual landscape of many arable rotations worldwide. The common flower color of *Brassica* species in U's triangle (Nagaharu, 1935) is yellow, although some white-flowered varieties exist in subspecies of the *B. oleracea* cytodeme. White flower has been reported in rapeseed lines re-synthesized through interspecific hybridization between *B. rapa* and white-flowered *B. oleracea* (Chen et al., 1988; Heneen et al., 1995; Zhang et al., 2002). Genetic analysis of flower color in *Brassica* has been conducted since 1929 (Pearson, 1929), and has been shown to be controlled by a single nuclear gene in *Brassica* species containing the C genome or sub-genomes, with white color dominant over yellow (Pearson, 1929; Zhang et al., 2002; Liu et al., 2004; Huang et al., 2014). Genetic mapping has identified a number of molecular markers linked to the flower color locus on chromosome C3 (Ramsay et al., 1996; Liu et al., 2004; Parkin et al., 2005; Geleta et al., 2012; Huang et al., 2014). However, to date the gene controlling flower color has not been identified, and the underlying molecular mechanisms and evolutional processes in *Brassica* remain elusive.

**Objectives:** In the present study, we will reveal its molecular mechanism that controls carotenoid accumulation and discuss the evolutionary history of flower-colored trait in *Brassica*.

**Methods:** We measured the content of total carotenoids in petals from fully opened flowers of a white-flowered rapeseed line '2127' and a yellow-flowered cultivar ZY821. Using HPLC, we also compared the carotenoid profiles in petals of '2127' and ZY821. To reveal the molecular mechanism of flower color formation in *Brassica* species, we attempted to identify the flower color BnaFC gene using a positional cloning strategy. The likely enzyme activity of BnaFC was tested through headspace-solid phase microextraction-gaschromatography-mass spectrometry (HS-SPME-GC-MS). The alleles of BnaFC were isolated and aligned in *B. napus*, *B. oleracea* and *B. carinata*.

**Results:** In *Brassica napus* yellow petals had a much higher content of carotenoids than white petals present in a small number of lines, with violaxanthin identified as the major carotenoid compound in yellow petals of rapeseed lines. Using positional cloning we identified a carotenoid cleavage dioxygenase 4 gene, BnaC3.CCD4, responsible for the formation of flower color, with preferential expression in petals of white-flowered *B. napus and B. oleracea* lines. Insertion of a CACTA-like transposable element 1 (TE1) into the coding region of BnaC3.CCD4 had disrupted its expression in yellow-flowered rapeseed lines. α-Ionone was identified as the major volatile apocarotenoid released from white petals but not from yellow petals. We speculate that BnaC3.CCD4 may use δ- and/or α-carotene as substrates. Four variations, including two CACTA-like TEs (alleles M1 and M4) and two insertion/deletions (INDELs, alleles M2 and M3), were identified in yellow-flowered *B. oleracea* lines. The two CACTA-like TEs were also identified in the coding region of BcaC3.CCD4 in *B. carinata*. However, the two INDELs were not detected in *B. napus* and *B. carinata.*

**Conclusions:** The results suggested that the insertions of TEs in BolC3.CCD4 predated the formation of *B.napus* and *B.carinata*, and that the two INDELs might occur outside of the origination centers of the two allotetraploids and not participate in their speciations.

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# Identification of a new cytoplasmic male sterility type NRO4270A in *Brassica napus*

**Background:** The production of the hybrid seeds through cytoplasmic male sterility (CMS) is the most important way of heterosis utilization in rapeseed (*Brassica napus*). CMS is often associated with novel open reading frames produced through the rearrangements of mitochondrial genome, and they interfere with the pollen development (Iwabuchi et al., 1999; L'Homme et al., 1997). Several CMS systems have been reported in rapeseed, such as ogu, pol, tour and nap. *B. napus* NRO4270A CMS was obtained in the progenies of distant hybridization of *Raphanobrassica* (RRCC) (Chen et al., 2006) and *B. napus* (AACC) by us.

**Objectives:** In the present study, the phenotypes of NRO4270A CMS would be investigated, and its restorer and maintainer relationship would be identified. In addition, its stage and characters of pollen abortion would be revealed, and its cytoplasm type would be tested.

**Methods:** The sterility degree and sterility rate of NRO4270A CMS in field were investigated in Wuhan (winter rapeseed production area) and Changyang or hezheng (spring rapeseed production area) in 2009-2013. Its restorer and maintainer relationship was compared with those of other CMS systems. The buds of NRO4270A CMS and its maintainer line were collected, and their paraffin sections were made and observed at different pollen development stages. The restriction fragment length polymorphism (RFLP) of NRO4270A mitochondrial DNA were analyzed and compared with those of other CMS systems.

**Results:** NRO4270A could not produce pollens and its male sterility was extremely stable, which could not be affected by environmental conditions, such as temperature and photo-period. Through restorer and maintainer relationship identification and RFLP analysis of mitochondrial DNA, NRO4270A is different from previous CMS systems, such as pol, ogu and kos. Microscopic structure analysis of NRO4270A anther development indicated that its pollen abortion occurred between the tetrad stage and mononucleate stage. At mononucleate stage, the microspore ektexine could not form, and tapetum vacuolated and expanded. Finally, tapetum and microspore completely degraded, and the pollen sacs became empty and could not produce pollen. Conclusions: It was suggested that NRO4270A was a new CMS system. Its discovery and utilization will overcome the problems of environmental sensitivity of some CMS systems and singleness of sterile cytoplasm type in rapeseed hybrid seed production.

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**POSTER THEME A**

# Anther-specific cysteine protease CP51 Is essential for pollen exine formation in *Arabidopsis* and *Brassica napus*

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**Background:** Cysteine proteases play important roles in intracellular protein degradation, programmed cell death and in responding to environmental stimuli.

**Objectives:** This study was to characterize a new cysteine protease.

**Methods:** Candidate genes were selected based on a microarray profiling study in which isogenic lines (the genic sterile and its backcrossing with normal fertility) were used . A putative cysteine protease named CP51 was transformed into *Arabidopisis* to study its function.

**Results:** We identified a new cysteine protease CP51 from *Brassica napus* and *Arabidopsis thaliana* which participates in exine formation and anther development. The gene encodes a papain-like subfamily (C1A) cysteine proteinase and is specifically expressed in anthers at stages 9-12 of A. thaliana, which was assessed by qRT-PCR and promoter-GUS fusion detection. RNAi transgenic *Arabidopsis* plants with reduced CP51 transcriptional levels exhibited male sterile phenotype with aborted microspores, shortened siliques and fewer seeds. Cytological analysis indicated that the tapetum degraded earlier and pollen abortion occurred due to defective pollen exine during the transition from the uninucleated stage to the binucleated stage. Scanning electron microscopy demonstrated that aborted microspores lacked complete or normal reticulate exine, and the intine membrane was extruded in pollens of CP51-RNAi plants. Transmission electron microscopy further revealed that the tapetum degeneration was initiated early and that normal tectum connections to the bacula were missing in anthers of CP51-RNAi plants. Taken together, these results suggested that CP51 participates in tapetum stability regulation and pollen exine formation.

**Conclusions:** CP51 is a member involved in the male gamete development.

# Evaluation of the Rfo introgression following recombination and mutation

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**Background:** The ogu-INRA CMS system is a cytological variant of the radish- (*Raphanus sativus L.*) derived ogu CMS (ogura; Ogura, 1968) system introduced through interspecific introgression into *B. napus* (Heyn, 1976). In this system, there are three different lines: A-lines are male sterile, B-lines are fertile maintainer lines for the respective A-lines and R-lines (restorer) are male fertile restorer lines.

The Rfo introgression is associated with poor agronomic performance due to a large unwanted piece of the radish chromosome that was introgressed with the Rfo gene. It contains 17 pentatricopeptide (PPR) motif repeats that confer fertility restoration (Hu et al., 2008). Previous reports have stated the Rfo locus and the PPR genes are likely to have evolved as a result of intergenic and intragenic recombination. Mutation is another strategy to induce change in the restorer genome. Ethyl methane sulphonate (EMS) has been extensively used to induce mutations in plants because it causes a high frequency of nucleotide substitutions (Talebi, et al., 2012). This approach to introduce variation has been used successfully in several *Brassica* species such as *B. napus, B. juncea* and *B. rapa* to induce fatty acid modifications in double haploids.

**Objectives:** This project will focus on evaluating the length and composition of the Rfo introgression present in restorer lines after undergoing recombination and mutation.

**Methods:** Four restorer (R-line) by non-restorer (B-line) crosses and four R-line by R-line crosses will be used for treatment with EMS. Seeds will be soaked in water for 12 hours, followed by soaking in EMS at concentrations varying from 0.5 to 1.2% for an additional 12 hours. These seeds will then be rinsed in water prior to planting. DNA will be extracted from vigorous, fertile M1 lines to sequence the restorer gene fragment and compare sequence variation between lines and to the control R-lines.

**Results:** Sequence differences in the PPR-B region will be observed when comparing the original R-lines and the mutagenized R-lines.

**Conclusions:** Recombination and mutation will generate changes that could potentially shorten the restorer fragment sequence and thus improve the agronomic performance of the restorer lines. Future work will focus on evaluating subsequent populations.

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**POSTER THEME A**

# Progress on self-incompatibility hybrid breeding in *B.napus L*

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**Background:** Self-incompatibility (SI) has been used widely for hybrid breeding in vegetables *B. rapa and B. oleracea*, but rarely utilized in *B. napus.* As *B. napus* is an oil crop, its hybrids should be fertile for harvesting seeds and a SI line must be propagated on a large scale to produce many hybrid seeds. The SI of line S-1300 is recessive in most accessions but dominant in some genetic backgrounds (Ma et al, 2003), and so it has been utilized for three-component hybrid breeding via SI F1 hybrids (Fu, 1981). With development of a method of propagating SI lines on a large scale by spraying salt solution, we have claimed a two-line hybrid breeding method by self-incompatibility. How the method works in practice is not reported.

**Objectives:** Field data were collected and summarized to show the high vigor of the two-line hybrids. To confirm the efficiency of breeding a SI line, Microspore culture and Gene-based PCR markers have been adopted. F1 fertility is predicted and its purity is checked by the Gene-based PCR markers. A protocol of seed production system has been claimed in order to extend the hybrids.

**Methods:** Field trials were conducted during rapeseed growing season of 2008-2014 in farm fields at three locations under normal condition for crop production in Hubei province, China. Performance of some hybrids was from provincial and National official trials from 2008 to 2014 in China. All trials were designed as randomized complete blocks with three replications in each environment. Each plot was 20 m2. DNA isolation and PCR were carried out as described by Gao et al (2013) and PCR primers were from the literatures by Gao et al (2013) and Tang et al (2009).

**Results:** Four hybrids have been delivered, three have elite performance on provincial and National official trials, and three are selected for further trials, showing that the two-line hybrid breeding method has high efficiency. Many diverse genetically SI lines have been bred indicating SI lines are easy and fast to be improved. Hybrid seeds with high hybridity have been produced by a protocol.

**Conclusions:** The two-line self-incompatibility hybrid breeding method is effective and fast with some distinguished advantages such that hybrids have high vigor, SI lines are easy to be bred by Microspore culture and SCAR markers and F1 fertility and purity can be told by SCAR markers.

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**POSTER THEME A**

# Haplotype diversity across quantitative resistance loci associated with sclerotinia resistance in *Brassica napus*

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**Background:** The omnivore fungal pathogen *Sclerotinia sclerotiorum* is a serious disease of many crop species including canola (*Brassica napus*). Resistance to sclerotinia is a desirable but rare trait. The Chinese *B. napus* cultivar Zhongyou 821 (ZY821) has a moderate level of quantitative resistance (He et al. 1987) and has been accessible to canola breeding programs world-wide since its release in the late 1980's. More recently, higher levels of resistance have been identified in *B. napus* germplasm lines from Pakistan, South Korea and Japan held at AAFC in Saskatoon. Bi-parental mapping populations of doubled haploid lines were developed from crosses with ZY821 and the new resistant sources with susceptible lines. Subsequently, the populations were phenotyped for stem rot resistance and genotyped with a combination of single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers. Numerous major and minor quantitative resistance loci (QRL) were identified through quantitative trait loci analysis, some of which mapped to the same chromosome and genomic block. In addition, QRL were identified by association mapping using a set of resistant and susceptible accessions in a world collection of *B. napus*. The resistant germplasm are mainly landraces and past breeding lines which are not adapted to the Canadian prairies and do not possess canola-quality seed traits.

**Objective:** The newly identified QRL are currently being introgressed into an elite breeding line to develop sclerotinia resistant germplasm. To target a specific QRL for introgression, it is imperative to elucidate whether genomic regions associated with sclerotinia resistance in different resistance sources are identical by decent or different allelic forms.

**Methods:** Existing SNP data at the previously detected sclerotinia resistance QRL of all lines used as parents of bi-parental mapping populations (PAK54, PAK93, DC21 and ZY821) and in association mapping is analysed to dissect relationships among the QRL by comparison with the larger association panel of lines. High density SNP array data is analysed using custom scripts and R packages to identify allele frequencies and haplotypes associated with the mapped QRL. The genetic relationships among the haplotypes underlying QRL-regions is further characterised through analysis of molecular variance and compared to genome-wide variation and population structure. Haplotypes of SNP markers across QRL is identified to infer allele frequency differences between the resistant lines. Additional haplotype characterization is conducted to look for signatures of selective sweeps.

**Conclusion:** Results will be presented identifying unique and shared alleles at previously mapped QRL conferring sclerotinia resistance in PAK54, PAK93, DC21 and ZY821. The analysis will reveal the presence/absence of conservation of haplotypes at the respective QRL among these resistant sources. This information is needed to prioritize resistance introgression targets in our canola breeding program.

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**POSTERS THEME A**

# Yield stability and adaptability of NS spring rapeseed genotypes based on GGE biplot analysis

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**Background:** Field experiments are usually being performed in different environments with an aim to evaluate yield stability of different crops under varying environmental conditions. Therefore, to reduce the possibilities of significant yield loss and to select specific cultivars for growing in target regions, the information of the effect of environmental factors on crop growth and development is essential (Marjanovic-Jeromela et al. 2011). The main environmental effects (E) and genotype environment interaction (GE) were recognized as the most important sources of crop yield variation (Yan et al. 2007). The GGE biplot technique (Yan 2001) is one of the tools used for GE interaction analysis. It can help to recommend genotypes for specific growing region taking into account the specificities of genotypes and growing conditions (Boshev et al. 2014).

**Objectives:** The objectives of the study were to evaluate grain yield stability, to graphically summarize the effects of genotype (G) and genotype by environment (GE) interaction, to identify "which won where", and to recommend rapeseed genotypes for a specific growing region using GGE biplot.

**Methods:** The study was carried out in 2009 and 2010 at two locations in Serbia (Sombor and Novi Sad) and two locations in Macedonia (Skopje and Bitola), with 9 spring rapeseed genotypes. In order to evaluate and to quantify the magnitude of genotype x environment interaction effects on spring rapeseed yield, GGE biplot analysis was used to depict the stability and adaptability of genotypes at different locations and discrimination ability of the testing locations.

**Results:** The first two principal components explained 86.5% of the G+GE variation for seed yield (PC1 64.46%, PC2 22.048%). Both locations in Serbia were clearly separated from both locations in Macedonia. Sombor and Bitola were closest to the ideal location for rapeseed growing. Rapeseed genotype JR-NS-36 was the most stable and was the closest to the ideal genotype. However, the most suitable genotypes for growing in Serbia are JR-NS-6 and JR-NS-11, while JR-NS-9 had the highest and the most stable yield in Macedonia.

**Conclusions:** This technique can serve as a useful tool for recommendation of rapeseed genotypes for specific growing region, taking into account the specificities of the genotypes and environmental conditions.

**Acknowledgment:** This work is a part of the project TR31025 supported by Ministry of Education, Science and Technological Development, Republic of Serbia.

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# Development of DuPont Pioneer proprietary Optimum® GLY Canola trait

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**Background:** Adoption of herbicide tolerant (HT) canola in North America has been rapid with almost 99% of Canada's 20 million acres of canola now planted to HT types which were first introduced 20 years ago. Glyphosate tolerance represents approximately 50% of the total acreage and is projected to increase to 60-65% in the next 10 years. Canola Optimum® GLY is a DuPont Pioneer proprietary HT trait that has advanced to the pre-commercial stage with expected commercial launch later this decade, subject to appropriate regulatory approvals

**Objectives:** To develop, obtain necessary approvals, and commercialize high-performing canola hybrids containing the Optimum® GLY herbicide tolerance event (DP-Ø73496-4) in North America and Australia. Secondly, to integrate current and future Protector™ traits into Optimum® GLY canola and tolerance to higher levels of glyphosate within an expanded application window (up to first flower).

**Methods:** The Optimum® GLY canola trait is based on the Glyphosate Acetyltransferase (GAT) gene that was optimized through the DuPont Pioneer proprietary DNA shuffling technology. The gene detoxifies glyphosate herbicide by acetylation to provide glyphosate tolerance. DuPont Pioneer was responsible for the development of the Optimum® GLY trait including a full range of trait development activities, including molecular characterization, indoor phenotypic and genetic characterization of events, trait introgression, field evaluation and seed production.

**Results:** The Optimum® GLY trait attributes include improved agronomics, better efficacy in the current label application window compared to current glyphosate herbicide tolerance, and commercial level glyphosate tolerance at late-stage plant development up to early flowering. Trials are currently being conducted to support a new label with higher levels of active glyphosate applied and an expanded application window to optimize weed control. Advanced pre-commercial hybrid testing and evaluations are ongoing. Optimum® GLY hybrids have been recommended for registration by the WCC/RRC in spring 2015 and will be candidates for commercial launch later this decade subject to appropriate regulatory approvals.

**Conclusions:** The commercialization of DP-Ø73496-4, Optimum® GLY Canola, will provide growers globally with a choice for glyphosate tolerant canola over a wide application window combined with high-yielding Pioneer genetics including key native traits, strong agronomics. In addition Optimum® GLY canola will provide additional integrated weed management options for growers.

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# Constructing a high density SNP genetic map and mapping QTL for yield-related traits using DH and IF2 populations in *Brassica napus*

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**Background:** Yield is the most important and complex trait for rapeseed (*Brassica napus*). Quantitative trait locus (QTL) analysis has proved to be an effective approach to dissect complicated quantitative traits. The newly developed *Brassica* 60 K Infinium BeadChip Array is a very effective tool for SNP genotyping, and it has been successfully exploited in rapeseed's seed fibre QTL analysis (Liu et al. 2013).

**Objectives:** To construct a high density SNP genetic map for mapping QTL for five yield-related traits in rapeseed using a double haploid (DH) population and an immortalized F2 (IF2) population derived from the DH lines.

**Methods:** Silique length (SL), thousand seed weight (TSW), seeds per silique (SPS), siliques per plant (SPP) and silique density (SD) of DH and IF2 populations were assessed in four different environments. Genome-wide single nucleotide polymorphism (SNP) of parental and DH lines were assayed by the *Brassica* 60 K Infinium BeadChip Array. Linkage analysis and SNP map construction were performed using QTL IciMapping V4.0 and JoinMap 4.0. WinQTLCart2.5 was used to detect QTLs.

**Results:** A 2217.2 cM SNP bin linkage map was constructed which contains 8876 SNP makers, and 7728 SNP makers were distributed among 900 bins. For QTL mapping, only one SNP maker of every bin was selected. The final high density linkage map for the DH population contains a total of 2046 non-redundant SNP makers, with an average distance of 1.08 cM between adjacent markers. Thirty-five and 23 significant QTLs were detected across environments for the five traits in the DH and IF2 populations, respectively. Amongst, 16 QTL were repeatedly detected in both populations. Eighteen QTL were repeatedly detected in the DH population and nine in the IF2 population across different environments. Two, two, two, one and one major QTLs were detected for SL, TSW, SPS, SPP and SD, respectively. Three of the major QTLs were repeatedly detected for two or three traits.

**Conclusions:** Major QTLs for five yield-related traits were successfully detected using a high density SNP bin map in DH and IF2 populations. The high density SNP bin map is very useful for QTL mapping, and SNP maker showing a great potential for marker-assisted selection. Some major QTLs for different traits were mapped in the same chromosome region, which gave a good explanation for significant phenotypic correlations of the traits at molecular level.

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# Cell and tissue-specific RNA sequencing and laser microdissection of the *Brassica napus* (canola) maternal seed subregions

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**Background:** *Brassica napus* (canola) contributes $19.3 billion to the Canadian economy each year due to its highly nutritive oil and protein reserves within the embryo. Embryo development and nutrient accumulation require the coordinated development and communication between all seed regions and subregions. The maternal tissues of the seed can be divided into a number of subregions including the outer and inner integuments surrounding the seed, the chalazal proliferating tissue (CPT) that subtends the chalazal endosperm, and the chalazal seed coat (CZSC) that serves as the first connection between the funiculus and the seed. While much research has been carried out on the filial embryo and endosperm, we have yet to fully understand the genes required to program the development of the maternal subregions at the cellular level in canola.

**Objectives:** Surprisingly, subregion-specific development and transcriptional circuitry of the maternal tissues of the canola seed has yet to be investigated. Our goal was to provide the first detailed anatomical description of the uncharacterized maternal seed subregions coupled with next-generation RNA sequencing to profile the genes and gene regulatory networks responsible for canola seed development.

**Methods:** The anatomy of the maternal subregions was studied using light and transmission electron microscopy across the mature ovule, globular, heart, and mature green stages of seed development. We then profiled each of the maternal subregions using laser microdissection coupled with next-generation RNA sequencing technology. These combined methods provide a high-resolution dataset of the transcriptional networks operative within the maternal seed. Hierarchical and fuzzy-K means clustering analyses combined with GO term enrichment were then used to predict and compare biological function and cellular processes within the CZSC, CPT, and inner and outer integuments.

**Results:** Vigorous bioinformatics analyses of RNA sequencing data revealed an impressive array of dominant expression patterns thought to control development of the maternal subregions in both space and time. Fuzzy-K means clustering analysis identified dominant patterns of gene activity between the different maternal subregions of the canola seed. Large numbers of transcripts were considered shared between all maternal subregions, while small numbers of genes were shown to be specific to each. Subsequent GO term enrichment showed that the CZSC has mRNAs associated with transport processes and also identified putative regulators of these processes operative in the CZSC and other maternal seed subregions. Light and electron microscopy identified vascular tissue of the funiculus, which supplies the seed with nutrients, terminating in the CZSC suggesting the CZSC is an unloading zone. In addition, plasmodesmata were identified between the cells of the CZSC, suggesting symplastic transport is likely present within this subregion.

**Conclusions:** Our combined anatomical and global transcriptomic datasets provides strong evidence that the maternal seed subregions in canola possess not only a structural and protective function but also serves a putative role in the transport of materials to the filial embryo and endosperm. Our data further provide a substantial informatics resource for those interested in oilseed genomics for improved seed development.

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# How duplications in oilseed rape (*Brassica napus L.*) genome impact the organization of genomic regions involved in quantitative resistance to *Leptosphaeria maculans*

**Background:** All crop species are recent or ancient polyploids and have more or less duplicated genomes. Following polyploidy events, structural and functional modifications result in differential gene content or regulation in the duplicated regions, which can play a fundamental role in the diversification of the genes underlying complex traits.

**Objectives:** To better understand the functioning of the genetic factors controlling complex agronomic traits, it is necessary to analyze them in the light of the duplications in the genome. We have addressed this issue in oilseed rape, a species with a highly duplicated genome, with the aim of studying the consequences of genome duplications on the structural and functional organization of the regions involved in quantitative resistance to blackleg. This resistance has been shown to be controlled by many genetic factors (1, 2).

**Methods:** The genetic architecture of quantitative resistance to blackleg was evaluated using several methods: (i) linkage analyses on bi-parental populations with Darmor-bzh as common resistant parent, (ii) a QTL meta-analysis on a set of 7-years phenotyping data on the 'Darmor-bzh x Yudal' population and (iii) genome-wide association analyses in two panels of oilseed rape varieties. High density genotyping SNP were used for these analyses allowing to synthesize all the results on a dense integrated genetic map. These data were used to assess the proportion of resistance QTL located at duplicated positions (3). We then took advantage of the recently released genome sequence of *Brassica napus* (4) for the structural analysis of the duplicated regions. *B. napus* – *Arabidopsis thaliana* relatedness was used to explore gene ontology categories and the function of genes located in the duplicated regions.

**Results:** Numerous genomic regions were identified, which confirmed the high polygenic nature of this resistance. Their distribution was quite equivalent between A and C genomes of oilseed rape but a bias was observed in relation with the subgenomes deriving from the ancestral triplication event of *Brassica* clade. At least 44% of the genomic regions corresponded to homoeologous duplicated regions of five *A. thaliana* syntenic blocks (3). Comparative genomic analysis with *A. thaliana* showed that few genes were conserved in all the duplications of a given ancestral bock and that many of these genes were involved in stress response. NBS-LRR genes or resistance gene analogs were also present in some of these regions.

**Conclusions:** Most of the identified resistance associated genes corresponded to genes retained in duplicated regions and were involved in response to stress. It has actually been demonstrated in various other species that genes over-retained after whole genome duplication were involved in stress response, indicating a common evolution pattern across species. Comparative genomics also allowed us to draw hypotheses on the function of genes underlying the QTL located in these genomic regions.

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# Improving early generation selection in canola breeding

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**Background:** Classical plant breeding has always relied on phenotypic selection as a keystone for any breeding program. Greatest advancement in genetic gains from plant breeding will be achieved by increasing heritability of traits under selection. The advancement of high throughput molecular markers have significant potential to assist plant breeders by providing a powerful tool for marker assisted selection (MAS). Similarly genome wide association studies (GWAS) will provide an additional tool to connect genotypic and phenotypic performance and for studying quantitative traits of interest such as yield and oil content. However, MAS and GWAS both require high quality and reliable phenotypic performance data.

**Objective:** Increase breeding methodology and selection efficiency in canola cultivar development programs.

**Methods:** Two studies were conducted. (1) Two hundred and ninety two spring canola and rapeseed (*B. napus*) accessions from a broad geographic distribution were grown in a greenhouse and leaf tissue analyzed with Sequenced-based Genotyping, producing more than 300,000 SNPs at genome-wide coverage. These accessions were grown in replicated field trials at one location in 2012 and two locations in 2013 and 2014. A wide range of morphological, agronomic, and quality traits were evaluated. (2) Nine commercial spring canola varieties were chosen from the germplasm collection (1) and crossed together in a 9x9 full diallel mating design. F1 plants were then self-pollinated to produce F2 seed, and these progeny were planted in a replicated covariance (reciprocal crosses) design in a greenhouse. Phenotypic data was collected (i.e. leaf characteristics, flower date, yield, pod density, etc.) for analyses.

**Results:** The plant traits days to flowering, leaf serration and lobing, and pod length showed high heritability between glasshouse plants and field evaluations, and between different sites and years under field testing. However, yield and oil content under glasshouse and field conditions was very poor. Heritability values for seed yield between field sites and years ranged between 0 and 0.4. Similarly, other important breeding traits had low heritability over locations and years. Greater variability of phenotypic performance over years and sites is perhaps the most limiting factor of success in plant breeding. In order for MAS, GWAS and quantitative trail loci to improve selection it is important that the genotype:phenotype relationship can be made reliably and have relationship over environments. Analyses from the diallel have yet to be completed at the time of submission. However, progeny performance of the diallel progeny will be predicted using available phenotypic and genotypic data available on the parent lines used and the results of these predictions discussed.

**Conclusion:** World-wide need for increased food production will necessitate larger genetic gains in our breeding efforts, which will require greater predictability in selecting superior cultivars. MAS and GWAS will have greater impact on cultivar development if these techniques can be applied at the parent level to select parents with greatest breeding value and thereafter identify specific individuals within progeny to be better cultivars.

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# Transcriptomics analysis of *Brassica napus* using de novo assembly methods

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**Background:** Canola (*Brassica napus*) is of significant global economic importance, this along with its unique evolutionary path that offers insights into polyploid evolution have ensured that it has been the focus of much genetic and latterly genomics research. All research in *B. napus* is complicated by the significant homoeology that exists between the subgenomes which resulted from the merger of the genomes of *B. rapa* (AA, n=10) and *B. oleracea* (CC, n=9), 7,500 to 12,000 years ago. This homoeology creates difficulties in determining the origin of gene transcripts and introduces ambiguity in assigning transcripts to a genomic location (Chalhoub et al, 2014, Harper et. 2012). This is exacerbated when using the widely adopted short read sequencing technologies that allow unbiased assessment of the plant's transcriptome, which is proving essential for the study of many agronomic traits. The current problem is how to adapt software tools designed for simpler genomes to provide efficient and effective analyses of transcriptome data of the complex *B. napus* genome.

**Objective:** To define robust protocols for analyzing short-read transcriptome data from *B. napus*, which are optimized for differentiating the homoeologous transcript pairs.

**Methods:** A transcriptome of *B. napus* morphotype 'DH12075' (leaf tissue) was created using experimental Illumina RNAseq data and the de novo transcriptome assembler Trinity (Grabherr et al. 2013). The resulting transcripts were compared by multiple sequence alignment to coding DNA sequence (CDS) data from ab initio gene prediction using MAKER (Cantarel et al. 2008). Simulated Illumina short-reads were generated from the same CDS data using ART software tool (Huang et al. 2012) and also assembled in Trinity. Accuracy of Trinity software was evaluated by multiple sequence alignment of the assembled CDS sequences to the original data.

**Results:** Initial assembly of simulated Illumina RNAseq reads resulted in 121,254 distinct transcripts (N50=423) and 125,586 total transcripts (including isoforms; N50= 432 bp) from the original 103,679 CDS, suggesting relatively good recovery. Assembly of experimental Illumina RNASeq data resulted in 56,025 distinct transcripts (N50 = 939 bp) and 87,545 total transcript isoforms (N50 = 1210 bp), this reduced number might be expected from a single tissue dataset. The assembled transcripts (both simulated and experimental) will be assessed for their integrity relative to the expected annotations. Multiple assemblies are currently being created with different k-mer lengths and additional software tools to study potential improvements.

**Conclusion:** The results of the different tools and assessment parameters will identify an optimal pipeline for transcriptome assembly in *B. napus*.

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**POSTERS THEME A**

# Development of molecular markers for Identification of *Fusarium* species

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**Background:** *Fusarium* wilt disease in canola primarily caused by *Fusarium oxysporum* and *Fusarium avenaceum* has caused substantial losses. Unlike other plant pathogens *Fusarium* species are making an association where involvement of more than one pathogen makes disease more complex.

**Objectives:** To control fungal hazards of plants, animals and humans, there is a need for a rapid, easy and accurate identification system of *Fusarium* isolates with molecular methods. Identification of *Fusarium* species has always been difficult due to confusing phenotypic classification systems.

**Methods:** We have developed a fluorescent-based polymerase chain reaction assay that allows for rapid and reliable identification of seven toxigenic and pathogenic *Fusarium* species.

**Results:** The species includes *Fusarium avenaceum, F. oxysporum, F. sambucinum, F. culmorum, F. equiseti, F. solani* and *F. graminearum*. The method is based on the PCR amplification of species-specific DNA fragments, which were designed based on sequences.

**Conclusion:** Besides providing an accurate, reliable, and quick diagnosis of these *fusarium* spp for canola this can also be leveraged for other crops.

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# High throughput phenotyping of OSR crops by Remote-, close range-, and in situ- sensing techniques: State of the art & first results

**Context:** The lack of suitable tools for rapid and non-destructive phenotyping methods may influence and hamper the physiological and genomic researches. Among the large number of high throughput phenotyping facilities at different scales (in situ, close range, and remote sensing), choosing the most appropriate data source, including the 'vector' and the 'sensor', remains a challenge.

At CETIOM, as part of the PHENOME (French phenotyping network) and RAPSODYN (optimization of the RAPseeds Oil content anD Yield under low Nitrogen input) projects, we establish, observe and follow the experimental platforms since 2012. These platforms have been designed for testing out the new phenotyping tools and methods. The final goal is to provide the high frequency measures of phenotypic traits of the plants at different growth stages using the data obtained from multiple sources. The expected results are mainly agronomical variables such as biomass, LAI, Nitrogen content, etc. The latters are basically estimated by combining statistical, physical and physiological models.

**Methodology:** We present, first of all, a summary of sensors and vectors tested at CETIOM experimental station in Dijon. Then some data, analysis methods and results of two different data sources will be shown: 1- the reflectance spectra obtained by a close range sensing device, the field VNIR spectrometer ASD (Advanced Spectrometry Device), 2- the RGB and multispectral images taken by a UAV (Unmanned Aerial Vehicle) system as a remote sensing tool. The pre-processed data has been used as input parameters of prediction models.

**Results and discussion:** The reliability of the estimated outputs, Green area index (GAI) and biomass, has been evaluated through the validation phase (lab traditional destructive methods). The first results show a good accordance between the data obtained from different sources and a reliable estimation of biomass and GAI ($R^2 \sim 0.7$).

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# Large scale SNP genotyping with optimized molecular marker sets for cost-efficient plant breeding in the *Brassica* species (*B. napus, B. oleracea and B. rapa*)

**Background:** Through the development of large genotyping arrays, it has now become routine to generate a wealth of genotyping data for individual plant lines. However, many of the genotype data generated in this way (e.g. with the *Brassica* 60K Illumina Infinium array) constitute of redundant information since the genotype data of many markers are in perfect linkage disequilibrium in breeding material and varieties.

**Objectives and methods:** With genotyping data generated from a large set of lines derived from various sources and countries in combination with mapping information for many markers, we have investigated in detail the extent of LD and marker haplotype groups in elite *Brassica napus* material as well as in *B. rapa* and *B. oleracea* to identify haplotype-specific markers of high quality. Taking all this information together, we have subsequently generated an optimized genotyping array for routine use in genetic analyses and breeding including genomic selection of oilseed rape and *Brassica* vegetables.

**Results and conclusions:** The array can now be used at much reduced costs compared to other arrays and without much loss of information compared to larger and more expensive arrays. In parallel and based on the same data set, we have generated an optimized marker collection based on individual SNP markers (KASP) for variety identification, variety purity analysis, marker-assisted backcrossing and other purposes.

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# Achieving significant increases in productivity by exploiting Genotype - Environment - Management Interactions (GEMI)

**Background:** Climate change and input reduction in agriculture lead to a diversification of cropping environments with a higher expression of biotic and abiotic stresses. In this context, adapting the choice of cultivars according to their cropping environment is of special importance to increase winter rapeseed productivity. Crop cultivar assessment programs aim to evaluate relative performance of new cultivars, by subjecting them to multi-environment trials (MET), which are a series of field trials conducted across a range of geographic locations and sometimes over several years. Choosing a cultivar according to its global performance can be risky because of GEMI, which induce significant variations in the relative performance of cultivars when they are submitted to various climate conditions and crop management practices.

**Objectives:** The aim is to bring further statistical analysis of the data collected on MET, with a view to enrich the current information on commercial cultivars, and therefore improve recommendations on cultivar use. More precisely, one issue is to quantify GEMI for winter rapeseed. Another is to understand these complex interactions, and define adapted mega-environments for each cultivar. This is done by characterizing environments through the identification of production constraints like weather, soil and management variables.

**Methods:** We applied methodologies which have been developed for GEMI analysis on a rapeseed MET. First, rapeseed GEMI are described through an Analysis of Variance (ANOVA) model, partitioning yield variability into components linked to different sources of variation: genotype, environment, and GEMI. Then, a cluster analysis on GEMIs matrix allows us to define mega-environments (characterized by weather, soil and management practices conditions), where cultivars perform similarly against each other. Lastly, an existing approach like DiagVar (Lecomte 2005), combining agronomic diagnosis and dissection of GEMI, was applied to rapeseed MET, in order to characterize cultivar resistance. The used model expresses GEMI as the sum of the cultivar's specific responses to each of those stresses and resources (Denis 1988).

**Results:** Winter rapeseed is an interactive crop and requires more understanding about GEMI. Their contribution to total yield is far less than the environment contribution but more than the genotype contribution. Choosing a cultivar solely on the global performance criterion is risky: in about 30% of cases it is a mistake. Cultivar characterization by GEMI is a good way to get information about cultivars.

**Conclusions:** Today rapeseed GEMI analysis is decisive for yield improvement, but it is not usual because of several rate-limiting steps in the approach. One of them is the characterization of environments which is difficult and not precise. In order to improve this step, diagnostic methods will be developed and shared. The use of existing crop models would be an additional option, which could make it possible to acquire non-measurable variables.

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# Characterization of senescence-associated proteases activities related to with N leaf remobilization of winter oilseed rape at the vegetative stage

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**Background:** Oilseed rape (*Brassica napus L.*) is a crop plant characterized by a weak N use efficiency (NUE) mainly due to a low N Remobilization Efficiency (NRE) during the sequential leaf senescence at vegetative stage.

**Objectives:** To characterize the key mechanisms able to improve the NUE of oilseed rape, the objective was to identify senescence-associated proteases activities implied in N remobilization during leaf senescence

**Methods:** Plants were cultivated at the vegetative stage in greenhouse under limiting or ample nitrate supply (0.375 mM, LN; 3.75 mM, HN). We investigated leaf senescence processes (chlorophyll and anthocyanin contents; soluble proteins quantity) and proteases activities of a mature leaf becoming senescent during the experiment. The characterization of proteases activities was performed firstly by using in gelo analysis of the RuBisCO (RBCL) degradation with or without inhibitors of specific proteases classes. Then, to determine which proteases are responsible of the RBCL degradation, we performed standard protease activity profiling using activity-dependent probes specific of proteases classes.

**Results:** As expected, the mature leaf became senescent regardless of the nitrate treatment but LN conditions amplified senescence processes associated with a high degradation of soluble proteins. Then, characterization of proteases activities showed that: (1) aspartic proteases were active during senescence regardless of nitrate supply and (2) serine proteases, proteasome and particularly cysteine proteases (Papain-like cys proteases PLCPs and vacuolar processing enzymes VPEs) activities were increased when senescence processes were amplified by a LN supply. Moreover, three PLCPs were identified as playing a key role during leaf senescence. Furthermore, it was suggested that VPEs proteases might be responsible of the activation of some PLCPs.

**Conclusions:** As soluble proteins degradation in senescing leaves of oilseed rape is crucial for the improvement of N remobilization, characterization of proteases activities is a key for comprehension of N remobilization. Moreover, the NRE genotypic differences in LN conditions observed by Girondé et al. (2015) might be associated to different or contrasted proteases activities during senescence. Investigations of genotype variability were in progress in order to better characterize the proteases activities associated with a high N leaf remobilization efficiency.

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# Characterization of the *Sclerotinia sclerotiorum* population from canola in western Canada needed for selection of partially resistant *Brassica napus* germplasm

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**Background:** *Sclerotinia sclerotiorum* is a fungal pathogen with a wide host range which includes canola (*Brassica napus*). The disease can be controlled by fungicide application, but its sporadic occurrence reduces the likelihood of economic returns. As an alternative, quantitative resistance in *B. napus* germplasm is being utilized to develop Canadian varieties with improved level of stem resistance. Since canola is planted on 800.000 hectares in western Canada and sclerotinia is wide spread, we sought to characterize the *S. sclerotiorum* population to ensure resistance in future canola varieties is effective against the prevailing pathogen population.

**Objectives:** To characterize genetic and pathogenic variability of S. sclerotiorum in western Canada, and examine whether quantitative resistance identified in *B. napus* germplasm is effective against the pathogen population.

**Methods:** In 2010, sclerotinia isolates were collected from canola fields in Alberta (AB), Saskatchewan (SK) and Manitoba (MB). A sub-set of 128 isolates and one isolate from1992 were selected for genotyping. DNA were screened with 35 simple sequence repeat (SSR) markers designed using the *S. sclerotiorum* genome (Broad Institute) and 12 SSRs from Sirjusingh and Kohn (2001). Genetic variability was described by analysis of haplotype (FaBox), molecular variance and gene flow (GenAlEx), linkage disequilibrium (Multilocus), population structure (Structure), and cluster analysis (NtSYS). Isolates representing 17 sub-populations were evaluated for pathogenicity by inoculating stems of six *B. napus* germplasm lines with mycelium followed by measurement of lesion length over time which was used in statistical analysis of phenotypic variation (SAS).

### Results and Discussion

Screening of fungal DNA yielded 446 polymorphic alleles ranging from 2 to 35 alleles per marker. Each *S. sclerotinia* isolate was a unique haplotype, and 97% of the genetic variation was ascribed to isolates, while 3% was ascribed to geographical location. Gene flow (Nm) was highest between neighbouring Provinces MB and SK (54.0), less between AB and SK (13.4), and low between the two distant Provinces AB and MB (7.9). Analysis of linkage disequilibrium (LD) clearly showed a clonal population. Two distinct populations were identified by Structure analysis, while cluster analysis identified 17 sub-clusters. Pathogenicity tests showed the 17 representative isolates were statically different in pathogenicity, and the level of partial resistance differed among *B. napus* lines. Furthermore, there was a significant *S. sclerotinia* by *B. napus* interaction indicating the presence of pathotypes in the Canadian population similar to findings in Australia by Ge et al. (2012). Germplasm line, PAK54, from Pakistan had the highest level of partial resistance across all isolates and is therefore a good candidate for transfer of quantitative resistance to Canadian canola varieties. The other lines ranked PAK93 (Pakistan), K22 (Japan), DC21 (South Korea) and Tanto (France) since resistance was lower to one or more of the isolates.

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# Transferring *Sclerotinia* resistance from *Brassica incana* into oilseed rape

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**Background:** Stem rot caused by fungal pathogen *Sclerotinia sclerotiorum* is a great threat for oilseed production in the world. In the previous studies, two resistance QTL totally explaining more than 30% and 60% of variances for stem and leaf resistance, were identified from *B. incana*, a wild *B. oleracea* with high level of resistance against *sclerotinia* (Mei et al, 2011 and 2013).

**Objectives:** the sclerotinia resistance should be transferred from *B. incana* into oilseed rape due to high colinearity between their genomes.

**Methods:** This line of *B. incana* as resistance donor was crossed with *B. rapa*, and the backcross progenies with *B. rapa* were selected with molecular markers linked with resistant loci together resistance evaluations.

**Results:** F1 was higher than *B. rapa* and partial to *B. incana* for the resistance, indicating that the behavior of resistance was partial dominant manner. Although low fertility was found in F1 and BC1F1 derived from parental *B. rapa*, the individuals of BC2F1 having the same chromosome number with *B. rapa* exhibited normal fertility. The lines with resistance loci from *B. incana* were significantly stronger than those without resistance QTL for resistance in BC2F1 and BC2F2. Further, 13 BC2F2 lines with 1.4- to 2-fold higher resistance in stem than a partial resistance line of *B. napus*, 'Zhongshuang 9', were chosen to develop new type *B. napus* via crossing with a hexaploid (AACCCC) derived from 'Zhongshuang 9' and resistant '*B. incana*' (Mei et al. 2015).

**Conclusion:** Our data suggest the resistance from *B. incana* is transferred into *B. rapa*. Now new type *B. napus* lines are being selected in order to pyramid resistance in both A and C subgenomes.

**References:**

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# Genome-wide analysis of population structure and linkage disequilibrium in Chinese semi-winter rapeseed

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**Background:** High-density SNP genotyping arrays are a powerful tool for genome-wide association studies and can give valuable insight into patterns population structure and linkage disequilibrium (LD).

**Objectives:** In this study we used the *Brassica* 60kSNP Illumina consortium array to assess the genetic diversity, population structure and the extent of LD and haplotype blocks in a diverse panel of 203 Chinese semi-winter rapeseed inbred lines.

**Methods:** Genome wide SNP data were used to calculate population structure. LD was calculated on the genome, subgenome and chromosome scale. Conserved homoeologous haplotype blocks were investigated for associations to known QTL for important traits.

**Results:** Population structure and principal coordinate analysis, using a subset of the SNPs, revealed diversification into three subpopulations and one mixed population, reflecting targeted introgressions from external gene pools during breeding. Pairwise LD analysis within the A- and C-subgenomes of allopolyploid *B. napus* revealed that mean LD, at a threshold of $r2 = 0.1$, decayed on average around ten times more rapidly in the A-subgenome (0.25-0.30 Mb) than in the C-subgenome (2.00-2.50 Mb). A total of 3,097 conserved haplotype blocks were detected over a total length of 182.49 Mb (15.17% of the genome). The mean size of haplotype blocks was considerably longer in the C-subgenome (102.85 Kb) than in the A-subgenome (33.51 Kb), and extremely large conserved haplotype blocks were found on a number of C-genome chromosomes. Comparative sequence analysis revealed conserved blocks containing homoloeogous quantitative trait loci (QTL) for seed erucic acid and glucosinolate content, two key seed quality traits under strong agronomic selection. Interestingly, C-subgenome QTL were associated with considerably greater conservation of LD than their corresponding A-subgenome homoeologues.

**Conclusions:** The data we present in this paper provide evidence for strong selection of large chromosome regions associated with important rapeseed seed quality traits conferred by C-subgenome QTL. This implies that an increase in genetic diversity and recombination within the C-genome is particularly important for breeding. The resolution of genome-wide association studies is also expected to vary greatly across different genome regions.

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# Comparative quantitative trait loci for silique length and seed weight in *Brassica napus*

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**Background:** Silique length (SL) and seed weight (SW) are important yield-associated traits in rapeseed (*Brassica napus*). Although many quantitative trait loci (QTL) for SL and SW have been identified in *B. napus*, comparative analysis for those QTL is seldom performed.

**Objectives:** With the release of reference genomes for *Brassica* crops, such as *B. napus, B. rapa* and *B. oleracea* (Chalhoub et al., 2014; Liu et al., 2014; Wang et al., 2011), it should be feasible to conduct genomic comparative analyses in *Brassica* crops.

**Methods:** A *B. napus* DH population, consisting of 261 lines, from a cross between the European winter cultivar 'Express' (female) and Chinese semi-winter line 'SWU07' (male), were evaluated together with an F2 population (RC-F2) derived from DH lines for silique length and weight at maturity in the experimental field of Southwest University, Chongqing, China, in 2010, 2011 and 2013. The QTL were identified with the composite interval mapping (CIM) procedure of the software WinQTL Cartographer 2.5 by integrating the data of field trial and SSR. The SL and SW QTL regions were aligned to the reference genomes of *B. rapa, B. napus* and *B. oleracea* by BLAST analysis of the sequences of SSR markers or SSR primers linked with QTL.

**Results:** 20 and 21 QTL for SL and SW were identified, totally explaining 55.1–74.3% and 24.4–62.9% of the phenotypic variations across three years, respectively. Of which, 17 QTL with partially or completely overlapped confidence interval on chromosome A09, were homologous with 2 overlapped QTL on chromosome C08 by aligning QTL confidence intervals with the reference genomes of *Brassica* crops. By high density selective genotyping of DH lines with extreme phenotypes, using a *Brassica* single-nucleotide polymorphism (SNP) array, the region of major QTL on chromosome A09 was aligned to a 1.14Mb and 1.05 Mb region on the reference genome of *B. rapa* and *B. napus,* respectively.

**Conclusions:** The alignment of QTL in rapeseed with *Brassica* reference genomes revealed homologous QTL on A09 and C08 chromosomes for SL. The major QTL on chromosome A09 was aligned to a ~1Mb region on the reference genome of *B. rapa* and *B. napus.*

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# Nine years canola breeding from scratch at North Dakota State University

**Background:** North Dakota is the leader in canola acreage and production with over 83% of U.S. acreage and produces about 84% of all U.S. canola (1.02 million acres and 1.7 billion pounds with a value of $364 million – 5 yr. average from 2009-2013; USDA-NASS). Canola ranked 4th most important field crops in North Dakota. Recently, construction of two canola based processing plants requires a three-fold increase of canola production in North Dakota. To meet the anticipated demand, it is necessary to improve the genetic potentiality of breeding lines adapted to this region. Without better performance and better adaptation, the demand for US canola will not be met, and the processing plants will need to look to foreign sources of feedstock. Basically, the spring canola varieties grown in this region are developed elsewhere and are may not highly adapted to this climatic and agronomic region. Therefore, North Dakota State University (NDSU) initiated a modern spring canola breeding program in 2006.

**Objectives:** The objectives of this program are to develop high oil per acre canola germplasm with better agronomy adapted to North Dakota.

**Materials and Methods:** A wide diversified *Brassica* germplasm with both roundup ready and conventional types have obtained from both private and public sectors across the world. Crossing and backcrossing have made between genetically diverse winter type with spring type canola, and spring type with spring type canola. Interspecific crosses were also made among *B. napus, B. rapa, B. juncea* and *B. carinata*. A modified pedigree breeding with early generation testing followed by multi-location field trials have been using in this program. A total of 367 diversified *B. napus* lines have been partially sequenced using GBS pipeline.

**Results:** A high oil variety (NDSU-662c) was released for the 1st time from this program in 2011. Three hybrids with high seed yield and high oil content were identified and are in process to release. Several hundreds of advanced breeding lines have been developed from the program. Canola double haploid production and molecular marker technology are already in place to accelerate the breeding line development program. The breeding program obtained a licensing agreement with INRA, France to utilize Ogura-CMS and restorer (R-2000) system for inbred line development for hybrid production. A collaborative breeding research has established with Monsanto and DL Seeds Inc. Both greenhouse and growth chamber are utilizing to grow canola in controlled environment. The seed quality lab is equipped with near infrared spectrophotometer for seed oil, seed protein and fatty acid profile analysis. Disease screening facilities are available both in greenhouse and in field under artificial inoculation system. The capacity of field plot testing and harvesting program is about 5,000 plots per year at seven trial locations across the state. Off-season (winter) nursery is located in Santiago, Chile that significantly reduces the breeding cycle. All necessary field and lab equipment have been purchased for this program.

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# Use of *Brassica rapa L.* to increase the genetic diversity in spring canola (*B. napus*)

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**Background:** Genetic diversity in Canadian spring *Brassica napus L.* (AACC, 2n = 38) canola need to be broadened (Juska et al. 1997, Rahman 2013). Some efforts have been made to broaden genetic diversity and productivity of spring canola by use of winter type (Kebede et al. 2010, Rahman and Kebede 2012); however, very limited efforts made to utilize genetic diversity of its allied species as interspecific cross often introduce many unwanted alleles and cause meiotic anomalies in segregating population (Falk 2010, Rahman 2013).

**Objective:** To increase the genetic diversity in Canadian canola germplasm and to study the inheritance of glucosinolates content and response to selection for low glucosinolates content in the subsequent progenies. To estimate the genetic diversity in the newly developed *B. napus* lines.

**Method:** A breeding research was undertaken to broaden the genetic base of the Canadian spring *B. napus* canola through introgression of allelic diversity from *B. rapa* (AA, 2n = 20). For this, interspecific hybridization between *B. napus* and *B. rapa* were done and pedigree breeding was applied to develop canola quality euploid *B. napus* lines. Agronomic and seed quality traits, such as silique length, number of seeds per silique and seed glucosinolates content, and ploidy level of the interspecific crosses derived populations was assessed in different generations. Genetic diversity in the population was estimated by SSR molecular markers.

**Results:** Repeated selection for glucosinolates content resulted many low glucosinolates families in advanced generation. Ploidy level of many plants had reached close to the *B. napus* parent. SSR marker analysis revealed that the new interspecific derived families are genetically distinct from *B. napus* parent.

**Conclusion:** Intensive selection cycles for canola quality traits and ploidy level over generations in interspecific crosses progenies can produce desired result. Genetically distinct and fertile *B. napus* type canola quality germplasm was developed from in this experiment from *B. napus* × *B. rapa* F2 derived population.

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**POSTERS THEME A**

# Genotyping-by-Sequencing, DArTseq platform for genome analysis in *Brassica napus*

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**Background:** Genotyping-by-sequencing based on genome complexity-reduction methods provides a cheaper alternative for genome analysis. In this study, we report the utilisation of DArTseq™ markers for assessment of genetic diversity, construction of ten linkage maps and a consensus map comprising ~100,000 markers. We will also report marker-trait associations identified using classical QTL and genome-wide association (GWA) approaches.

**Objectives:** To test the usefulness of DArTseq markers for molecular breeding applications in canola.

**Methods:** DArTseq analysis was carried-out as described previously (Raman et al. 2014). Phylogenetic analysis among 219 accessions of *B. napus* and related species was performed using ~25,000 SNPs. A consensus map from ten DH populations originating from Australia, Canada, China and Europe was constructed as described previously (Raman et al. 2013). Mapping populations and a diversity panel comprising 180 lines of *B. napus* were phenotyped for agronomic traits such as resistance to blackleg, NDVI, CID, WSC, and flowering time for QTL and GWA analysis.

**Results:** DArTseq markers discriminated all accessions of *Brassica* into distinct groups representing different species/origin. We constructed the first consensus map consisting of 95,663 markers, comprising mainly DArTseq, and SNP markers present in 60K illumina infinium array. Generally the consensus map was in agreement with component maps of individual populations and the physical map positons of DArTseq markers on the published reference genomes of *B. napus, B. rapa* and *B. oleracea*. Data on trait-marker associations using classical QTL and GWA analyses will be presented. Some of the loci detected with GWA were confirmed by QTL mapping. Putative candidate genes implicated in target traits were identified within up to 40kb genomic regions.

**Conclusions:** DArTseq markers provided a suitable platform for molecular breeding applications such as assessment of genetic diversity, construction of genetic linkage maps, identification of molecular markers associated with traits of interest and delineation of genome structure based on chromosomal ancestral blocks of *Brassicaceae*.

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# Candidate gene based association mapping for introgressed resistance against *Sclerotinia sclerotiorum* in *Brassica juncea*

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**Background:** *Sclerotinia* stem rot caused by *Sclerotinia sclerotiorum* is a major threat to Oilseed *Brassica* production systems across the world. Complete resistance against this pathogen has never been reported. We had previously reported success in introgression of the high level of resistance to Sclerotinia stem rot in *B. juncea* from wild crucifers (Garg et al. 2010). For present communication we used *B.juncea-fruticulosa* introgression set to carry out candidate gene based association mapping to genetically characterize resistance by focusing on the genes of known function. The introgression set is known to carry varying proportions of genomic segments from *B. fruticulosa*.

**Objectives:** To associate the allelic variation at selected functional candidate loci, with introgressed resistance to sclerotinia stem rot.

**Methods:** A set of 208 BC1S5genotypes were assessed for stem rot resistance. The resistance responses were evaluated by stem inoculation method described by Buchwaldt et al. (2005). These lines were sorted into five different classes based on stem lesion length. We downloaded the genomic sequences of 14 genes previously reported to be associated sclerotinia resistance in *Arabidopsis.* Sixty primers were designed for 14 candidate genes. These primers were used to amplify genomic region of the candidate gene in an association mapping set (91 genotypes). Proc glimmix in SAS 9.4 (SAS Institute Inc., Cary North Carolina, USA) is used for statistical analysis of phenotypic data. Marker-trait associations were investigated by using P3dem, P3demma models performed in TASSEL V 2.1 (Bradbury et al. 2007).

**Results:** Seven significant marker-trait associations could be recognized at a Bonferroni-corrected threshold (–log 10 (P) > 3.388). Of these two markers, NPR-4_1 (4.413) and CYP450-2_2 (20.532) (common in two models, P3dem, P3demma) were found to be significantly associated with candidate genes NPR and CYP450 that are known for defensive response against sclerotinia stem rot. Other markers MYB-1_3, IGMT5-1_2, CYP450-4_1, PAD3-1_1 and PAD3-2_2 also showed significant associations with sclerotinia resistant MYB domain, IGMT and PAD3 genes.

**Conclusions:** The candidate gene markers associated with sclerotinia stem rot resistance can potentially be exploited by breeders for screening of resistant genotypes, which further will improve the efficiency of MAS programmes and cloning of genes help in trait transfer to commercial mustard genotypes.

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**POSTERS THEME A**

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# Development of ultra-high erucic acid *Brassica carinata* using interspecific hybridization

**Background:** The competitiveness of Canadian agriculture and related industries depend on Canada's ability to exploit new opportunities, particularly as they relate to the emerging bio-economy. Crop development and species diversity are an important aspect of the bio-economy, as is maximizing crop value through total crop utilization in the value chain. Ethiopian mustard has long been identified as a viable industrial oil crop for the hotter and drier regions of western Canada. Also, owing to its inherent mid to high erucic acid content it is an ideal candidate to be a superior feed stock for high value bio-products such as bio-fuels and lubricants.

**Objectives:** To develop an ultra-high erucic *B. carinata* strain

**Methods:** During the development of canola quality *B. juncea* a FAD2B mutant was identified which had high levels of oleic acid. This mutation was subsequently introgressed into *Brassica carinata*. The breeding line VR10-183 was developed as a stable FAD2B mutant after several backcrosses. Work to further increase erucic acid levels was undertaken via a second round of interspecific hybridization using *B. rapa* spp. yellow sarson cv. R500 (C22:1 content >50%) and the FAD2B mutant. The breeding line VR13-156 was identified after several backcross generations. It was field tested in 2014. Molecular markers were developed to aid in the selection process.

**Results:** The FAD2B *B. carinata* line VR10-183 contains 53% erucic acid. The FAE gene was isolated from R500 and compared to the FAE alleles in VR10-183. A CAPS marker was developed for screening purposes to ensure that all three copies of FAE were maintained during the crossing scheme. Subsequently VR13-156 was identified as being homozygous for both the FAD2B and R500 FAE alleles. The C22:1 content of greenhouse produced seed was 57.5%. Progeny from this material was tested in the field in 2014 with an average erucic acid content of 58.25%. A KASP marker was developed to aid in the introgression of the two genes into more genetically diverse backgrounds.

**Conclusions:** The successful development of an ultra-high erucic acid *B. carinata* was accomplished by interspecific hybridization and the successful transfer of the FAD2B and FAE alleles. A *B. carinata* line with high seed oil, early maturity and high seed yield will ensure that this species becomes the crop of choice for industrial oil applications.

# Comparative transcript profiling of the bilocular and triolocular shoot apical meristems and ovarys in *Brassica juncea L.*

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**Backgrounds:** Compared with bilocular plants, multilocar ones shown a higher yield per plant attributed by the increase in the number of seeds per silique. In recent years, some genetic analysis works in *Brassica juncea* have significantly widen our knowledge about the trait. However, the genes related to multilocular trait have not been cloned and the molecular basis of the locule number determination of *B. juncea* still remained unknown. The plant materials used in this study were BC5 and BC6 populations constructed by two *Brassica juncea* lines, J163-4 with triloculus and J248 with biloculus.

**Objectives:** The aim of this work was to identify the differences between the bilocular and trilocular shoot apical meristem (SAM) and ovary of *Brassica juncea L.* at the transcriptional level, and find out the different genes involved and their related functions. These results might be helpful to understand the molecular basis of the locule number determination.

**Methods:** Bilocular and trilocular SAM of BC5 populations and ovary of BC6 were used in this study. After the work of RNA isolation and purification, cDNA library construction, the libraries were paired-end sequenced with 100 bp on the Illumina Hiseq 2000. Clean reads were de novo assembled into transcripts by Velvet/Oases. Functional annotation of transcripts was performed using BLASTX searches against the *Arabidopsis*, KEGG, eggNOG databases and the non-redundant unigenes were obtained (E-value < 1e-5). The GO annotations for the unigenes were determined and the differentially expressed unigenes were obtained. The Real-time quantitative PCR vertification was employed to verify these unigenes.

**Results:** A total of 139,339,803 sequences were successfully obtained and assembled into 168,783 transcrits, which composed the transcriptomes of the SAM and ovary. After functional annotation, 23,412 unigenes were got. A total of 825 and 709 unigenes were obtained with significant expression difference for SAM and ovary, separately. In SAM, these expression different unigenes were mainly enriched in metabolism, membrane transport, signal transduction pathways, while these unigenes in ovary were mainly enriched in energy metabolism, genetic information processing, cell growth death and some organismal pathways. Some unigenes involved in SAM homeostasis, phytohormone responses and signal transduction were significantly differently expressed.

**Conclusions:** These results showed that phytohormone responses and signal transduction may play a part in trilocular silique formation. In SAM, IAA19 and SAUR42 were significantly down expressed in triloclar sample and ARR16 up regulated. In our results, CLV3 and ROP9 were up regulated signicantly in SAM.

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# Tribenuron-methyl induced male sterility resulted from anther-specific acetolactate synthase inhibition in *Brassica napus*

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**Background:** Male sterility induced by chemical hybridizing agents (CHA) is a potent tool for utilizing crop heterosis. Sulfonylurea herbicide tribenuron-methyl (TM) inhibits branched-chain amino acid (BCAA) biosynthesis in plants by targeting acetolactate synthase (ALS) (Binder, et al. 2007), and low dose TM has been widely used as an effective CHA in rapeseed (*Brassica napus*) (Yu et al., 2006).

**Objectives:** Although TM-induced male sterility is wildly used for utilization of heterosis, the molecular basis of this trait remains unknown.

**Methods:** Targeted expression of csr1-1 (McCourt et al., 2006) and CYP81A6 (Liu et al., 2012), TM daubing in special branches, and silencing of genes in BCAA biosynthesis pathway ALS and KARI (Binder, et al. 2007) were executed. Several previously defined promoters were used, including Elongation factor-1α, 35S, APETALA3, SUCROSE TRANSPORTER 2, LIGHT-HARVESTING COMPLEX B2.1, and ABORTED MICROSPORES. At least 10 independent transformed lines were generated for each construct, and the pollen viability of rapeseed treated or control were analyzed by aceto-carmine staining.

**Results:** Both over expression and anther-specific expression of target-site resistance gene csr1-1 reversed the TM-induced male sterile phenotype in rapeseed. Targeted expression of csr1-1 in vegetative tissues or the early stages of stamen and petal development remained show male sterile phenotype after TM treatment. Sterile anthers only occurred when the side branches daubed with TM or in all higher branches above main stem daubed with TM, while the rest of the branches were fertile. The male fertile phenotype of lines expressing metabolism-based resistance gene CYP81A6 in mesophyll, phloem or vegetable tissues, and TM daub-stem experiment uncovered evidence that TM was mainly polar-transported from leaves to anthers through mesophyll and phloem. The percentages of BCAAs were significantly decreased after CHA TM spraying. Silencing of genes in BCAA biosynthetic pathway also led to low male fertility.

**Conclusions:** We reported a polar transportation of TM and anther-specific inhibition of ALS based mechanism to understand the selective male sterility in TM-induced rapeseed. As sterile anthers could be induced in special branches by daubing TM to selected branches, the costly and time-consuming manual emasculation in near-isogenic lines and construction of hybrid combinations would be replaced by daubing stem with TM.

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**POSTERS THEME A**

# Fine mapping of the trilocular gene *Bjmc1* and identifying the candidate gene in *B. juncea*

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**Background:** In rapeseed breeding, the most important goal is to develop cultivars with high yields. Previous studies have proved that multilocular rapeseed plants generally have higher yield than bilocular plants (Bechyně, 1995; Katiyar et al. 1998; Lv et al. 2012).

**Objectives:** In this study, we aim to investigate the inheritance of a landrace with trilocular siliques in *Brassica juncea* in China, and to clone the trilocular gene *Bjmc1*.

**Methods:** Based on several populations described by Xu et al (2014), BSA method were used to developed SSR and AFLP markers, and BAC clones of the purple-leaf mustard were used to develop SSR and SCAR markers. Finally, a large population (9300 individuals) of *Bjmc1* constructed in BC4, BC5 and BC6 population was used to construct the map of *Bjmc1* gene.

**Results:** The result showed that the trilocular trait was controlled by two independent recessive nuclear genes, *Bjmc1* and *Bjmc2*. *Bjmc1* was preliminarily mapped by 24 AFLP markers and 7 SSR markers. Five AFLP markers linked to the target gene were successfully converted into SCAR markers (Xu et al, 2014). In order to fine mapping the *Bjmc1* gene, the BAC library of purple leaf mustard (provided by Prof. Zhongsong Liu) was screened, and the positive clones were sequenced. According to the sequence, a SCAR marker (SC40) and two SSR markers (SR52 and SR151) were developed further. All the molecular markers that we have identified were used to screen the large population (9300 individuals). We found that EC14MC14 and SR151 were the closest markers in the map, and the genetic distance of these two genes were 1.1 and 0.04 cM respectively. In addition, the result indicated that SC40 was co-segregated with *Bjmc1* gene. We sequenced the open reading frames (ORFs) around the SC40 ranging of 75kb in bilocular and trilocular plants respectively. Interestingly, no sequence difference was found in all the ORFs except the ORF40.

**Conclusions:** Since only the ORF40 showed sequence difference between the bilocular plants and trilocular plants and the homologous gene mutant of ORF40 in *Arabidopsis* also exhibited multilocular trait, we predicted that the ORF40 was the candidate gene of *Bjmc1*. And now the genetic complementation experiment is performing to test the candidate gene.

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# Differential expression of small RNAs in the shoot apical meristem regulate the plant architecture in *Brassica napus*

**Background:** Increasing crop yield and mechanized harvesting are major challenges for modern agriculture. Plant architecture is an important agronomic trait, strongly influencing the suitability of a plant for cultivation, its yield and the efficiency with which it can be harvested (Reinhardt and Kuhlemeier, 2002). MicroRNAs (miRNAs) are endogenous small RNAs that play crucial regulatory roles in various developmental processes (Bartel, 2004). The molecular genetic bases of plant architecture focused on the activity of its shoot apical meristem (SAM), and initiates outgrowth of axillary meristem (AM), when AMs start to develop lateral organ branches, and correct timing for reproduction and senescence(Li, 2008). Although several genes have been found to regulate these traits, there is a lack of information on the expression profile of miRNAs regulate plant architecture in the oil crop *Brassica napus*.

**Objectives:** Desirable plant architecture greatly improve yield. The purpose of this research is to find miRNAs that regulate the development of SAM, and discovery some new miRNAs that have not been reported in *Brassica napus*.

**Methods:** A near-isogenic line of plant architecture was constructed. Squaring shoots from the normal plant architecture (Normal) and the mutant rod-like plant architecture (Rodlike) were sampled, respectively. Then two small RNA libraries and their corresponding degradome libraries were constructed and sequencing in Beijing Genomics Institute (BGI, Shenzhen, China). The bioinformatics analysis of sequencing data were analyzed as previously described. The stem-loop RT-PCR method was used in the quantitative RT-PCR experiments of miRNAs. To examine the miRNA-directed cleavage of their predicted targets in vivo, the RLM-5′ RACE was used to find the cleavage site.

**Results:** Small RNA sequencing identified 108 known *Brassica* miRNAs and 261 novel miRNAs, including 53 novel miRNAs that were highly homologous to other plant species. To our surprise, only six known miRNA families were found to be differentially expressed, but more than 130 novel miRNAs were identified differentially expressed and most of them just expressed in one of the samples. In addition, a total of 258 transcripts in Normal and 239 transcripts in Rodlike were found to be targets identified through degradome sequencing. Analysis of correlated expression between differentially expressed miRNAs and their targets demonstrated that plant hormone signal transduction and many transcription factors cooperate to balance the stem cell maintenance and differentiation.

**Conclusions:** Our approach identified potential key regulators of miRNAs in the SAM of *Brassica napus* during shoot development. The results provide novel insight into the regulatory roles of miRNAs related to *Brassica napus* plant architecture. The discovery of novel miRNAs will sever as a foundation for further research in *Brassica napus* miRNAs.

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# Characterizing growth physiology, flowering phenology, yield components and seed quality attributes in founder lines of a spring *Brassica napus* Nested Association Mapping population

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**Background:** The Nested Association Mapping (NAM) strategy facilitates the dissection of complex traits important for crop improvement (McMullen et al, 2009). Recent field and greenhouse studies have focused on describing physiological and seed quality characteristics of the founder and reference lines from an annual *Brassica napus L.* NAM population. Both above- and below- ground growth characteristics were measured to characterize growth physiology, flowering and maturation phenology, yield attributes, non-target metabolite profiles of leaves and seeds and seed quality variation. Results of this study will also establish the links between phenotypes and molecular variants to accelerate genetic improvement of spring canola through association mapping.

**Objectives:** The objectives of the study were to: i) examine if differences between lines exist for photosynthetic assimilation rate (A), stomatal conductance (gs) and water-use efficiency (WUE) ii) to study the extent of plasticity in flowering phenology iii) to profile non-target metabolites of foliar and seed components, iv) to investigate the differences in root architecture that may exist among spring *B. napus* founder lines and v) to compare the results obtained from the greenhouse study with field evaluation of founder lines in 2014.

**Methods:** A total of 61 lines that included 50 NAM founder lines and the reference line, four additional diverse lines, historic commercial cultivars Tower, Westar and Midas and three representative commercial lines were used in this study. Four replications of the plants, grown under standard greenhouse conditions, were characterized throughout development for several growth physiology, flowering and yield-related traits. Chlorophyll content was measured using SPAD-502 chlorophyll meter, stomatal conductance using a leaf porometer, leaf area using a Licor LI-3100. Root systems were examined for root architectural traits. In the 2014 field season, four replications of the lines were grown at two locations and characterized for a range of plant, canopy and seed related characteristics.

**Results:** The results obtained from the 2014 field experiment demonstrated significant phenotypic variation for growth and developmental and seed quality traits among the lines. The present study further quantified this variation and explained the extent of genetic variability existing among the founder and reference lines in growth physiology, flowering phenology and yield related attributes.

**Conclusions:** The results from this study will be used in canola breeding programs to develop cultivars with improved agronomic and nutritional characteristics with the ultimate goal being identification of yield-stability related traits under variable environmental conditions. These results will be used to target phenotypic traits for study within the NAM population where the underlying components of the traits will be dissected and associated with genomic regions, identifying markers linked with economically important traits.

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**POSTERS THEME A**

# An *Arabidopis* gene (*AtGRP1*) increases seed yield when overexpressed in *Brassica napus*

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A gene encoding a protein (GRP1) of unknown function was isolated from *Arabidopsis* and characterized. Overexpression of *AtGRP1* (35S::*AtGRP1*) in Arabidopsis resulted in plants with increased number of and taller racemes than the wild type and resulted in increased seed yield. An *AtGRP1-YFP* fusion introduced into tobacco protoplasts suggested that *AtGRP1* was located in the nucleus. Expression of the 1.9 kb *AtGRP1* promoter fused to GUS in *Arabidopsis* indicated that the gene was expressed strongly in vascular elements of leaves, stems, floral tissues and siliques but not in seeds. Generation of homozygous lines knocked out for *AtGRP1* was unsuccessful suggesting that *AtGRP1* may be essential for plant development. DNA sequences orthologous to *AtGRP1* were not detected in *B. rapa* or *B. napus* genome sequences so *35S::AtGRP1* was introduced into *B. napus* cv Westar in order to assess if an observable phenotype similar to *GRP1* overexpression in Arabidopsis could be generated and to provide insights into the role of *AtGRP1* in plant related processes. Homozygous lines of transgenic *B. napus* overexpressing *AtGRP1* indicated an increased proliferation of racemes than the wild type and under strictly controlled growth cabinet conditions yielded 15-20% more seed than the wild type. Elucidation of the role of *AtGRP1* in plant development is in progress.

# Development of genic microsatellite markers and analysis of genetic variability in *Brassica juncea L.*

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**Background:** *Brassica juncea L.* is the second major oilseed crop in India. Since the turn of the last century more than 150 high yielding varieties have been released. They possess a great deal of similarity with respect to morphological descriptors available for their identification and differentiation. Therefore, unequivocal identification of increasing number of varieties enforces to look for alternatives. We report the development of a comprehensive set of genic microsatellite (SSR) markers through deep transcriptome sequencing, and evaluation of its efficacy in revealing genetic variability in *B. juncea* varieties.

**Objective:** Development of a comprehensive set of genic SSR markers in *B. juncea.*

**Methods:** Total RNA isolated from *B. juncea* cv. CS-52 was used for preparation of pair end cDNA sequencing library. Expressed sequence reads were generated by whole transcriptome sequencing on Illumina MiSeq platform. High quality filtered sequence reads were assembled using CLC Genomics Workbench 7.5. The unigenes were used for mining genic SSR markers, and their primers were designed using BatchPrimer3 v1.0 software. Genic SSR loci with SSR lengths ≥ 18 bp were tested for amplification using genomic DNA from *B. juncea* cv. CS-52. The optimized SSR primers were then used for PCR amplification in nine *B. juncea* genotypes of diverse nature. The genotype profiles produced by SSR markers were used for calculating PIC values (Botstein et al. 1980). Thirty highly polymorphic SSR markers were used for analysis of genetic variability in 70 *B. juncea* varieties. The genetic similarity was estimated based on Jaccard's similarity coefficient. All the 70 *B. juncea* varieties were clustered with the UPGMA analysis and SAHN procedure of the NTSYS-PC v2.10t (Rolf 2000).

**Results:** The Illumina MiSeq sequencing generated 47962057 expressed sequence reads. These reads were assembled into 45,280 unigene contigs from which a total of 4,100 SSR loci were identified. Trinucleotide was the most common repeat unit with a frequency of 59.88% followed by di- (38.73%), tetra - (0.71%), hexa - (0.44%) and pentanucleotide repeats (0.24%). PCR primers were designed from the unique sequences flanking 3,141 SSR loci out of which 460 were selected for primer synthesis. A total of 340 genic SSR loci amplified successfully of which 42.6% exhibited polymorphism among nine *B. juncea* genotypes with PIC values ranging from 0.18 to 0.81. Thirty highly polymorphic markers, selected on the basis of PIC values, were used for analysis of genetic variability in 70 commercial varieties of *B. juncea.* The dendrogram obtained by the UPGMA analysis clearly distinguished them in definitive groups corresponding well with their published pedigree.

**Conclusions:** We developed 4,100 genic SSR markers in *B. juncea* by whole transcriptome sequencing. Out of this 340 were validated using a set of nine diverse *B. juncea* genotypes. Genetic variability study among 70 commercial B. juncea varieties using 30 highly polymorphic markers selected from the 340 validated markers effectively distinguished even the closely related cultivars.

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# Evaluation, characterization and classification of toria germplasm

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**Background:** *Toria* [*Brassica rapa (L.)* var. *toria*] holds promise among all the *Brassicas* due to its significance as a valuable donor source for high temperature stress at seedling stage. This crop is gaining importance globally due to its advantages over other oilseeds, viz., higher yield potential, low moisture requirement, higher return at low cost of production, wider adaptability for various farming conditions, etc., which is important towards having the next yellow revolution. Narrow genetic base of the crop for component traits is the main constraint for the cultivation of this crop.

**Objectives:** Evaluation of phenotypic diversity usually reveals key traits of interest to the plant breeder. Broadening of genetic base through utilization of diverse genetic resources and relevance of broad genetic base in evolving new cultivars by incorporating new genes in the existing ones is quite vital.

**Methods:** Agronomic characterization continues to be a useful tool for the classification of germplasm, as it allows plant breeders to select valuable genetic resources to be utilized later in different breeding programmes. Ninety two germplasm accessions of toria collected from different sources were grown evaluated for eight quantitative variables and 14 qualitative traits by giving scores in accordance with the standard DUS descriptor.

**Results:** Among all the 92 accessions studied, 76 lines were observed to have medium number of lobes (3-5) in the leaf except 16 lines which had many lobes (>5). Majority of the germplasm (79 lines) had narrow leaves (<7.0 cm). A total of 52 lines were early in flowering (31-40 days) whereas, all the germplasm was medium in maturity (81-100 days). On the basis of branches, most of the lines (89) were classified under intermediate category (8-14 branches). Four accessions were classified as short (<51 cm) on the basis of height, whereas, 11 lines were characterized as tall (76-100 cm). Half of the accessions were having medium main shoot length (41-60 cm) and siliqua density was found high in 48 lines. Moreover, most of the genotypes possessed short siliqua length (<4.5 cm). A large number of rapeseed mustard germplasm was also evaluated and characterized for various agro-morphological traits and biotic stresses by Misra et al. (2004) and Misra and Kumar (2009). In the present study, 12 lines were having few no. of seeds/ siliqua (<11), 62 lines recorded intermediate no. of seeds/ siliqua (11-20) and 18 lines exhibited many seeds/siliqua (>20). Medium seed size (3.0-4.0 g) was most common and observed in 52 lines. Seed yield/ plant grouped all the germplasm into 2 categories viz. Low (<10.0 g) and medium (10.0-20.0 g) of which 32 lines were grouped into low and remaining 60 in medium. On the basis of oil content, majority of the lines (65 lines) were characterized into medium (38-42 %) and the remaining 27 lines were having high oil content (43-46 %). Zada et al. (2013) also reported sufficient genetic variation in 134 germplasm collections of Ethiopian mustard on the basis of characterization for 33 agro-morphological characters ranging from seedling emergence to crop maturity.

Qualitative traits like leaf hairiness, leaf color and dentation of leaf margin were examined 45 days after sowing. The difference for hairiness grouped all the accessions into 3 categories (dense, sparse and absent). Leaf colour of more than 40% genotypes was dark and purple green, whereas 17 lines were having purple leaves. Lyrate dentation of leaf margin was the most common followed by auriculate type. Yellow and light yellow colour was most dominant for petal, whereas 5 lines had white petal colour. On the basis of siliqua surface texture all the genotypes were classified into three categories viz. intermediate (53 lines), constricted (28 lines) whereas, 11 lines had smooth siliqua surface texture. Semi-appressed siliqua angle with main shoot was most common (44 lines) followed by open (30) and appressed type (18 lines).Seed coat colour divided all the germplasm into five categories (yellow in 2 lines, dull grey in 16, reddish-brown in 37 lines, brown in 28 lines and black in 9 lines).

**Conclusions:** The characterization of germplasm will provide valuable information for strengthening of future breeding programme on *toria*. It will be helpful in formation of data base and reference lines/core collection for further use.

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# Genetic enhancement of *Brassica carinata* through interspecific hybridization and population improvement

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**Background:** *Brassica carinata* originated in highlands of Ethiopia is a natural alloploid of *B. oleracea* and *B. nigra*. It has been found to be a potential oilseed crop in different agro-ecological conditions due to its inherent properties (Malik 1990, Rakow and Getinet 1998). However, its cultivation has not been commercially feasible in India due to long maturity duration, tall plant stature and low oil content in comparison to widely grown *Brassica juncea*. It would be desirable to reshape the existing genotypes through introgression of earliness, short plant height and high oil content from *Brassica juncea*.

**Objectives:** to develop *Brassica carinata* genotypes which may be acclimatized in Indian agro ecological situations through introgression of earliness, short plant height, bold seed size and high oil content from *B. juncea* with desirable traits of *Brassica carinata* (profused branching, more siliquae and tolerance/resistance against abiotic and biotic stresses)

**Methods:** Two approaches; interspecific hybridization and population improvement were followed to enrich the *B. carinata* gene pool. In interspecific hybridization; Pusa Swarnim (a variety of *B. carinata*) was crossed with Indian mustard variety 'NRCHB 101'. Resulting F1 (ABBC) were grown and diploidization of genome was realized through colchicines treatment. Subsequent generations (F2-F5) were advanced through pedigree selection. In population improvement; one *B. carinata* population "MCB 1" was grown in vicinity to *Brassica juncea* gene pool and open pollination was allowed. Seed set on *B. carinata* genotype was harvested and random bulk was drawn to raise next population in vicinity of *Brassica juncea* gene pool. Three cycles were repeated and subsequent generations were advanced through pedigree selection. Developed genotypes of *Brassica carinata* and *Brassica juncea* were evaluated alongwith Pusa Swarnim and NRCHB 101 of *B. carinata* and *B. juncea,* respectively, as check cultivars. Observations were recorded on days to flower initiation, days to maturity, plant height, siliquae per plant, 1000-seed weight, shoot tip siliquae bearing, seed yield and oil content.

**Results:** Developed *Brassica carinata* genotypes exhibited genetic gain of -15, -30, 26, 2.5, and 50 percent for maturity duration, plant height, seed weight, oil content and seed yield, respectively, over check variety (Pusa Swarnim).

**Conclusions:** Developed *B. carinata* genotypes possess desirable traits of both species; *B. carinata* (tolerance against abiotic and biotic stresses, profused branching, shoot tip siliqua bearing and more siliquae) and *B. juncea* (earliness, high oil content, bold seed size, short plant height). High genetic gain for seed yield could be realized due to significant reduction in maturity duration making it acclimatized in prevalent conditions of high temperature at maturity.

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# The use of a new gene pool for obtaining forms of winter oilseed rape (*Brassica napus L.*) with changed quality characteristics

**Background:** Due to the non-food use of rapeseed oil, high oleic and low linolenic (HOLL) genotypes are under development and investigation worldwide in addition to obtaining breeding forms without antinutritive alkenyl glucosinolates. Allele-specific functional markers are very useful for selection of new forms with changed seed oil fatty acid composition by making the process more time- and cost-effective.

**Objectives:** The aim of this work was to develop new mutant oilseed rape HOLL genotypes with changed composition of the mono- and polyunsaturated C18 fatty acid in seed oil and characterized by high seed oil content and extremely low glucosinolate content in seed protein accompanied by MAS for detecting the wild-type and mutated alleles of FAD2 and FAD3 desaturase genes.

**Methods:** The plant material used in this study comprised the HO and LL oilseed rape mutant lines developed at the PBAI-NRI, Poznan, Poland (Spasibionek, 2006) as well as HO x LL recombinants and the selected lines with high seed oil and low alkenyl glucosinolate content.Total oil content was measured in whole dried seeds by NMR, whereas seed oil composition and glucosinolate content were determined by gas chromatography. Genomic DNA was extracted from young leaves using the Doyle method; allelic variants of FAD2 and FAD3 desaturase genes were determined by specific functional CAPS markers (Falentin et al., 2007) and by SNaPshot analysis (Mikolajczyk et al., 2010), respectively.

**Results:** New genotypes of oilseed rape were developed: HO-type, characterized by up to 82% of oleic acid in seed oil as well as HOLL recombinants with 81% of oleic acid and 1.9% of linolenic acid. Molecular analyses confirmed the presence of homozygous mutated alleles of fad2 and fad3 genes responsible for the oleic and linolenic acid content in seed oil of the obtained rapeseed lines. New lines of high, up to 53%, oil content as well as low alkenyl glucosinolate content – up to 0.2 µM g-1 of seeds and low total glucosinolate – up to 2.0 µM g-1 were selected.

**Conclusions:** The obtained lines make a valuable plant material for further study and field experiments. The new HO mutants and HOLL recombinants have been introduced into hybrid breeding to improve their agronomical value, whereas the selected forms with high oil content have been crossed with other valuable sources of genetic variability among winter oilseed rape cultivars.

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# Association of domestication related genes with phenological periodicities in the available germplasm constellations of *Brassica juncea* and its diploid progenitors

**Background:** *B. juncea* (2n=36;AABB) is an amphidiploid that arose from multiple natural hybridization events between *B. rapa* (2n = 20, AA) and *B. nigra* (2n = 16, BB).The crop was first domesticated as a vegetable crop and only later as an oilseed crop. Domestication related directional selection for specific alleles is known to occur at genes which control the major adaptation traits like initiation and duration flowering. These results in genetic bottlenecks in the genes and genomic regions subject to natural selection compared to the unselected ones. We have previously discovered evidence for selective sweep in flowering and shattering related loci (FLC,FT and SHP) in multiple populations of *B. juncea* and its diploid donors using the FST-based method (Kaur et al. unpublished).

**Objectives:** To study the association of the genes/genomic regions responsible for adaptation to domestication with the phenological periodicities in *B. juncea* and diploid progenitor species.

Methods: A world wide germplasm collection comprising land races , historical and modern cultivars of *B. juncea* (88), *B. rapa* (83) and *B. nigra*(19) formed the basic germplasm. Data were recorded for initiation of flowering and completion of flowering. DNA markers were developed from the gene sequence and the genomic region around flowering associated genes (FLC and FT). These markers were used to genotype entire germplasm. Association analysis was then carried out using software Tassel to document association between the phenotype and the molecular markers. Unique amplified products were also sequenced to understand allelic variation at a given genomic region.

**Results:** In general *B. rapa* germplasm was earliest to bloom and complete flowering. *B. juncea* behaved more like *B. rapa. B. nigra* was not only late to flower and also continued to flower for a longer duration than both the *B. rapa* and *B. juncea.* Polymorphism generated by gene-based microsatellite markers helped to identify selective sweeps for the target genes in the three test species. Selective sweeps were primarily recognizable in a very small genomic region around the gene under selection. Analysis of these showed differential responses to the directional selection for flowering habit. The associations between the genomic region and flowering phenotypes differed across *B. juncea* and parental diploids. To some extent, same was true for comparisons in multiple populations with in a same species.

**Conclusions:** *B. juncea* and its progenitor species showed differential responses to domestication related selection pressure for the same target trait.

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# Copy number variation generated by homeologous rearrangement creates phenotypic diversity

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**Background:** Polyploidisation and subsequent diploidisation as a means of genome stabilization are acknowledged mechanisms in plant speciation. Rapeseed as an example for an amphidiploid crop has undergone several rounds of genome duplication, and genome stabilization is an ongoing process ever since. In the EraCAPS project "Evo-Genapus: Evolution of genomes: structure-function relationships in the polyploid crop species *Brassica napus*" we are investigating the influence of genome rearrangements on selection for adaptive and agronomic traits.

**Objectives:** We hypothesize that the genome restructuring observed in resynthesised *B. napus* represents an accelerated form of genome evolution that is ongoing in naturally derived, cultivated *B. napus*. We are testing this hypothesis on a genome-wide scale by investigating mapping populations derived from crosses between a winter oilseed rape inbred line variety and three different resynthesized rapeseed accessions. Observed structural genome variation related to trait variation of relevance for rapeseed as a crop will improve our general understanding of functional genome evolution in polyploid crops.

**Methods:** In this subproject we are quantifying different mechanisms of genomic changes, such as homeologous chromosome exchanges, to identify specific examples demonstrating the influence of genomic rearrangements on phenotypic variation. Comparative mapping of IlluminaTM *Brassica* 60k SNP markers and a large set of phenotypic data will enable us to associate phenotypic variation with the genomic constitution of the mapping parents. Whole-genome resequencing of the mapping parents allows us to calculate copy number variation along the genome and differentiate segments of altered copy number in comparison to the assembled genome of the European winter oilseed rape cultivar 'Darmor-bzh'. These segments represent candidates for insertions/deletions and will be investigated in detail.

**Results:** As an example, homeologous rearrangements are common between chromosomes A9 and C8. We will present preliminary data suggesting that important seed quality QTL on chromosome A9 result from homeologous rearrangements that generated interesting variation for selection and breeding. We expect that knowledge about copy number variation and rearrangements will help to improve and focus selection processes in breeding for yield and quality traits in modern rapeseed lines.

# Asexual propagation of the sterile plant from the recessive genic male sterility and utilization for production of the fully sterile line

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**Background:** The three-line system of the recessive genic male sterility (RGMS) is one of very important waysfor utilization of heterosis in winteroilseed rape (*Brassica napus*)in china. Compared with CMS,it is more difficultyto produce the fully sterile line (the100% sterile line), because 50% fertile plants from the homozygous two-type line need be thoroughlyremoved, and 50% sterile plants need be reservedto produce the fully sterile line. This step would result in reduction of output andlowerphysical purity. In order to overcome the questions, the study hopedthat a large number of the sterile plants were obtained to productthe fully sterile line by tissue culture instead of bythe homozygous two-type lineof RGMS.

**Objectives:** The homozygous two-type line "20118AB" of RGMS and the temporary maintainer line were used in this study.The homozygous two-type line "20118AB" is including 50% fertile plants (Genotype: AabbRfRf) and 50% sterile plants (Genotype: aabbRfRf). The genotype of the temporary maintainer line is aabbrfrf.

**Methods:** The fertility segregation of the homozygous two-type line "20118AB" and the offspring derived from sib-crossing and test-crossing with the temporary maintainer line were investigated in order to identify the genotype of the sterile plants "20118A". Young brancheswere used as the materials for tissue culture from the sterile plants with the genotype aabbRfRf.

**Results:** The youngbranches were thoroughly sterilizedandplaced on the B5 medium with 3.0 mg/l 6-BA and 0.5 mg/l NAA. After about 30 days, the shoots wouldcome out from the petiole orcallus tissue. The new shoots could be inoculated into the new B5 medium with 1.0 mg/l 6-BA and 0.5 mg/l NAA for asexually propagation. The reproductive cycle is 2 weeks, and the coefficient is 6-8 times.

**Conclusions:** The oilseed rape has high propagation coefficient, so it is feasible to use asexual propagation for production of the fully sterile line. This way could simplify the stepsandsignificantly increase production. The purity ofthe fully sterile line is up to 99%, so the article plants need no longer removein production of hybrid seed. In conclusion, this study was very practical for production of GMS hybrid seed.

**References:**

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# Seed quality development in *Brassica napus*

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**Background:** *Brassica napus L.* is an intensively bred and highly valuable crop in Canada's agricultural industry. A significant problem with *B. napus* breeding programs is the limited genetic diversity available. Therefore, great effort is required to continue the growth of the Canadian canola industry by utilizing diverse germplasm within a breeding program. A set of 314 *B. napus* lines derived from re-synthesis between *B. rapa* and *B. oleracea*, in conjunction with ethyl methanesulfonate (EMS) mutagenesis and doubled haploid methodologies, has developed genotypes with increased diversity for seed quality. The discovery of modified seed quality provides new genetic sources for introgression into future breeding lines and cultivars.

**Objectives:** The objectives of this research are to phenotypically and genotypically analyze a diverse set of *B. napus* breeding lines for oil content, seed protein content, fatty acid content, and amino acid composition.

**Methods:** Phenotypic analysis was conducted on agronomic traits in 2014 field trials. Additionally, seed quality was analyzed using near-infrared spectroscopy and gas chromatography. Amino acid analysis will also be performed to reveal amino acid composition profiles. Genotyping will be performed using multiple markers on an ABI genetic analyzer along with genotype-by-sequencing (GBS).

**Results:** Phenotypic data has shown diversity for seed quality, including oil content (34 to 52%), seed protein content (23 to greater than 30%), and diverse fatty acid profiles. Populations will be analyzed using over 200 molecular markers and amino acid profiles will also be discussed.

**Conclusions:** Comparison of the phenotypic data with the genotypic data has helped to identify the genomic regions associated with these diverse seed quality traits. This data will be useful in QTL analysis and aid in further efforts to diversify *B. napus*.

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# Introgression of resynthesized *Brassica napus L.* into parental lines of winter oilseed rape F1 hybrids

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**Background:** Incorporation of resynthesized, (RS), *Brassica napus L*. genotypes in the breeding programs can be used to increase the variation in double low oilseed rape gene pool. But the RS lines of oilseed rape reveal poor performance for many agronomic traits and shows inferior seed yield as compared to current breeding material. However, after the crossing of resynthesized *Brassica napus* with 00-quality oilseed rape and then androgenesis in vitro of obtained F1 hybrid, it should be possible to select doubled haploids (DH) with improved seed yield and seed quality as well as significant genetic distance to present cultivars.

**Objectives and methods:** Until now resynthesized oilseed rape was obtained as a result of reciprocal crosses between three subspecies of Brassica rapa and two subspecies of *Brassica oleracea* using embryo rescue technique (Sosnowska, Cegielska-Taras 2014).

**Results:** As expected, the RS lines obtained in the study have been characterized by a high content of erucic acid in oil and glucosinolates in seeds. Several resynthesized oilseed rape lines were crossed with lines of double low quality winter oilseed rape. Populations of large number of androgenic plants semi-resynthesized (semi-RS) were developed from F1 hybrids using isolated microspore in vitro culture method. The seeds of all obtained semi-RS DH lines were analyzed biochemically with regard to 00-quality of seeds. Among the population of the semi-RS DH lines of winter oilseed rape with zero erucic acid content and low amount of glucosinolates were selected. Moreover, in this study the genetic similarity among some resynthesized lines, *B. oleracea* and *B. rapa* as parents and collection of winter oilseed rape genotypes was evaluated using AFLP markers.

**Conclusions:** Four selected semi-RS DH lines were characterized by either the zero erucic acid content in oil and glucosinolate content lower than 15 µmol g-1 seeds. The dendrogram based on AFLP markers indicated that as well RS lines as semi-RS lines created distant group among studied 101 parental lines of winter oilseed rape F1 hybrids.

**References:**

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# Agronomic and seed quality traits of DH populations of winter oilseed rape obtained from reciprocal crosses between black- and yellow-seeded DH line

**Background:** Oilseed rape is very important source of high nutritional quality oil with a balanced fatty acid composition, a valuable material for many industrial branches and a protein-rich meal for livestock feed. The yellow-seeded oilseed rape has thinner seed coat and thus higher oil and protein content as well as lower fibre content than black-seeded types. However, the introduction of this trait to oilseed rape involves reduction in yield and lowering of agronomic performance. Genetic information about the gene expression of quantitative traits can help to create an effective breeding strategy to develop new varieties.

**Objectives:** In the present study, the influence of maternal effects were evaluated on the basis of differences between the two DH line populations HZ and ZH obtained from reciprocal crosses between black-seeded DH H2-26 and yellow-seeded DH Z-114, derived from natural mutant with bright seeds and spring line of *B. napus* with segregating seed colour (Bartkowiak-Broda et al. 2009).The population, marked as HZ, consisted of 27 DH lines, derived from F1 hybrid DH H2-26 × DH Z-114 and the population marked as ZH consisted of 30 DH lines, derived from F1 hybrid DH Z-114 × DH H2-26.

**Methods:** Field experiments in a randomized complete block with four replications design were conducted in two seasons. Seed colour was determined with spectrophotometer Color Flex on the scale from 0 (black) to 5 (yellow). The experimental data were analyzed with uni- and multivariate statistical methods.

**Results:** Parental forms differed significantly in terms of seed yield, number of seeds per pod, thousand seed weight, protein, neutral detergent fibre (NDF), acid detergent fibre (ADF) and glucosinolate content, and seed colour. However, the maternal effects were revealed in DH line populations only for the thousand seed weight. In contrast, the influences of the paternal form were found on content of neutral and acid fibre and seed colour. The direction of crossing played a role in the frequency of the occurrence of transgression effects, and this was particularly in protein, NDF and ADF content. Positive transgression effects for protein content and negative transgression effects for NDF and ADF content were noted only among DH lines of ZH population, so in this population, which the paternal parent contained more protein, NDF and ADF. The use of multivariate statistical analysis allowed the simultaneous characterization and grouping of tested lines of HZ and ZH populations in terms of several traits.

**Conclusion:** Application of the statistical methods revealed the influences of the maternal parent on the thousand seed weight and paternal parent on content of fibre and seed colour.

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Bartkowiak-Broda I., Piotrowska A., Hernacki B., Cegielska-Taras T., Michalski K. 2009. Development of yellow seeded winter oilseed rape (*Brassica napus L.* var. *oleifera*). GCIRC, Technical Meeting in Manesar, India, 2009.

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# Genetic diversity for morphological and physiological traits in diverse rapeseed germplasm

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Genetic diversity is the back bone of any plant breeding program. Seed companies and plant breeders involved in the development of crop varieties for specific consumer and industrial uses are often limited by either the lack of desired trait in existing genetic stocks or the narrow variability in the available germplasm for the trait of interest. It is always desirable to obtain or develop germplasm which possess the genes for economically important plant and seed traits. The Plant Genetic Resources Institute (PGRI) located at National Agriculture Research Center, Islamabad, Pakistan is holding a large number of *Brassica* accessions (land races) collected from diverse regions of Pakistan. Dow AgroSciences in Collaboration with PGRI has characterized the germplasm for various morphological and physiological traits in tiple environments of Pakistan and Canada. This research information will focus on explaining the genetic diversity for some traits such as physiological maturity, plant type, plant height, seed characteristics. The distribution patterns and conservation of traits will also be discussed. The data will provide insight into the geographical diversity of the *Brassica* germplasm.

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# Introgression of imidazolinone tolerance into spring turnip rape (*Brassica rapa L*) and the launch of Aurea CL

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**Background:** Imidazolinones (IMIs) are a family of herbicides that control weeds effectively. IMIs inhibit the function of acetolactate synthase (ALS), enzyme required for the synthesis of branched chain amino acids. Tolerance to IMI was developed in microspore mutagenized oilseed rape (*Brassica napus L.*) (Swanson et al.1989). Mutants, carrying PM1 and PM2 ALS alleles, were found to have a superior tolerance to IMIs. Previously the imazamox, an imidazolinone herbicide, was shown to be very good in controlling the most troublesome weeds in oilseed fields in Finland (Haukkapää et al. 2005).

**Objectives:** The aim of this study was to incorporate the IMI tolerance into turnip rape (*Brassica rapa L.*) and oilseed rape lines well suited to northern climates and to measure the actual level of tolerance of IMI tolerant lines to imazamox.

**Methods:** IMI tolerant lines were developed by crossing an oilseed rape line (originated from BASF Corporation, USA), containing both PM1 and PM2, to several turnip rape and oilseed rape breeding lines. The breeding work was done at the University of Helsinki in 2003-2007. Hybrids were backcrossed until the $BC_6$ generation in turnip rape and $BC_4$ generation in oilseed rape. After each backcross, the progeny was tested for the presence of the tolerance genes using a 30 g ai $ha^{-1}$ application rate of imazamox. PCR analyses were used for monitoring the presence of PM1 and $PM_2$ alleles. IMI tolerance of the newly formed breeding lines was tested in field and greenhouse experiments. The level of IMI tolerance was tested with increasing doses of imazamox, and the activity of ALS enzyme and dry matter accumulation were measured as well. Results: Introgression of PM1 and PM2 into several oilseed rape lines was successful whereas only $PM_2$ was stable in turnip rape lines. Both field and greenhouse experiments showed that turnip rape carrying only the PM2 mutation withstood imazamox well. Furthermore the data suggest that turnip rape was more tolerant to imazamox than oilseed rape. IMI tolerance did not show any negative impact on the agronomic parameters of both *Brassica* species.

**Conclusions:** Based on these trials IMI tolerant turnip rape (*B. rapa*) line no: 4003 went through the official variety tests and was accepted on the official variety list in Finland as Aurea CL in 2011. Later it was approved on the EU list of cultivars as well. Since that it has been grown successfully on the farmers' fields.

**References:**

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# QTL controlling pod shattering resistance in yellow-seeded *Brassica napus* line YN01-429

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**Background:** The majority of the Canadian canola crop is swathed prior to combining, in part, due to susceptibility to mature pods shattering. A key element in optimizing straight cutting or direct combining of canola is a better understanding of the genotypic and physiological contributing factors to pod dehiscence. An initial investigation into variation in Canadian canola cultivars, hybrids and breeding lines showed that yellow-seeded lines possessed less shatter potential than many commercial black-seeded lines (Wang et al. 2007).

**Objectives:** 1) Mapping of the genomic regions controlling reduction of pod shatter observed in YN01-429; 2) Determine if regions controlling shatter reduction were interspecifically-derived during the introgression of yellow seeded genes from *B. carinata* and *B. rapa* through linkage; and 3) Assess if pleiotrophy exists between reduction in shattering potential and other agronomic or seed fiber-related traits.

**Methods:** The elite yellow seeded breeding line was crossed to two different shatter susceptible, black-seeded lines and doubled-haploid populations were developed. Both populations were evaluated in full for one season at one or two sites and lines with extreme phenotypes were evaluated in a second field season. Days to flowering and maturity were recorded in each trial. Pod shattering phenotypes were assessed in non-harvested trials that were visually rated for pod shattering throughout the fall season. Linkage maps of the populations were developed with either SSR or SNP markers and QTLs for each site-year were determined. The trial was arranged in a split-plot where a harvested main-plot treatment produced seed that was analyzed for standard canola seed quality parameters and fiber fractions using NIR.

**Results:** In the first population evaluated, several QTL on various linkage groups were consistently detected across two sites and explained 6.4 to 16.1% of the variation. A QTL on N13 was confirmed the second season on population extremes. QTL analysis on the second population showed a unique single locus on N2 which explained 22.5% of the variation which was not detected in the first population examined. The shatter reduction QTL on N13 was shown to be derived from black-seeded pedigree parents used in the development of YN01-429 when surrounding SSR markers were assessed on all pedigree parents. In both populations, a major QTL for seed coat colour and fiber fractions was found on N9 and mapped to different linkage groups than shatter reduction QTL. Similarly, flowering and maturity QTLs mapped to different genomic regions. There were very little to no associations between shattering and these seed quality related traits, flowering or maturity ratings.

**Conclusions:** Uncharacterized variation for pod shatter exists within the *B. napus* genepool; however, several QTL control the trait and detection of these QTL is highly dependent on background genotype and environment.

**References:**

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**POSTERS THEME A**

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# Characterization of genomic regions responsible for microspore embryogenic potential and direct embryo to plant conversion of a doubled haploid oilseed rape population

**Background:** Microspore culture is a very powerful technique in breeding of oilseed rape. Despite the progress achieved in optimizing in vitro culture protocols, tremendous differences remain in the microspore embryogenic potential among genotypes (Ferrie and Möllers 2010). Furthermore, breeding progress is hampered by a highly variable direct embryo to plant conversion of breeding lines. The Swedish spring cultivar Topas has been extensively studied for its excellent microspore embryogenic potential and the derived line DH4079 showed an outstanding embryo production of many thousand embryos per experiment, which has made DH4079 a standard in many investigations. In contrast, a very low embryo yield is obtained from inbred line 617 of winter oilseed rape cultivar Express, in the range of nil up to 50 embryos per experiment, under comparable conditions. Moreover, a moderate and a good direct embryo to plant conversion were found for DH4079 and Express 617, respectively. The genetic basis of such differences in microspore culture response and plant regeneration is still largely unknown.

**Objectives:** To identify genomic regions and candidate genes related to microspore embryogenic potential and direct embryo to plant conversion through the analysis and QTL mapping of both traits in a DH population derived from the cross DH4079 x Express 617.

**Methods:** In vitro propagated F1-plants of the cross DH4079 x Express617 were used as microspore donors to generate a DH population of 200 lines. DH lines were grown and used as source of microspores, which were cultured following a standard protocol (Möllers et al. 1994). The number of regenerated embryos (MDE) per experiment was recorded. MDE were transferred to Gamborg B5 medium and following a cold treatment at 2 °C, direct embryo to plant conversion was scored. Experiments are repeated four times and mean values will be used for QTL mapping based on an Illumina Infinium *Brassica* 60K SNP molecular linkage map.

**Results:** So far 100 lines of the DH population were characterized for their embryogenic potential and direct embryo to plant conversion. Large and significant differences in microspore embryogenic potential were observed among DH lines, ranging from a few embryos to more than forty thousand as a mean of four experiments. Significant differences were also found for direct embryo to plant conversion, which ranged from 5 to 85%. Heritabilities for both traits were high (85% and 93%). A linkage map based on 3,142 SNP markers was developed and will be used for QTL mapping and candidate gene identification.

**Conclusions:** The DH population showed a continuous and significant variation for microspore embryogenic potential and direct embryo to plant conversion between genotypes, indicating that both traits are quantitatively controlled by several genes.

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# Broadening of genetic diversity in spring canola (*Brassica napus L.*) by the use of the C-genome of *B. oleracea* var. *italica* and *B. oleracea* var. *capitata*

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**Background:** Canola (*Brassica napus,* AACC, 2n = 38) is one of the important edible oil in the world after soybean and palm. In Canada, spring *B. napus* canola is one of the most important crops, contributes about $19 billion per year to the Canadian economy (Canola Council of Canada, 2013). Presence of genetic variability in breeding population is pre-requisite to develop new cultivars with improved yield to meet the demand of ever growing population in the world. Genetic diversity in spring *B. napus* canola is low; therefore, breeding efforts must be taken towards broadening of genetic diversity in this crop (Cowling 2007, Rahman 2013). This can be accomplished through introgression of genetic diversity from diploid progenitor species *B. rapa L.* (AA, 2n = 20) and *B. oleracea L.* (CC, 2n = 18), and other allied species of the family *Brassicaceae*.

**Objectives:** The research project was undertaken to develop canola-quality spring *B. napus* inbred lines with allelic diversity introgressed from the C-genome of *B. oleracea* var. *italica* (broccoli) and *B. oleracea* var. *capitata* (cabbage).

**Methods:** *B. napus* × *B. oleracea* interspecific crosses were made and the F1 plants were self-pollinated for F2 seeds. The F2 populations were self-pollinated for several generations with selection for different agronomic and seed quality traits. F4 plants were genotyped with SSR markers.

**Results:** All F5 generation families were free from erucic acid (0.18 ± 0.02 SE percent of total fatty acids) and mean of glucosinolate content in F6 was less than 20 µ mol g-1seed. About 69% of the F6 plants had partec value similar to the *B. napus* parent A04-73NA.

**Conclusions:** Results suggest that spring *B. napus* canola quality lines can be developed from *B. napus* × *B. oleracea* interspecific crosses for introgression of allelic diversity from broccoli and cabbage into *B. napus*.

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# Pod trait comparisons between a pod-shatter resistant accession and a pod-shatter susceptible accession in *Brassica napus L.*

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**Background:** During rapeseed harvest, pod shatter loss proportion usually reaches up to 10% to 30%. In order to decrease the yield loss, it is necessary to breed varieties with pod-shatter resistance. Except for molecular assisted selection, it is more practical for breeders to differ the pod-shatter resistant accessions from pod-shatter susceptible accessions via pod traits.

**Objectives:** This article tried to find pod trait differences between pod-shatter resistant accession H155 and pod-shatter susceptible accession Qva (Wen et al. 2008). The pod traits included pod agronomic traits, valve anatomic structures and fiber content in pod valves.

**Methods:** Random impact test (RIT) for pod-shatter resistance of oilseed rape was developed by Morgan et al. (2000) and improved by Wen et al. (2008). Pod traits (pod length, pod width, beak length, valve thickness and seed number per pod) were obtained by a trial including three replications. Additionally valve anatomic structures were investigated by laser microscope and by transparent electron microscope. Total fiber contents in valves were tested according to the methods of Van Soest.

**Results:** Pod shatter resistance index difference between H155 and Qva reached 1% significant level. Pod length, beak length, valve thickness and seed number per pod of H155 were 8.71 cm, 0.50 cm, 1.84 cm, 0.22 mm and 32.8 seeds, respectively. Compared with H155, Qva had much shorter pod (3.74 cm), shorter beak (0.72 cm), thinner valve (0.15 mm) and less seeds per pod (10.2 seeds).

Results of anatomic studies of valve revealed that there were valve anatomic structural differences between H155 and Qva. In H155, cells in endocarp were long, perpendicular to endocarp and arranged tightly.The cell walls in endocarp, mesocarp and vascular bundle were seriously lignified. On the contrary, cells in endocarp of Qva were round and arranged loosely. Cell walls in endocarp of Qva were slightly lignified, and cell walls in mesocarp and vascular bundles were hardly lignified. Fiber contents in valves between H155 and Qva reached significant difference. Fiber content in H155 valves was higher by 5.37 times than that in Qva. Consequently, H155 had much stronger valve than that of Qva in mechanical strength.

**Conclusions:** Pod traits affects pod-shatter resistance in *Brassica napus L.* and accessions with longer and thicker valve have better pod-shatter resistance. Pod-shatter resistant susceptible accession have shorter, thinner and weaker valves than pod-shatter resistant accessions. Anatomic structure is another affecting factor of pod-shatter resistance. Those accessions with tightly arranged cells in endocarp and seriously lignified cell walls in endocarps, mesocarps and vascular bundles have strong mechanical strength and are pod-shatter resistant. These conclusions can help breeders to select pod-shatter resistant accessions without molecular assisted selection.

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**POSTERS THEME A**

# Genome-scale diversity for prediction of hybrid performance in winter oilseed rape

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**Background:** High-density single-nucleotide polymorphism (SNP) genotyping provides a powerful platform to investigate heterozygosity on a genome-wide scale and investigate the genomic basis of heterosis. Analysis of genome diversity patterns within and between divergent gene pools can potentially provide valuable input to statistical models for genomic prediction of hybrid performance. Ultimately, genome-based prediction could help breeders to introgress novel variation into hybrid gene pools and select the most promising parental combinations to maximize trait performance.

**Objectives:** In this work we are evaluating different approaches for predicting hybrid performance in winter oilseed rape. The data generated also provides insight into genome-wide diversity patterns caused by intense selection for important traits in breeding populations. Genomic selection strategies can potentially help breeders to enrich genetic diversity in depleted chromosome segments, with potential gains in hybrid performance facilitated by genome-based prediction models.

**Methods:** We are investigating genome-scale diversity by genotyping large breeding populations, representing divergent winter-type oilseed rape gene pools, with the *Brassica* 60kSNP Illumina consortium genotyping array. Patterns of diversity are analysed at the population and chromosome level using population genetic parameters and assessment of linkage disequilibrium. Phenotype data from F1 test hybrids, generated using these individuals as pollinators of four different male-sterile (MSL) mother lines and tested in multiple field environments, are being used to develop statistical models for prediction of hybrid performance based on parental genome profiles.

**Results:** Genome-wide patterns of linkage disequilibrium (LD) and diversity (FST) reveal chromosome regions with strongly eroded diversity in different winter oilseed rape breeding pools. These regions represent key targets for improvement of hybrid performance by introgressions of novel diversity into heterotic pools. Preliminary results of hybrid prediction in a large population of winter oilseed rape test hybrids demonstrate the power of genome-based modeling approaches for selection of high-performing hybrid combinations.

**Conclusions:** Genome-wide diversity data enable targeted improvement of parental pools for winter oilseed rape hybrid breeding and facilitate genomic prediction of hybrid performance.

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# Global DNA methylation dynamics during the microspore reprogramming to heat stress induced embryogenesis in *Brassica napus L.*

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**Background:** Microspore embryogenesis is a unique and facilitating phenomenon in plants, and consists of the inducible reprogramming of microspores deviating their original pathway toward embryogenesis (Seguí-Simarro and Nuez, 2008). Although fruitful achievements have been made in the past, few studies have examined the relationship between DNA methylation and heat stress, which is a crucial triggering factor for microspore embryogenesis induction (Shariatpanahi et al., 2006), on a genomic scale.

**Objectives:** To reveal whether DNA methylation is as an crucial epigenetic regulatory way to switch the microspore cell fate to microspore embryogenesis after heat induction. If so, How it is working? Simultaneously, we hope to seek the important DMRs (Differential Methylation Regions) and the related genes that were corresponding to the heat treatments.

**Methods:** The high/low embryogenic potential cultivar Topas and Westar were chosen for uninucleate microspore isolation and in vitro treatments with 18°C and 32°C for 6 h, respectively. Then collected all these heat treated and untreated samples for total DNA isolation. The genomic DNA of each sample was sent to BGI (BGI Tech Solutions Co., Ltd, Shenzhen, China) for bisulfite sequencing. The qualified sequencing data was analyzed by Bismark, methylKit, and some other bioinformatic tools.

**Results:** DNA methylation status of the microspores originated form the cultivar Topas that with high embryogenic potential was more sensitive to heat stress compared with the low embryogenic potential cultivar Westar. And heat treatment definitely triggered DNA hypomethylation occurring on each Topas chromosome, especially on the CpG and CHG contexts. Additionally, in Topas microspores, the C genome DNA methylation status in CpG and CHG sites was more sensitive to 32°C heat treatment compared with that of A genome. Actually, our prediction also showed that the *Brassica napus* C genome possess more CpG islands frequency throughout the chromosomes, suggesting the evolution of C and A genome were asymmetric in allotetraploid plant *Brassica napus*. Furthermore, we identified the DMRs and the related genes, which were corresponding to the heat treatments.

**Conclusions:** The present study uncovered the evolutionary disequilibrum of the subgenome on DNA methylation pattern as well as their asymmetric response to heat during the microspore reprogramming to embryogenesis, meanwhile the heat stress induced DNA hypomethylaiton may be essential for microspore cell fate changing in *Brassica napus L.*.

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# Fine mapping of a yellow seeded gene in *Brassica juncea L.*

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**Background:** Yellow seed of rapeseed is a very important trait; sometimes the yellow seed oil content is 1-3% higher than that of brown seed with the same genetic background (Liu et al. 1991). So yellow seed breeding is considered as one of the effective ways to improve the oil content. A yellow mustard is the main rapeseed variety in the northwest of China. The yellow seed of this yellow mustard was controlled by a major gene, and the gene was mapped to A09 Chromosome in *Brassica* (Xu et al. 2010; Huang et al.2012).

**Objectives:** In order to fine map the yellow seeded gene, we made use of the genome sequences of *B. rapa* to develop the SCAR and IP markers, fine map the yellow seeded gene. This study will provide a useful clue for cloning the yellow seeded gene.

**Methods:** A BC8S1 population consisting of 1256 individuals derived from a yellow seeded landrace 'Wuqi mustard" and a brown seeded landrace "Wugong mustard" was developed for gene mapping. Through the analysis of previous markers' sequences, we found that the gene was located between 23.304 and 28.224M in A09 chromosome in *B. rapa*. We randomly selected some genes in this region, and designed primers according to the sequences of these genes; at the same time, we also used the markers' sequences of this region published in website (http://brassicadb.org/brad) to develop new SCAR markers. MAPMAKER/EXP 3.0 program was used for linkage analysis, a minimum LOD score of 3.0 was used for map construction.

**Results:** Five SCAR (sequence characterized amplified region) and five IP (intron polymorphism) markers were successfully developed, and all of IP markers were co-dominant. The markers IP4 and Y1 were located in the either side of the yellow seeded gene at a distance of 0.1 and 0.3 cM, respectively. The gene was mapped in a region of 0.54M in A09 chromosome. In this region, three IP markers IP1, IP2 and IP3 co-segregated with the targeted gene.

**Conclusions:** Thanks to the power of next generation sequencing technology, *B. rapa* genome has been completely sequenced and published in public domain. The sequence information can be unlimited employed in developing markers linked to genes of interest, fine mapping or cloning targeted gene in *Brassica*. Developing IP markers is a very effective way for gene mapping. The fine mapping of the yellow seeded gene will lay a solid foundation for the yellow seeded gene cloning and gene function research.

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# Genome wide identification and expression profiling of *B. rapa* annexins

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**Background**: Annexins are multifunctional proteins first discovered in plants and are present in both eukaryotes as well as in prokaryotes. In plants, annexins have been shown to play vital roles in growth and development under normal and stress conditions (Jami et al. 2012). Whole genome sequencing of *Brassica rapa,* an important species of the genus *Brassica* with many subspecies grown all over the world as vegetable and oilseed crops, has made identification of gene families easier.

**Objective**: In the present study, we planned to identify the annexin family members from *Brassica rapa* genome database; characterize them through bioinformatics tools and study their expression patterns in response to different abiotic stress condition and signaling molecules.

**Methods**: Genome-wide identification of annexin gene family was achieved by performing a search with keyword "annexin" in *B. rapa* genome database (http://www.plantgdb.org/BrGDB). For the prediction of motifs and domains, SMART program (http://smart.embl-heidelberg.de) was used and the GSDS server (http://gsds.cbi.pku.edu.cn) was used for gene structure prediction. For expression profiling of the annexin gene family, 3d old seedlings were treated with salicylic acid (SA) 100 µM, sodium chloride (NaCl) 200 mM, methyl jasmonate (MJ) 100 µM, hydrogen peroxide ($H_2O_2$) 10 mM, methyl viologen (MV) 10 µM and abscisic acid (ABA) 100 µM. Total RNA was isolated from whole seedling and different tissues by Trizol (Invitrogen, USA), which was followed by cDNA synthesis using total RNA (2 µg). Real time PCR was performed and data was analyzed by ΔΔCt method.

**Results**: In a search with keyword "annexin" in BrGDB, we found 13 annexin like sequences (Bra034402, Bra036764, Bra039578, Bra031890, Bra024346, Bra017102, Bra000091, Bra017103, Bra000090, Bra033961, Bra009049, Bra009048 and Bra008892) in *B. rapa* genome. However, *A.thaliana* has only eight annexin in its genome. Further analysis of *B. rapa* genome by PLAZA (http://bioinformatics.psb.ugent.be/plaza/versions/plaza_v3_dicots/) WGMapping tool showed that this expansion of annexin family in *B. rapa* genome happened due to block duplication and tandem gene duplication. In a protein sequence analysis by SMART program we found two annexin domains in Bra000090 and Bra017103, while Bra039578 showed three annexin domains and one N terminal transmembrane region in its sequence. Other annexin members showed the presence of 4 annexin domains. Gene expression analysis of eight members of the *B.rapa* annexin family showed a spatial and temporal regulation under different stress treatments like NaCl, MV, $H_2O_2$, SA, MJ and ABA. In every treatment, Bra034402 expression level was very high in comparison to other annexins. In tissue specific expression Bra017102, Bra000090, Bra009049 and Bra009048 expressed at higher level in leaf than in other tissue. Bra034402 and Bra008892 showed more expression in stem followed by leaf and then in root. Bra024346 showed higher expression in root than other tissue. Bra033961 showed equal expression in stem and leaf which was higher than root.

**Conclusion:** Genome-wide identification and expression analysis will help in further functional characterization of annexin family members of *B. rapa*.

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# Characterization of FAE1 in the zero erucic acid germplasm of *Brassica rapa L.*

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**Background:** The modification of erucic acid content in seeds is one of the major goals for quality breeding in oil-yielding *Brassica* species. However, few low-erucic-acid (LEA, <2%) resources are available, and novel LEA genetic resources are being sought. Fatty acid elongase 1 (FAE1) is the key gene that controls erucic acid synthesis. However, the mechanism for erucic acid synthesis in *B. rapa* lacks systematic study.

**Objectives:** Most LEA cultivars of *B. rapa* were developed by the introduction of recessive alleles from the donor varieties SPAN, or their derivative lines. To expand the genetic resource of LEA genes, we attempt to isolate the zero erucic acid lines from Chinese landraces and the mechanism of LEA formation in *B. rapa* was explored.

**Methods:** The erucic acid contents of 1981 Chinese landraces were analysed and the accessions with erucic acid content lower than 20% were selected to isolate zero erucic acid lines by half-seed fatty acid analysis (Gupta et al. 2004). The sequences and expression profiles of FAE1 at different development stages were analysed among the *B. rapa* accessions with different erucic acid contents. The molecular marker based on the deletions in the promoter sequences of LEA accessions was designed and the association with erucic acid content was detected in the segregating population.

**Results:** We isolated lines with zero erucic acid from 1981 Chinese landraces of *B. rapa*. The variations FAE1 coding sequences were not correlated with the erucic acid content of *B. rapa* as reported for *B. napus*. The FAE1 gene transcript was more abundant in the high-erucic-acid (HEA) than in the LEA accession during the seed development. Moreover, the FAE1 promoter sequences of LEA and HEA materials shared 95% similarity. 28 bases deletions (containing a 24 bases AT-rich region) were identified at approximately 1300 bp upstream from the FAE1 start codon in zero erucic acid cultivars and landrace, which may cause the decrease of the expression. The molecular marker based on the deletions was designed, and the genotype with the deletions was co-segregated with the LEA trait in the segregating population in the segregating population.

**Conclusions:** The formation of LEA is not attributable to variations in FAE1 coding sequences, but may be attributable to the decrease of FAE1 expression. The promoter variations might modify the expression level of FAE1 and the results shed light on novel regulation mechanisms for erucic acid synthesis.

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# Preliminary study on difference of main carotenoid contents in the petals with different colors in *Brassica napus L.*

**Background:** The color of rapeseed (*Brassica napus L.*) flower petals is commonly yellow, but it can also be in other colors, including white, milky white, pale-yellow, golden-yellow or orange, etc. Different colors of flower petals in oilseed rapeseed can be a useful indication character and also an agronomic character, which is of important values for breeding, genetic studies, and modern touristic agriculture. Some genetic studies have been made to understand the inheritance of flower petal colors in *Brassica napus L.*( Tian et al. 2007; Wen et al. 2010) However, no researches have been found to investigate the differences in pigment contents in the flower petals with different colors in the species. Carotenoid contents and compositions were observed to be associated with the yellow-to-orange colors of inflorescence in *Calendula officinalis L.* (Pintea et al., 2003). The yellow color of flower petals in Japanese morning glory (Ipomoea nil) was also found to be related to the carotenoid compositions and the patterns of carotenogenic gene expression during the petal development (Yamamizo et al., 2010) [2]. In Citrus, the difference in fruit skin colors was also observed to be correlated to the compositions of carotenoid contents in the peel. (Tao et al., 2003) In the present study, we investigated the difference in the contents of three main carotenoids (xanthophyll, lycopene and β-carotene) in the petals with different colors in *Brassica napus L.*

**Objectives:** The aim of the study was to investigate the difference in main carotenoid contents in the flower petals with different color in *Brassica napus L.*, and to observe the relationship between carotenoid contents and flower colors in *Brassica napus L.*

**Methods:** Fifteen *Brassica napus* lines in 4 types of petal colors, white (5 lines), pale-yellow (2 lines), yellow (4 lines) and golden-yellow (or orange) (4 lines), were selected to investigate the contents of carotenoids in the flower petals. The fresh petals were taken from each line at the full blossom stage and stored in plastic tubes under liquid nitrogen temperature for use. Fresh petals were ground into powder with liquid nitrogen, and near 0.5mg of powder was taken to extract the total carotenoids by referring to the earlier studies (Parlog et al. 2009, Pintea et al. 2003, Yamamizo et al. 2010) with minor modifications. Contents of the 3 main carotenoids (xanthophyll, lycopene and β-carotene) were determined with the HPLC method (Agilent 1200, USA), referring to the protocols adopted in previous studies (Parlog et al. 2009, Pintea et al. 2003, Yamamizo et al. 2010) with necessary adjustments, and were quantified based on the standard curves established with their standard samples.

**Results:** The average contents of xanthophill were 5.27, 6.84, 8.29 and 58.30 μg/g-1 of fresh petal in the 4 flower color types of rapeseed lines, i.e., white, pale-yellow, yellow and golden yellow petal lines, respectively; The average contents of lycopene were 2.94, 33.15, 16.77 and 1267.0 μg/g-1, and the average contents of β-carotene were 2.68, 7.06, 27.59 and 9.87μg/g-1 in the 4 different types of lines, respectively. The total contents of the 3 main carotenoids were 10.89, 47.05, 69.90 and 1363.99 μg/g-1 of fresh petal in the 4 types of rapeseed lines, respectively. It was evident that the golden-yellow petal lines contained an extremely high content of lycopene and also a high content of xanthophill in the flower petals; the yellow petal lines contained a high β-carotene content in the petals; and the white petal lines were low in all the 3 main carotenoid contents. The total contents of the 3 main carotenoids in the petals were shown in the order of white petal < pale-yellow petal < yellow petal < golden-yellow petal lines. The difference in contents of the 3 main carotenoids was small among different rapeseed lines with the same type of petal color.

**Conclusion:** The total contents of the 3 main carotenoids in petals were positively related to the shade of flower color in *B. napus L.* Flower petals with the same type of color contained close contents of carotenoid compositions. The yellow petal color and the golden-yellow petal color of flowers were highly correlated to the high contents of β-carotene and lycopene in the petals, respectively. White flower petals were in low contents of nearly all the kinds of carotenoids.

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# Inheritance and molecular marker identification of the yellow seed character in Ya'an Yellow Rape (*Brassica rapa L.*)

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**Background:** Ya'an Yellow Rape is a landrace cultivar of *Brassica rapa L.* long and widely grown in the Ya'an district, Sichuan Province, Southwest of P. R. China. This landrace has a high rate of yellow-seeded plants, containing high oil content, maturing early and adapting especially to the wet and sunshine-scarce mountainous regions. It is an elite landrace cultivar of *B. rapa* with potential uses in yellow-seeded cultivars breeding in both *B. rapa L.* and *B. napus L.*. However, the genetic rules and gene markers of the yellow seed character are not clear. Studies have demonstrated that, compared to black and brown seeds, yellow seed in *Brassica* species has a higher oil content, a thinner seed coat, and a higher protein content in the meal (Jonsson et al. 1977; Liu et al. 1992). A research on the yellow seed trait of Ya'an Yellow Rape will facilitate the utilization of this special landrace resource in *B. rapa L.*

**Objectives:** We carried out the studies to: (1) investigate the inheritance pattern of the yellow-seeded trait in Ya'an Yellow Rape, and (2) identify SSR molecular markers tightly linked to the seed color gene in the line, and furtherly anchor the seed color gene to specific linkage group in *B. rapa L.*

**Methods:** One yellow-seeded inbred line (P1) and one brown-seeded inbred line (P2) developed from Ya'an Yellow Rape were selected and the six genetic populations, including P1, P2, F1, F2, BC1 and BC2, were prepared for the genetic study on seed color of the land cultivar. The populations were planted on the experimental station for rapeseed breeding in Dayi, Chengdu in 2013. Seed colors were investigated visually with 55, 66, 54, 316, 295 and 152 random plants for P1, P2, F1, F2, BC1 and BC2, respectively. Segregation ratios were calculated in F2, BC1 and BC2 populations, respectively, and X2 tests were made to evaluate the goodness of fit to the expected ratios. Two hundred and forty-nine individual plants were taken from BC1(P1/P2//P1) to identify the SSR makers associated with the yellow seed color trait . Fifteen pairs of SSR primers were selected from previous reports (Li et al. 2012; Liu et al. 2009; Padmaja et al. 2013) and one pair of SSR primers, TT8-SSR, was designed based on the sequence of BrTT8 gene that was reported to be the yellow seed color gene in *B. rapa L.* (Li et al. 2012). PCR amplifications were performed with these SSR primers and the SSR markers associated with the seed color genes were analyzed.

**Results:** Seeds from all the $F_1$ plants, including reciprocal cross $F_1$ hybrid, were in brown color, showing that the brown color seed trait was dominant, without any cytoplasmic effects. The ratio of brown-seeded plants : yellow-seeded plants in $F_2$ generation was 245 : 71, well fitted to the expected ratio 3:1 (x2= 0.949, = 3.84). The ratio of brown-seeded plants : yellow-seeded plants in $BC_1$ was 161:134, fitted to the expected ratio 1:1 (x2= 2.292, = 3.84). The $BC_2$ generation (152 plants) comprised only brown-seeded plants, fitted to the expected ratio 1:0 (x2= 0, = 3.84). These observations indicated that only one Mendelian gene locus was involved in control of the seed color trait in Ya'an Yellow Rape.

Four SSR markers (gsr23, gsr29, gsr44 and TT8-SSR) showed specific band patterns for each seed-color trait and were found to co-segregate with the seed color genes. The other two SSR markers, OL12-F02 and Na10-A08, were identified to be 0.8 cM away from the seed color gene and located on the same side of the gene.

**Conclusions:** The present study showed that the yellow-seeded trait in Ya'an Yellow Rape was controlled by a single recessive gene. The 4 SSR makers (gsr23, gsr29, gsr44 and TT8SSR) were completely associated with the seed color trait and can be used as good molecular makers for selection of the trait. The two SSR markers OL12-F02 and Na10-A08 were 0.8cM apart from the gene and located on the same side, indicating that the two SSR makers were dramatically close to each other and on the same locus.

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# Microsporogenesis of reverse thermosensitive male sterility Huiyou50S in *Brassica napus*

**Background:** Thermo-sensitive genic male-sterility (TGMS) has great advantages in hybrid crop production. Reverse TGMS line Huiyou50S in B. napus was bred from a spontaneous semi-sterile plant found in our breeding nursery in April of 2000 in a Chinese cultivar Huiyou50. Different from common TGMS, the reverse TGMS Huiyou50S is sterile when cultured in low temperature but fertile in high temperature.

**Objectives:** A comprehensive understanding of the development mechanism is essential for the efficient utilization of a male sterility before it is used to produce of F1 hybrids. The objectives of this study were to make further explanations for the developmental aberrations leading to male sterility.

**Methods:** Floral buds representing a wide range of developmental stages were collected from plants of male fertile and sterile. Anther development of Huiyou50S was observed by light and electron microscopy.

**Results:** Microstructural evidences indicated that the anther abortion occurred at the tetrad to early uninucleate microspore stage. The protoplast of uninucleate microspore condensed and then degraded, eventually, only the distorted shells remained. The tapetal cells vacuolated at tetrad stage and degenerated rapidly before microspore disintegration. Observed under transmission electron microscope, tapetum abnormality exhibited at the tetrad stage and then the cytoplasm of microspores was apparently disintegrated at mid-microspore stage. The tapetum was disrupted rapidly at vacuolated microspore stage, without elaioplast formed. Subcellular alterations in Huiyou50S anthers included undeveloped primexine in tetrads and microspores, dysfunctional plastids and a loss of recognizable elaioplast in the tapetal cells. The evidences suggested that tapetum abnormality was associated with microspore abortion in Huiyou50S.

**Conclusions:** The cytological evidence found in this investigation were important for other theoretical research, for example, right time for sampling in the differential analysis on the transcriptome, proteome, and metabolome, and some biological pathway involved in male sterility. Thereafter, it will promote the research on the development of plant male gametophyte and broaden the range of heterosis utilization of rapeseed.

# Introgression of the clubroot resistance gene *Rcr1* from *Brassica rapa* into *B. napus*

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**Background:** Clubroot disease, caused by *Plasmodiphora brassicae*, poses a serious threat to canola production in Canada. Clubroot resistance (CR) gene Rcr1, which confers resistance to pathotype 3, was identified in a pak choy cultivar "Flower Nabana" (FN) (Chu et al., 2014). Resistance to pathotypes 2, 5 and 6 in FN was also mapped into the Rcr1 region and SNP markers tightly linked to *Rcr1* were developed (Yu et al. 2014).

**Objective:** We introgessed *Rcr1* from *B. rapa* into *B. napus*, which could enable canola breeders to accelerate incorporation of *Rcr1* into their canola varieties. Furthermore, a common *B. napus* line carrying *Rcr1* will be part of a set of isogenic spring type *B. napus* lines for identification of pathotypes of *P. brassicae* and monitoring in situ changes in race structure of *P. brassicae* within canola fields.

**Methods:** An interspecific cross between FN and a *B. napus* doubled haploid line DH16516 originating from the cultivar Topas was conducted. The resulting F1 was crossed with an elite AAFC breeding line SV11-17667 to produce a $BC_1$ generation. The genetic composition of the A-genome and the number of C-genome chromosomes in the BC1 population was determined using a genome-wide SNP (INFCanSeq_6K_illumina) analysis applied to the $BC_1$ resistant plants. One $BC_1$ resistant plant was selected for further backcross with DH16516 to produce $BC_2$. The resulting $BC_2$ plants were analyzed with a gene-specific SNP marker SNP_A03_13 for *Rcr1* and self-pollinated to produce $BC_2S_1$.

**Results:** One BC1 plant (N66) was obtained that carried *Rcr1* with limited amount of FN genetic background out of the 77 BC1 plants and a full set of C-genome chromosomes most similar to natural *B. napus*. Resistance to pathotype 3 was evaluated in 45 $BC_2$ plants, which were also analyzed with the SNP_A03_13. The SNP marker co-segregated with the disease reaction phenotypes. Eleven $BC_2$ plants heterozygous at the Rcr1 locus were self-pollinated to produce $BC_2S_1$. A total of 140 plants in one $BC_2S_1$ family were tested for resistance to pathotype 3 and they showed a 3:1 ratio for resistance: susceptibility. Also, *B. napus* plants homozygous at the Rcr1 locus were identified by using SNP_A03_08. In addition, the reaction of 36 $BC_2$ plants to a new pathotype of *P. brassicae*, 15 of 36 plants were highly resistant to this new pathotype.

**Conclusions:** Spring type *B. napus* lines carrying the clubroot resistance gene *Rcr1* from *B.rapa* were developed by employing a combination of molecular genetic approaches and conventional breeding methods.

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# Separation of antifreeze protein and expression analysis of Copper and Zinc superoxide dismutase (*Cu/Zn-SOD*) gene from winter turnip rape

**Background:** Winter turnip rape (*Brassica campestris L.*) has been a valuable ecology and oil crops in cold and arid region of northwest China. Cold and extremely low temperature cannot make winter turnip rape successfully overwinter and limit its production

**Objective:** "Longyou 6"and "Longyou 7" are two ultra cold-tolerance winter rape cultivar, which can resist an extremely low temperature(-32°).our objective is to separated antifreeze protein and clone related gene from winter tTurnip rape.

**Method:** Antifreeze protein was separated from "Longyou 6" by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and mass spectrometry. The cDNA sequence of *gene* was isolated by RT-PCR, the obtained cDNA sequence was analysed. Real time RT-PCR was used to assess the expression of related gene in response to lower temperature stress. The enzyme activity was measured by NBT deoxidization method in leaves and roots.

**Results:** Results showed that copper and zinc superoxide dismutase (*Cu/Zn-SOD*) was an important antifreeze protein. The cDNA of *Cu/Zn-SOD* was cloned from Longyou7 by RT-PCR, using the primers designed according to the published crucifer *Cu/Zn-SOD* cDNA sequences. The sequence of *Cu/Zn-SOD* from winter turnip rape was 459 bp, encoding a predicted protein of 152 amino acid residues. It contained specific sequence characteristics and conserved domain of *Cu/Zn-SOD* superfamily. The expression analysis showed that the *Cu/Zn-SOD* was a differential expressed gene induced by lower temperature.

**Conclusion:** *Cu/Zn-SOD* was was an important antifreeze protein. The gene had genetic characteristics similar with other known species and it could provide reference for functional research, and it might play a role in cold tolerance of the Winter Turnip Rape

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# Study on the heterosis of photosynthetic traits of *Brassica napus*

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**Background:** High photosynthetic efficiency combined with heterosis may be an effective way to break through the yield bottleneck of rapeseed. Studying the photosynthetic physiology and breeding for high photosynthetic efficiency should be important contents for the breeding of rape at present an in the future.

**Objectives:** The heterosis of photosynthetic traits of *Brassica napus* in different growth stages and different cross combinations were compared. In addition, the correlations of photosynthetic traits between hybrids F1 and their parents were analyzed in order to reveal the true circumstance of photosynthetic traits and provide basis for high photosynthetic efficiency breeding.

**Methods:** Four cytoplasmic hybrids F1 of *B. napus* (Zayou 59, Qinyou No.7, Qinzayou No.1, and Qinzayou No.3) and their parents and 19 pairs of orthogonal and reciprocal combinations were used as research materials in this experiment. By using some measurement instruments photosynthetic traits such as photosynthetic area, main photosynthetic parameters, net assimilation of total plant, net population photosynthetic rate and the chlorophyll content were determinate at different stages, and the heterosis of F1, correlations between parents and offspring and genetic effect of photosynthetic traits were also be studied in this experiment. At last, all the data were statistical analysis with SPSS 13.0 software.

**Results:** The photosynthetic area and the net assimilation of total plant of hybrids F1 were obvious positive heterosis in the whole growth stage. The main photosynthetic parameters were obvious positive heterosis in specific stage. The chlorophyll content was little heterosis and the crop growth rate was obvious negative heterosis in the whole growth stage. The heterosis of photosynthetic traits were that: the photosynthetic area > the net assimilation of total plant > the main photosynthetic parameters > the chlorophyll content > the crop growth rate; the net photosynthetic rate and chlorophyll content of orthogonal combinations were higher than reciprocal combinations by 2.34% and 1.26% respectively. The over-stand heterosis, mid-parents heterosis and over-parent heterosis of net photosynthetic rate of 10 orthogonal combinations were higher than corresponding reciprocal combinations and the three heterosis of chlorophyll content of 11 orthogonal combinations were higher than corresponding reciprocal combinations; the photosynthetic traits of parents, especially female parent, value of mid-parent and high parent were high correlation with hybrid F1. It was showed that the photosynthetic traits were controlled by both environment and gene, and it was play an important role in photosynthetic traits genetic that genetic effect of add and dominant.

**Conclusions:** The photosynthetic traits of *B. napus* had heterosis and the hybrid F1 was influenced greatly by parents on photosynthetic traits. In the breeding for high photosynthetic efficiency of rape, the photosynthetic traits of parents should be improved so as to change the photosynthetic traits of hybrid F1 and the high value parent should be as female parent.

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# A beautiful story about the *AP3* gene mutations and petals development

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**Background:** The emergence of core eudicot petals makes the world more various and colorful. However, origin and evolution of the petals is still mysterious. Despite more results suggest that evolution of core eudicot petals is more relate to APETALA3 (AP3) lineage duplication, but until now it is still unclear what happened in the lineage bringing the brilliant evolution.

**Objectives:** Our previous research distinguish that two kinds of higher homologous *AP3* genes, *B.AP3.a* and *B.AP3.b,* specified petals and stamens development of *Brassica*. *B.AP3.a* regulates petal and stamen normal development, whereas *B.AP3.b* losing 24-bp sequence (8 residues) only specifies stamen development and has little effect on petals development. Due to the 24-bp difference existing natively and exhibiting two statuses before and after *AP3* mutation, we inferred that the 24-bp foreign insertion probably led to the petals evolution of core eudicots. Further explore what bring functional differentiation of the *AP3* genes will help us to uncover the secret of the origin and evolution of core eudicot petals.

**Plant Materials and Methods:** A series of *AP3* related mutants of *Brassica rapa* and *Brasscia napus* were used. In addition, the *ap3-3* mutants, with homeotic identity of petals to sepals and stamens to carpels, of *Arabidopsis thaliana* (landsberg erecta) were used as the transgenic recipient. A series of molecular biological techniques were used.

**Conclusions:** The two higher homologous *AP3* genes of *B.rapa* had obvious functional differentiation in the petal development: *B.AP3.a* as a major gene generated wild-type normal petals, while *B.AP3.b* as a redundant gene generated small dosage petals; In *B. rapa*, the mutations of *B.AP3.a* and *B.AP3.b* genes generated the sepal carpeloid mutant HGMSa. *Brassica napus* contained two *B.AP3.a* and two *B.AP3.b,* loss of the two *B.AP3.a* functions was the key reason for the apetalous mutation, however, the loss-of-function in all four *AP3* genes led to the sepal carpeloid mutant AMSa; The 24-bp special sequence was the key loci to causing functional differentiation of the *B.AP3.a* and *B.AP3.b* genes; The *euAP3* motif and PI-derived motifs of *AP3* are irrelevant to petals formation, and the 8 amion acids (the 24-bp sequence) of *AP3* protein were the key loci to specify normal petal development; The 24-bp special sequence insertion probably gave rise to the functional differentiation and promoted great evolution of core eudicot petals.

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# The comparison analysis of transcriptome in waterlogging *Brassica napus L.*

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**Background:** Rapeseed is the second largest oil crop worldwide. In China, the largest planting country in the world, 80% of rapeseed is planted along the Yangtze River. In this area, rapeseed is always planted in the paddy field as rotation crop just after rice and rainfall occurs a lot during the season, leading to serious waterlogging (Zou et al., 2013). Most researches were focused on physiological and morphological traits of the response to waterlogging in rapeseed. However, there is little study on molecular mechanism. The only research was carried by Zou using a tolerant variety, ZS9 (Zou et al., 2013).

**Objectives:** Studies reveal there is natural variation of waterlogging tolerance in rapeseed (Zou et al., 2013; Zou et al., 2014). One way of exploiting waterlogging tolerance is to unravel genetic mechanisms beyond natural variation. It will be helpful to perform comparative transcriptome analysis between tolerant and sensitive varieties under waterlogging.

**Methods:** By the comparison between transcript profiles of ZS9 and GH01, 2977 genes with similar expression patterns and 17 genes with opposite expression patterns were identified. Besides, 1438 genes and 1861 genes were indicated to be specifically regulated in ZS9 and GH01, respectively. Analysis of overlapped genes between ZS9 and GH01 revealed that waterlogging tolerant ability is kindly decided by the regulation ability of genes with same expression patterns. Moreover, the opposite gene expression patterns revealed ABA signal might contribute to waterlogging tolerance.

**Results:** By the comparison between transcript profiles of ZS9 and GH01, 2977 genes with similar expression patterns and 17 genes with opposite expression patterns were identified. Besides, 1438 genes and 1861 genes were indicated to be specifically regulated in ZS9 and GH01, respectively. Analysis of overlapped genes between ZS9 and GH01 revealed that waterlogging tolerant ability is kindly decided by the regulation ability of genes with same expression patterns. Moreover, the opposite gene expression patterns revealed ABA signal might contribute to waterlogging tolerance.

**Conclusions:** Thus, this study reduces the number of candidate genes evidently, and helps to focus on a limited number of the genes to be investigated by more specific and detailed functional analysis, which it can be useful for further study.

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# Genetic analysis of seed yield in *Brassica napus L.* by mixed major gene and polygene inheritance model

**Background:** Seed yield is a very complicated quantitative trait. Many researches have been performed on its inheritance in *Brassica napus L.*. However, the genetic basis still is one of research focuses. The early opinion showed that seed yield was dominated by many minor-effect genes which genetic effects were assumed equal to each other. Other studies argued that many quantitative traits including seed yield were simultaneously controlled by few major genes and many polygenes. This latter is called the mixed major gene plus polygene inheritance model (or mixed inheritance model/ mixed genetic model).

**Objectives:** To dissect the genetic components involved in seed yield per plant and their heritability. It will improve the selection efficiency for good genotype of seed yield.

**Methods:** The joint segregation analysis method of mixed major gene plus poly-gene genetic model was used to study the inheritance of yield per plant. According to the theory that major gene effects in a segregating generation is an independent normal distribution modified by the polygene and the environment, four populations of the parents $P_1$, $P_2$, $F_1$ and $F_{2:3}$ (derived from $F_2$) were investigated. The most suitable genetic model was selected using Akaike's Information Criterion and the fitness of the selection was tested by a set of fitness tests.

**Results:** The frequency distributions of yield per plant in F2:3 family populations showed the characteristics of mixture normal distribution, which indicated that the inheritance of seed yield per plant followed the major gene plus poly-gene model. Results indicated that genetic model C-0 was the most fitted genetic model for the seed yield per plant, no major gene controlled seed yield. In another word, yield per plant in oilseed rape was controlled by additive-dominance-epistasis polygenes. The additive effect of major gene was -3.88, which indicated locus of the allele from female parent decreased the yield per plant, on the contrary, the locus of the allele from the male parent increased it. The dominant effect of polygene was 20.73. The polygene heritability in $F_{2:3}$ was 30.29%.

**Conclusions:** Genetic model C-0 was the most fitted genetic model for seed yield per plant. Seed yield per plant in in *Brassica napus L.* was controlled by additive-dominance-epistasis polygenes. This indicated that additive-dominance-epistasis polygenes played a crucial role in the control of seed yield per plant. Dominant effects were higher than additive effects. The results provided strong evidence that seed yield per plant was a complicated quantitative trait; additive, dominant, and epistasis effects were the genetic basis of heterosis in rapeseed

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# Genetic mapping of a single co-localizing QTL for clubroot resistance to five pathotypes of *Plasmodiophora brassicae* in *Brassica rapa*

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**Background:** Clubroot disease, caused by *Plasmodiophora brassicae*, was identified in canola fields in central Alberta in 2003 and is continuing to spread on the Canadian prairies, posing a serious threat to canola production in western Canada. There are at least five pathotypes of *P. brassicae* (2, 3, 5, 6 and 8) identified (Strelkov & Hwang 2014). Most resistance sources in *Brassica* species are race- or pathotype-specific (Diederichsen et al. 2009).

**Objectives:** We aimed to evaluate the resistance to different Canadian pathotypes of *P. brassicae*, map a clubroot resistance (CR) gene using tunable genotyping-by-sequencing, and develop SNP markers tightly linked to this CR gene in *B. rapa*.

**Methods:** One *B. rapa* canola breeding line T19, highly resistant to five pathotypes of *P. brassicae*, was crossed with a susceptible *B. rapa* canola DH line ACDC, and the resulting $F_1$ was backcrossed to ACDC to generate a $BC_1$ population. $BC_1S_1$ lines were produced by self-pollinating individual $BC_1$ plants. The parents and 92 $BC_1S_1$ lines with 12 plants each were inoculated with single-spore isolates of pathotypes 2, 3, 5, 6 and 8, respectively. Plant inoculation, disease rating and index of disease (ID) calculation followed the methods described by Strelkov et al. (2006). DNA was extracted from young leaves following the protocols of the DNeasy Plant Mini Handbook (Qiagen). DNA sequencing and genotyping were performed at Data2Bio (Ames, Iowa, US).

**Results:** All ACDC plants developed severe galling, with IDs of 100% in response to each pathotype, while T19 exhibited a resistant response with IDs of 0. The distribution of IDs in the BC1S1 lines was divided into two classes: 43 lines were susceptible (ID > 60) and 49 lines were resistant or partially resistant (ID < 60), which fitted a 1:1 ratio (x2= 0.53, P > 0.5). A genetic map consisting of 10 linkage groups with 1,438 high quality SNPs was constructed with an overall genetic length of 1,165 cM. A single co-localizing QTL namely *Rcr4* on chromosome A03 was detected for all 5 pathotypes, and 7 SNPs tightly linked to the QTL were identified.

**Conclusions:** CR in the *B. rapa* breeding line T19 to all five pathotypes of *P. brassicae* was completely associated. It is controlled by a single co-localizing QTL *Rcr4* on chromosome A03 of *B. rapa*. SNPs in the vicinity of *Rcr4* could be used for marker assisted selection in canola breeding.

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**POSTERS THEME A**

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# High-density single nucleotide polymorphism (SNP) array mapping for yield-related quantitative trait loci in *Brassica napus L.*

**Background:** Seed weight (SW) and silique length (SL) are two important yield-related traits in oilseed rape. In the last two decades, large efforts have been devoted to unravel these complex traits through quantitative trait locus (QTL) mapping. While most studies were based on low density linkage maps constructed by limited molecular markers (e.g. RFLP, AFLP and SSR), they were insufficient to provide accurate QTL information that controls the traits. Nevertheless, large number of markers can be created by single nucleotide polymorphisms (SNPs), which are commonly used for the detection of genetic variations and construction of high-density genetic maps in rapeseed.

**Objectives:** Our goals are to construct a high density SNP map of *B. napus* and mapping QTL for SW and SL, using SGZ-DH population across eight environments. This approach would provide adjacent markers for rapeseed breeding and obtain the precise QTL intervals for map-based cloning of target genes.

**Methods:** A newly segregated DH population was obtained from the cross between "SG-DH198" (one line from SG-DH population) (Zhao et al. 2005) and a modern Chinese variety "Zheshuang 72" (SGZ-DH population 161 lines ). Field experiments were conducted in eight environments, in which SW was from open-pollinated bulked seeds and SL obtained from measuring 10 siliques of 5 plants (main inflorescences). The *Brassica* 60K SNP Bead Chip Array was used to genotype 161 DH and parental lines. Imaging of the arrays was performed using an Illumina HiSCAN scanner, whereas allele calling for each locus was conducted by the GenomeStudio genotyping software v2011. The SNP marker data was scored according to the definition of JoinMap 4.0. WinQTLCart 2.5 software was applied to carry out QTL analysis.

**Results:** We constructed the SGZ genetic map that comprised of 1333 bins corresponding to 3516 SNP markers. The map spanned 1883.5 cM across nineteen chromosomes with an average interval of 1.41 cM between bins. Using composite interval mapping, four (qSWA2, qSWA7, qSWC1 and qSWC9) and six (qSLA6-1, qSLA6-2, qSLA7, qSLC4-1, qSLC4-2 and qSLC9) significant QTLs for SW and SL were repeatedly detected across environments, which together explained 41.61% and 66.55% of the phenotypic variations in population, respectively. Two major loci that co-localized on A7 (qSWA7/ qSLA7) and C9 (qSWC9/ qSLC9) showed pleiotropic effects for both traits, with "Zheshuang 72" as the favorable alleles. These results explained the significant correlations between SW and SL (R=0.20-0.36) as observed in population. The peak position of these two co-localized QTLs spans the physical regions of 18.21-18.23 Mb and 26.23-26.31 Mb in the *B. napus* genome sequence.

**Conclusions:** The SGZ-SNP map developed in the current study serves as an important tool for future comparative map study. The identified physical positions of QTL could benefit the cloning of candidate genes underlying QTL and assist the high yield breeding.

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# Physical mapping of the *OilA7* region and identification of a candidate gene in *Brassica napus*

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**Background:** Oil content in rapeseed is one of the most important economic traits. Despite a large body of QTL information, map-based gene cloning in *B. napus* is rare. In our initial QTL analysis, *OilA7* was detected in all eleven environments, showing the largest additive effect for oil content in SG population (Zhao et al., 2005, 2012). Using substitution mapping, *OilA7* was validated and fine mapped in a reduced genomic interval (Zhao et al., 2011). Here, we present our work on further fine mapping and candidate gene analysis.

**Objectives:** Based on the fine mapping of *OilA7* by developing locus specific markers and creating higher generations of substitution populations, we aimed to isolate the candidate genes in delimited QTL region by combining bioinformatics analysis and gene expression profile. To further validate the candidate genes, we adopted real-time PCR, allelic comparison of gene structures and transgenic analysis.

**Methods:** According to the flanking marker sequences of *OilA7*, homologous genomic regions from *Brassica* database (BRAD) (http://brassicadb.org/brad) were selected for marker design. Fine mapping was performed based on the marker genotypes of $BC_4F_2$ / $BC_4F_3$ plants and trait phenotypes of $BC_4F_{2:3}$ / $BC_4F_{3:3}$ families. Oil content was determined by Soxhlet extraction with two replicates. We used RNeasy mini kit (QIAGEN) to isolate the total RNA from developing seeds (5-7, 15-17 and 25-27days after flowering) of two parents (Sollux and Gaoyou) and from $BC_4F_4$ NILs. RNA samples were sequenced on an Illumina HiSeq 2500 with 100 cycle pair-end run. RNA-Seq reads were mapped to a reference genome using TopHat. The transcript abundances were measured in FPKM (fragments per kilobase of exon per million fragments mapped) by Cuffinks 2.1.1. The differential expressions (FDR ≤ 0.05) were analyzed with Cuffdiff. To further validate the allelic difference of candidate genes, real-time PCR was adopted with NILs. The recombinant plasmids that contain target alleles from NILs are being sequenced.

**Results and Conclusions:** *OilA7* locus was delimited in a genomic region on A7 ca. 300kb, where 8 of 60 genes (according to the BRAD) showed significant difference of transcript abundances between parents in at least one of three developmental stages. Bioinformatics analysis suggests two genes as the candidates for *OilA7*, one dictates the thickness of seed coat and the other codes a key enzyme in the fatty acid biosynthesis passway. Further, real-time PCR analysis on $BC_4F_4$- NILs suggests one of them to be the more potential candidate, since Gaoyou allele (increasing oil) behaved 25 times higher in gene expression than Sollux in the seed stage of 17-20 days after flowering. Sequencing two candidate genes and transgenic analysis are underway.

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**POSTERS THEME A**

# Utilization of a novel recessive glossy mutation in hybrid breeding

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**Background:** The dominant sterility, controlled by epistatic interaction between the dominant GMS gene (Ms) and the suppressor gene (Rf), was reported by Li et al. (1985). Base on this GMS, the three-line system has been developed (Li et al. 1995), and its two hybrid cultivars, such as Heza No.7 and Heza No.9 have been registered in China recently. It is time-consuming and laborious to remove false hybrids from the 100% sterile population during hybrid seed production. In order to overcome this problem, the novel recessive glossy genes were transferred into the homozygous two-type line.

**Objectives:** The homozygous two-type line of the dominant GMS was used in this study including 50% fertile plants (Genotype: MsMsrfrf) and 50% sterile plants (Genotype: MsMsRfrf). The novel recessive glossy mutation was as the donor controlled by two recessive genes (g1and g2).

**Methods:** Traditional plant breeding methods, such as crossing, self-pollination and test crosses were used. Fertility survey in segregating population was made during flowering period.

**Results:** The cross was made between the fertile plants (Genotype: MsMsRfrfG1G1G2G2) among the homozygous two-type line and a novel recessive glossy mutation (Genotype: msmsrfrfg1g1g2g2), and the sterile line with glossy trait (Genotype: MsMsrfrfg1g1g2g2) was obtained from the generation of self-pollination. The fertile plants from a cross the sterile line with glossy trait (Genotype: MsMsrfrfg1g1g2g2) × the homozygous two-type line fertile plants (Genotype: MsMsRfrfG1G1G2G2) backcrossed with the sterile line with glossy trait (Genotype: MsMsrfrfg1g1g2g2), and then obtained the fertile plants with glossy trait (Genotype: MsMsRfrfg1g1g2g2). The new homozygous two-type line with glossy trait was produced by continuous sib-crossing between the sterile line with glossy trait and the fertile plants with glossy trait. The 100% sterile population with glossy trait was obtained from the sterile line with glossy trait × the novel recessive glossy mutation.

**Conclusions:** There are great differences between glossy trait and the normal trait in *Brassica napus*, which could be visually distinguished. Base on the glossy trait, it is easy and convenient to identify the purity of the 100% sterile population. More importantly, this trait was used to get rid of the false seeds mixed in the 100% sterile population in early stage. Because the false plants were removed before being planted for hybrid seed production, it is unnecessary to get rid of the false plants in hybrid production fields. So, there was significant savings in labor.

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# Genetic dissection of seed yield per plant in *Brassica napus L.* by QTLs mapping

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**Background:** In crop breeding, high yield is the major objective. Therefore, seed yield is central to the analysis of genetic rules and exploration of approaches for crop breeding improvement. Among the approaches, heterosis utilization is the most important method for seed yield improvement. Seed yield of rapeseed is a complicated quantitative trait. To explore the genetic basis, many theoretical researches have been performed on heterosis. However, the genetic basis of heterosis continues to be a contentious issue. Nevertheless, QTLs mapping and genetic effects analyses of seed yield can help us to understand the origins of heterosis and improve the selection efficiency of superior genotypes for seed yield.

**Objectives:** To elucidate the genetic basis of heterosis better and improve the selection efficiency and accuracy of the superior genotypes for seed yield, QTLs and the genetic components involved in seed yield per plant were detected and dissected.

**Methods:** A linkage map is constructed with SRAP , AFLP and SSR markers by F2 populations. QTLs and epistasis are likewise detected. The statistic software of Windows QTL Cartographer Version2.0 and CIM were applied to identify the QTLs of seed yield.

**Results:** Four QTLs, yp13, yp9, yp12 and yp3 were detected and located on linkage groups 13, 9, 12 and 3, respectively. These QTLs each accounted for 7.26%, 7.53%, 6.99% and 10.27% of the phenotypic variation, respectively, alleles from female parent increase the effects of QTLs yp13, yp12 and yp3. On the other hand, the alleles from the male parent decrease the effects of QTL yp9. Genetic effects of all of the four main QTLs showed positive over-dominance. The 10 significant two-locus combinations controlling seed yield per plant were detected by two-way ANOVAs among seventeen co-dominant markers, which have significant effects on seed yield per plant in rapeseed. The interaction effects analysis also showed that DD and DA were the most significant type of interaction in seed yield per plant.

**Conclusions:** The results indicate that the number for two-loci combinations involving the entire genome detected in seed yield per plant was greater than that of one-locus. Epistasis interaction included loci of both QTL and non-QTL, in which the later was in the majority. The results also provided strong evidence that epistasis plays a significant role as the genetic basis of heterosis. In general, additive, dominant, and epistasis effects are the genetic basis of heterosis in *Brassica napus L.*

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**POSTERS THEME A**

# Restriction enzyme digestion of chloroplast DNAs, PCR amplification of specific genes and molecular cloning of rbcL genes on Polima CMS and Shan 2A CMS

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**Background:** Polima CMS and Shan2A CMS are two of the classic and effective cytoplasmic male sterility(CMS) lines in *Brassica napus L.,* and the two famous CMS lines have played very important roles in study and utilization of rapeseed heterosis in China even in the world. In recent years, in cytoplasm study of the two sterile lines, about comparative study of mitochondrial DNAs(mtDNAs) of the two has been much more reported(Yang et al. 1998; Wang et al. 2001; Lin et al. 2006). However, about comparative study of chloroplast DNAs(cpDNAs) has been seldom reported.

**Objectives:** This research compared Shan2A CMS with Polima CMS in their chloroplast genomes through restriction enzyme digestion of cpDNAs, PCR amplification of the specific genes and molecular cloning of rbcL genes about the two CMS syetems.

**Methods:** In accordance with the method of Liang et al.(1995), cpDNAs were extracted by seedling leaves from four materials including Polima CMS and its maintainer line PolB, Shan2A CMS and its maintainer line Shan2B, and several special gene fragments of cpDNAs(Accession number: AF267640, AY752707, AY752722, AY752708, AY752724) were amplified by PCR. The RubisCO large subunit(rbcL) genes(Accession number: AF267640), which were related to photosynthesis in the 5 special gene fragments, were further cloned and sequenced in the 4 materials. Restriction enzyme digestion and gel electrophoresis detection were also performed respectively to cpDNAs of the 4 materials using an excess of 4 kinds of enzymes including *BamH*, *EcoR*, *Hind* and *Pst*.

**Results:** The results were as follows: The same one target band was found respectively in horizontal submarine agarose gels in the 4 materials when 5 pairs of the special gene primers of cpDNA in rapeseed were used, and amplified products were consistent with expected target fragments. The sequencing results showed that the DNA sequences of amplification products of the 4 materials were exactly the same, their gene sizes are respectively 1733 bp, and the sequences are in accordance with that of the forecast purpose fragment(rbcL gene). The results of comparative analysis of enzyme fragment polymorphism indicated that the band types in the 4 materials are same and the enzyme digestion products have no difference, respectively.

**Conclusions:** The above results reflect the conservatism of chloroplast genomes of the two sterile systems in a certain extent.

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