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ORAL PRESENTATION THEME D

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

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

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

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

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

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

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

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

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

Methods: The experiment was carried out at four sites in France from 2011 to 2014. Winter oilseed rape *(Brassica napus)* -subsequently referred to as 'WOSR'- was grown as sole crop and intercrop with three different legume mixtures: (i) faba bean *(Vicia faba)* + lentil *(Lens culinaris)* -subsequently referred to as 'FL'-; (ii) grass pea *(Lathyrus sativus)* + fenugreek *(Trigonella foenum-graecum)* + lentil -subsequently referred to as 'GFL'-; and (iii) purple vetche *(Vicia benghalensis)* + common vetch *(Vicia sativa)* + berseem clover *(Trifolium alexandrinum)* –subsequently referred to as 'VVT'. The experimental plots (120 to 200 m^2) were set up in a randomized block design with WOSR, WOSR+FL, WOSR+GFL, WOSR+VVT as treatments with three replicates. In each trial, sole WOSR was managed according to local agriculture guidelines and conditions. The management of intercrops differed from sole WOSR in terms of nitrogen fertilization rates which were reduced by 30 kg ha-1.

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

Conclusions: Intercropping frost-sensitive legume crops with winter oilseed rape is thus a promising way to reconcile yield and reduction in pesticides and fertilizer use and perhaps to benefit more widely to the cropping system.

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Gene flow depends on the initial transgene location in the crop: *Brassica napus-Raphanus raphanistrum* model

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

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

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

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

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

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

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Pinolene-based compounds to reduce oilseed rape drought-induced yield losses

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Background: Oilseed rape (Canola, *Brassica napus L.*) shows strong yield decreases when water stress periods occur at the reproductive stages: the total seed production can be affected up to 50% (Champolivier and Merrien, 1996). Whereas the breeding for a drought tolerant oilseed rape variety seems far to be achieved, the need to meet suitable yield under water deficit conditions force to study further agronomic techniques to achieve the purpose. Nearly fifty years ago, film-forming antitranspirants were considered a promising agronomic tool to preserve water in plants and thus avoid yield decreases under water deficit: their effectiveness was limited as their stomata-blocking property was strongly related to a drastic decrease in CO_2 assimilation (Kettlewell, 2014). More recently, they have been shown to be effective in improving yield under drought conditions when applied at the most drought-sensitive stage (Kettlewell, 2014).

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

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

Results: The results showed improvements in most of the physiological traits assessed: Gs was significantly decreased by the treatments ($p<0.001$) accompanied with a significant increase in CO_2 assimilated by droughted-sprayed plants compared to the un-sprayed, leading to a strong increase in water use efficiency. On the contrary the well watered-treated plants showed suppression in both Gs and CO_2 assimilation. LWP and RWC were significantly increased, proving the effectiveness of the treatments in improving plants water status under drought conditions. Thermal imaging analysis showed a significant increase in Lt under water stress conditions ($p<0.001$): the two compounds demonstrate different ability on blocking stomata with a stronger increase in Lt of the Di-1-*p* menthene compared to the Poly-1-*p* menthene treated plants. Gs and Lt were significantly correlated ($p<0.001$). Yield components were statistically increased by the film-forming treatments with a significant increase in pods per plant and seeds per plant.

Conclusions: Despite the common idea that a film forming antitranspirant treatment suppresses CO_2 assimilation and fixation leading to a decrease in photosynthetic efficiency and thus productivity, we demonstrate that applying film-forming compounds at 1L/ha over the most drought-sensitive stage on oilseed rape could be effective in avoiding drought-induced yield losses.

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

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

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

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

Results: The main contributors to the selected environmental impacts were field emissions (N_2O in air for Global Warming, NH_3 in air for Acidification, NO_3^- nitrate in water for Marine Eutrophication). Experimental data, from a French network (the NO GAS project), showed that the direct emissions calculated with the tier 1 IPCC method (2006) tended to be overestimated: the calculated N_2O emissions of rapeseed crops were 2.2 times higher than the measured emissions. The assessment of the effect of improved practices, such as organic fertilization or the introduction of legume crops in the rotation, showed some improvements on environmental impacts but it quickly reached its limits due to the use of models that were too simple to simulate all their environmental benefits.

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

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Improving nitrogen-use efficiency in oilseed rape *(Brassica napus)*

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

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

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

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

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Enabling european farmers to grow oilseed rape sustainably

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Oilseed rape is an important crop in Europe and since 1990 the harvested area continues to expand1 mostly due to an increased demand for vegetable oil for human consumption and industrial purposes. Maintaining this growth sustainably on a finite amount of land requires a holistic approach to crop production, combining good agronomic practices with crop protection systems and support tools. BASF's commitment to sustainable oilseed rape production is reflected in innovative solutions like it's modelling tool, AgBalanceTM, and plant protection systems such as the Clearfield® Production System and AgCelence®.

AgBalance analyzes all segments of the value creation pipeline from agricultural inputs, production, processing, and retail, ending with the consumer. This tool uses a well-established statistical modelling system2 to evaluate ecological, economic and social impacts, and compares the impact of decisions to improve sustainability in agriculture. AgBalance is currently used by stakeholders in 15 EU countries, and is a key tool in the evaluation of efficient solutions for reducing greenhouse gas emissions during the oilseed rape production process for biofuels, as well, for the assessment of winter oilseed rape (WOSR) production systems in Germany.

One of the assessed WOSR production systems is the Clearfield Production System which combines elite herbicide tolerant hybrid varieties with regionally tailored herbicides (HRAC group B) for season long control of broadleaf and grassy weeds, resulting in increased yields. In Europe, the combination of imazamox-based herbicides in combination with e.g. metazachlor (HRAC group K3) provides a cross spectrum herbicide with a dual mode of action which avoids herbicide resistance development. Clearfield oilseed rape is internationally recognized as non-GMO, and therefore can be freely grown and traded. Through collaboration with the vast majority of oilseed rape breeders, a broad portfolio of herbicide tolerant spring and winter hybrids have been launched and are in continuous development, including varieties intended for specialty markets, such as high oleic low linolenic (HOLL).

An increase in oilseed rape acres in Europe resulting in narrower crop rotations will favour disease development, such as Phoma lingam and Sclerotinia sclerotiorum. For this reason, BASF is offering a number of tools to help the farmer better understand the diseases that affect their crop and enable them to adopt the best crop management practices for sustainable crop production. BASF's AgCelence plant health products have been carefully developed to provide disease control coupled with improved plant health. Pictor® and Efilor® each have a combination of two active ingredients and have been developed for in flowering control of Sclerotinia and Altenaria. Caryx®, a combination plant growth regulator and fungicide, and Alterno®, a combination of two actives, are products developed for autumn and spring disease control which also improve winterhardiness, root enhancement, plant architecture and lodging control in the spring.

To ensure the high yield potential of today's oilseed rape varieties, BASF, through broad innovative solutions, strategic partnerships, and a holistic method for assessing agricultural sustainability, has brought and will continue to deliver a number of key products to the European marketplace in the areas of plant health and weed management.

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Genetic control of seed germination and vigour in rapeseed *(Brassica napus)*

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Background: Rapid and uniform seed germination is a crucial prerequisite for the achievement of high and stabile yield levels in rapeseed production. Furthermore, enhanced germination facilitates a reduced time span between sowing and emergence and thus a gain in growing time. For breeding of vigorous cultivars with fast and uniform field emergence it is important to understand the genetic factors contributing to adequate germination performance and seedling growth.

Objectives: The genetic control of seed germination and vigour was studied in diverse *Brassica napus* materials using different methodical approaches. Linkage analysis was carried out in a segregating doubled-haploid winter oilseed rape population, and genome-wide association studies were performed in a genetic diversity panel to define genomic regions harboring promising genes affecting seed germination and early seedling growth. Furthermore a systems biological approach was performed to identify regulatory networks affecting germination performance and seedling vigour.

Methods: Linkage analysis was performed in a bi-parental winter oilseed rape population (n=250) and a winter type diversity panel (n=248). All lines were genotyped with the Illumina® *Brassica* 60k SNP array. Seeds from different production environments were subjected to extensive automated in vitro phenotyping of germination related traits, such as germination speed, absolute germination rate and radicle elongation rate. Marker sequences were mapped onto the *B. napus* Darmor-bzh reference genome. Candidate genes within trait-associated regions were identified by functional annotation followed by gene ontology analysis. Weighted gene co-expression network analysis (WGCNA) was used to explore co-expression networks involved in seed germination and early seedling development.

Results: The data obtained underscore the high dependency of seed germination and seedling growth on environmental factors, but nevertheless reveal substantial potential for improvement by genomic based breeding. Several candidate genes could be identified within genomic regions associated with germination speed, absolute germination rate, radicle growth and thousand seed weight. A number of promising candidates could be validated by sequencing based transcriptome analysis or physiological experiments.

Conclusions: Large-scale automated phenotyping revealed broad phenotypic variability for germination performance. Marker-trait associations and candidate genes provide a solid basis for the establishment of reliable genomic selection tools for improved seed germination and seed vigour in rapeseed.

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Increasing canola yields under climate variability through genetic engineering of heat and drought stress tolerance

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Canola crop productivity is ultimately defined by its yield. Recent agronomic and economic studies indicate that yield losses are most significantly attributable to unfavourable environmental conditions such as those imposed by drought and heat, and those conditions can occur throughout the growing season. The effects of these stresses are particularly damaging to canola yield when they coincide with the transition to flowering in Canola. Exposure to either drought or heat stress independently has a significant negative impact on crop yield and this effect is compounded when these stresses occur in combination. Therefore, improvement of dual stress tolerance to heat and drought in crop plants has become a top priority to stabilize yields in canola varieties. Aiming to solve this problem, we employed unique forward genetic screens and a range of genomic approaches to discover and characterize genes that are involved in the regulation of heat and drought tolerance. In particular, we have identified and completed the functional analysis of a subset of target genes that constitute a novel transcriptional regulatory cascade that controls the plant's responses to these combined stresses. In the laboratory conditions, *Arabidopsis* and Canola plants with mis-sense expression of these regulatory genes were able to tolerate higher temperature or drought treatment beyond their control lines. More importantly, these plants produced higher seed yield than their controls when both stresses were applied simultaneously. The dual stress tolerance and yield enhancement properties of these transgenic plants were further confirmed in large-scale, multiple season and location Canola field trials. These results represent a significant breakthrough in canola crop improvement. Technologies derived from this research could enable canola farmers around the world to obtain higher yield and productivity over variable and adverse environmental conditions.

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Nested association mapping for drought tolerance

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Background: *Brassica napus* is the most important oilseed crop in Europe and second one worldwide. Due to the climate change, it is foreseen, that drought events will take place more often in many areas of the world, like middle Europe. So it is important to ensure high yield and quality even under unfavorable conditions like drought.

Objectives: The main goal of the project is to dissect complex traits, like drought tolerance, based on a double haploid nested association mapping population (BnNAM-DH). This population should be phenotyped for drought tolerance in a pot experiment under semi controlled conditions. Before running the association mapping, preliminary analyses have to be done: calculation of the population structure or the linkage disequilibrium in the population. For the association mapping itself, a fitted mixed linear model has to be developed. Furthermore, epistatic effects and haplotypes will be be calculated.

Methods: For the construction of BnNAM-DH, 21 genetic diverse parents were crossed to one elite line. 210 genotypes of the population (10 genotypes per family) were grown in a pot experiment with two treatments: 1. well watered and 2. water scarcity for 28 days after flowering. Phenotyping was done for two years in 2013 and 2014. During growing season, flowering time and plant length were scored. After harvest yield per plant and yield related traits and seed quality traits were estimated. Genotyping was carried out using the 60K *Brassica* Infinium® SNP array. LD and population structure were calculated using the statistical software R. Association analysis for identification of QTL is performed using a mixed model approach with conditional analysis (forward/backward selection), cross validation and haplotype blocks. Also epistatic effects are calculated.

Results: Analysis of linkage disequilibrium revealed differences of linkage disequilibrium among the chromosomes. Population structure was calculated using principle component analysis. The first two principle components explain a small portion of the genetic variance (≈ 10%). The fitted mixed model is able to detect significant markers, with and without treatment interaction. For the first year, 12 significant marker by treatment interactions could be found after conditional analysis. Threshold was set at a LOD-score of 3. Several significant markers are located near to candidate genes, for the relevant trait.

Conclusions: The nested association mapping population in addition to a well fitted model is a useful tool to dissect complex traits and to understand the genetic basis behind each individual trait. Through extra features like cross validation and conditional analysis, it is possible to define a really small genomic region for the QTL. This makes it easier and faster in future, to find the corresponding functional single gene for each trait being analyzed.

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Assessing QTLs for yield and their stability under limiting nitrogen supply using a wide field trial network

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Background: Rapeseed is a major oil crop with a relatively low Nitrogen Use Efficiency making its production highly dependent on N inputs. Improve yield and guarantee yield stability under low N input are new issues for rapeseed breeders in Europe. This addresses the question of the stability of yield genetic determinants under various nutrition regimes.

Objectives: The objectives of this work were to identify the genomic regions associated with yield in rapeseed and to assess their stability under contrasted environmental conditions, including N contrasted conditions.

Methods: A field trial network of seven locations was defined across France to represent the diversity of pedo-climatic rapeseed growing areas. For each location two contrasted N regimes were carried out (N+ optimal and N- low). This design allowed the field characterization of plant material during six years for linkage disequilibrium-linkage analysis purposes. Yield and yield-related traits were scored and N-responsive traits were calculated as N-/N+ and ΔN/N+. The plant material consisted in a diversity set of 93 accessions and two doubled-haploid populations that were all densely genotyped with the Illumina® *Brassica* 60k SNP array.

Results: N contrasted conditions were obtained across our field trial network. Very few genotype x N interactions were detected and a great stability of the QTLs was assessed between the N conditions. On the contrary, strong genotype x site interactions were found with most of the QTLs specific to one site only. This work came up with the detection of several QTL regions of interest with a particular dense one on the A5 linkage group with QTL stable across sites and N conditions. Candidate genes underlying this region were identified by functional annotation and gene ontology analysis.

Conclusions: A field phenotyping network was set up in the frame of the French project RAPSODYN (www.rapsodyn.fr/en) and validated for contrasting N conditions. Promising QTLs were detected and are currently transfered to elite lines and hybrids.

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ORAL PRESENTATION THEME D

Differences on photosynthesis between leaf and silique of winter oilseed rape *(Brassica napus L.)* to potassium deficiency

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Background: Leaf and silique are two main photosynthetically active organs of winter oilseed rape *(Brassica napus L.)*, both of which play key roles in vegetative architecture and yield formation (Bennett et al., 2011). As one of the essential plant elements, the potassium (K) uptake of oilseed rape is substantial. Potassium is involved in many physiological processes, such as photosynthesis. Differ from leaf, silique is a non-foliar photosynthetically reproductive organ. It may result in distinct physiological and photosynthetic properties, along with their different responses to K deficiency.

Objectives: Changes on physiological and photosynthetic properties of silique under K deficiency were always neglected. Thus, the objectives were to analysis the differences on photosynthesis between leaf and silique to K deficiency, which will facilitate a better understanding of photosynthesis of oilseed rape and improve seed yield according to optimal potassium fertilizer management.

Methods: Measurement of gas exchange parameters was carried out on leaf and silique during their steady stage of photosynthetic function at field condition with K sufficient supply treatment (+K) and K deficiency treatment (-K). Light- and CO2-response curves, combined with imaging-PAM analysis were applied to reveal the distinguishing response characteristics and chlorophyll fluorescence of leaf and silique to K deficiency.

Results: The results showed that leaf K concentration was lower than silique, and under K deficiency, it decreased more than silique. Net photosynthetic rate of leaf was three times higher than that of silique. K deficiency significantly decreased the net photosynthetic rate; the average reduction rate was 28.0% and 26.2% for leaf and silique, respectively. The light compensation (LCP) and saturation (LSP) point of silique were higher than those of leaf. In contrast to the +K treatment, the LCP values of those two organs were significantly increased, however, the LSP values were declined in K-deficient organs. The reduction of the light utilization capacity of leaf was more than that of silique under K deficiency. Silique performed a higher capacity of CO_2 utilization. The CO_2 compensation point (CCP) of silique was lower than that of leaf; however, the CO_2 saturation point (CSP) of silique was higher than leaf. K deficiency mainly increased the CCP values of those two organs. Furthermore, imaging-PAM analysis indicated that minimum fluorescence (F0) of silique was considerably lower than leaf. But the maximum quantum yield of PSII (Fv/Fm) and non-photochemical quenching (NPQ) of silique was higher than those of leaf. Greater heterogeneity emerged in leaf, especially the mesophyll near leaf margin under K deficiency.

Conclusions: Leaf organs equipped with the stronger photosynthetic capacity, however, pod could make the better of high light intensities and low internal CO_2 conditions. Potassium deficiency imposed constraints on CO_2 assimilation of two organs, yet leaf was more sensitive. Optimal K supply improved utilization efficiency of high light intensities and low CO_2 concentration.

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ORAL PRESENTATION THEME D

Developing a non-destructive method for assessing canola genotypic differences in tolerance to heat and drought stresses

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Background: Canola is a crop sensitive to drought and heat stresses that usually occur with different frequency and duration almost every summer during canola flowering in Canada and elsewhere in the world. The performance of any crop depends strongly on its root architecture and function. Canola roots may play a central role in overcoming the constraints to growth and development imposed by heat, drought and other environment-induced stresses. An electrical capacitance method was reported to be sensitive in delineating stressed plants from healthy plants in hydroponic or sand-based growth media, but investigation is needed to test its suitability for use under field or potted soil conditions.

Objectives: A controlled study was conducted to (i) examine canola genotypic differences in root electrical capacitance, (ii) determine if the measured differences in root electrical capacitance can be used to delineate responses of canola genotypes to heat and drought stresses, and (iii) develop an electrical capacitance method to screen for root biomass and root-related traits.

Methods: The experiment was arranged in a split-plot design with temperature regime in the main-plot units, and combinations of genotype, drought, and growth regulator treatments in the subplots. The seeds were sown in plastic pots filled with soil mixtures (clay loam/peat moss and vermiculates). There were two treatments: high (27/17 oC) and normal (23/17 °C). Two water levels (sub-plot), 85% (i.e., control) and 45% SWC were applied to the plants. At 31 DAS, electrical capacitance, biomass of stem, leaf and root from each pot were determined.

Results: Compared to the control, high temperature and drought stresses significantly suppressed root and aboveground biomass, with large differences in measured parameters among canola genotypes. Changes in root electrical capacitance values reflected the responses of canola genotypes to the stress factors. High temperature and drought stress had a significant synergetic/ interaction effect on decreasing root biomass and root capacitance. There was a linear relationship between electrical capacitance and root biomass, with larger R2 values ($P < 0.01$ n=63) in stress-tolerant genotypes across high temperature and drought stresses.

Conclusions: Our results indicate that drought stress severally restricted growth and dry matter accumulation in all genotypes, but there was an interaction between genotype and heat stress on canola biomass. Measuring electrical capacitance in soil-based pot study is a promising tool to estimate root biomass in examining canola genotypic differences in response to high temperature and drought stresses.

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Management of oilseed rape (OSR) volunteers to secure low alpha-linolenic acid content in High Oleic Low Linolenic (HOLL) OSR crop

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Background: HOLL winter oilseed rape (WOSR) has high oleic acid content (C18:1, >75 %) and low alpha-linolenic acid content (C18:3, <3.5%). HOLL oil is therefore heat-stable and can be used for frying without hydrogenation. The presence of conventional WOSR volunteers from previous crops can significantly increase C18:3 content, with a critical threshold density of two volunteers m-2 (Baux et al. 2011).

Objectives: To consolidate the HOLL market, it is important to secure oil quality, and therefore to limit volunteer density. We tested different management strategies to control volunteers, and analyzed the consequences on oil quality.

Methods: A field trial was carried out for harvest season 2013 in Agroscope, Changins. An imidazolinone-tolerant (IT, Clearfield® technology) HOLL variety was sown at a rate of 50 seeds m-2, together with a conventional WOSR variety (25 seeds m-2) to simulate volunteers. Six herbicide treatments were applied in fall 2012: i) control (no-herbicide), ii) pre-emergence herbicide (Devrinol® Top, 345 g/l napropamide, 30 g/l clomazone) , iii) Cleranda® (17.5 g/l imazamox, 375 g/l metazachlor, IMI), at early post-emergence (CD 11), iv) at standard post-emergence (CD 12-13), v) at late post-emergence (CD 16), and vi) in two split ½ dose applications (CD 11 and 14). Two other field trials took place in 2013. First an IT-HOLL variety was sown with an increasing density of conventional volunteers (0, 1, 2, 5, 10 or 25 seeds m-2) and two herbicide treatments (pre- or post-emergence, as before). Secondly, two imidazolinone-sensitive HOLL varieties (hybrid or OP-line) were sown with an increasing conventional volunteer density (0, 1, 2 or 5 seeds m-2). In 2015, three methods to control weeds and volunteers (pre-, post-emergence herbicides, or mechanical treatment) have been integrated using an IT-HOLL variety in a long-term experiment comparing plough or superficial tillage since 1967. In all experiments, we assessed volunteers, weeds and crop density, as well as yield and oil quality.

Results: The Clearfield® system totally eliminated the volunteers, independently of the initial density and the method of application and hence secured HOLL oil quality (C18:3 <3%). Linolenic acid content of the OP-line variety increased significantly with two volunteers m-2. The hybrid variety was less affected by the presence of volunteers (no significant effect of two volunteers m-2), had a lower C18:3 content and a higher yield potential. Our results show that superficial soil tillage allows a 47% reduction of volunteers as compared to ploughing. In fall 2014, mechanical treatment and IMI-herbicide application were efficient to reduce volunteer density.

Conclusions: We identified three efficient strategies to guarantee low C18:3 content in HOLL WOSR. Varieties with high competitive ability and yield potential are less affected by the presence of volunteers. Mechanical weed control and adapted soil tillage can secure low volunteer densities. The Clearfield® system can efficiently eliminate conventional volunteers. However, there is currently a tradeoff between IMI-tolerance and variety competitiveness in terms of yield and oil quality.

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ORAL PRESENTATION THEME D

Effects of N fertilization on the carbon footprints of canola and mustard under diverse environments

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Background: Canola and mustard plants require large amounts of nitrogen fertilizers to be applied in order to increase their seed yield and improve seed quality. However, nitrogen fertilization is one of the major contributors to greenhouse gas emissions in crop production. It is unknown how nitrogen fertilizer can be managed in canola and mustard to optimizing crop productivity while, at the same time, minimizing carbon emission from the crop production.

Objectives: This study was conducted to (i) provide a quantitative estimate of total greenhouse gas emissions and the carbon footprint of canola and mustard grown on the semiarid northern Great Plains and (ii) determine the effects of environments and N fertilizer rates on the carbon footprint of canola and mustard.

Methods: Five oilseed crops, napus canola, rapa canola, juncea canola, juncea mustard, and alba mustard, were grown under the N rates of 0, 25, 50, 100, 150, 200, and 250 kg N ha-1 at eight environmental sites (location x year combinations) in Saskatchewan. Those sites represent the major oilseed production ecoregions in western Canada. Straw and root decomposition and various production inputs were used to calculate greenhouse gas emissions and the carbon footprints.

Results: On average, emissions from N fertilization were 9 times the emission from the use of pesticides, and 11 times that of various farming operations. Straw and root decomposition emitted 120 kg CO2 eq ha-1, contributing 10% to the total emission. Emissions from the production, transportation, storage, and delivery of N fertilizer to farm gates accounted for 42% of the total greenhouse gas emissions, and the direct and indirect emission from the application of N fertilizer in oilseed production added another 31% to the total emission. Carbon footprint increased slightly as N rates increased from 0 to 50 kg N ha-1; but as N rates increased from 50 to 250 kg N ha-1, carbon footprint increased substantially for all five oilseed crops evaluated. Oilseeds grown at the more humid Melfort site emitted 1355 kg CO2 eq ha-1, 30% greater than emissions at the drier sites of Scott and Swift Current. Oilseeds grown at Melfort had a carbon footprint of 0.52 kg CO2 eq kg-1 of oilseed, 45% greater than that at Scott (0.45 kg CO2 eq kg-1 of oilseed) and 25% greater than that at Swift Current (0.45 kg CO2 eq kg-1 of oilseed).

Conclusions: Carbon footprint of oilseeds was a function of the rate of N fertilization applied to the crop, and the magnitude of this effect varied with environments. Environmental variation contributed 10% to the variation in carbon footprint. Nitrogen manufacture and application together contributed as high as 74% of the total emission accumulated during the course of the crop production. Key to lower carbon footprint in oilseed production is to improve N management practices.

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ORAL PRESENTATION THEME D

Legumes in rotation affect hybrid canola and malting barley

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Background: The high and unpredictable cost of fertilizer nitrogen (N) in western Canada has generated interest in alternative N sources. Legumes produce N through fixation, and may increase soil residual and mineralizable N, thus reducing the need for fertilizer N in subsequent crops. Hybrid canola *(Brassica napus L.)* has a high N requirement for optimum yield, but knowledge of the rotational effects of legumes on canola is limited.

Objective: The objective was to determine the effects of legume and non-legume preceding crops on yield, quality and net revenue of canola grown the following year and malting barley *(Hordeum vulgare L.)* grown after canola.

Methods: Field pea *(Pisum sativum L.)*, lentil *(Lens culinaris Medik.)*, faba bean *(Vicia faba L.)*, canola and wheat *(Triticum aestivum L.)* harvested for grain, and faba bean grown as a green manure were direct-seeded at seven locations in western Canada in 2009. Canola was seeded in 2010 and barley in 2011, with fertilizer N applied at 0, 30, 60, 90 and 120 kg/ha.

Results: On average, all legumes, except faba bean harvested for grain, produced higher canola and barley yields compared to when wheat was the preceding crop. Faba bean green manure produced the highest yields, while canola on canola produced the lowest canola yield. The legumes had little or no negative effect on percent canola oil or barley protein. Yields of both crops increased with increasing N rate, but percent canola oil decreased, and barley protein increased. The study showed that basing N management on a target yield, 25% less fertilizer N was required to maintain a given canola or barley yield when canola and then barley followed field pea or lentil rather than wheat. An economic analysis indicated that over the entire 3-yr crop sequence, lentil and field pea harvested for grain provided the greatest net revenue. Although the faba bean green manure produced the highest yields, this was insufficient to compensate for the loss of crop revenue and resulted in the lowest overall net return.

Conclusion: The results indicate that growing lentil or field pea for grain prior to hybrid canola can improve canola yield, subsequent barley yield and overall net revenue without negatively affecting canola oil or malting barley protein.

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ORAL PRESENTATION THEME D

Indirect N_2O emissions: Model-based quantification of N leaching and NH_3 emissions in OSR fertilized with mineral and organic fertilizers

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Background: Emissions of nitrous oxide (N_2O) from arable land are a major contribution to the global GHG balance. To quantify their amount, direct and indirect N_2O emissions have to be considered. Direct N_2O emissions can be measured directly at the place of origin. Indirect N_2O emissions result from displaced reactive nitrogen compounds like ammonia or nitrate. They account for one third of the total global agricultural N_2O source and approximately two thirds of the uncertainty in the total source [1]. Fertilizing OSR with organic fertilizer may lead to high amounts of ammonia volatilization. The high N content in the plant residues enables a fast mineralization, which can cause N leaching after OSR harvest.

Objective: Using a model-based N balance approach, indirect N_2O emissions should be quantified by simulating ammonia emission and nitrogen leaching.

Methods: Starting in autumn 2012, a field experiment including a crop rotation (OSR, wheat, barley) was carried out two years in the northern part of Germany. In spring OSR was fertilized with 180 kg N, either as mineral (CAN) or organic (180 kg NH4-N) fertilizer. Wheat and barley were fertilized with CAN (220 kg N and 200 kg N respectively). NH_3 emissions were detected after organic fertilizing events by Draeger tube measurement technique [2]. Direct N_2O emissions were measured weekly using manual chambers. Soil mineral N (SMN) samples were taken three times per growing season.

To quantify indirect N_2O emissions we used measured data, a dynamic simulation model to calculate N leaching and a semi-empirical model for NH3 volatilization. SMN data at the beginning of the experiment were used as initial model data. Fertilization (experimental data) and modelled mineralization were used as system input, whereas modelled N uptake by plants, nitrogen leaching and gaseous N emissions (N_2O, NH_3 (measured); NO_x, N_2 (modelled)) were regarded as system output. Finally we calculated indirect N_2O emissions using corresponding IPCC TIER 1 emission factors.

Results: In 2013 and 2014 we measured 45 and 40 kg NH3-N per ha total NH_3 emissions, respectively. This results in calculated indirect N_2O emissions of about 0.45 kg N per ha and 0.4 kg N per ha, respectively, originating only from ammonia volatilization. That means, for example, indirect N_2O emissions in a range of up to 64% of the measured direct N_2O emissions in 2014. In autumn 2013, a SMN value of 25-30 kg N ha- 1 in a depth of 60-90 cm under wheat following OSR gives indication of a high N leaching potential which leads to considerable indirect N_2O emissions.

Conclusion: Fertilizing OSR with organic fertilizer leads to relatively high amounts of indirect N_2O originating from ammonia compared with measured direct N_2O emissions. High SMN values after OSR potentially lead to high N leaching.

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ORAL PRESENTATION THEME D

All together now – comparative analysis of genome-wide associations for flowering time, plant height and seed yield in winter-type *Brassica napus*

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Background: Several studies have documented colocalization of QTL for flowering time, plant height and seed yield in *Brassica napus*. Interestingly, flowering genes like Bna.FT and Bna.TFL1 have been found to determine seed traits, and similar results have been found in *Arabidopsis thaliana* and *Brassica rapa*. In this study we tested the hypotheses (1) that climatic adaptation may be orchestrated by some general adaptation loci and (2) that some of those loci may be part of the flowering network.

Objectives: We studied flowering time as a measure of reproductive adaptation, plant height as a biomass parameter and seed yield as a measure of reproductive success. Through a genome-wide association study (GWAS) we aimed to identify chromosome regions potentially responsible for the simultaneous regulation of two or three traits. Genes underlying such regions are candidates to be overall adaptation loci and may represent important breeding targets.

Methods: A total of 140 European winter-type *B. napus* inbred lines were genotyped with 21,623 unique, single-locus single-nucleotide polymorphism (SNP) markers using the *Brassica* 60K-SNP Illumina® Infinium consortium array. Phenotypic associations were alculated over the years 2010-2012 for flowering time, plant height and seed yield in 3 locations in Germany using a mixed model with PC-adjustment. Gene ontology enrichment analysis was performed using the R package "GOstats" and the web-based platform REVIGO.

Results: We identified 68 cross-trait regions with potential adaptive value. Within these regions, *B. napus* orthologs for a number of candidate adaptation genes were detected, including central circadian clock components like CIRCADIAN CLOCK-ASSOCIATED 1 (Bna.CCA1) and TIME FOR COFFEE (Bna.TIC) along with the important flowering-time regulators FLOWERING LOCUS T (Bna.FT) and FRUITFUL (Bna.FUL). Gene ontology (GO) enrichment analysis of candidate regions revealed strong enrichment for response to environmental factors like abiotic, biotic or endogenous stimuli, hyperosmotic response or response to heat. Moreover, there was enrichment for biosynthesis of both small and large molecules, (e.g. flavonoids), immune system processes and macromolecule methylation.

Conclusions: Our results provide a valuable framework to further improve the adaptability and yield stability of this recent allopolyploid crop under changing environments. By performing the study in adapted germplasm representing the broader genepool of modern winter oilseed rape, we demonstrate the existence of considerable untapped potential for exploitation in breeding.

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ORAL PRESENTATION THEME D

Characterization of morph-physiological responses associated with water stress tolerance in *Brassica napus*

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Background: Adaptation to moisture stress is a complex outcome of constitutive or induced responses to the external stimuli. *Oleiferous Brassicas* are frequently subjected to moisture stress as these crops are cultivated primarily on light textured soils under low moisture conditions in many parts of the world, including India and Australia. Yield losses can be heavy if drought stress coincides with the reproductive growth (Lawlor and Cornic 2002). Identifying genetic variation for drought tolerance is critical as climate changes and anthropogenic activities are projected to further limit water availability in the southern hemisphere.

Objectives: Genetic variations for tolerance to water stress are yet to be adequately documented in *Brassicas*. Availability of international germplasm under longstanding ACIAR/GRDC funded projects allowed undertaking elaborate studies to quantify drought tolerance in canola *B. napus* and to identify associated morpho-physiological traits.

Methods: An assembled set of 30 genotypes was first field evaluated for drought tolerance under three irrigation modules: i) no irrigation) ii) one irrigation (45 days after sowing) and iii) two irrigations (45 and 85 DAS). Based on the initial evaluation, 12 genotypes were further investigated for another two years, for photosynthesis and leaf traits (90DAS), water potential, SPAD, RWC (periodically), chlorophyll fluorescence, and root traits (100DAS), stomatal frequency/size (120DAS), and biochemical estimations (90DAS). Drought susceptibility/tolerance/ efficiency were also worked out using standard protocols.

Results: Moisture restriction reduced stomatal conductance (Cs), transpiration (Tr) and consequently, photosynthesis (Pn). Cv. Tarcolla registered lowest Pn (4.8umolm-2s-1), suggesting low Cs (0.110molm-2s-1) and Tr (2.57molm-2s-1).Stomatal frequency and size were maximum in GSC6. Garnet had longest roots while cv. Karro showed maximum root area. Damage to PSII was maximum in cv. Karro (0.668) and least in cv. Ruby (0.728) under restricted moisture. Photochemical efficiency was comparable in Opal and RT-057.Leaf area/plant was lowest in Ruby and highest in EC609303. SPAD chlorophyll, RWC and water potential were high during vegetative phase and declined thereafter. Moisture availability improved LA by 33.8% and 53.8%. Water deficit up regulated sugars and proline in the elite genotypes and decreased seed storage products. Variations existed in drought susceptibility index (DSI) for growth and yield components. DSI computed on moisture stress and one irrigation for SY (0.05- 2.07, mean 0.979) and DTE1 (69.9-99.3% mean 85.7%) was higher than calculated on two irrigations DSI2 (0.140-1.94, mean0.968) and DTE2 (54.6-96.6%mean77.1%). Overall, Cv. Opal and RT-057 (DSI ≤0.53, DTE ≥87.0%, DTI≥0.513) showed maximum tolerance to moisture restriction.

Conclusions: Test genotypes showed varied responses to moisture restriction. Cv. Opal and RT-057 showed maximum drought tolerance. Elite genotypes had higher Pn, lower damage to PSII, greater root length, RWC, sugars, proline lower DSI and higher DTE. Pn coupled with fluorescence, RWC along with DSI and DTE appeared reliable to monitor genetic variation for responses to moisture stress.

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ORAL PRESENTATION THEME D

Effect of S deprivation on osmotic potential components and N metabolism in oilseed rape leaves: identification of a new early indicator

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Background: Compared to other plant species such as cereals and like many *Brassicacea*, winter oilseed rape *(Brassica napus)* requires a relatively large input of mineral nutrients such as sulfur (S). As a consequence, oilseed rape is especially sensitive to S limitation. Identification of early sulfur deficiency indicators is of prime importance for *Brassica napus* which yield and the nutritional quality of seeds are negatively affected by S deficiency (McGrath and Zhao, 1996; D'Hooghe et al., 2014).

Objectives: S is mostly stored as sulfate in leaf cell vacuoles and can be mobilized during S deficiency. So, this study investigated the impact of S deprivation on leaf osmotic potential in order to identify the mineral and/or organic compounds that contribute osmotically during sulfate mobilization. The objective was to identify early events during S deprivation, well before growth reduction and with kinetics close to the induction of sulfate transporters that could be potentially usable under field conditions as indicators of S nutrition levels.

Methods: *Brassica napus* were grown at vegetative stage during four weeks and then were exposed to S deprivation for 28 days (Control: 508.7 µM sulfate vs S deprivation: 8.7 µM sulfate). Plant samples were harvested kinetically. Each plant was sampled as emerged leaves, corresponding to leaves present at the beginning of treatments application (at d0) and new emerging leaves which correspond to leaves appearing during treatments. Plant samples were analyzed for osmotic potential, water content, mineral and organic solute contents, total S and N contents, 15N-nitrate uptake, nitrate reductase activity and transcript levels of sulfate and nitrate transporters.

Results: *Brassica napus* revealed two response periods to S deprivation. The first one occurred during the first 13 days during which plant growth was maintained as a result of vacuolar sulfate mobilization. In the meantime, leaf osmotic potential of S-deprived plants remained similar to control plants despite a reduction in the sulfate osmotic contribution, which was fully compensated by an increase in nitrate, phosphate and chloride accumulation. The second response occurred after 13 days of S deprivation with a significant reduction in growth, leaf osmotic potential, nitrate uptake and nitrate reductase activity, whereas amino acids and nitrate were accumulated.

Conclusions: This analysis of S deprivation suggested that a ([chloride]+[nitrate]+[phosphate]) :[sulfate] ratio could provide a relevant indicator of S deficiency, modified nearly as early as the over-expression of genes encoding sulfate tonoplastic or plasmalemmal transporters, with the added advantage that it can be easily quantified under field conditions.

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ORAL PRESENTATION THEME D

Genetic variation for nitrogen use efficiency in oilseed rape *(Brassica napus L.)*

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Background: Oilseed rape *(Brassica napus L.)* is the most important oil crop worldwide after perennial oil palm and soybean. For oilseed rape production nitrogen (N) is the plant nutrient that has to be fertilized in the highest quantities, and meeting future human demand for oil yield will also require N fertilization. At the same time, N that is not taken up by the vegetation can escape from the agricultural production system, potentially causing severe damage in other ecosystems. Examples include nitrate leaching into groundwater, nitrogen run off into rivers, lakes and oceans, and losses of volatile NOx and ammonia into the atmosphere. Therefore, increasing nitrogen use efficiency is becoming a major global megatrend in agricultural production, in particular for crops used as a biofuel source. Besides more exact fertilizer applications coupled to plant nitrogen demand, the use of genetic diversity to breed for N use efficiency (NUE) is a promising strategy towards a more sustainable agriculture.

Objectives: Although breeding progress for resistance and quality traits is well described, there is still little information about plant traits that can be considered as key drivers of increased nitrogen use efficiency. In this project we are comparing winter oilseed rape varieties from different years of market release in order to identify NUE-related traits underlying breeding success that can be used to select for improved N efficiency in ongoing breeding programs.

Methods: Independent experiments were used to assess genetic variation for NUE:

1) During the 2012-2013 growing season a set of 30 highly diverse *B. napus* genotypes, including older and more recent varieties and resynthesized lines, was investigated in Mitscherlich pots at a high and low N level. By separating plant biomass at flowering (BBCH 67-69) and seed maturity into leaves, stems and pods the trait interrelationship for N uptake (NupE) and utilization efficiency (NutE) was studied in detail.

2) During the 2014-2015 growing season a set of 30 elite winter type oilseed rape varieties, including older and modern hybrids and lines were cultivated in the field at a high (220 kg N ha-1) and a reduced (120 kg N ha-1) N fertilization. These were subject to non-destructive and destructive nitrogen measurements during spring.

Results: The results give first insights into the ability of elite genotypes to acquire N during vegetative growth stages, into differences between inbred and hybrid cultivars, and into breeding progress in European winter oilseed rape. Furthermore, the pot experiments indicated a huge phenotypic variation for NupE until flowering within diverse *B. napus* materials, based on different N quantities in plant organs. While the genotypes also differ in their NutE during the generative phase, both trait complexes seem to be completely independent inherited. Therefore, our results suggest a great potential to improve total NUE.

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ORAL PRESENTATION THEME D

Strategies for Improving Winter Survival in U.S. Southern Great Plains Winter Canola

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Background: Winter canola cultivar evaluation began in the late 1980s in the southern Great Plains of the United States. Initially, European cultivars were introduced into the region, but winter survival was poor because the materials were un-adapted to the harsh climatic conditions. In 1993, Kansas State University (K-State) started a winter canola breeding program and began coordinating the National Winter Canola Variety Trial (NWCVT). In the beginning, acreage growth did not exceed a few thousand hectares. However, in recent years, renewed efforts by public and private entities have led to a greater interest and dramatic acreage increases. An estimated 125,000 hectares were seeded in the southern Great Plains in fall of 2014.

Enhanced winter survival has been observed in the NWCVT over the past 22 years because of breeding efforts and refined production practices. Although adaptability has improved, work remains as producers face the effects of variable temperatures, drought, and climate change. In the 2013/14 and 2014/15 growing seasons, winter survival was a significant issue in many U.S. locations because of extreme cold temperatures, excessive fall growth, and a short winter acclimation period.

Objectives: Genetics and production management play critical roles in enabling a winter canola crop to survive winter weather conditions. Comparisons between top K-State cultivars, industry standards, and new winter canola cultivars will be made. Traits, including the semi-dwarfing trait and prostrate growth habit, will also be examined. The effects of seeding date and density will be evaluated in studies conducted in Kansas.

Methods: Head-to-head comparisons for winter survival and yield will be made between new cultivars and commercial checks across NWCVT sites in the southern Great Plains. Fall plant stand, spring vigor, winter survival, and yield will be assessed in the seeding density studies. A three-year planting management study designed as a cultivar by production practice factorial will be summarized (Assefa et al., 2014).

Results: Local and international canola cultivars show substantial improvement in winter survival compared with the check cultivars Sitro and Wichita. KS4549, an experimental cultivar from the K-State canola breeding program, offers enhanced winter survival and possesses a prostrate growth habit. Lower seeding rates reduce in-row plant-to-plant competition, resulting in greater winter survival than higher seeding density.

Conclusions: Adjustments to production practices may be necessary to enhance winter survival. New winter hardy cultivars from K-State will be released within the next two to three years. Cultivars possessing the semi-dwarfing trait have greater winter hardiness than the first hybrids introduced in the region. With the recent licensing of the OGURA-INRA hybrid breeding system, opportunities exist to incorporate winter survival traits from K-State germplasm into hybrid parent lines to be licensed by industry.

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ORAL PRESENTATION THEME D

Phenological and physiological traits to explore G×E interaction in canola in south-western Australia

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Background: While genotype (G) × environment (E) interaction (G×E) complicates broad crop adaptation, understanding its causes facilitates breeding for specific adaptation. Knowledge of phenology in the adaptation of canola to different environments and the contribution of phenotypic traits to yield under these environments is necessary to explore the G× E interaction and enable breeders to select specific traits for a targeted environment.

Objectives: The major objective of this study is to i) evaluate the significance of the G×E interactions on seed yield and investigate whether there is a repeatable G×E interaction pattern which may lead to the identification of canola growing mega-environments; ii) identify the phenological and phenotypic traits that can explore the G×E interaction for targeted breeding.

Methods: A multiple-environment trial (MET) with a large number of genotypes over four year (2006-2009) was used to partition G×E interactions and investigate the role of phenology in canola adaptation to the environment. Another two year (2009 and 2010) detailed study was used to quantify the impact of phenological and physiological traits on yield in the low and high rainfall environments.

Results: Two mega-environments (ME) were identified. ME1 combines > 330 mm seasonal rainfall with a cooler, longer post-anthesis growing period. ME2 is more terminally drought-prone, with higher temperatures and < 300 mm rainfall, resulting in a short growing season. There were significant crossover yield responses to environment changes: the medium flowering genotypes produced significantly higher yield than the early flowering genotypes in ME1 but yielded poorly in ME2, and vice versa. Principle component analysis in two contrasting years confirmed that yield was positively correlated to flowering time in the high rainfall year and negatively in the drought year. Yield was highly correlated with increased biomass at vegetative stage and maturity, plant height, crop growth rate (CGR) during the linear growth period, leaf mass per unit area (LMA), pod number per unit area, and water soluble carbohydrate stored in stem in the high rainfall environment. In contrast, yield showed weaker or no correlation to any of these traits in the drought year. The key outcome of this G×E interaction study is the importance of phenology to the adaptation of canola and different role of phenotypic traits in the low and high rainfall environments. It is suggested that breeding for specific adaptation to each mega-environment should be targeted with a strategy focusing on drought and heat tolerance in ME2 and high biomass in ME1. In ME1, traits associated with achieving high biomass, including higher LMA, greater CGR, early vigour, and mid-late flowering could be used as selection criteria to improve yield. In the low rainfall environment, early flowering allows canola to escape drought and ensures enough water for grain filling that improves harvest index.

Conclusion: Canola genotypes showed significant G×E interactions in yield response to the environment. This interaction can be explored by target breeding through matching phenology to rainfall and growing season length and selecting traits contributing to the difference in biomass and HI.

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ORAL PRESENTATION THEME D

Understanding the biochemical basis of abiotic stress induced lipid pathway adjustments

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Background: Modification of membrane glycerolipid profile is an important part of plant metabolic adaptation to unfavorable environmental conditions. Profound changes of fatty acid desaturation in the lipid bilayer occur under temperature stress. Similarly, proportions of glycerolipid classes in membrane systems alter extensively under conditions of nutrient limitation. When plants encounter phosphorus starvation, for example, the quantity of phospholipid decreases, whereas the level of galactolipid species increases. These metabolic changes are associated with rebalancing of glycerolipid pathways located in the chloroplast and the cytosolic ER membrane systems. How such metabolic coordination is mechanistically modulated, and more importantly, what metabolic factors are involved remain to be fully explored.

Objectives: We were interested in identifying key biochemical junctures and metabolic factors influencing membrane lipid adaptation to abiotic stresses.

Methods: 1D and 2D TLC were standard techniques used for lipid separation, and gas chromatography was employed for fatty acid composition analysis. Lipidomic profiling through ESI-MS/MS analysis was performed at the Kansas Lipidomic Research Center. RNAseq were conducted on the Illumina HiSeq2000 at the National Research Council, Aquatic and Crop Resource Development (ACRD)-Saskatoon, Canada. Transgenic *Brassica* plants were generated through agrobactrium-mediated transformation.

Results: In a plant cell, glycerolipid synthesis relies on two major pathways located in the chloroplast and the endoplasmic reticulum (ER), known as the prokaryotic and eukaryotic pathway, respectively. Through comprehensive lipid profiling and lipidomics analysis, we show that the chloroplast prokaryotic pathway is up-regulated in response to cold stress, while high temperature promotes the ER eukaryotic pathway. Furthermore, under heat stress, C34 diacylglycerol (DAG) moieties of 16:0/C18 (sn-1/ sn-2) generated by the ER pathway are preferentially transported to the chloroplast over that of C36 (C18/C18) DAG, leading to a reduction in the overall capacity of fatty acyl desaturation in membrane lipids (Li et al., 2015). Through introducing a feedback-resistant Gly-3-P dehydrogenase gene (gpsAFR) from Escherichia coli, we generated *Brassica* transgenic plants with an augmented chloroplast lipid pathway (Shen et al., 2010). Significantly, these gpsAFR transgenic plants have a higher input from the chloroplast lipid pathway, resulting in a lipid phenotype resembling that of plants under phosphorus starvation. We will discuss the potential of such a transgenic approach in improving crop phosphorus utilization efficiency.

Conclusions: Our study highlights the significance of glycerolipid pathway coordination and the utility of lipid metabolism genes in improving plant adaptation to abiotic stress

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ORAL PRESENTATION THEME D

Sustainability & carbon footprint of canola production in Western Canada

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The biofuel markets in some regions of the world such as Europe, the United States and California apply qualifying sustainability criteria to the biofuel feedstock production. A common component of the sustainability criteria is the carbon footprint for producing the feedstock. The published carbon footprints of canola production exhibit significant variation and range from 334 to 979 kg CO_2eq/tonne. Carbon footprints are known to be sensitive to spatial and temporal influences. This work is the first to be based on actual data collected from Canadian canola producers and it produces emission results one third lower than those reported by Shrestha, two thirds lower than those reported by Gan a decade earlier, and lower than the default value in the RED in Europe and the values used by CARB for the LCFS system. The lower footprint is the result of several differences in production practices including an increase in reduced and no till agriculture, and a reduction in summerfallow. Some of the ecozones of western Canada also exhibit relatively low N_2O emissions which is a function of the low levels of natural precipitation.

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ORAL PRESENTATION THEME E

Comparison between Canadian canola harvest and export surveys

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Canola is the major oilseed crop in Canada, in 2014 the canola production was 15.496 million metric tonnes, the second highest production recorded in Canada –17.876 million metric tonnes in 2013 – with a 5 year average production of 14.332 million tonnes. Every year, about 50% of this production is exported mainly towards Japan, China, and Mexico.

The Oilseeds section of the Grain Research laboratory reports canola quality data based on an annual voluntary harvest survey of western Canadian canola involving producers, grain companies and oilseed crushing companies. Quality parameters such as oil, protein, chlorophyll, glucosinolates, free fatty acids and the fatty acid composition are analyzed and reported. At the same time, monthly exports are monitored and the same testing is done on the individual Canadian canola shipments leaving the Canadian ports.

The results suggested that overall harvest survey data can help to predict the exports quality for oil, protein, free fatty acid and fatty acid composition. However, chlorophyll data of the harvest survey and export chlorophyll data showed large differences. Factors such as dockage and green seeds counts contributed to the differences between the two surveys for chlorophyll.

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ORAL PRESENTATION THEME D

Identifying climatic limitation to oilseed rape yield in Switzerland

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Background: Climate plays an important role in agriculture. Crops are submitted to various stresses such as water stress, heat stress, frost, that may decrease yield or quality. The aim of this work was to determine the most important climatic limiting factors of oilseed rape yield in Switzerland, in order to better understand yield variation among location and years.

Methods: We adopted a knowledge- and data-based climate suitability evaluation approach, where several climate indices estimated over relevant phenological phases are used to quantify climate-yield relationships. Four phases were calculated dynamically based on Habekotté (1997) phenology model: emergence, vegetative growth, anthesis, and seed filling. For each phase, response functions to climatic indices were pre-defined based on literature and expert knowledge, and automatically refined based on observed data as described for maize by Holzkämper et al. (2013). The chosen indices were maximum temperature, minimum temperature (below 0°C, or below -12°C for the vegetative growth phase), photothermal quotient, length of the phase and water availability. A different weight was attributed to each phase in order to account for the fact that climatic stresses can have different impacts on yield depending on the growth stage. The maximum yield, chosen as the best yield observed in Switzerland in the last 20 years, was set to 6.6t/ha. For each phase, only the strongest limitation was taken into account. The approach was applied to oilseed rape *(Brassica napus L.)* production in Switzerland and compared to yield data gathered from the main oilseed production regions in Switzerland.

Results: Comparison with independent yield data showed a good agreement of estimated crop-specific climatic suitability with scaled yields. The calculation of phase weight showed that the flowering phase was the most sensitive to climatic stresses, followed by seed filling (anthesis to maturity phase).

Analysis of climatic limitations on two locations in Switzerland showed that a low radiation, resulting in low photothermal quotient was the most important limiting factor for both phases, with more impact during flowering. This parameter is highly variable among years, but only smaller differences could be registered among locations in Switzerland, resulting in greater yield variability among years than among locations.

This approach gave a good estimation of oilseed rape yield variation in Switzerland. Further possible applications of this work could be either to map best climatic areas for oilseed rape growth, or even to adapt variety choice to regional climatic limitations.

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ORAL PRESENTATION THEME E

Enhancing production and productivity of oilseed *Brassica:* Research need for 21st century

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Background: India is one of the largest cultivators of oilseeds crop. Per capita consumption of vegetable oils has increased from 6.2 kg/year in 1986-87 to14.2 kg/year during 2012-13. This eventually pushes the demand for oil significantly.

Objectives: Rapeseed-mustard is the third most important source of edible oil after soybean and oil palm. In India, it is commonly referred to as Sarson. It is an important cooking medium and dietary fat of the majority of northern, north-western, central, eastern and north-eastern states of India. It is also the most common medium of pickling and food preservation. Domestic production of edible oil has remained almost stagnant during last five years. Oilseed production in the country is facing several challanges related to biotic as well as abiotic stresses, natural resources, climate change and fragmented land holdings.

Methods: The projected annual requirement of total vegetable oil by 2025 A.D. is 27 m tonnes with the production target of 14.03 mt tonnes to meet enhanced per capita consumption 16.98 kg/year. This is challenging, nevertheless it is possible to achieve the goal by adopting vertical and horizontal growth. Immediate research need for vertical growth would conventional breeding with emphasis on sustainability, genetic engineering of through exploitation of available genetic variability. Heterosis breeding should be the major focus. Furthermore, augmentation or identification of trait specific germplasms, pre breeding and genetic enhancement, allele mining, proteomics, marker assisted breeding and gene pyramiding would facilitate better exploitation of the available gene pools in order to overcome the production constraints. Reducing the yield gap and additional area under cultivation are the viable approaches for horizontal growth. An estimate area of 1.08 mha could be brought under rapeseed-mustard cultivation from Eastern Uttar Pradesh, Bihar, West Bengal, NEH region, Madhya Pradesh, Jharkhand, Odisha and Chattisgarh where the fields remain fallow after rice cultivation. Non traditional area such as Karnataka, Southern Rajasthan and Vidarbha region of Maharashtra could contribute about 0.3 mha area for rapeseed-mustard cultivation.

Conclusion: It is possible to minimise the demand and supply gap by proper technology interventions for improving the yield. Also, bringing non-traditional area for cultivation will assist in achieving the target.

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ORAL PRESENTATION THEME D

Global change adaptation: What future for OSR crops and products? A foresight study for oilseed chains at 2030 horizon

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Background: The French oils and proteins organizations wonder about the future of their productions, particularly OSR, at 15-year horizon. The evolution of the world is accelerating with a population rising from 7 billion today to 7.9 to 8.9 in 2030. The climate change effects on agriculture are largely subject to assumptions, also future mitigation/adaptation policies. Lifestyles and diets are changing, often richer in meat. The development of non-food uses is questioned by the changes in food needs. The flow of production and trade of oils and proteins evolve, particularly in Asia (China's soybean imports, massive palm oil production in Indonesia-Malaysia). Some scientific and technical achievements are questioned, such as biotechnologies.

Methods: A panel of experts worked according to the French school of foresight methods: system's description, retrospective study, hypotheses' formulation and aggregation of assumptions in 4 macro-scenarios on global and European context (then quantified) that have no predictive value when taken alone, but which together form a framework for understanding the issues and challenges, helping in strategic options' evaluation.

Results:
Scenario1: "towards chaos": international economic and political crisis, tensions on food due to high population, stagnating agricultural yields and impoverishment. The major challenge is to produce vegetable protein for human consumption. Policies aim at restraining price volatility while maintaining food security and standards of living.

Scenario2: "Regional policies and bilateralism": in the absence of international consensus, countries including Europe implement unilateral policies for climate change mitigation, leading to a latent protectionism. Veganism increases in Europe while consumption of animal protein progresses in China and Africa. Europe is strengthening its protein self-sufficiency and non-GMO policies, and exports top quality end-products.

Scenario3: "trust": international cooperation and standardization to prevent climate change: "green growth" scenario favored by a moderate population, a dynamic agricultural production, marked by animal protein consumption's growth and by energy transition policies. Global demand of vegetable protein for feed explodes in a very open and competitive market.

Scenario4: "cooperation forced by climatic and food tensions": climate change puts agricultural production under pressure in a context of high population growth, leading to international cooperation. The main challenges are reaching sustainable food production and changing eating patterns. Policies focus on climate change, food security, and resource efficiency.

Conclusions: Massive development of palm oil production is expected in addition to oil from soybeans, whose production is supported by protein requirements. Without sanitary disasters on oil palm plantations, all scenarios highlight oil surplus and high plant proteins deficiency. Non-food uses of oils could therefore have a key role. The deficit of protein is a strong trend but needs to be qualified with regard to demands for human nutrition and food/feed industries.

The future OSR competitiveness is questioned particularly on three aspects: crop's efficiency regarding resources (nitrogen, energy...), ability to better use the protein fraction in animal feed and to valorize it as human food, development of new industrial processes and uses of the oil fraction.

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The use of precision gene editing to develop new non-transgenic traits in canola

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Background: New techniques continue to be discovered to develop desirable traits in plants. Different geographies and markets do not accept transgenic traits so alternative technologies must be developed to provide farmers with new and desired traits. Precision gene editing is a rapidly growing field which allows for specific targeted changes to be made in the DNA of a target organism. Gene editing can be done in a way to avoid the introduction of foreign genetic material and as such, will develop non-transgenic plants that are acceptable in those geographies and markets which prefer non-transgenic plants.

Methods and Results: RTDS™ (Rapid Trait Development System) developed by Cibus is a gene editing technology which can be used to target precise changes in genes of interest. Herbicide tolerance (HT) in both canola and flax were targets for the technology. Robust cell culture systems were developed to allow RTDS converted cells to be identified and regenerated into plants. Molecular screening confirmed the presence of the targeted changes and plants with the HT targeted change(s) were regenerated and are in various stages of commercial development.

Conclusions: Gene editing as demonstrated in applying RTDS to both canola and flax is a powerful new technique to develop traits in plants that are non-transgenic and can develop commercially valuable crops that fit into geographies and markets where other traits are currently not accepted.

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ORAL PRESENTATION THEME D

Towards a Canadian government model policy for management of low-level presence of genetically modified crops in imported grain, food and feed

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Governments as well as public and private institutions around the world are actively seeking ways to increase agricultural productivity and make other useful improvements to crops. In support of these efforts, it is expected that the number and variety of genetically modified (GM) products commercialized will continue to increase. Once a GM crop is authorized for commercial use in a foreign jurisdiction, trace amounts of that crop may become mixed with other varieties of the same crop or other crops in that jurisdiction. This can happen during the cultivation, harvest, transportation, and storage of the GM crop. Even when best management practices are strictly followed, it is often difficult to prevent this from occurring. As a result, a GM crop that is not approved in the importing jurisdiction may unintentionally be present at low levels in the grain, food or feed products exported to that jurisdiction. This is what is called low-level presence (LLP).

Under the Canadian regulatory framework, the presence of an unauthorized GM crop in Canada constitutes non-compliance. The Government of Canada is developing a model that provides a more predictable, pragmatic approach to managing LLP in imported grain, food and feed.

Governments as well as public and private institutions around the world are actively seeking ways to increase agricultural productivity and make other useful improvements to crops. In support of these efforts, it is expected that the number and variety of genetically modified (GM) products commercialized will continue to increase. Once a GM crop is authorized for commercial use in a foreign jurisdiction, trace amounts of that crop may become mixed with other varieties of the same crop or other crops in that jurisdiction. This can happen during the cultivation, harvest, transportation, and storage of the GM crop. Even when best management practices are strictly followed, it is often difficult to prevent this from occurring. As a result, a GM crop that is not approved in the importing jurisdiction may unintentionally be present at low levels in the grain, food or feed products exported to that jurisdiction. This is what is called low-level presence (LLP).

Under the Canadian regulatory framework, the presence of an unauthorized GM crop in Canada constitutes non-compliance. The Government of