

Proceedings

13th International Wheat Genetics Symposium

April 23-28, 2017, Tulln - Austria



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Proceedings of the 13th International
Wheat Genetics Symposium

April 23-28, 2017 - Tulln, Austria



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Wheat Genetics
Symposium



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Editors

Hermann Buerstmayr, Christina Lang-Mladek, Barbara Steiner, Sebastian Michel, Maria Buerstmayr, Marc Lemmens, Johann Vollmann, Heinrich Grausgruber

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Welcome from the organizers

With great humility and pleasure my team and I took over the responsibility to host the 13th International Wheat Genetics Symposium for the first time in Austria. The 13th IWGS takes place 64 years after the first IWGS convened August 11-15, 1958 in Winnipeg, Canada and has traditionally been held in 5 year intervals.

It is you who make this Symposium a successful and memorable one. My team and I welcome you and thank you for attending this event: we are close to 500 participants from all over the world, representing 46 countries. We will witness 72 oral and 320 poster presentations, among which 64 presenters are students.

The IWGS 2017 aims to highlight the current status of wheat genetics, wheat genomics, wheat phenomics and wheat breeding. The year 2017 marks the release of the wheat reference genome assembly version 1, which allows an unprecedented insight into the wheat genome. At the same time the next steps are well underway, such as sequencing and assembling several key cultivars, thus paving the way for even deeper analysis of structural and functional diversity. Equally important is that novel and high throughput genotyping and phenotyping as well as biometrical methods for genomics assisted breeding are constantly being refined, and sequenced mutant populations have been developed. Recently evolved gene editing tools appear promising for functional analysis and for developing novel traits. So there is a lot to discuss and a lot to learn from each other. I trust that this symposium will be of benefit for the whole community to share novel ideas and approaches.

All these developments and innovations will deepen our understanding of this so important crop plant and will find their way into knowledge based crop improvement. This requires constant communication between the research and the breeding community. The difficult and complex task to implement innovations in their day to day breeding work remains with the practical breeders.

Innovations in technology can only be as good as the germplasm available. Germplasm evaluation and searching for useful alleles in the wheat gene pool, including wild relatives, genebank accessions, and mutant populations will be a major endeavor for future wheat improvement, particularly for adaptation to stressful environments. Access to germplasm and open germplasm exchange among researchers and breeders must be maintained and stimulated, in order to allow for long term mutual progress in wheat improvement.

Advancement in research and improvement of cultivars depends on people. We all know that a crop plant like wheat needs to be adapted to specific growing regions and cultivation practices: there is no 'best' wheat cultivar. Crossing, selecting and cultivar development need to be done for each region individually. Therefore, regional breeding and testing is compulsory. This means that we need many well trained and enthusiastic people who put this work into practice in their home countries or regions. Capacity building, training, exchange of ideas, tips and tricks are key components and must be deepened and widened. IWGS 2017 encourages and stimulates young researchers' involvement *e.g.* by allocating 10 oral presentation slots for a specific students' session.

Let me thank the local organizing committee, first and foremost Mrs. Susanne Weber, our awesome symposium secretary, whom many of you had contact with. Without Susanne's support I would have gone crazy. I wholeheartedly thank our webpage designer and administrator Max Böck (<https://mxb.at>) who always was at hand with quick and efficient solutions, the fantastic local organizing committee and all student helpers!

My sincere gratitude goes to all members of the international organizing committee, who were responsive, supportive and encouraging throughout the last two years. In addition I thank the reviewers, to a large extent but not exclusively overlapping with the IOC, who took over the difficult task to read, evaluate and suggest submitted abstracts for the oral or the poster sessions. This was not a trivial task, since the requests for oral presentations were four times higher than the number of slots available. I think they did an excellent job and shaped a magnificent oral and poster program,



considering a combination of scientific excellence, regional and gender balance in their evaluations. The IWGS 2017 is possibly the first one with a dedicated students' session which empowers young researchers to present and discuss their work among each other and with senior scientists. Among all scientific contributions 64 will be presented by students, I am sure all of you highly appreciate this. We will allocate enough time and space for the poster sessions. Presenting your research as poster has the great advantage that you can communicate with your audience even more personally and more interactively.

Without sponsoring and industry support this Symposium could not be accomplished. Therefore, my sincere appreciation to all supporters, let me particularly express my gratitude to the city of Tulln, with city mayor Peter Eisenschenk, who was always enthusiastic about hosting this Symposium in Tulln.

Hermann Buerstmayr, April 23, 2017, Tulln, Austria.
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KEYNOTE OPENING LECTURE

The option space for feeding the world in 2050 without deforestation: exploring the role of diets and agricultural technology

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Key message: Extended abstract for the keynote lecture at the 13th International Wheat Genetics Symposium, 23rd-28th April 2017, Tulln, Austria

Land is a key resource for human societies and for all other organisms (animals, plants and microorganisms) inhabiting terrestrial ecosystems worldwide. Humans use land for at least three purposes, i.e. resource supply, waste repository and living space (i.e., the area required for production, consumption, transport, recreation and many other activities). Increasingly, land use policies and strategies also aim at optimizing other “ecosystem services”, such as the conservation of habitats, species or ecosystems or increased carbon sequestration. Maximization of one function or service, such as biomass supply, often affects other functions, such as carbon sequestration or conservation. Because global land is finite and predominantly human-used (particularly the naturally fertile regions), almost any extension of area use for one purpose implies a reduction in the available area for other functions or services. Along with the growth of the world population and its per-capita consumption, trade-offs among different functions, respectively competition for land arising from attempts to maximize land’s specific functions, are therefore becoming more important (Haberl, 2015). In this context, conserving the world’s remaining forests is a high-priority goal.

In this lecture I will discuss the biophysical option space for feeding the world in 2050 in a hypothetical zero-deforestation world. The work presented builds on long-standing research aiming to quantify and map global land use (Erb et al. 2007) and biomass flows (Haberl et al. 2007; Krausmann et al. 2008, 2013; Erb et al. 2016a). Based on this research, we have been able to construct the diagnostic model BioBaM (“biomass balance model”; see e.g. Haberl et al. 2010, 2011; Erb et al. 2016b). This model allows researchers to analyze future option spaces related to land use (change) and the supply of biomass or other land-based services. In BioBaM realistic assumptions on future yields, agricultural areas, livestock feed and human diets and assess are systematically combined to assess the biophysical feasibility of “scenarios”, i.e. unique combinations of assumptions on the future development of these variables.

For each scenario, BioBaM determines whether the supply of crop products meets the demand and whether the grazing intensity stays within plausible limits. In a recent article (Erb et al. 2016b) we have analyzed the options to meet the global food supply in 2050 without deforestation. We find that a broad range of options exist, some even involving low levels of crop yields, such as those that would result from global adoption of organic farming. Within the option space, individual scenarios differ greatly in terms of biomass harvest, cropland demand and grazing intensity, depending primarily on the quantitative and qualitative aspects of human diets (Figure 1). Grazing constraints also strongly limit the option space. Without the option to encroach into natural or semi-natural land, trade volumes will rise in scenarios with globally converging diets, thereby decreasing the food self-sufficiency of many developing regions.

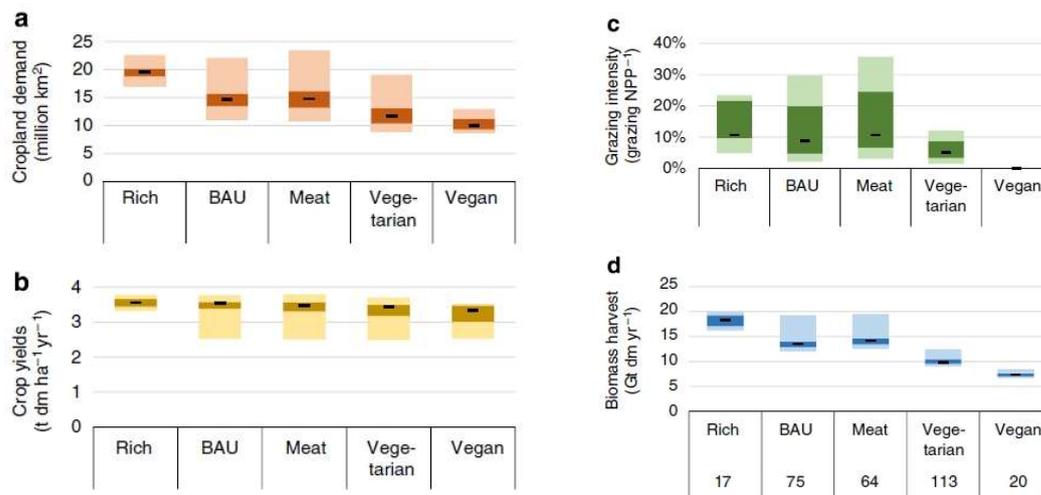


Figure 1: Characterization of feasible and probably feasible scenarios, broken down into human diets. (a) Cropland demand, (b) average cropland yields, (c) grazing intensity, measured as grazing harvest in percent of actual NPP, (d) biomass harvest, i.e., total biomass harvested globally for food supply, given as dry matter. Dark boxes indicate the two inner quartiles (>25 and <75%) of all feasible scenarios; light grey-shaded boxes indicate the minimum and maximum values. Small lines indicate the median. Numbers below diets indicate the number of feasible scenarios. The total number of scenarios per diet is 120, with the exception of the VEGAN diet, with 40 scenarios. ‘Rich’ denotes a conversion toward rich diets globally. BAU denotes a ‘business as usual’ diet similar to expectations published by FAO. ‘Meat’, ‘vegetarian’ and ‘vegan’ are diets derived from recommendations for a healthy diet, with and without meat, respectively without any animal products in the ‘vegan’ case (Source: Erb et al. 2016b).

According to this study, human diets are the strongest determinant of the biophysical option space. Unsurprisingly, vegan diets and diets with a low share of livestock products show the largest number of feasible scenarios. High yields or intensive livestock systems do not show such a strong effect on the number of feasible scenarios and do not necessarily reduce cropland demand or grazing intensity because the land-sparing effect can be annihilated by shifts towards rich diets.

Yields show a smaller effect than human diets on the overall option space, but low yield levels limit the number of feasible scenarios, particularly for diets with meat, which are affected primarily by cropland constraints. Even in a zero-deforestation world, low-yielding agriculture such as organic farming is feasible if paired with a vegetarian or vegan diet. The expansion of cropland does not critically influence the option space, with the exception of the zero-cropland expansion variants, where approximately half of scenarios are not feasible. Cropland area and grazing intensity are found to be strongly interlinked. Under “zero deforestation,” large cropland entails smaller grazing lands and thus higher grazing intensity.

The option space analyzed is delineated solely on the basis of a biophysical balance between supply and demand. The study does not aim at exploring probabilities, and it does not support straightforward conclusions regarding the desirability, political practicability or sustainability performance of different scenarios.

A central trade-off relates to the area savings resulting from increased yields. These savings may help increasing carbon storage, but this effect can potentially be compensated for by emissions from increased energy and resource demand in agriculture or increased biomass use. Analogous trade-offs can be suspected with issues such as nitrogen leaching, phosphorus depletion, or biodiversity loss.

The identification of preferred future options would require additional analyses beyond biophysical analyses and the assessment of fundamental and complex economic, political and social effects



associated with envisaged changes, such as the structural change in diet trajectories, farming practices, the replacement of land use systems and economic effects, e.g., rising food prices. Integrated assessment models (IAMs) enable researchers to assess the cost-benefit structures of future developments, often based on optimization approaches and conducted in detailed, economic sector-specific manner. Complementary to IAMs, simple, transparent and data-based approaches enable scrutiny of the biophysical conditions, constraints and effects of anticipated changes in the land system, e.g., by contextualizing results or by providing reality checks. Both research strands are required for advancing our scientific understanding of the trade-offs related to land use and for identifying political strategies that allow developments to stay within planetary boundaries.

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Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Identifying sequence features impacting recombination in wheat: lessons from chromosome 3B

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Key message: We performed a fine-scale mapping analysis of crossovers in bread wheat and found that crossovers occur more frequently in gene-rich regions and share similarity with recombination hotspots in human.

Meiotic recombination is a process that allows reshuffling of diversity by reciprocal exchange of DNA called crossover (CO). This phenomenon is conserved in most eukaryotes and is highly regulated since at least one CO per chromosome is absolutely required to ensure a correct segregation of chromosomes during meiosis. In bread wheat (*Triticum aestivum* L.), COs are almost limited to subtelomeric regions of chromosomes resulting in a substantial loss of breeding efficiency in the proximal regions though these latter carry $\approx 60\text{-}70\%$ of the genes. Identifying sequence and/or chromosome features impacting recombination occurrence is thus relevant to improve and drive recombination in a near future. With the recent release of a reference sequence of chromosome 3B and of the draft assemblies of the 20 other wheat chromosomes, we were able to perform a fine-scale mapping analysis of COs. We genotyped a large segregating population of 1270 F₆ lines with 774 SNPs highlighting 74 small intervals (<26 kb) on chromosome 3B that precisely delineate 252 crossover events. Additional genotyping of two collections of 180 varieties representative of the Asian and European genetic pools with the same SNPs, revealed a common location for ancestral recombination events (predicted through Linkage Disequilibrium analyses) and the previously-identified hotspots. We also analyzed 596 COs in 476 intervals of less than 26 kb distributed on the 21 chromosomes. Ninety percent of COs were delineated in intervals of less than 10 kb with 81% of intervals presenting only 1 CO. Similar results as those observed for chromosome 3B were found. In both cases (chromosome 3B and whole-genome analysis), surrounding-crossover-sequence analysis showed that COs occur more frequently in gene-rich regions and around genes expressed during meiosis. We also observed differences in transposable element content and we identified an original associated motif (carried by *TIR-Mariner transposon*) that might drive recombination. This motif shares high degree of similarity with the one associated with recombination hotspots in human and carried by *THE1A/B* retrotransposon, suggesting a common ancestral mechanism for recombination control in eukaryotes.



Topic: Cytogenetics; Diversity and Evolution of the Triticeae

***Tritico nihil est fertilius* – nothing is more prolific than wheat. The cultural and cultivation history of wheat**

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Key message: From the domestication in the Fertile Crescent, wheat was considered the superior grain. However, in adapting to new climates, it was integrated into new cuisines and competed with other cereals.

The main cereals in the Fertile Crescent were barley, emmer and gradually free threshing tetraploids and hexaploids. The relative whiteness of leavened and baked bread made wheat the preferred cereal for the socially privileged. Wheat had twice the value of barley. The primacy of wheat was enshrined in all three monotheistic religions and persists in the Middle East to this day. The Anatolian expansion to Europe mainly involved tetraploids, mostly emmer, spelt during the Bronze Age. The Roman Empire established free threshing hexaploids in the north and west. *Tritico nihil est fertilius* - Nothing is more prolific than wheat, wrote Pliny (*Natural History* 18: 94). However, ecological limits eastwards bordered with (leavened) rye and northwards with (unleavened) barley and oats. For nearly two thousand years this determined the European bread map (Bjørnstad 2016). The frequently hostile border between France and Germany was symbolically expressed as wheat versus rye. Despite bread preferences between West and East, wheat remained *the* cereal in church (Vermander 2015). Outside Europe, wheat met with established cereals. In China, cuisine was based on boiling soft cereals like millets and rice. Soft wheats were adopted, but not hard wheats nor baking, and (like in India) as a spring crop. Coming to America, the Europeans suffered agronomic and cultural frustrations when growing wheat. In a few decades spring wheat succeeded in Mexico and Peru, but took centuries in North America before Hard Red Spring and Hard Red Winter wheats and roller mill technology, provided a wheat bread basket, erasing the European bread map. The different names of wheat either emphasize hardness or grinding or whiteness (cf. wheat/white). The latter is both chemical (oxidation and betacarotene) and physical, due to light dispersion in the crumb. Millers 'improved' whiteness by elaborate sifting or chemicals to satisfy discriminating customers, and breeders used genetics. In the USA and in South Africa whiteness at times had racist overtones. Today artisan bread has reversed this preference for well-paying wheat eaters.

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Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Genome evolution in *Aegilops* evaluated using molecular-cytogenetic analyses

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Key message: Evolution of diploid *Aegilops* was characterized by extensive C-banding and low translocation polymorphisms. Most significant genome modifications occurred at the stage of formation of tetraploid forms.

Genome evolution in *Aegilops* was studied using C-banding and FISH with 10 DNA probes. Diploid species were characterized by diverse karyotype structures and C-banding patterns (Figure 1). Distribution of most FISH probes was more conserved across related genomes compared to C-banding/(GAA)_n patterns. Position of (GTT)_n signals did not always coincide with the location of C-bands, and in some polyploidy species this sequence allowed discrimination of chromosomes belonging to different genomes (Figure 2). The distribution of 5S and 45S rDNA loci was a conserved feature of all genome types, although variation in the number and distribution of minor rDNA loci has been observed. Interspecific divergence of diploid *Aegilops* was caused by amplification, elimination and re-distribution of repetitive DNA families and by chromosomal rearrangements. An enhancement of chromosome asymmetry and an increase of heterochromatin content were general trends in genome evolution. Diploid species were characterized by extensive C-banding polymorphisms, but lower number of chromosomal rearrangements as compared to polyploid taxa. The polyploidy species of the D-genome cluster showed relatively high karyotype stability. Chromosome modifications during speciation involved both parental genomes. We found evidences of multiple origins of *Ae. juvenalis*, in which two distinct cytotypes have been discovered (Figure 3a,b). Significant genome modifications in one cytotype (Figure 3a,c,d) and relatively 'intact' genome structures in another (Figure 3b) suggested that the latter one emerged recently from hybridization of *Ae. crassa* with *Ae. umbellulata*, while the former one arose much earlier from hybridization of the same parental species and further underwent significant genome modifications. Species of the U-genome cluster were characterized by high genome variability. Karyotype evolution occurred via single and multiple translocations and, in certain cases, introgressive hybridization. Our analysis strongly suggested that at least *Ae. columnaris*, and *Ae. biuncialis* originated as a result of multiple hybridization events. Our data suggest that most significant genome modifications occurred at the stage of formation of tetraploid forms, whereas the emergence of hexaploid species usually did not lead to structural genome alterations.

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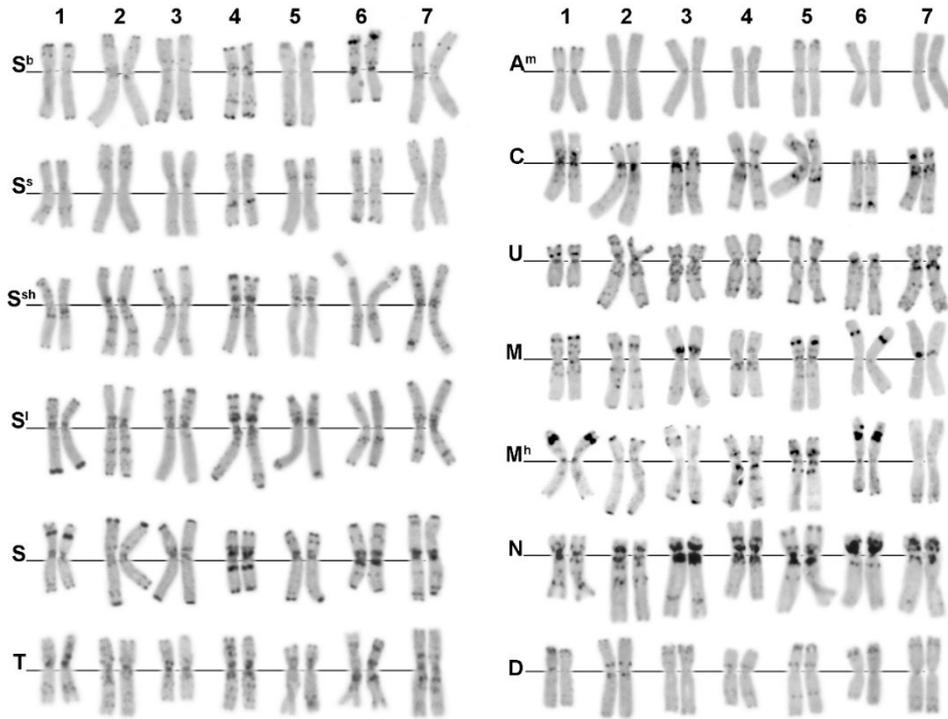


Figure 1: Comparison of karyotype structures and C-banding patterns of diploid wheat and *Aegilops* species: Sb, *Ae. bicornis*, IG 47619; Ss, *Ae. searsii*, G7.12; Ssh, *Ae. sharonensis*, original accessions collected in Atlit, Israel; Sl, *Ae. longissima*, original accession, collected in Ha Bonim, Israel; S, *Ae. speltoides*, original accession, collected in Katzir, Israel; T, *Ae. mutica*, PI 598388; Am, *Triticum monococcum*, PI 428282; C, *Ae. caudata*, TA 2095; U, *Ae. umbellulata*, TU04; M, *Ae. comosa*, 2110003; Mh, *Ae. heldreichii*, 2110002; N, *Ae. uniaristata*, PI 554418; D, *Ae. tauschii* ssp. *strangulata*, K-1461. Chromosomes are arranged according to genetic nomenclature.

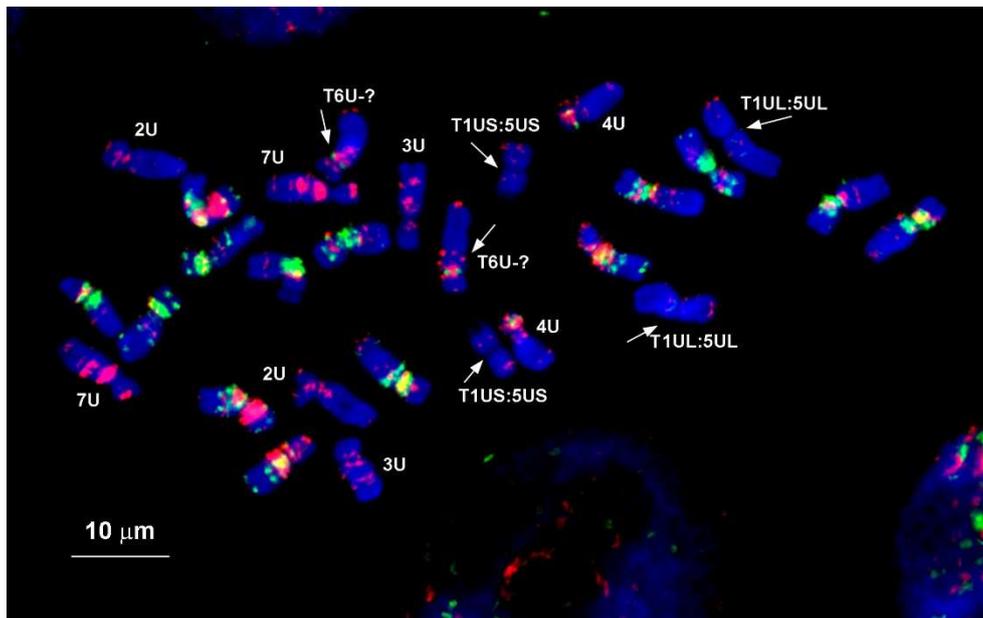


Figure 2: Metaphase cell of *Aegilops columnaris*, K-1193, labeled with (GTT)10 and (CTT)7 probes (green and red colors respectively). The U-genome chromosomes are numbered according to genetic nomenclature; chromosomes involved in reciprocal translocations are indicated with arrows.

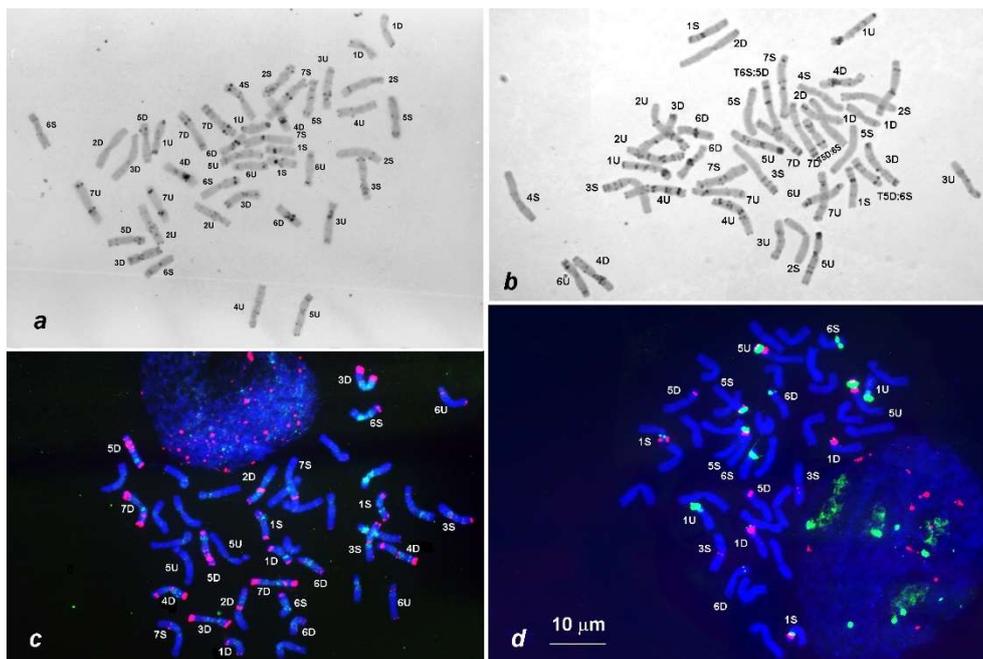


Figure 3: C-banded (a,b) and FISH labeled (c,d) metaphase cells of *Aegilops juvenalis*, I-571695 (a,c,d) and K-1380. Chromosomes are numbered according to genetic nomenclature.



Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Introgression of a new stem rust resistance gene from *Aegilops markgrafii* into wheat

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Key message: A new stem rust resistance gene against race Ug99 has been transferred from *Aegilops markgrafii* into the wheat genome by using marker-assisted chromosome engineering.

In a previous study, we reported that a wheat *Alcedo/Aegilops markgrafii* disomic addition line, AIII(D) (2n=44), was resistant to three Ug99 lineage races and five North American races of the stem rust pathogen (*Puccinia graminis* f. sp. *tritici*), and that the resistance originated from the alien chromosome. The objective of this study was to translocate a small alien segment carrying the stem rust resistance gene to a homoeologous wheat chromosome. The *Ae. markgrafii* chromosome in AIII(D), based on SSR marker analysis, was identified as homoeologous to a group 6 chromosome but with its telomeric region in the long arm homoeologous to a group 7 chromosome. Therefore, AIII(D) was crossed to Chinese Spring (CS) monosomic 6A (M6A) and resistant F₂ progeny were screened for Robertsonian translocations using genomic *in situ* hybridization (GISH). A plant carrying a Robertsonian translocation was identified and was backcrossed to CS *ph1b* (Figure 1a). Resistant BC₁F₁ plants homozygous for *ph1b* were backcrossed as the male parent to CS. A BC₂F₁ population tested with race TMLKC segregated 471 resistant and 566 susceptible. The resistant plants were genotyped with seven SSR markers specific for the *Ae. markgrafii* chromosome. Dissociation of the alien segment carrying the stem rust resistance gene was detected in 17 plants. Marker and GISH analysis of the dissociation lines indicated that the stem rust resistance gene was located near the telomere (Figure 1b) and tightly linked to *Xwmc232*, which was previously mapped near the 7BL telomere. Stem rust gene *Sr25* is located near the 7DL telomere, and because the AIII(D) gene has similar infection type to *Sr25*, we tested marker BF145935 linked to *Sr25* on the 17 dissociation lines. BF145935 amplified a 200-bp amplicon in AIII(D) and all dissociation lines, but was absent in CS and five rust susceptible progeny. As reported in a previous study, BF145935 also amplified 187-bp and 207-bp amplicons located to wheat chromosomes 7A and 7D, respectively. In one dissociation line, a plant known to be homozygous for the AIII(D) gene lacked the 7A amplicon, suggesting that the AIII(D) gene may be translocated to chromosome 7A in that line. We are working to confirm if the AIII(D) gene has been transferred to chromosome 7A in all lines, or has been translocated to other group 7 chromosomes. The new wheat introgression lines carrying the gene and linked markers provide new resources for improving wheat for resistance to stem rust.

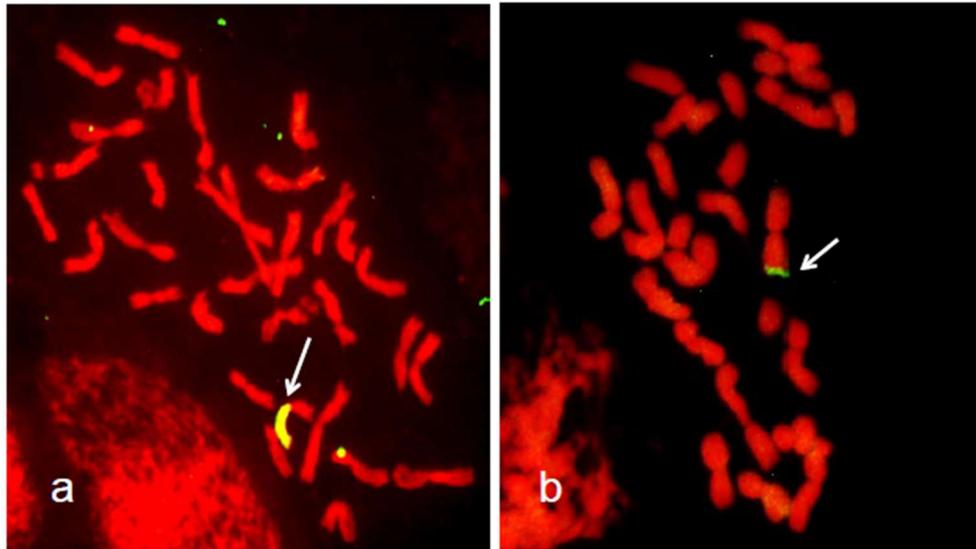


Figure 1: Images from genomic *in situ* hybridization of the plants carrying new wheat-*Aegilops markgrafii* chromosome translocations: (a) Robertsonian translocation; (b) a new wheat line carrying the stem rust resistance gene on shortened *Ae. markgrafii* chromatin. The alien chromatin from *Ae. markgrafii* (yellow-green color) is indicated by arrows.



Topic: Harnessing Diversity for Triticeae Improvement

A step change in the transfer of interspecific variation into wheat from its wild relatives

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Key message: New technology and introgression strategies are enabling the systematic exploitation of genetic variation from wild relatives for exploitation in the production of high-yielding wheat varieties adapted to environmental change.

The objective at the start of the introgression programme at the University of Nottingham was to transfer the whole genomes of a range of wild relatives (e.g. *Amblyopyrum muticum*, *Aegilops speltoides*, *Triticum urartu*, *T. timopheevii*, *Secale cereale*, *Thinopyrum bessarabicum*, *Th. elongatum* and *Th. intermedium*) into wheat in small, overlapping chromosome segments. In this work we have generated F₁ interspecific hybrids between wheat (carrying the *ph1* mutant or carrying *Ph1* suppressors - the *ph1* mutant and *Ph1* suppressors enable interspecific recombination to occur between the chromosomes from different genomes) and a number of its wild relatives. The interspecific F₁ hybrids were effectively haploid for the three wheat genomes, i.e. A, B and D and also the genome(s) of the wild relatives. In the absence of homologous chromosomes from the same genome high levels of homoeologous recombination were expected to occur between the chromosomes from the three genomes of wheat and those of the wild relatives in the interspecific F₁ hybrids. In order to recover introgressions resulting from homoeologous recombination in the gametes, each of the F₁ hybrids were recurrently backcrossed to the wheat parent and the progeny analyzed. In the past one of the major bottle necks in wheat/wild relative introgression has been the absence of technology that facilitates the high throughput detection/characterisation of introgressions. In order to overcome this problem we have used a new 35K Axiom SNP array specifically designed to detect introgressions. The introgression strategy we have employed coupled with the new Axiom array led us to believe that we would detect new introgressions. However, the number of introgressions we detected was far higher than we had predicted from previous work and represents a step change in the field of wheat/wild relative introgression, i.e. over 1000 new introgressions have been generated and detected from a range of species. This work has enabled us to develop genetic maps of the wild relatives based on the introgressions generated and in addition allowed the determination of the syntenic relationship between wheat and each of the wild relatives. In collaboration with a network of global partners, work is presently underway to target introgressions carrying genetic variation for agronomically important traits. Targetted introgressions will be incorporated into breeding programmes for the development of superior wheat varieties that are adapted to climate change.



Topic: Harnessing Diversity for Triticeae Improvement

Advances in alien gene transfer in wheat

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Key message: An overview of alien gene transfer in wheat covers advances in screening for variability, intergeneric hybridization, induction of recombination, advances in linkage map construction and application of new classes of markers.

The primary gene pool of wheat contains extensive variability for most traits that are used for wheat improvement. However at times the variability is not sufficient because of excessive biotic or abiotic stresses. The screening of secondary and tertiary gene pools for variability in biotic stresses, abiotic stresses, value-added traits and agronomic traits continues to generate useful germplasm. Variability for virtually any trait can be found with sufficient screening. Intergeneric hybridization has continued at a steady pace. Progenies have been advanced by backcrossing, doubled haploids or single seed descent resulting in a variety of cytogenetic stocks such as chromosome addition/substitution lines or various types of translocations. These would have been verified by GISH analysis and screened with various fungal pathogens. The *Ph* system has been employed to induce recombination between alien and wheat chromosomes. Large numbers of progeny need to be screened for resistance and efficient marker systems need to be deployed to select segregates with minimal alien chromatin. Alien genes have had an impact on most biotic stresses in wheat. For example resistance to wheat stem rust and particularly the new variant, Ug99 has relied heavily on alien genes. Six of the seven resistance genes currently being deployed have come from alien sources. In screening various materials for additional genes, resistance has been detected in species with R, V, D, E1, E2, and St genomes. Integration processes are underway. Synthetic hexaploids are proving to be good sources of disease resistance with some combinations provided resistance for up to three diseases. Linkage maps in alien species and new classes of molecular markers have had a major impact on the isolation, introgression and deployment of alien genes. Detailed linkage maps have been prepared in some of the most significant donors of alien genes such as *Lophopyrum ponticum*, *Thinopyrum intermedium*, *Triticum monococcum* and *Aegilops tauschii*. Other maps are currently being developed. These markers will greatly facilitate the process of gene mapping and deployment from these species. Two new chip-based SNP markers systems have recently become available. These are the Illumina Infinium 9000 chip and where sequence information is available, the genotype-by-sequencing (GBS) method. Large numbers of low cost markers are provided by these methods. SNP technology has been successfully applied to the mapping of introgressed genes for resistance to Fusarium head blight, stem rust and stripe rust. KASP technology, a complement to SNP technology had been used to screen F₁ hybrids and BC progeny to detect recombinants and isolate desired introgressions.



Topic: Harnessing Diversity for Triticeae Improvement

The Wheat Gene Catalogue: 50 years on

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Key message: As 2018 marks 50 years of the Catalogue a decision needs to be made as to whether wheat should remain the main focus.

I was asked by the third IWGS to prepare a gene catalogue for wheat. Prior to 1968 there was a single 17-page publication summarizing wheat genetic nomenclature (Ausemus et al. 1946). The first report and catalogue proposal was presented at, and accepted by, the fourth IWGS in 1973. Since then, annual supplements were published and 5-yearly updates were presented to the IWGS. It is appropriate at the outset to acknowledge the people who contributed very significantly to the catalogue over many years; in particular Mike Gale, Gary Hart, Katrien Devos, John Rogers, Jorge Dubcovsky and Yukiko Yamazaki who built the current MacGene database in the early 2000s and maintained it since that time. Thanks are due to the many others who contributed in various ways, including the early supporters Ernie Sears, Ralph Riley, Douglas Knott and Irvine Watson. The basic Wheat Catalogue is no more than orderly lists of symbolized names and numbers aimed to avoid duplication. Over the years we introduced lists and information regarding DNA markers, genetic nomenclature based on homoeology, QTL, and currently sequence data and possibly a more universal nomenclature covering a wider range of species. Wheat is one of the most important world crops providing about one-quarter of human sustenance; it is also a model polyploid organism with a huge genome. Given that we are about to have entire genetic sequences at our fingertips it is timely to consider the future of the catalogue – a focus to germplasm, including related species, and the use of genetic resources in agriculture with attempts to address the increasing complexity of restricted international exchange of seed and IP issues, and a closer link to proteins, gene function and expression across the Triticeae and beyond.

Reference

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Topic: Harnessing Diversity for Triticeae Improvement

Mobilizing wheat biodiversity from gene banks to breeding programs

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Key message: CIMMYT-Seeds of Discovery project aims to characterize and mobilize genetic diversity from gene bank to wheat, to address challenges from changing climate and provide food and nutrition security.

New molecular and biometric technologies have made it possible and practicable to identify and 'package' previously untapped diversity from wheat's gene pool for use in modern wheat breeding programs. One way to achieve this is through 'bridging germplasm' – semi-finished lines from which desirable genes can be introgressed into elite germplasm with minimal transfer of undesirable traits (hitherto a major constraint to the use of raw genetic resources in breeding programs). Through the 'Seeds of Discovery' (SeeD) project, funded by Mexico since 2011, scientists have been using cutting-edge DNA tools and informatics to identify and use high-value traits from CIMMYT and ICARDA large wheat germplasm collections, with particular emphasis on heat tolerance, drought tolerance and disease resistance. More than 60 000 accession (40%) of CIMMYT's wheat germplasm bank, and 30 000 accessions from ICARDA have undergone genotyping-by-sequencing-DArTseq technology. Best-bet subsets of exotic landraces were selected using Focused Identification of Germplasm Strategy (FIGS) allowing to capture new allelic variation for heat and drought tolerance. A total of 1000 landraces and synthetics were used in this study to provide breeders with a 'genetic resources utilization toolkit' to enhance the use of the diversity contained in these germplasm banks. These exotic parents (landraces, wild relatives and synthetics) have each been crossed in three-way fashion with elite lines to generate bridging germplasm (75% elite, 25% exotic). More than 10 000 advanced-generation bridging lines have been developed from these crosses for testing and use. Seeds of germplasm bank accessions and bridging lines selected or evaluated for heat, drought, yellow rust and powdery mildew along with their respective genotypic and phenotypic characterization data, constitute unique resources for wheat improvement. The derived and selected pre-breeding germplasm have been evaluated by national breeding programs in India, Iran, Mexico and Pakistan and potential lines have selected for further large scale evaluation.



Topic: Harnessing Diversity for Triticeae Improvement

How can genome organization impact wheat breeding (and *vice versa*)?

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Key message: Wheat has a complex genome as well as a complex history of evolution that includes domestication and breeding, both being intricately linked.

Wheat is one of the most important crops worldwide, providing food for ≈30% of the world population. While significant advances have been made since the Green Revolution, today's agriculture is facing unprecedented challenges: to keep pace with the growing human demand in an environmentally and socially sustainable manner and in a context of climate change. It has long been argued that a better knowledge of the wheat genome should help facing this challenge. To this aim, huge efforts have been made by the wheat scientific community to produce a high quality reference sequence of the wheat genome. From a basic research viewpoint, the detailed analysis of these sequences revealed unexpected features. These include an uneven distribution of recombination events, a strong structural and functional partitioning, a higher proportion of non-syntenic and duplicated genes compared to other crops, a correlation between gene and genome structure and expression as well as the existence of chromosomal domains enriched in co-expressed genes. These characteristics are the result of wheat evolution. However, compared to wild species, wheat has a complex history of evolution that also includes domestication and breeding. This presentation will illustrate with examples the intricate links between the wheat genome organization and wheat breeding, showing how one can impact the other.



Topic: Structural and Functional Wheat Genomics

The wheat genome in the 21st century

Rudi Appels on behalf of the International Wheat Genome Sequencing Consortium (IWGSC)

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Key message: The IWGSC has achieved the establishment of a reference genome assembly based on DNA isolated from Chinese Spring. This milestone and its relevance for wheat improvement will be discussed.

A reference genome assembly of Chinese Spring became available recently. Telosomic genetic stocks were produced by E.R. Sears and reported at the 5th IWGS 40 years ago (Sears & Sears 1978). Flow sorting and the capacity to produce BAC libraries, established in the Dolezel lab (Olomouc, Czech Republic) provided the basis for a global network to contribute to sequencing of the 40 telosomes from 20 chromosomes – chromosome 3B was determined as a whole chromosome and was the first chromosome to be assembled in the Feuillet lab (INRA, Clermond-Ferrand, France). A flexible strategy with short- and mid-term goals to meet the challenges of sequencing the large, hexaploid genome, initially focussed on physical maps anchored to genetic maps for each of the 21 bread wheat chromosomes. Subsequent projects included sequencing of minimal tiling paths of mapped BACs, a chromosome-based survey sequence of the genome, and a whole genome assembly using the NRGene DeNovoMAGICTM software. The whole genome assembly was combined with POPSeq and HiC data to produce IWGSC v0.4 in June 2016 which was then used as a template for integrating whole genome and chromosome data from BAC-based resources (sequences, physical maps, WGP tags) to produce IWGSC ver1.0 in October 2016. Publication of a well annotated, reference sequence of hexaploid bread wheat Chinese Spring is occurring in 2017. The IWGSC is currently driving a project to engage colleagues within its extensive network to contribute to validating and refining genome annotations. The Apollo platform (Lee et al. 2013) provides a dynamic environment to capture new annotations which ideally would interface with the global data bases where the automated annotations of genome sequences are located. Specific examples to illustrate the integration of complex datasets (including the whole genome sequencing of different varieties) using the reference genome will utilize an analysis of the prolamin coding gene family and the fructan accumulation related gene families. Translation of genome analysis outputs to the wheat production chain will also be discussed.

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Topic: Structural and Functional Wheat Genomics

Genome assemblies of elite cultivars provides insights into the wheat pan-genome

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Key message: This study represents novel insights into the pan-genome of elite cultivars and is expected to impact wheat biology research and breeding.

Wheat (*Triticum* spp.) remains one of the last major crop species for which a fully assembled and annotated genome is not yet available. This has slowed identification of genes underlying phenotypic expression of agriculturally important traits. Recently, the International Wheat Genome Sequencing Consortium (IWGSC) announced the most comprehensive and contiguous assembly of the Chinese Spring hexaploid ($2n=6x=42$; AABBDD) wheat genome. This sequence will represent the gold standard reference of hexaploid wheat and is already paving the way for innovations in wheat biology and breeding. Another valuable resource is the pan-genome, which refers to the complete genomic composition of a species, including structural variation, copy number variation, and genes that maybe absent from a single reference genome. Several methods have been proposed to characterize the pan genome, including comparative analysis of complete *de novo* genome sequences from multiple lines. Here we report on highly contiguous assemblies of four elite wheat cultivars and their comparative analysis as a step towards characterizing the wheat pan-genome. Early results suggest that at least 6-8% of the genes show presence-absence variation in these cultivars. Two of the cultivars assemblies carry a 20 Mbp translocation on chromosome 2A representing the *Vpm-1* introgression from *Aegilops ventricosa*. This study represents novel insights into the pan-genome of elite cultivars and is expected to impact wheat biology research and breeding.



Topic: Structural and Functional Wheat Genomics

High quality assembly of the durum wheat genome cv. Svevo

Luigi Cattivelli on behalf of The International Durum Wheat Genome Sequencing Consortium

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Key message: The genome of durum wheat (cv Svevo) has been sequenced, assembled according to the highest quality standard and anchored to a genetic map.

Durum wheat with a total production of about 35 million tons is the 10th most important crops worldwide. It is an integral component of the Mediterranean diet and one of the historical foods that followed the birth of civilization, likely to be one of the first domesticated crops in the Fertile Crescent. An international consortium has generated a high quality reference sequence of the modern durum wheat cultivar Svevo. Whole genome libraries were sequenced with Illumina short paired-end (2×250 bp) and long mate pair (up to 8-10 kb) protocols and the reads were assembly with the NR-Gene DeNovoMAGICTM pipeline. In total, a 270× coverage was obtained, and the reads were assembled into 10.5 Gb of sequence with an L50 and L90 length of 6 and 1 Mb, respectively (N50: 493; N90: 2019). The assembly scaffolds have been anchored to a high-density genetic map based on Svevo×Zavitan RIL population and merged into superscaffolds using Hi-C data; 95% of the scaffolds have been anchored along the chromosomes and 90% oriented. The annotation of the assembled genome implemented with extensive transcriptomic data has been completed and more than 60 000 gene models were annotated. The availability of the durum wheat genome allows to highlight the genomic variations associated with the transition from wild emmer to durum wheat as well as the genome rearrangements between the A and B genomes of durum and the corresponding genomes of the hexaploid wheat.

Acknowledgement

The work was supported by the Flagship project Interomics.



Topic: Structural and Functional Wheat Genomics

Dissection of the wheat flowering pathway using sequenced mutant populations

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Key message: Combinations of null-mutations in critical regulatory genes of the vernalization and photoperiod pathways reveal unique features and interactions in the regulation of wheat heading time

We used a sequenced EMS mutant population of the tetraploid wheat variety Kronos to identify truncations or deleterious amino acid changes in critical wheat flowering genes. We back-crossed the mutations into the non-mutagenized Kronos and then combined loss-of-function mutations in the A and B homoeologs to generate null-mutants. We also generated crosses among different null-mutant lines to understand their epistatic interactions. Null-mutants for the vernalization gene *Vrn1* demonstrated that this gene is not essential for flowering, and that it negatively regulates the flowering repressor *Vrn2* in the leaves. *Vrn1* alleles for winter growth habit are induced by vernalization, and this response is modulated by polymorphisms at a Grp2 binding site in the first intron. Lines combining null-mutations in *Vrn1* and its closest paralogs *Ful2* and *Ful3* revealed a limited effect of the last two genes on wheat flowering time. *Vrn2*-null mutants in tetraploid and hexaploid wheat were sufficient to induce a spring growth habit, and resulted in the early upregulation of *Ft1* (= *Vrn3*). *Ft1*-null mutants showed a one-month delay in flowering suggesting redundant florigenic functions of other *Ft-like* genes. We showed that the encoded Ft1 protein is part of an activation complex (including 14-3-3 and Fdl2) that binds to the *Vrn1* promoter and induces flowering. Based on these results we propose a model in which a feedback regulatory loop involving *Vrn1*, *Vrn2* and *Ft1* represses flowering during the fall and irreversibly induces flowering in the spring. The photoperiod gene *Ppd1* accelerates wheat flowering under long days (LD), and Kronos *ppd1*-null mutants flower roughly two months later than the wild-type. Phytochrome null-mutants demonstrated that the light-mediated induction of *Ppd1* requires functional *PhyC* and *PhyB* genes. Both *phyc*-null and *phyB*-null mutants flowered later than *ppd1*-null mutants, suggesting effects on additional flowering genes. Using night break experiments we demonstrated that the *Ppd1* effect is dependent on the length of the night and that at least 15 consecutive short nights are required to trigger a high transcriptional activation of *Ft1* and to accelerate flowering. We also detected significant interactions between *Ppd1* and the clock gene *Elf3*. In the *elf3*-null mutants, the differences in heading time between the constitutively active *Ppd-A1a* and the wild-type *Ppd-A1b* alleles disappeared under both long and short days. Integration of photoperiod and vernalization signals determine the levels of Ft1 protein delivered to the shoot apical meristem, which regulates transcription levels of *VRN1* and GA biosynthetic genes, important for spike development.



Topic: Structural and Functional Wheat Genomics

Irradiation induced deletion mapping allows high-resolution mapping of the Fusarium head blight resistance QTL *Qfhs.ifa-5A* residing in the low-recombining peri-centromeric region of chromosome arm 5AS

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Key message: Using two γ -radiation derived wheat panels an unprecedented 300-fold increased map resolution was achieved in the severely recombination-suppressed peri-centromeric region of chromosome arm 5AS.

Fusarium head blight (FHB) is a threat for wheat production. Growing resistant cultivars is the best strategy for controlling FHB. The QTL *Qfhs.ifa-5A* resides in the peri-centromeric interval of chromosome 5AS (Buerstmayr et al. 2003). Recombination around centromere is strongly suppressed rendering fine-mapping of *Qfhs.ifa-5A* challenging. Even among 3650 near isogenic recombinant inbred lines (NI-RILs) used for genetically fine-mapping, solely four lines recombined within the C-5AS1-0.40 bin covering 40% of the physical length of 5AS. To improve fine-mapping of *Qfhs.ifa-5A* we supplemented recombination based fine-mapping with radiation hybrid (RH) mapping technique. RH mapping utilizes radiation to generate double strand breaks (DSBs) leading to deletions; breaks occur randomly and evenly distributed across the chromosomes. Overlapping regions of jointly deleted/retained markers are used for map construction. We developed two gamma irradiated wheat panels. The radiation selfing (RS) panel was generated by selfing plants from irradiated seeds of the experimental line NIL3 containing the *Qfhs.ifa-5A* resistance allele in the background of the cultivar Remus. Among the RS-NIL3-panel we identified 28 sister lines showing deleted sequences across the *Qfhs.ifa-5A* support interval. Beyond fine-mapping, identified mutant lines will be used for phenotyping. We enhanced fine-mapping with an RH-panel generated by pollinating Chinese Spring (CS) Nullisomic-5A/Tetrasomic-5B plants with gamma-irradiated CS pollen. Marker data of both panels were used to construct a RH-consensus map. The marker order was consistent among meiotic maps, physical bin maps and the RH-consensus map. Unlike genetic linkage maps, where markers clustered at a few recombination points, DSBs in the radiation induced deletion maps were evenly spaced. The QTL interval in the recombination derived NI-RIL map with *Xbarc186* and *Xcfa2250* as flanking markers comprised seven loci, with clusters of completely linked markers. The same interval in the RH-consensus map was separated by 60 loci and covered a distance of 300.3 cR. There was a more than 8-fold increase in number loci and the ratio of cM/cR translates into a 312-fold increased map resolution of the QTL interval for the RH map compared to the meiotic map. RH mapping, a powerful tool for *de novo* mapping, demonstrated to be particularly efficient for high-resolution mapping of recombination poor stretches, such as pericentromeric regions.

Acknowledgement

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Topic: Structural and Functional Wheat Genomics

A TRIM insertion led to a gene resurrection event that causes male sterility in wheat

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Key words: wheat, male sterility gene *Ms2*, map-based cloning, TRIM, gene resurrection

The male sterile *ms2* mutant has been known for 40 years and has become extremely important in the commercial production of wheat. However, the gene responsible for this phenotype has remained unknown. We here report the map-based-cloning of the *Ms2* gene. The *Ms2* locus is remarkable in several ways that have implications in basic biology. Beyond having no functional annotation and clearly having undergone pseudogenization, we find that the *Ms2* allele in the *ms2* mutant acquires a terminal-repeat retrotransposon in miniature (TRIM) element in its promoter. This TRIM element is responsible for the anther-specific *Ms2* activation that confers male sterility. The identification of *Ms2* not only unravels the genetic basis of a historically-important breeding gene and therefor accelerates its application in crop breeding more widely, but also illustrates pseudogenization at the population level and shows that resurrection of an unfixed pseudogene in the population can contribute to genetic novelty and phenotypic plasticity.



Topic: Structural and Functional Wheat Genomics

GrainGenes: supporting the small grains community

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Key message: GrainGenes is a digital platform that serves small grains research communities as their central data repository and as a facilitator for community activities.

Funded by the US Department of Agriculture, GrainGenes provides long-term data sustainability for small grains researchers and hosts a range of community newsletters, databases, and digital workspaces for wheat, barley, rye, and oats. GrainGenes is a gateway for integrated access to several types of peer-reviewed and curated genomic, genetic, and phenotypic data, along with QTL and other experimental outcomes. The availability of reference genome assemblies of wheat and barley, along with their diversity data, is making a significant impact at GrainGenes and we are creating genome-centric views on our interface with rich links to data that are already housed at GrainGenes, curated over decades. We recently updated the GrainGenes Genome Browser with JBrowse, and are creating training videos for our users to smooth the learning curve for the new interface. GrainGenes will continue creating/implementing new tools and views for the small grains community, supporting them in their research, and providing them a long-term repository for their peer-reviewed experimental and computational data.



Topic: Structural and Functional Wheat Genomics

Explore IWGSC reference genome data thanks to URGI resources

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Key message: I will present wheat genomics data hosted at URGI, especially the IWGSC reference genome.

The wheat genome is a complex genome, its analysis is a key challenge of agronomy and modern bioinformatics. As the international wheat sequence repository, URGI (INRA research unit in genomics and bioinformatics dedicated to plants and crop parasites) provides tools and browsers to explore those wheat genomics data. URGI provides access to the IWGSC reference genome through a JBrowse (https://urgi.versailles.inra.fr/jbrowse/gmod_jbrowse/). Thanks to this browser the wheat community can get annotation data like genes, markers and transposable elements. We also set-up a Blast instance with all the wheat sequences (https://urgi.versailles.inra.fr/blast/?dbgroup=wheat_all_&program=blastn). Moreover an InterMine instance containing annotation data of the IWGSC reference genome is available. Users can make data mining thanks to complex tools like query builder or region search. The URGI website provides access to all wheat physical maps (https://urgi.versailles.inra.fr/gb2/gbrowse/wheat_phys_pub/) including many tracks like contigs, scaffolds or markers. There are also WGP tags and links to order specific BAC libraries. We provide a strong interoperability between data sources by linking the Blast results, the annotation JBrowse and the physical maps. There are also many links to genetics and phenomics data integrated in GnpIS (<https://urgi.versailles.inra.fr/gnpis/>; Steinbach et al. 2013), a multi-species integrative information system that we developed at URGI.

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Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Pathogen-informed strategies for wheat disease resistance improvement

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Key message: Pathogen adaptation to resistance is a major problem for genetic improvement of wheat disease resistance. Identification of pathogen molecules involved in adaptation provides novel tools to use resistance more sustainably.

Major resistance (R) genes in wheat are of great importance in many wheat breeding programmes. However, R-gene based resistance can be short-lived under agricultural conditions due to pathogen adaptation. Therefore, there is a need to develop more sustainable uses of R genes in plant breeding. In our research we explore possible ways how to make R-gene based resistance more durable. Our experimental work is mostly done in the wheat-powdery mildew pathosystem as a model for the interaction of wheat with an obligate biotrophic pathogen. On the host side, we are studying and modifying R genes encoding nucleotide-binding, leucine-rich repeat immune receptors (NLRs) with the goal to engineer protein variants which have increased resistance spectra towards pathogen isolates. In a first step towards achieving this goal, we are identifying novel R genes at the molecular level based on recently developed genomic tools. The encoded NLRs are then functionally analyzed by studying their interactions with the cognate avirulence factors in order to determine the molecular basis of specificity. Using this approach we have identified NLRs that recognize additional haplotypes of fungal Avr genes in transient expression assays. Furthermore, a strategy based on resistance gene combination (pyramidization) using over-expressed, transgenic forms of R genes is tested. On the pathogen side, we are identifying avirulence gene products in the pathogen that are recognized by NLR proteins in the host. Transient expression experiments are performed in the heterologous plant species *Nicotiana benthamiana* to functionally study the molecular determinants of specificity of interactions in host as well as pathogen genes. The goal of this work is to (i) improve our molecular understanding of the molecular interactions of host and pathogen molecules and (ii) in the development of pathogen-informed strategies to achieve broader recognition spectra of NLR proteins. Ultimately, resistance breeding based on the molecular understanding of the interactions should result in a more sustainable use of the precious R gene resources.



Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Quantitative trait loci for heat tolerance in wheat

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Key message: Different heat tolerance QTL were found for floret fertility and grain size effects. The *Sr2* rust resistance gene may act as a heat susceptibility gene for grain size.

In various parts of the world such as southern Australia and around the Mediterranean, wheat crops are damaged by short heat waves (>30°C for several days) that occur during the sensitive reproductive stages of development. Effects (grain number, size, or quality) depend on the time and intensity of exposure, which are complications that hamper efforts to directly select for tolerance in the field. Therefore the aim of our research is to identify molecular markers and knowledge that can be used to assist in the selection of new heat tolerant varieties. Heat treatments (3 d, 37/27°C day/night) were applied using a growth chamber, either at around meiosis or at 10 days after anthesis, to target responses of floret fertility and final grain size, respectively. In a Waagan×Drysdale doubled-haploid population, separate major QTL were detected for these responses (on 2BS for floret fertility and 3BS for grain filling). At the 2BS locus, floret fertility tolerance from Waagan showed dominant sporophytic inheritance. Susceptibility in Drysdale was accompanied by an absence of starch in mature pollen, but pollen grains had the normal number of nuclei (three), indicating that meiosis had proceeded. Crossing experiments suggested female gametogenesis was not affected. We defined the most heat susceptible stage of microsporogenesis in Drysdale florets to be from meiosis to the young microspore stage. At the 3BS locus, grain filling heat susceptibility from Drysdale was associated with accelerated chlorophyll loss (Shirdelmoghanloo et al. 2016a) and the presence of the *Sr2* stem rust resistance gene. Grain size and chlorophyll heat responses were also found to be correlated across a sample of 36 wheat genotypes (Shirdelmoghanloo et al. 2016b). An examination of grain filling dynamics showed that our treatment reduced final grain size by prematurely terminating grain filling (as a delayed response), not by affecting initial grain filling rate (Shirdelmoghanloo et al. 2016c). We are proceeding with haplotype analysis and trialling of near-isogenic lines to establish the potential significance of these QTL as tools in wheat breeding.

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Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

A genetic mechanism for adaptation of wheat to variable growth temperatures

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Key message: Through identifying and understanding genes which are regulated by ambient temperature we aim to provide a mechanism to adapt wheat plants to be more robust to variable temperatures.

Absolute temperature and seasonal variations in temperature are essential signals that enable wheat to develop according to a predictable developmental plan, and are important determinants of yield. However, with increasingly variable and unpredictable temperatures coupled with more extreme absolute temperatures, wheat growers face significant challenges to maintain current global yield. To address this challenge we conducted a screen on more than 100 genetically diverse wheat cultivars to identify differences in growth under two, realistic ambient growth temperatures. This screen identified a range of approximately 25 days for flowering time existed in ambient temperature responses between these hexaploid wheat cultivars. Detailed physiological and genetic analysis of cultivars that display extreme responses has uncovered key genes that facilitate adaptation and increased robustness of wheat to changes in ambient temperature, a number of these genes are involved in the wheat vernalization response. Additionally, we have identified that the double ridge stage in the wheat ear development is a critical regulatory point for temperature adaptation, and show that altered growth temperatures at this developmental stage modify architecture and fertility of the mature inflorescence. The results presented here provide novel understanding about the influence of ambient growth temperature across the life-cycle of a wheat plant and reveal information about the molecular mechanism that underpins adaptation of wheat to variable growth temperatures. By understanding the ambient temperature aspect of vernalization regulation and the role of temperature at defined stages of inflorescence development, we propose a strategy by which wheat yield could be stabilised under a changing climate.



Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Functional genomics to improve wheat disease resistance

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Key message: One promising approach to accelerate yield increases in wheat is the application of next generation sequencing technologies to facilitate the discovery of natural and induced genetic variation underlying economically-important traits.

One promising approach to accelerate yield increases in wheat is the application of next generation sequencing technologies to facilitate the discovery of natural and induced genetic variation underlying economically-important traits, such as disease resistance. The goal of the current project is to advance fungal resistance of wheat by isolation of novel resistant pathways induced by EMS mutagenization. A large *Triticum turgidum* cv 'Kronos' TILLING population was phenotyped under natural pressure of yellow rust (*Puccinia striiformis* f. sp. *tritici*) (Pst) in the field in the USA. As the EMS-induced mutation repertoire in the genic space of this population has already been described using sequencing data obtained from exome-capture experiments (Krasileva et al. 2017), it is advantageous to use it in forward genetics screens. Subsequent phenotyping of 'Kronos' mutant lines derived from that TILLING population in the field and chamber experiments in the UK with UK natural Pst populations yielded several 'Kronos' mutant lines with increased adult resistance and complete seedling and adult resistance to Pst. These mutant lines were backcrossed to 'Kronos' wt to develop F₂ populations segregating for Pst resistance for further mapping and cloning of causative mutations. Our cloning strategy combines modern sequencing approaches, exome-capture, and mapping-by-sequencing with the recently produced in-house *T. aestivum* wheat assembly TGACv1 mapped to a POPSEQ genetic map. Using these approaches, we were able to identify the chromosomal region linked to the causative mutation in our first segregating population containing partial adult rust resistance. F₃ families of that population were phenotyped in the field for Pst resistance and homozygous resistant and susceptible F₂ bulks were produced. The gene space of these bulks was isolated by exome-capture and sequenced. Using EMS mutations as polymorphic markers between 'Kronos' mutant and 'Kronos' wt we mapped the causative mutation on chromosome 1BL. We are currently screening the F₂ population with KASP markers to reduce the genetic interval containing the causative mutation. We are investigating annotated genes in the TGACv1 wheat assembly to identify putative genes of interest associated with disease resistance that were mutated in the resistant Kronos mutant line. F₃ families derived from several additional populations are currently grown in UK field and will be phenotyped during the Pst season in 2017. The same approach of causative mutation isolation will be applied to isolate the causative mutation confirming Pst resistance in these additional 'Kronos' mutant lines.

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Topic: Applying Novel Tools to Practical Wheat Improvement

Potential of molecular markers for hybrid wheat breeding

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Key message: We describe three genomic selection approaches facilitating largely hybrid wheat breeding in the future.

Hybrid breeding is currently one of the major topics in the wheat community. Recent large experimental studies have confirmed a high commercial heterosis for hybrid wheat. Across all quality classes, a commercial heterosis larger than 1 ton per hectare was observed. Furthermore, yield stability of hybrids was found to be higher than that of line varieties. However, many questions regarding the design and optimization of breeding schemes are yet to be discussed. Here, we present three approaches based on molecular markers that allow improving substantially the efficiency of hybrid wheat breeding. First, good male lines are a major bottle neck for efficient hybrid seed production. High anther extrusion is one necessary prerequisite for a good male line. In a genome-wide association mapping approach, we found the trait to be quantitatively inherited, but with a strong effect of *Rht-D1*. Consequently, a weighted genomic selection method achieved high prediction accuracies for anther extrusion (Boeven et al. 2016). Second, the expensive hybrid seed production in wheat hinders the development of heterotic groups based on phenotypic evaluation of general combining ability. We therefore developed an algorithm for a genomics-based prediction of heterotic groups taking into account minimal group sizes in order to warrant long-term selection gain (Zhao et al. 2015). Finally, we investigated the potential to integrate genomic selection in routine elite breeding schemes. From these simulations, a breeding scheme based on genomic selection followed by one-stage phenotypic selection on hybrid performance was found to maximize the annual selection gain (Longin et al. 2015).

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Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Identification of new allelic variants for freezing tolerance in winter wheat *WRKY71* and *ZCCT2* genes

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Key message: Induced mutation in *WRKY71* gene significantly decreased mutant plant survival at low-negative temperature in comparison to wild type in winter wheat.

Common wheat (*Triticum aestivum* L.) is a staple crop species with a worldwide cultivation. However, climate change and unpredictable temperature fluctuations cause a considerable damage in winter wheat, especially at northern latitudes. Many genes responsible for cold-regulation are being activated during cold acclimation which subsequently increases plant's tolerance to freezing. Better understanding of the mechanisms involved in cold acclimation may lead to germplasm with better overwintering capacities. The aim of this work was to study the effect of induced mutations in genes associated with cold acclimation. Mutagenized populations of two winter wheat cultivars (Kena DS and Gaja DS) were developed using ethyl methane sulfonate treatment. Point-mutations were subsequently detected by high resolution melting (HRM) analysis with gene-specific primers. DNA sequencing was used to confirm the induced mutations. Two genes, vernalization gene *ZCCT2* and transcription factor gene *WRKY71*, known to take part in cold acclimation control, were chosen for HRM screening. A total of 229.8 kb and 235.5 kb of genomic DNA were screened for *ZCCT2* and *WRKY71* gene, respectively. Five novel alleles of *ZCCT2* gene in exon 1 were identified, of which four were missense (G137A; G145A; G137A; G245A) and one silent mutation (C160T). Further 7 novel alleles were detected in exon 1 for *WRKY71*, of which two were missense (G494A; C436T) and five silent mutations (C381T; G426A; C444T; G456A; G471A). Mutant plants, with new allelic variants, were propagated to generation M₃ in order to validate mutation phenotypes. Artificial freezing test revealed that mutations in *ZCCT2* gene had no effect on freezing tolerance. However missense mutation (G494A) in *WRKY71* gene significantly ($p < 0.05$) decreased mutant plant survival at low-negative temperature in comparison to wild type genotype having LT50 (temperature with 50% survival) values of -10.74°C and -12.02°C, respectively.



Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Genetic and epigenetic controls of heat tolerance in wheat (*Triticum aestivum* L.)

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Wheat production is estimated to fall by 6% for one centigrade temperature increase. To adapt wheat production to the global climate change, we undertake genetic and epigenetic studies on heat tolerance in wheat (*Triticum aestivum* L.) since 1990s: GWAS is used to identify genomic regions associated with heat tolerance. A total of 1286 world-wide collections of wheat lines, including registered cultivars, landraces, synthesized wheat, mutants, introgression lines and other genetic resources, are evaluated for their heat tolerance performance based on yield loss under high temperature conditions in Xinjiang, Ningxia, Hebei and Shanxi provinces of China. By using 90K wheat SNP array, we are genotyping \approx 500 wheat germplasms with heat tolerance difference, and several genomic regions are found to be closely associated with heat tolerance. In addition, by using RIL population combined with QTL analysis, we identified one QTL for TKW (*QTKw.cau-5D*) located on chromosome 5DL using wheat mutant line Fu4185, which was detected under high temperature conditions (Cheng et al. 2015). To unravel the underlying molecular mechanisms responding to heat stress, we perform expression response analysis to the heat, drought or combined stresses, and identify several regulatory pathways involved in stress responses (Liu et al. 2015). We also established heat responsive miRNA long-non coding RNA profiling and identified 12 and 77 candidate miRNAs and long-non coding RNA involved in heat tolerance or sensitivity (Xin et al. 2010, 2011). We observed that the TamiR159 overexpression rice lines were more sensitive to heat stress relative to the wild type, indicating that the down-regulation of TamiR159 in wheat after heat stress might participate in a heat stress-related signaling pathway, in turn contributing to heat stress tolerance (Wang et al. 2012). In addition, we found that *GCN5*, a histone acetyltransferase, plays a key role in the preservation of thermotolerance via versatile regulation using *Arabidopsis* as a model system, and expression of the wheat *TaGCN5* gene re-establishes heat tolerance in *Arabidopsis gcn5* mutant plants (Hu et al. 2015). In wheat breeding program, we registered four wheat cultivars with enhanced heat tolerance. Among which, Nongda 211 contributes to nearly half planting area of Beijing wheat belt. Whereas Nongda 5181, an excellent representative with enhanced heat tolerance, exhibited more than 10% increase of wheat yield under normal and hot environments, and developed a famous reputation in the Northern Winter Wheat Region.

Acknowledgements

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Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Unraveling the mechanisms of stem rust resistance conferred by the *Sr35* gene against the *Puccinia graminis* pathogen

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Key message: A fungal effector identified by the next-generation sequencing of the EMS mutants and natural isolates of *Puccinia graminis* is recognized by the *Sr35* gene and triggers resistance response

The identification of genetic factors in wheat and its pathogens that define the outcome of infection is critical for developing varieties with long lasting resistance. We have used the recently cloned *Sr35* gene (Saintenac et al. 2013) as entry point to unravel the molecular bases of resistance conferred by this gene to wheat stem rust caused by *Puccinia graminis* f. sp. *tritici* (Pgt). One of its recently emerged broadly virulent races, Ug99, posed a substantial threat to global wheat production. Microscopic analyses revealed that in the presence of *Sr35*, the avirulent Pgt races undergo haustoria development inhibition associated with the single cell death. We have performed the whole genome comparative analyses of multiple *Sr35*-virulent and -avirulent Pgt strains including natural field isolates and chemically mutagenized strains that acquired virulence to the *Sr35* gene. The *Sr35*-virulent strains were developed by treating the spores of *Sr35*-avirulent RKQQC Pgt race with EMS. Except for a *Sr35*-carrying wheat line, all mutagenized strains showed similar virulence response on the panel of lines carrying different stem rust resistance genes. This result suggested that the *Sr35* avirulence phenotype in the RKQQC race is likely controlled by a single gene. We have used a forward genetic screen to identify an avirulence factor that is detected by *Sr35*. Whole genome sequencing of fifteen *Sr35*-virulent Pgt mutants detected strong effect mutations in a single candidate gene. This effector candidate gene is constitutively expressed in the infected wheat leaves and encodes a protein with a predicted N-terminal secretion signal. *In planta* experiments showed that the candidate gene can trigger a *Sr35*-dependent hypersensitive reaction. The experiments with the fluorescent protein fusions suggest that the products of both effector candidate gene and *Sr35* likely interact. The candidate effector's sequence diversity explained most of the virulence of Pgt isolates on the *Sr35*+ and *Sr35*- wheat lines. Based on our results we conclude that the identified fungal candidate effector is *AvrSr35* that is responsible for triggering the defense response by the *Sr35* gene.

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Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

***Stb16q*-mediated resistance against *Zymoseptoria tritici* is conferred by a new class of R gene**

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Key message: Identification of a new R gene involved in broad-spectrum resistance against the devastating wheat disease *Septoria tritici* blotch.

Zymoseptoria tritici, the causal agent of *Septoria tritici* blotch (STB) represents a global threat to wheat production. The fungus has acquired widespread fungicide resistance thereby limiting the efficacy of chemicals for disease management and requiring the development of wheat cultivars with broad-spectrum and durable resistance. The synthetic wheat lines M3 and TA4152-19 have been shown to be immune to all *Z. tritici* isolates tested and represent the only sources of resistance not yet overcome by virulent strains. QTL mapping led to the identification of a major gene in TA4152-19 designated *Stb16q* that controls necrotic leaf area, latent period and region-bearing pycnidia (Tabib Ghaffary et al. 2012). To determine the mechanism of resistance, we initiated the map-based cloning of *Stb16q* using a large F₂ population derived from the cross between TA4152-19 (*Stb16q*) and the susceptible wheat line ND495. BAC-based physical mapping and sequencing revealed a cluster of receptor-like kinase genes (RLKs) near *Stb16q*, but only one of these genes, *Rlk6*, was present in the genetically defined interval. To functionally validate this gene, we developed a population of mutants using EMS and identified one M₂ family where a point mutation in *Rlk6* co-segregated with STB susceptibility. In addition, introduction of the *Rlk6* genomic sequence into the susceptible cultivar Courtot by stable transformation conferred resistance to *Z. tritici* isolates. Re-sequencing this gene from 184 wheat accessions identified diagnostic markers to study the distribution of the gene among different wheat populations. Here, we report a new class of R gene involved in plant pathogen resistance and provides a new resource for broad-spectrum resistance against STB in wheat.

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Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Detection and stacking of new genes for tan spot (yellow spot) resistance results in genetic gain

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Key message: Two major quantitative trait loci for tan spot resistance were detected on chromosomes 2A and 1A and were successfully combined along with *tsn1* into fixed lines resulting in genetic gain.

Although good progress has been made internationally to understand tan spot (syn. yellow spot and yellow leaf spot), caused by *Pyrenophora tritici repentis*, resistance in wheat, relatively few resistance genes have been identified and mapped in the Australian germplasm. Only one (*tsn1* on chromosome 5B, which confers insensitivity to the fungal effector ToxA) is in general and known use in Australian breeding programs. Under a current national project a major effort was made to improve the understanding of genetics of tan spot resistance in present and future donors, identify novel quantitative trait loci for resistance and develop a series of fixed lines, each carrying tan spot resistance genes from 2 or 3 resistance sources in elite Australian backgrounds that can be used as parents in resistance breeding. Two doubled haploid mapping populations Calingiri/Wyalkatchem (247 DH lines) fixed for the ToxA-insensitivity allele *tsn1* and IGW2574/Annuello (97 DH lines) fixed for the ToxA-sensitivity allele *Tsn1* were used in this study. The populations were screened for tan spot resistance at various growth stages, environments and national sites. Phenotypic frequency distributions for tan spot severity traits were continuous, which could indicate that resistance was conditioned by several genes with minor effects. Nevertheless, major quantitative trait loci (QTL) were detected on chromosomes 2A and 1A. Each of these QTL had effects at various growth stages and in experiments conducted at various locations in Australia. The QTL on 2A explained up to 29.2% of the genotypic variation in the Calingiri/Wyalkatchem population (with the resistance allele contributed by Wyalkatchem). The QTL on 1A explained up to 28.1% of the genotypic variation in the IGW2574/Annuello population (with the resistance contributed by Annuello). These resistance loci were stacked along with *tsn1* using single seed descent and marker assisted selection. Thirty two F₅ lines were developed which are triple homozygotes at the three tan spot resistance loci and are also fixed for the *vrn1* locus. Sixteen of these F₅ lines were screened for tan spot resistance at various growth stages and environments in South Perth, Western Australia. Lines expressed significantly higher resistance than parents and grandparents at both seedling and adult plant stages that was effective in various environments. These lines are important resources that can be used by breeders for rapid development of varieties with high levels of resistance.



Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Combining QTL-based markers and genomewide prediction with phenotypic screening for efficient selection of Fusarium head blight resistance in wheat

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Key message: We show that selection using a combination of known QTL, genomewide prediction, and phenotypic screening can efficiently select for Fusarium head blight resistance in wheat.

Resistance to Fusarium head blight in wheat is quantitatively inherited with no documented race specificity of *Fusarium* species isolates. Dozens of QTL mapping experiments have identified a few major QTL but most of the phenotypic variance is accounted for by minor QTL, each explaining less than 5% of the variance. Field-based screening efforts can be effective, but are time and resource consuming, requiring inoculum application at critical growth stages and mist irrigation to provide an environment conducive to disease development. We routinely track two QTL, *Fhb1* and *Fhb5* (chromosome 5AS), in our parents and pre-yield trial lines, and screen more than 3000 lines in two field-based nurseries, including about 2000 pre-yield trial lines. We are experimenting with genomewide predictions to complement our QTL-based marker and phenotypic screening, with a desired outcome of replacing the field-based screening of 2000 pre-yield trial lines with a combination of QTL and markers. To assess prediction model accuracy, a panel of 383 F₇-derived lines from the UMN breeding program were phenotyped in five or more environments between 2009 and 2013, and genotyped at high density for model training and prediction. Accuracies of 0.30-0.54 were achieved when using genomewide selection alone and 0.45-0.65 when used in combination with 6-11 QTL identified through association mapping on the panel. Current investigations are utilizing an optimized subset of approximately 500 of the 2000 pre-yield trial lines to predict FHB reaction of the remaining 1500 lines, saving considerable phenotyping effort at this early stage of FHB evaluation.



Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Silencing of a lipase maturation factor 2-like gene by wheat-mediated RNAi reduces the survivability and reproductive capacity of grain aphid (*Sitobion avenae*)

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Key message: Lmf2-like genes can be used as a target gene for the control of grain aphids and show that feeding of aphids with wheat expressing lmf2-like RNAi resulted in significant reductions in survival, reproduction, and molting of grain aphid.

Lipase maturation factor (LMF) family proteins are thought to be required for the maturation and transport of active lipoprotein lipases through the secretory pathway. However, the specific roles of LMF2 remain unknown. In this study, a grain aphid lmf2-like gene fragment was cloned; it was highly similar in sequence to a homologous gene in pea aphid. An RNAi vector was constructed with this fragment and used for wheat transformation. The expression of the lmf2-like gene in aphid, as well as the growth and reproduction of the aphids, was analyzed after feeding on the transgenic wheat. There were no significant differences in the expression of the lmf2-like gene at the different growth stages of grain aphid. The expression of the lmf2-like gene was significantly reduced by 27.6% on the fifth day, and 57.6% on the tenth day after feeding. The total number of aphids produced on the transgenic plants was less than the number produced on control plants, and the difference became significant or highly significant after two weeks; the extent of molting was also reduced in the aphids reared on the transgenic plants. Our findings indicate that lmf2-like genes can be used as a target gene for the control of grain aphids and show that feeding of aphids with wheat expressing lmf2-like RNAi resulted in significant reductions in survival, reproduction, and molting of grain aphid.



Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Mapping a complementary gene to *LrSV2* for adult plant specific leaf rust resistance identified in the durable resistant variety Sinvalocho MA

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Key message: A pair of complementary genes for adult plant race-specific leaf rust resistance were identified and mapped in the durable resistant traditional variety Sinvalocho MA.

Resistance genes are frequently studied in a single contrasting cross. However, in order to uncover genetic interactions between unlinked genes, the analysis of different crosses becomes necessary. *LrSV2* was first detected in the cross of the traditional Argentinian variety Sinvalocho MA and the experimental line Gama 6 and mapped to subdistal chromosome 3BS (Diéguez et al. 2014). Later, the analysis of the F₂ population from the cross of R46 (Recombinant Inbred Line derived from Sinvalocho carrying the *LrSV2* gene) and the commercial variety Relmo Sirirí allowed the detection of the unlinked complementary gene *LrcSV2*. The two genes act complementarily, meaning that the presence of at least one dominant allele of both is necessary to express the *LrSV2* race-specific adult plant leaf rust resistance. This complementary gene was located on chromosome 4BL, distal to the previously identified adult plant resistant gene *Lr12* (Singh & Bowden 2011). Using 100 RILs (recombinant inbred lines) from the cross Sinvalocho MA × Purple Straw, a genetic map with 25 molecular markers was constructed; including two microsatellites developed from wheat 4BL sequence contigs identified using the ESTs (expressed sequence tags) in the syntenic *Brachypodium distachyon* chromosomal region. *LrcSV2* was mapped within a 1cM interval, with one cosegregating microsatellite. The analysis of 466 F₂ individuals from the same cross allowed the identification of nine recombinants between the closest flanking markers which will be used to narrow down the target interval by developing new markers in the region that will be genotyped in a greater number of F₂ for the construction of a fine map. According to our experience with the project of positional cloning of *LrSV2*, the analysis of 3404 F₂ individuals allowed the definition of a 0.03 cM interval that spans about 110 kb in a wheat subtelomeric bin like this, a physical distance which is suitable to find few overlapping BAC (bacterial artificial chromosomes) clones spanning the region. A Sinvalocho MA non pooled genomic BAC library was already developed through collaboration with the CNRGV-INRA (Toulouse, France).

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Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

The molecular basis of the durable disease resistance conferred by *Lr34*

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Key message: We will discuss the latest insights into the molecular basis of the durable and partial disease resistance that is conferred by the *Lr34* gene coding for an ABC transporter.

Plant diseases, particularly those conferred by pathogenic fungi, are a serious problem for wheat production. On average, more than 10% of the globally produced wheat is lost to diseases each year. The most sustainable strategy to prevent crop losses caused by pathogens is to explore the plant's natural resistance mechanisms in breeding. The wheat gene *Lr34* (=Yr18/Sr57/Pm38) confers durable and partial field resistance against the fungal rust diseases and powdery mildew. Reports about *Lr34*'s use in breeding and agriculture data back to the beginning of the 20th century and despite its extensive use in breeding and agriculture for more than a century, no increase in pathogen virulence towards *Lr34* has been reported. The *Lr34*-mediated multi-pathogen resistance is conferred by a single gene encoding an ATP-binding cassette (ABC) transporter. The resistance-conferring *Lr34res* allele evolved from an ancestral gene version as a result of two spontaneous gain-of-function mutations that occurred only after wheat domestication. An *Lr34*-like disease resistance has not been described in other globally important cereals, which is in agreement with the very recent emergence of the *Lr34res* allele, long after wheat shared its last common ancestor with other related cereal species. Nevertheless, *Lr34* is functionally transferrable as a transgene into all major cereals, including maize, rice and barley, where the gene confers partial resistance against various biotrophic and hemi-biotrophic pathogens. In rice for example, expression of *Lr34* resulted in partial resistance against rice blast (*Magnaporthe oryzae*), which is the most devastating rice disease worldwide. Besides an important cereal crop, rice is also a model organism for cereal genetics and genomics. The phenotypic similarity of *Lr34* in wheat and rice in combination with the advancements in rice genomics make the *Lr34*-expressing rice lines an ideal tool to elucidate the molecular function of durable, broad-spectrum disease resistance. We used RNA sequencing to study the transcriptional reprogramming induced by *Lr34* and we defined a '*Lr34*-responsive core gene set' in rice. Interestingly, the changes in expression pattern in wheat and rice were very similar and resulted in a constitutive induction of a multiple stress response. Here, we will discuss how the whole transcriptome analysis and physiological experiments in rice and wheat allowed us to get a deeper insight into the molecular function of the durable, broad-spectrum disease resistance conferred by *Lr34*.



P 314 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Pore-forming toxin-like gene provides resistance against *Fusarium* head blight in wheat

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Key message: Pore-forming toxin-like gene in the *Fhb1* QTL interval confers resistance against *Fusarium* head blight in wheat. The gene was functionally validated using TILLING, RNAi-based gene silencing and transgenic over-expression.

Fusarium head blight is a serious disease of wheat, affecting not only yield but also quality of the affected grain due to the associated mycotoxins. *Fhb1* is a major QTL conferring type 2 resistance against the causal fungus, *Fusarium graminearum*, in wheat. We performed map-based cloning of *Fhb1* locus from Chinese wheat cultivar Sumai 3. Using a Sumai 3 BAC library with $\approx 3\times$ genome coverage, candidate genes were identified in the *Fhb1* interval. A *pore-forming toxin-like (PFT)* gene at *Fhb1* locus was found to confer FHB resistance using mutation analysis, gene silencing and transgenic overexpression. PFT is predicted to encode a chimeric lectin with two agglutinin domains and an ETX/MTX2 toxin domain. Transgenic plants under native-promoter control were also generated, and are currently being evaluated for their disease responses. The results will be presented in the meeting, and details have been published (Rawat et al. 2016).

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P 316 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Map-based cloning of *Fhb1* revealed unique mutation of a well-conserved gene resulting in resistance to wheat Fusarium head blight

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Key message: *Fhb1* gene for type II resistance to Fusarium head blight was fine mapped and cloned. It was shown that this gene is conserved among plants, but a unique mutation of the 3BS homolog causes shift of its start codon, consequently, the *FHB1* resistance.

Head or ear blight caused by *Fusarium* spp. fungi can devastate staple cereal crops, particularly wheat. Deployment of resistant cultivars is currently the best control measure, but lack of understanding of resistance mechanisms has greatly hindered the breeding efforts. In the FHB-resistant germplasm Wangshuibai, we previously identified a major QTL on chromosome 3BS conferring resistance to spread within spike, the type II resistance. To clone this gene, NIL isogenic lines of this QTL and the Sumai3 *FHB1* QTL, developed using FHB-susceptible lines Mianyang 99-323 and PH691, were used in secondary segregation population construction. Using recombinants identified in these populations, both QTL were fine mapped. In the constructed BAC physical map, they were confined to a common 26.8 kb interval containing only three ORFs. Sequence-based analysis demonstrated that Wangshuibai and Sumai 3 contain an identical FHB resistance gene in the target interval. The *FHB1* candidate was determined based on sequence comparison and transcriptional profiling analysis, and was confirmed through haplotyping and transgenic studies. This gene is conserved among plants, but a unique mutation of the 3BS homolog causes shift of its start codon, consequently, results in the function in FHB resistance.

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P 318 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

TaHRC is the key gene underlying *Fhb1* resistance to Fusarium head blight in wheat

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Key words: *Fhb1*, Fusarium head blight, haplotype analysis, TaHRC

Fusarium head blight (FHB), mainly caused by *Fusarium graminearum*, is a devastating wheat disease worldwide. FHB not only significantly reduces grain yield, but also affects grain quality due to Fusarium damaged kernels and mycotoxin contamination. Although FHB resistance is controlled by quantitative trait loci, *Fhb1* on the chromosome 3BS shows a consistent major effect on reducing FHB symptom spread within a spike in different genetic backgrounds. Recently, several *Fhb1* candidate genes have been cloned, including *GDSL lipase* (*GDSL*, Schweiger et al. 2016), *pore forming toxin-like protein* (*PFT*, Rawat et al. 2016), and *His-rich Ca-binding protein* (*TaHRC*, Su et al. 2006). Among these genes, *GDSL* and *PFT* are characterized as presence (resistance allele) and absence (susceptible allele) of the genes, whereas the resistance allele of *TaHRC* has a ~752 bp deletion, and the susceptible allele does not have the deletion. To determine most possible candidate for *Fhb1*, we evaluated an association-mapping panel for FHB resistance, and found that FHB resistant Ning 7840 haplotype contains all resistance alleles at the three loci, whereas FHB susceptible Dahongpao haplotype contains only *GDSL* and *PFT* loci. Sequence analysis of the Dahongpao and Ning 7840 haplotypes found that the three genes share identical sequences between the two haplotypes except that Dahongpao haplotype has the 752 bp deletion in *TaHRC*. RNA interference and gene editing experiments confirmed that loss of function of *TaHRC* significantly enhances FHB resistance. Screening 1165 wheat landraces and cultivars from 53 countries for the three genes found that *GDSL* and *PFT* are present together (70 accessions) or independently (61 accessions) in 10% accessions from many countries where *Fhb1* has never been detected. *PFT* gene has also been identified in durum wheat. In Chinese landraces, *GDSL* and *PFT* were detected in 500 landraces collected nationwide, and the deletion mutation in *TaHRC*, however, is present only in the accessions from southern China. Haplotype analysis and geographic distribution data of candidate gene alleles reveal that *TaHRC* is the key candidate for *Fhb1* and the *Fhb1* resistance allele was evolved from a single deletion in the Dahongpao haplotype. The Dahongpao haplotype occurred in landraces worldwide, whereas Ning7840 haplotype with *Fhb1* is present only in southern China and Japan, suggesting *Fhb1* is a relatively new gene that was evolved in these regions.

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P 320 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Functional identification of the wheat gene enhancing mycotoxin detoxification of the major Fusarium resistance QTL *Fhb1*

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Key message: The Fusarium resistance QTL *Fhb1* confers resistance to the mycotoxin deoxynivalenol. Fine-mapping resulted in an 860kb region harboring 28 candidates. Forward and reverse genetics approaches are conducted for gene identification.

Fusarium head blight (FHB) is one of most destructive diseases of wheat. The contamination with mycotoxins such as deoxynivalenol (DON) constitutes a serious threat to food safety. The development of resistant varieties is the most effective approach to control the disease. The best validated resistance QTL is *Fhb1*, conferring resistance to fungal spread and to DON by detoxification into the less toxic DON-3-O-glucoside (Lemmens et al. 2005). Recently a pore-forming toxin-like gene was identified as the gene behind spreading resistance but it does not confer resistance against DON suggesting that this trait is under different genetic control in the same region (Rawat et al. 2016). In order to identify the DON detoxifying gene we have established the genomic sequence of the resistant line CM-82036 (*Fhb1* carrier) and fine-mapped the QTL interval to 860 kb comprising 28 genes. Yet, no DON-glucosyltransferase was annotated. We added expression data to the contig to further substantiate gene predictions thereon and to identify promising candidate genes. For functional validation a TILLING population (EMS) of CM-82036 comprising 6000 lines was generated. Mutant lines were identified for several candidates and phenotyped for FHB and DON susceptibility. In a forward genetics approach 1500 mutant lines were infiltrated with DON solution. Toxin application to flowering heads induced typical straw-like color in DON-sensitive lines whereas DON-resistant lines did not show any bleaching symptoms. Twenty-five mutants displayed DON-induced bleaching, five of these developed similar symptom severity as the susceptible control lines. These DON-sensitive mutants are being characterized for all *Fhb1* candidate genes to identify common mutations in the causal gene. In addition, DON and DON-3-O-glucoside contents are determined to compare conversion rates of mutants differing in *Fhb1*. Knowledge of the DON resistance gene underlying *Fhb1* is of major interest to biologists and moreover has potential benefit for plant breeding.

Acknowledgement

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Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

International collaboration to improve wheat quality for processing and health

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Key message: The Expert Working Group on 'Improving Wheat Quality for Processing and Health' of the Wheat Initiative aims to maintain/improve the quality of high-yielding wheat under varying environmental conditions.

The Expert Working Group (EWG) on Improving Wheat Quality for Processing and Health of the Wheat Initiative, established in 2015, aimed to maintain/improve the quality of high-yielding wheat under varying environmental conditions. This EWG focuses on wheat quality in the broad sense, including grain compositional factors (proteins, allergens, carbohydrates), nutritional quality, grain processing, food safety, genetic resources and gene nomenclature as shown in Figure 1. The EWG also promotes the sharing of genetic resources and the standardisation of nomenclature of genes related to grain quality. The first meeting of the EWG was held in Paris in 2016, with 31 researchers from 18 countries. We are working on the following globally important topics: (i) standardising methods to determine gluten protein composition, while unifying the nomenclature to define allelic diversity of gluten proteins, and improve the understanding of the role of gluten proteins on dough processing and end-product properties; (ii) germplasm screening for the identification of sources of variation for various quality component traits; (iii) a deep understanding of the inheritance and genetic factors controlling the bioavailability of grain bioactive compounds, including micronutrients and dietary fibre, to improve the nutritional and health value of wheat and cereal-based foods; (iv) a deep understanding of the nature and content of proteins and other factors, such as fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) of wheat showing negative effects on health and toxic reactions and developing low-allergen and low FODMAP wheat suitable for patients suffering various wheat related food disorders; (v) understanding the effects of food manufacturing processes on the digestibility of wheat proteins, bio-availability of nutrients, and the interaction with gut micro-organisms; (vi) fine-tuning gluten, starch properties and grain hardness according to specific (and diverse) end-uses by understanding genotype × environment × management interactions; (vii) reducing mycotoxins and toxic minerals in wheat and wheat products; (viii) development of low cost biomarkers for the above determinants of wheat quality and safety.

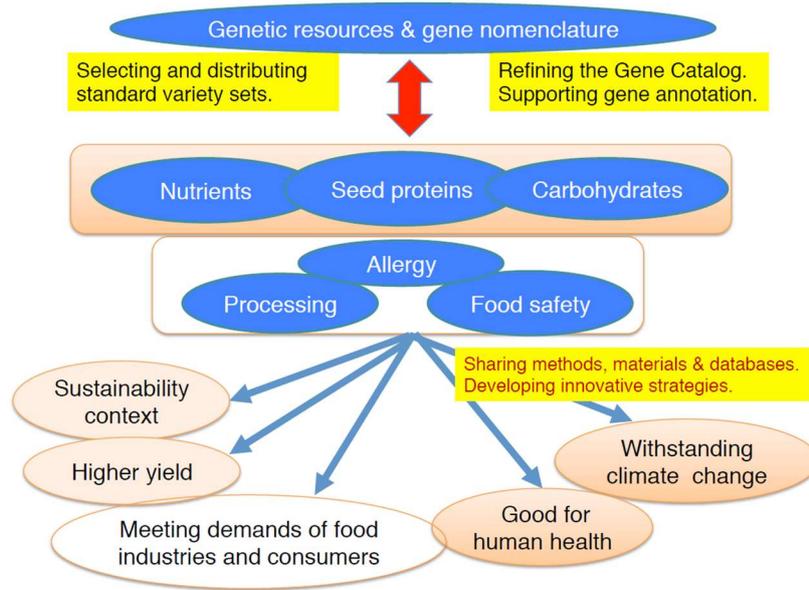


Figure 1: Outline of the Expert Working Group on 'Improving Wheat Quality for Processing and Health' of the Wheat Initiative.



Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Improving nitrogen use efficiency in wheat by genome wide and candidate genes targeted association studies

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Key words: Nitrogen use efficiency, wheat, genome wide association studies

Breeding new varieties adapted to limited nitrogen application and with an improved Nitrogen Use Efficiency (NUE) is a crucial challenge in a context of limitation of inputs, especially fertilizers, for ecological and financial issues. The main goals of the study are to rank genotypes according to their NUE and identify traits and markers associated to a better tolerance to low N levels. It will help breeders selecting and developing varieties able to answer farmers' demand in the future. BreedWheat project has develop a field trials network during 3 years with 12 experiments conducted by French breeding companies using a panel of 220 European elite lines grown at two contrasted nitrogen applications. A classification based on an environmental characterization allowed to determine nitrogen stress duration and intensity in each experiment. Phenotypic measurements involved yield, its components and nitrogen contents at harvest allowing to compute NUE, N uptake, utilization and remobilization. Using this phenotypic dataset, classification methods were applied to identify contrasted variety behaviors regarding nitrogen stresses. Using the TaBW420k Axiom array develop within the BreedWheat project, a Genome Wide Association Study (GWAS) was carried out using 250k polymorphic markers. 28 000 marker traits associations were detected representing 1649 LD blocks. Among them, five were selected for SNP densification and confidence interval reduction. Candidate genes produced in the project using RNASeq data will also be analyzed by association studies using specific SNPs develop in their sequences and will also be mapped into the genome and linked to previous results by colocalization. *In fine*, we developed a set of markers associated with traits of interest and also identified varieties with contrasted behaviors regarding different types of nitrogen stresses. These tools will help breeders perform a selection of varieties with a better NUE in the future.

Acknowledgements

All the BreedWheat partners (ANR-10-BTBR-0003) involved in field trials and Etienne Paux and co-workers for genotyping information on the 220 varieties panels using TaBW420k Axiom Array.



Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Getting good genes and high-throughput phenotyping for nutritious wheat breeding

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Key message: High-throughput phenotyping and genotyping techniques are being implemented to enhance breeding efficiency and selection accuracy for grain zinc concentration in wheat. Several promising genomic regions were identified for enhanced zinc accumulation.

Wheat is not only a source of energy for many wheat based consumers, but is also a major source of protein and essential micronutrients such as zinc (Zn) and iron (Fe). Developing wheat varieties with enhanced levels of micronutrients in adapted elite genetic backgrounds offers a cost-effective and sustainable solution to the rural households. The rich genetic diversity for Zn and Fe concentrations in different wild species and landraces provides novel alleles for genetic enhancement of Zn and Fe in wheat. Significant progress has been made through targeted crossing for the incorporation of several novel alleles for grain Zn and Fe in the elite germplasm (Velu et al. 2014). This breeding effort led to the release of biofortified wheat varieties in South Asia, 'Zinc-Shakti (Chitra)' and 'Zincol-2016' which were developed by using synthetic hexaploid wheat (SHW) and *Triticum spelta* as donors, respectively. Our QTL mapping studies identified several promising genomic regions for increased grain Zn. Of these, two major loci identified from the PBW343×KenyaSwara and a locus from Seri82×SHW population (Figure 1) (Crespo-Hererra et al. 2016) were converted into breeder-friendly SNP marker for use in early generation MAS. In addition, a GWAS study was conducted using the HarvestPlus Association Mapping (HPAM) panel of advanced biofortified wheat lines derived from diverse progenitor species, and the panel was genotyped using 90K Illumina iSelect SNP array. The HPAM panel showed large variation for grain Zn across 8 different locations in Mexico and India (Velu et al. 2016) (Figure 2). The multi-site GWAS analyses identified some common loci from adapted wheat and several rare alleles from diverse wild relatives for increased grain Zn in wheat. To make faster progress in breeding for Zn biofortified wheat, fixing and accumulation of those common loci and introduction of new rare alleles into the high-yielding elite germplasm represents a promising way. The high-throughput phenotyping and genotyping techniques are being implemented to enhance breeding efficiency and selection accuracy. For instance, X-Ray based scanning techniques allow fast-track screening of thousands of samples for grain Zn and Fe concentrations which is non-destructive, cost and time-effective approach. The low cost unmanned aerial vehicles (UAV) are being optimized for rapid proximal measurements of grain protein and micronutrients.

Acknowledgements

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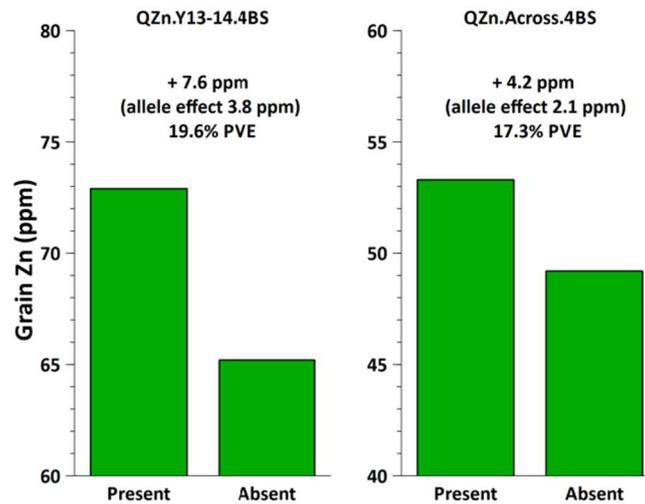


Figure 1: Effect of a QTL on chromosome 4BS identified from Seri 82×SHW mapping population for grain Zn concentration

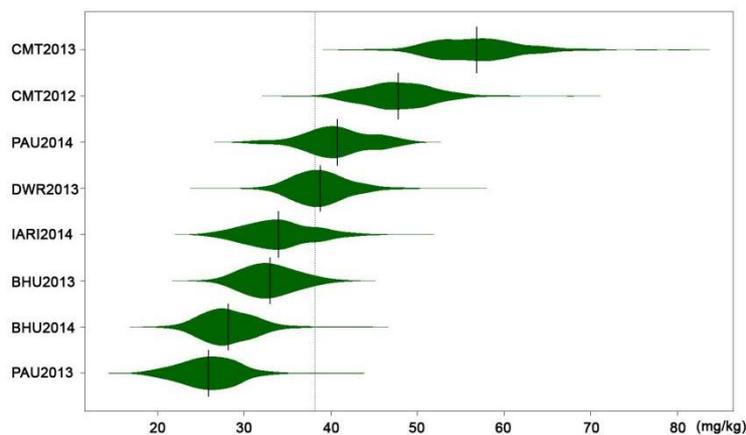


Figure 2: Frequency distribution of grain Zn in the HPAM panel evaluated across 8 different environments of India and Mexico

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Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Dough mixing properties of wheat flours with high levels of HMW-glutenin subunits Dx5 and Dy10

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Key message: Increasing wheat dough strength with balanced levels of HMW-glutenin subunits Dx5 and Dy10.

Genetics and genetic engineering have established that high-molecular-weight (HMW)-glutenin subunits are important in determining the elastic strength of doughs made from wheat flour. In particular, the subunit pair Dx5+Dy10 is associated with superior bread-making potential in cultivars where it is found. Since the genes for these subunits are closely linked, it has been difficult to ascertain whether Dx5 or Dy10 or the combined action of both is needed for stronger gluten. When we over-expressed each subunit separately in transgenic wheat, we found that increases of subunit Dy10 up to 4× improved mixing strength and tolerance with little effect on loaf volume, but increases of Dx5, even as low as 2.3-fold, depressed loaf volumes. Higher levels of Dx5 resulted in flours too strong to mix effectively in mixographs. To test the hypothesis that a balanced level of x and y-subunits could mellow the over-strengthening effects of higher-than-wild-type levels of Dx5, we crossed transgenic lines that have similar over-expression levels of either Dx5 or Dy10. We derived lines homozygous for the transgenes and found that expression levels are additive in the doubly transgenic lines. We will present the results of small scale mixing experiments of flours with balanced over-expressed Dx5+ Dy10 and of flours with balanced Dx5+Dy10 content that also exhibit reductions in all other HMW-glutenins due to transgene-mediated suppression. Mixing behaviors of flours from these lines will be compared to those of their parents. These results will help to differentiate between the roles of x- and y-type subunits in determining gluten strength.

Acknowledgement

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Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Root-specific elimination of the *Aegilops speltoides* B chromosomes and the possible mechanism behind

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Key message: B chromosomes occur as an optional addition to the standard chromosome set. They are generally considered as non-functional and without any essential genes, possessing however some peculiar features.

In most species the number of B chromosomes (Bs) is constant in all somatic cells of a B-carrying organism. However, a tissue-type specific distribution of Bs is known for certain species, like *Aegilops speltoides* (Mendelson & Zohary 1972). The 550-600 Mbp large Bs of *Ae. speltoides* are absent in the roots, but stably present in all other organs and tissues of the same individual. In order to elucidate the process behind the tissue-type specific distribution of Bs two B-specific repeats were identified first using *in silico* cluster analysis of WGS reads, which were generated from genomic DNA of plants with and without Bs. Paired end reads of both libraries (20-fold sequence coverage) were used to construct a *de novo* WGS assembly resulting in a total length of 2.7 Gbp comprised by over 3.5 million contigs. Subsequently, this genomic reference was used to perform a comparative analysis based on *k*-mer frequencies between both datasets. The approach enabled the extraction of B-derived sequences and revealed additional B-specific repeat sequences. In addition, a B-specific accumulation of mitochondrial- and plastid-derived sequences was found (Ruban et al. 2014). FISH of tissue sections prepared from developing embryos revealed that the elimination of Bs starts with the beginning of radicle formation fifth day after pollination. This process occurs mostly in the meristematic area in between apical and basal parts of the embryo via micronuclei formation after nondisjunction of B-sister chromatids at anaphase. Immunostaining with the centromere-specific marker anti-CENH3 revealed centromere activity of lagging Bs. Thus it is likely, that extended cohesion of B sister chromatids is responsible for the tissue-type specific elimination Bs during embryogenesis. Whether nondisjunction of Bs plays also a role in the postmeiotic drive mechanism of *Aegilops* Bs as previously demonstrated for the Bs of rye (Banaei-Moghaddam et al. 2012), remains to be analysed.

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Topic: Harnessing Diversity for Triticeae Improvement

Introgressing genetic diversity into wheat from the wild relative *Thinopyrum elongatum*

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Key message: Stagnating yields are an increasing problem for the ever-growing global population. Introgression of wild relative genetic material increases genetic variation within crop species, for breeders to produce higher-yielding lines.

By 2050 the world population is predicted to exceed 9 billion people, a growth of over 34%. This growth would require an increase of global food production by approximately 70%, or an additional 1 billion tonnes of cereal, simply to maintain our current ability to feed the global population (Rosegrant et al. 2001). Most of the increased food production must be achieved on the same land; as populations expand and need living space, potential new agricultural land diminishes. To increase yield in wheat, its genetic variation needs to be expanded. *Thinopyrum elongatum* is a wild, distant relative of wheat that has already been used as a source of wheat gene pool improvement by providing many disease and pest resistance genes (Gill et al. 2011). A study conducted by Reynolds et al. (2001) also showed that introgression from *Th. elongatum* resulted in hybrids showing increased vigour with significantly higher yield and biomass from higher flag-leaf photosynthesis during grain filling. F₁ hybrids were created by crossing *Th. elongatum* with common bread wheat with a *ph1* deletion, allowing pairing and recombination at meiosis between homoeologous chromosomes. These F₁ hybrids were further backcrossed with common bread wheat (without the *Ph1* deletion) to generate offspring with small chromosome segments of alien DNA present in a mostly wheat genetic background. Initial genotyping was achieved using the Affymetrix 35k SNP array (Figure 1). Genomic *in situ* hybridisation (GISH) is being used to validate this genotyping (Figure 12). KASP markers are currently being developed to allow higher throughput monitoring of introgression location and number within every back-crossed line. Once identified, plants with desirable, single introgressions will be self-fertilised to ensure the new genetic material is homozygous. These plants can then be sent to breeders for phenotypic analysis. Initial phenotyping measurements are being taken on a large population of BC₂-F₂ individuals and results suggest variation in chlorophyll fluorescence, flowering date and plant height.

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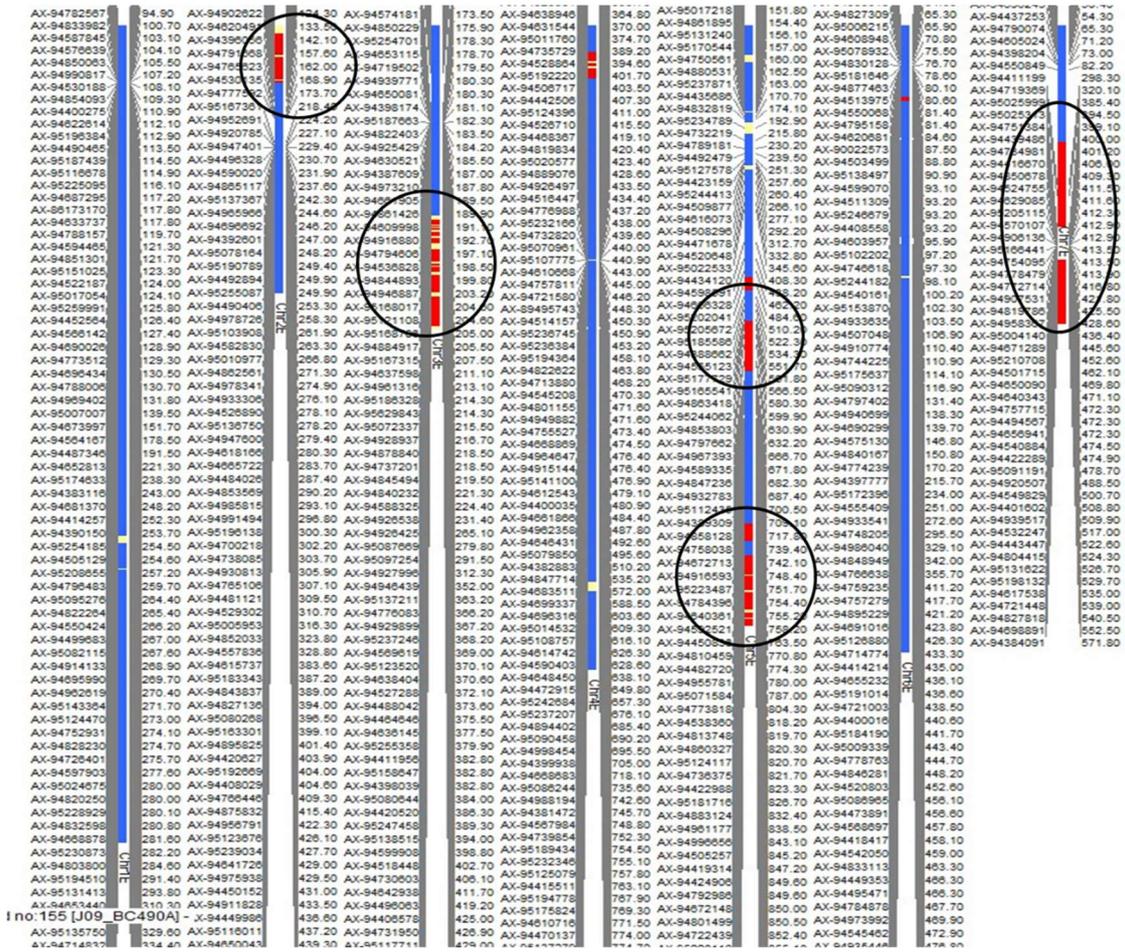


Figure 1: Initial genotyping for back-crossed line BC4-90A.

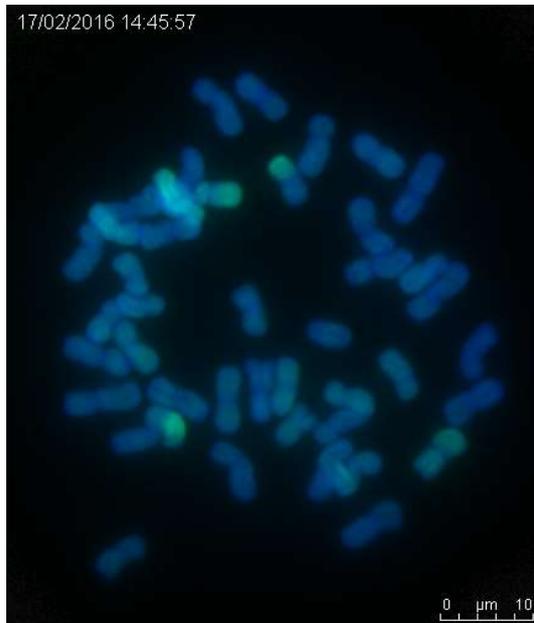


Figure 2: GISH results of back-crossed line BC4-90A.



Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Identification of novel candidate genes of bread wheat involved in early salt stress tolerance through the integration of genomics and transcriptomic resources

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Key message: The integration of genomics and transcriptomic resources in bread wheat allowed to identify a novel gene with homology to bHLH-type transcription factors to validate its role in salt stress tolerance.

Soil salinization is a constraint for global food security. Bread wheat is an important crop for human diet but soil salinity can cause important yield reductions. Salinity tolerance is a complex trait and the understanding of the genetic mechanisms involved in the early stress responses is necessary to develop germplasm with increased tolerance to salt exposure. Therefore, the leaf transcriptome of genotypes contrasting in their response to salinity was studied under stress conditions to identify candidate genes participating in pathways that lead to salt tolerance. The early salt stress response was studied in the cultivar 'Zentos' (tolerant) and the synthetic genotype Syn86 (susceptible), which are the parents of an advanced backcross population. The continuous measurement of the photosynthesis rate in a hydroponics experiment with the contrasting genotypes under stress, allowed the identification of the inflection points during the stress response (Figure 1a). Leave samples from four control and stressed plants were collected in the time points shown in Figure 1b for the transcriptomic analysis. Leaves from each control and stress condition were pooled to generate 14 libraries of sequences using the Massive Analysis of cDNA-3' Ends strategy (MACE). High quality reads were retained and the Tophat/Cufflinks protocol was followed to assess the expression levels of the genes (Trapnell et al. 2012). The improved assembly of the wheat reference genome was used for mapping the reads (Clavijo et al. 2016) and only the uniquely mapped were used for further analyses. We analyzed the genes with normalized expression values greater than 1 across samples and used a fold change ≥ 3 to select the differentially expressed genes. Three groups of differentially expressed genes were identified as shown in Figure 2. One differentially expressed gene discovered in the chromosome 3B co-localized with a QTL for shoot height under salt stress conditions. This gene presents higher levels of expression in 'Zentos' under stress conditions (Figure 3). NCBI Blast results show homologies with *bHLH* -type transcription factors from barley. The expression of this gene is being validated in additional time-points of stress response using qRT-PCR.

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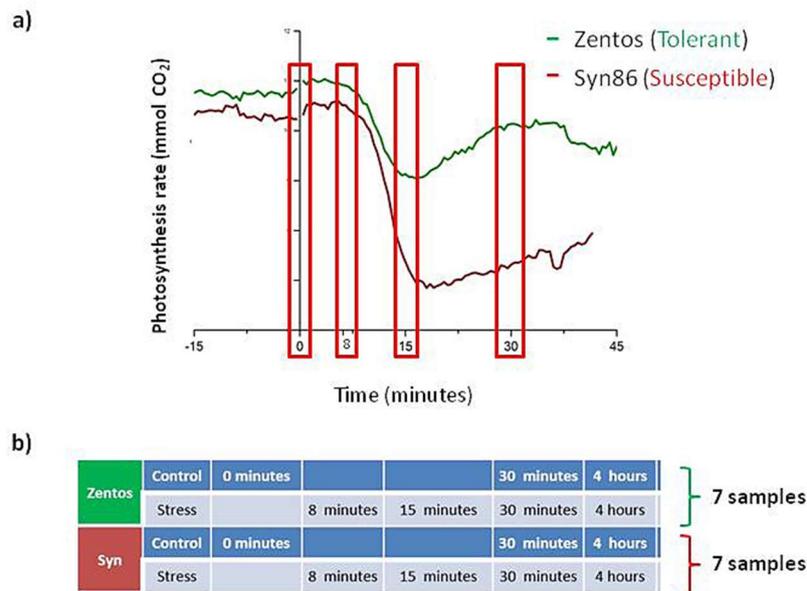


Figure 1: Transcriptomic experimental design: (a) Inflection points of photosynthesis rate identified during early salt stress response assessment after 150 mM NaCl treatment in a hydroponics experiment. The photosynthesis rate starts to decrease at 8 min and drops to the lowest level after 15 min of stress. Then a period of recovery begins and after 30 min the photosynthesis become stabilized in the tolerant genotype. (b) Time points under control and stress conditions used to prepare the libraries for transcriptomic analysis.

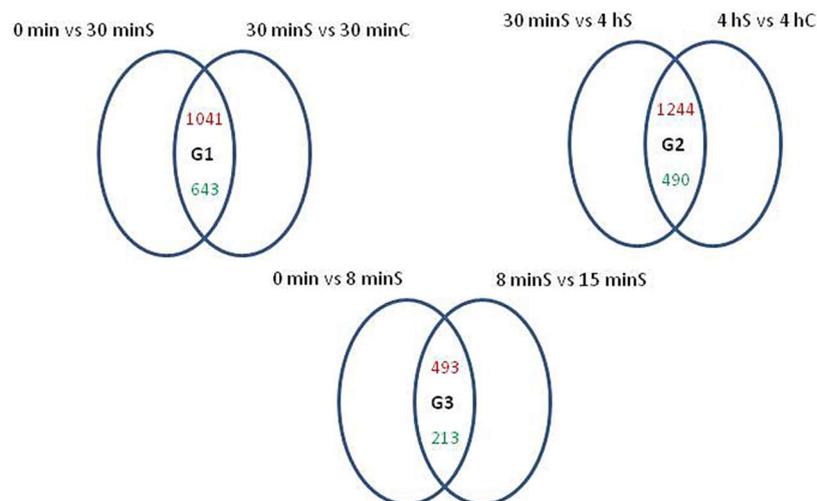


Figure 2: Venn diagrams showing the overlaps between comparisons of gene expression levels of two samples defined to establish the three groups of differentially expressed genes with a fold change ≥ 3 . The number of identified genes were highlighted in red and green for Syn86 (susceptible) and Zentos (tolerant), respectively. C, control; S, stress; G, group of differentially expressed genes.

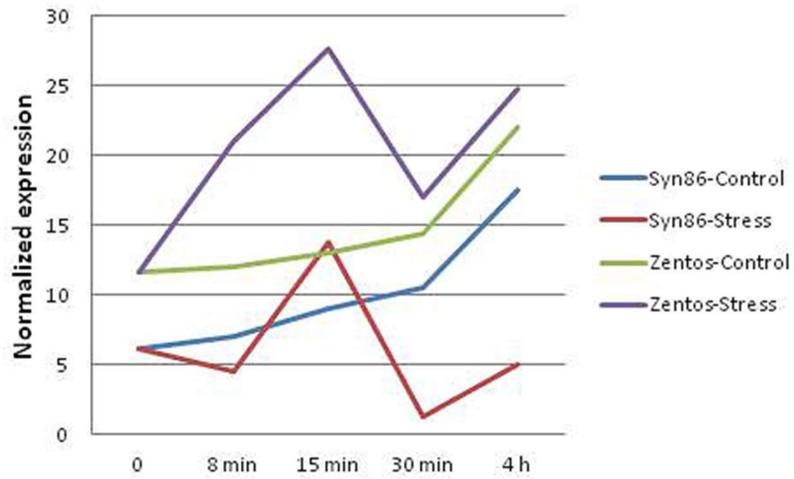


Figure 3: Expression profile of the differentially expressed gene that co-localizes with a salt-tolerance QTL, shown in the contrasting genotypes (salt-susceptible Syn86, salt-tolerant Zentos) under control and salt-stress conditions in the time points studied.



Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

New strategy to get durable disease resistance in wheat

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Key message: Genes involved in resistance to the wheat powdery mildew pathogen, *Blumeria graminis* f. sp. *tritici* (Bgt) in barley can confer host resistance once transferred into wheat.

Nonhost resistance (NHR) is the immunity displayed by all genotypes of a plant species towards all pathotypes of a potential pathogenic species. NHR is the most common form of disease resistance in plants, and this resistance is durable. From previous research at IPK (Rajaraman et al. 2016, research group of Dr. Patrick Schweizer, Gatersleben, DE) using a transient expression system, two barley receptor-like kinases (RLKs) genes have been shown to play a role in NHR to the wheat powdery mildew pathogen, *Blumeria graminis* f. sp. *tritici* (Bgt). Our current research aims to validate the phenotype observed in the transient assay through stable over-expression studies in wheat. Assessment of these barley genes in wheat transgenic lines for response to Bgt inoculation will determine whether genes involved in NHR in barley could confer host resistance when expressed in wheat.

Acknowledgements

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Topic: Structural and Functional Wheat Genomics

Identification and analysis of wheat *TaMSH7* gene promoter region

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Key message: Identification of wheat *TaMSH7* gene promoter localization and its structure analysis for further evaluation of methylation status.

Introgressive hybridization is a powerful tool in wheat improvement and this process has been linked to the functionality of DNA mismatch repair (MMR) system. It has been shown that plant MSH7 protein (MSH6 paralog of other eukaryotes) plays an important role in maintaining the genome integrity and meiotic recombination (Tam et al. 2009). Besides, wheat *TaMSH7* gene links to the *Ph2* locus, which controls recombination rate between homeologous chromosomes (Dong et al. 2002). The impact of methylation on the expression level of MMR system genes in plants is not investigated. However, studies of the methylation effect on promoter regions of *MSH2* and *MSH6* genes in cancer cells indicate that methylation causes a reduction of the expression of relevant genes and leads to increasing number of errors in the genome. Investigation of *TaMSH7* methylation level within the genomes of introgressive wheat lines may indicate an alteration in MMR system functioning under stress conditions caused by the introduction of foreign genetic material. Current work is devoted to the determination of *TaMSH7* promoter region sequence and primers designing for subsequent bisulfite sequencing PCR for *TaMSH7* promoter region methylation state evaluating. Previously, *TaMSH7* gene was localized on the short arms of 3A, 3B, and 3D wheat chromosomes. However, only a partial mRNA of the particular gene is currently sequenced (Dong et al. 2002). Comparison of *TaMSH7* gene sequence with 3B chromosome sequence revealed that full-length gene contains more than 8 kb and at least 15 introns. Comparison of the wheat *TaMSH7* gene sequence with ten *MSH7* genes from another plant species, including maize and rice, revealed that *TaMSH* cDNA is probably truncated from the 5'-end. Comparison data analysis of the region with a length of about 1 kb before the beginning of partial *TaMSH7* cDNA indicates that the likely start codon candidate is ATG located in 433-435 position upstream to cDNA beginning. Search for CpG islands in this region indicates the presence of more than 75 tightly located CpG methylation sites upstream from the putative ATG start codon and almost no CpG sites downstream. Therefore, the 5'UTR region of *TaMSH7* gene can potentially be hypermethylated. Primers covering 31 CpG sites of 5'UTR were designed. Further investigation of this region and using bisulfite sequencing PCR to verify the *TaMSH7* promoter methylation level can reveal the particular expression of the gene within the genome of wheat introgressive lines and its effect on genome stability.

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Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Regulation of inflorescence architecture in wheat by *TEOSINTE BRANCHED1*

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Key message: We show that inflorescence architecture and spikelet development in wheat is regulated by the transcriptional regulator *TB1*, which is able to alter arrangement of spikelets in a dosage dependent manner.

Inflorescence architecture contributes significantly to grain production in cereals, and it has been modified during domestication to increase yields and facilitate harvesting. A unique attribute of grass inflorescences is the arrangement of flowers (or florets) on reproductive branches known as spikelets. In wheat, spikelets are arranged in an alternating phyllotaxy on opposite sides of the central rachis; despite their importance for grain production, little is known about the genes that underpin spikelet development in wheat. Here, we investigate the genetic regulation of inflorescence architecture using a pair of near-isogenic lines (NIL) that display contrasting arrangements of spikelets, and we show that formation of ectopic supernumerary spikelets in one NIL is caused by tetrasomy for chromosome 4D and increased expression of the transcriptional regulator *TEOSINTE BRANCHED1* (*TB1*). We show that the additional copy of *TB-D1* suppresses outgrowth of tillers, delays the progression of inflorescence development, and facilitates formation of supernumerary spikelets by reducing the expression of floral meristem identity genes during early developmental stages. Through generation of transgenic plants that ectopically express *TB-D1* and use of suppressor mutagenesis experiments, we demonstrate that *TB1* regulates plant and inflorescence architecture in wheat *via* a dosage-dependent manner. We also show that allelism for *TB1* may contribute to diversity for inflorescence architecture amongst winter wheat varieties grown in the United Kingdom, suggesting that *TB1* has been important during wheat domestication. These results show that *TB1* has a conserved role in regulating plant architecture traits in wheat, as is the case in maize, rice and barley, and therefore provides a new genetic target to be used by breeding programs to enhance wheat yields.



Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Waking up too early: TaMKK3-A underlies the major 4AL pre-harvest sprouting resistance locus, *Phs-A1*, in global germplasm

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Key message: Our work supports TaMKK3-A as the causal gene for the pre-harvest sprouting resistance locus *Phs-A1*, and highlights the ancient origin and increase of the causal TaMKK3-A mutation through domestication.

Pre-harvest Sprouting (PHS) is an important cause of quality loss in wheat grains and is characterised by too early germination of seeds before harvest. Although PHS is a complex multi-genic trait, a significant proportion of the natural variation for sprouting is controlled by a few major QTL, including *Phs-A1* on chromosome arm 4AL (Figure 1a). In this study, we show that *Phs-A1* confers resistance to grain sprouting by affecting the rate of dormancy loss during dry seed after-ripening. To identify the causal gene underlying *Phs-A1*, we fine-mapped (Figure 1b) and constructed the physical map of the *Phs-A1* interval in hexaploid and tetraploid wheat. This revealed 16 genes (Figure 1c) including the tandem *Plasma Membrane 19 (PM19)* genes and *TaMKK3-A*, which were previously shown to affect seed dormancy and proposed as candidate genes for *Phs-A1* in two independent studies (Barrero et al. 2015, Torada et al. 2016). Our fine-mapping in independent UK populations supports *TaMKK3-A*, and not the *PM19* genes, as the major gene conditioning *Phs-A1* effect (Shorinola et al. 2016). In addition, association analysis in diverse germplasm segregating for *Phs-A1* shows perfect linkage of the *Phs-A1* effect with *TaMKK3-A*, but not *PM19*. Using diverse progenitor, historic and modern germplasm, we show that the causal *TaMKK3-A* polymorphism originates from the diploid A genome progenitor *Triticum urartu*, and that this allele has increased in frequency in modern breeding lines (Figure 1d). Finally, analysis of exome-capture data of diverse hexaploid wheat populations highlights four distinct haplotypes at the *TaMKK3-A* locus (Figure 1e). The sequence, marker and haplotype information reported in this study will facilitate breeding for improved grain quality through the deployment of *Phs-A1*.

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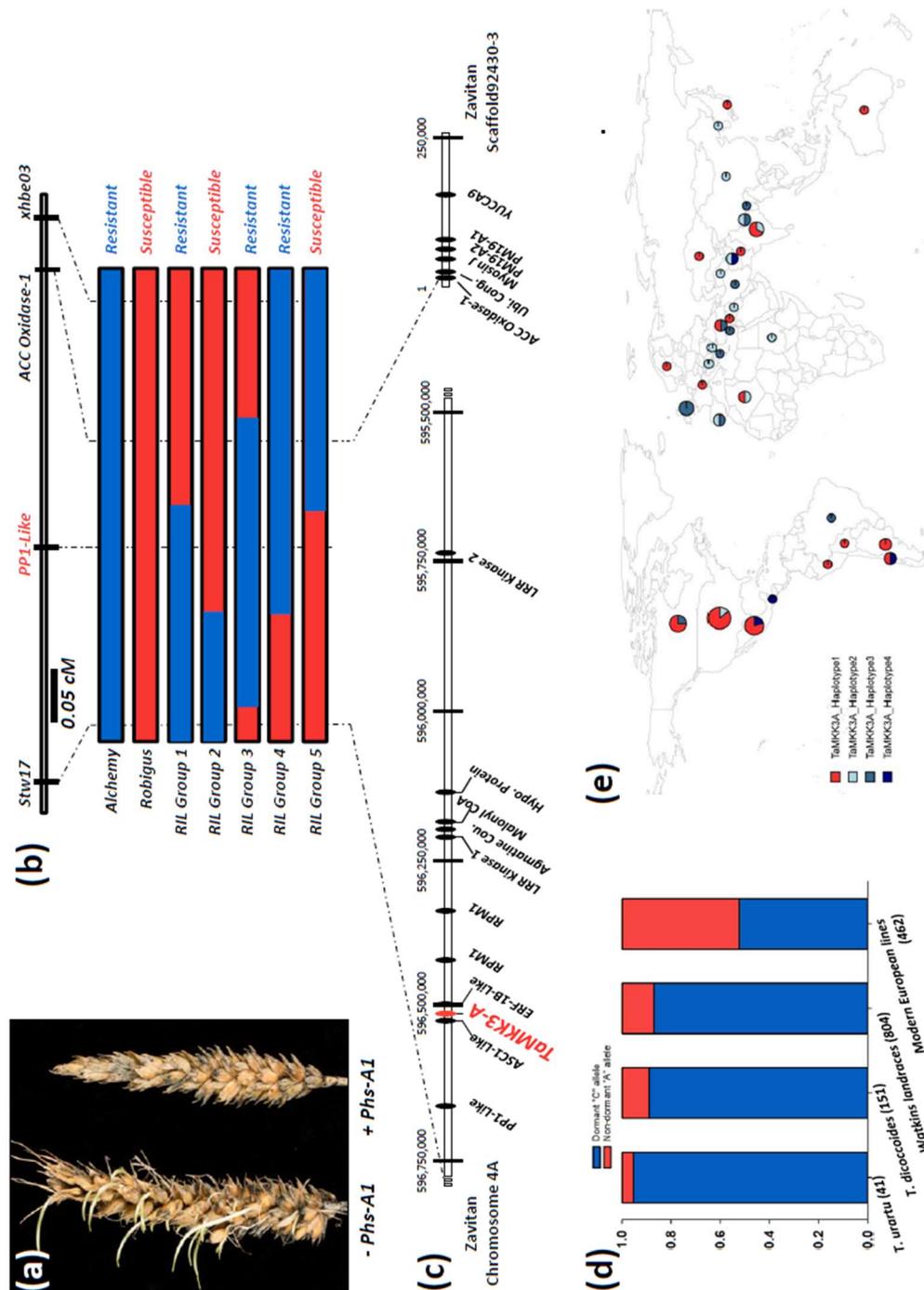


Figure 1: Characterisation, fine-mapping and diversity study of the *Phs-A1* sprouting resistance locus: (a) Lines with *Phs-A1* show increased resistance to sprouting compared to lines without *Phs-A1*; (b) Fine-mapping of *Phs-A1* in Alchemy (resistant)×Robigus (susceptible) RIL population. Alchemy and Robigus alleles and phenotypes are depicted with blue and red, respectively. The PP1-Like marker, linked to *Phs-A1*, is highlighted in red; (c) Physical map of *Phs-A1* interval in tetraploid emmer wheat Zavitan. Genes are represented with ovals including the causal gene *TaMKK3-A* gene highlighted in red; (d) Allele frequency of causal C>A mutation in *TaMKK3-A* in *Triticum urartu* and *T. turgidum* ssp. *diccoides*, Watkins landrace collection and modern European Gediflux collection. Numbers in parenthesis represent number of lines genotyped; (e) Geographical distribution of the four distinct *TaMKK3-A* haplotypes. The sizes of the pie charts represent the sample size. Susceptible and resistant *TaMKK3-A* haplotypes are coloured red and blue, respectively.



Topic: Applying Novel Tools to Practical Wheat Improvement

Comparing strategies to select crosses using genomic prediction in two wheat breeding programs

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Key message: Evaluation of crosses prediction methods with and without accounting for progeny variance. Mid-parent values was a much larger factor determining genetic gain than increasing the progeny variance of a cross.

In wheat breeding programs, a critical decision is to determine crosses that have high probability to deliver progenies with higher genetics gains (Zhong & Jannink 2007, Bernardo 2014). We present an application of genomic models for predicting parental cross combinations for grain yield, grain protein, and loaf volume across two wheat-breeding programs, INIA-Uruguay and CIMMYT. We evaluated three methods for selecting the ‘best’ crosses based on (1) mid-parents, (2) top 10% of the progeny within a cross, and (3) maximizing mean and variance within progeny using thresholds. The last two methods were evaluated with the predicted variances obtained through progeny simulation using the PopVar (Mohammadi et al. 2015, Tiede et al. 2015) package in R software. The first two methods showed 82% of crosses in common for yield, 55% for loaf volume and 53% for grain protein, even though only the second method accounts for the variance of the progeny (Figure 1). While the expected variance of the progeny is important to increase chances of finding superior individuals from transgressive segregation, we observed that the mid-parent values of the crosses selected was a much larger factor determining genetic gain than increasing the progeny variance of a cross (Figure 2). Overall, the genomic resources and the statistical models are now available to plant breeders to predict both the performance of breeding lines per se as well as the value of progeny from any potential crosses, but further understanding on optimizing the cross combinations is needed.

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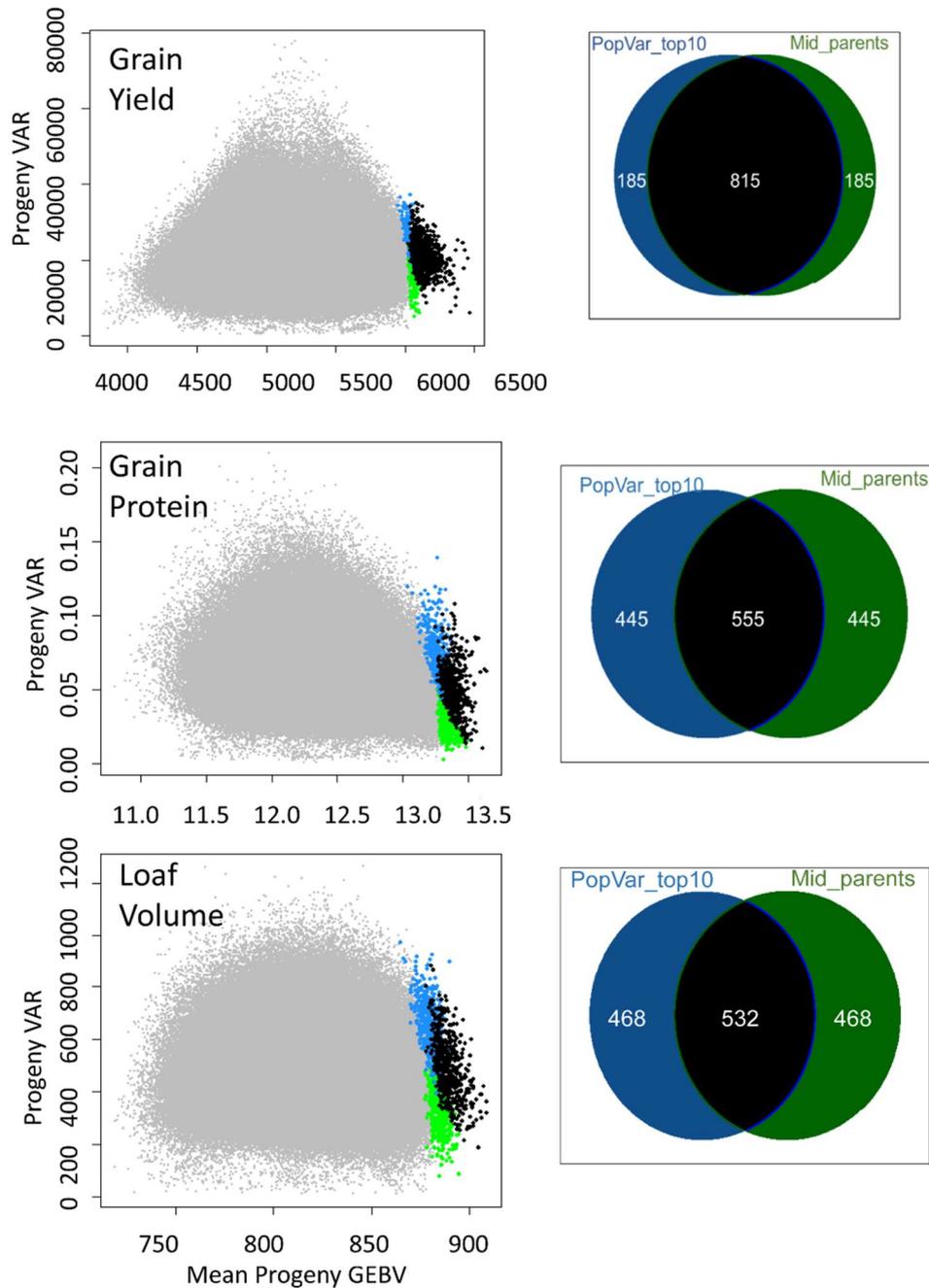


Figure 1: Plots on the left are PopVar predicted progeny mean GEBV plotted against variance within each trait. The best 1000 crosses are indicated with blue when are selected using mean top 10% of the progeny, with green when are selected using mid-parents predictions, and with black when are selected by both. On the right, Venn diagrams represent the number of common and non-common crosses selected by mean top 10% of the progeny and mid-parents value (within one thousand crosses referred before).

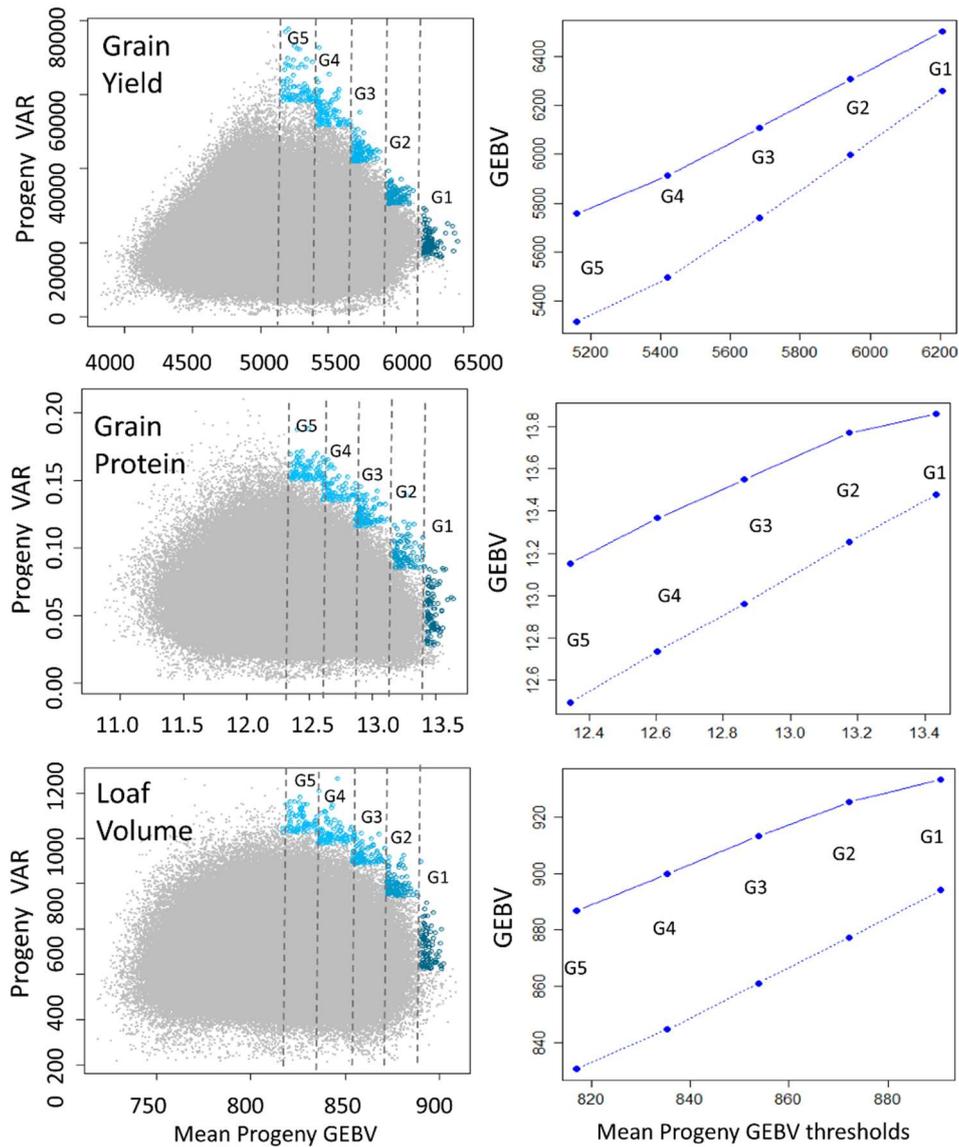


Figure 2: Plots on the left are PopVar predicted progeny mean GEBV plotted against variance within each trait. The best 100 crosses selected using five progeny mean GEBV thresholds (G1>G2>G3>G4>G5) and maximum variance are indicated with. Plots on the right are mean GEBV value of 100% (broken line) and 10% (solid line) of top progeny for PopVar for each selection group (G1-G5).



Topic: Applying Novel Tools to Practical Wheat Improvement

The relevance of epistasis to genome-wide prediction in wheat

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Key message: Genomic prediction abilities for complex traits such as grain yield in wheat can be efficiently improved by modeling main and epistatic effects.

Genomic selection is a powerful tool to predict complex traits which are regulated by many genes. Prediction models based on additive and dominance effects have been successfully applied to predict complex traits in human, animal and plant populations. In contrast, epistatic interaction effects have been often ignored because of high computational load. A solution to reducing the computational load is extended genomic best linear unbiased prediction (EGBLUP), which models interaction effects through an epistatic relationship matrix. The necessary theoretical background of EGBLUP and its relationship with the well-known reproducing kernel Hilbert space regression model has been elaborated recently (Jiang & Reif 2015). Using a diverse population of 3816 wheat elite lines evaluated in multi-environment field trials for a number of important agronomic traits, we investigated the potential and limits to enhance the prediction ability by modelling additive and additive-by-additive epistatic effects (Mirdita et al. 2015, He et al. 2016). All 3816 lines were genotyped with 15k SNP markers. We performed genome-wide association mapping for additive and additive-by-additive epistatic effects for grain yield. Under a liberal threshold (FDR<0.2), we detected 34 markers with significant main effects. But most of them explained less than 1% of phenotypic variation. Moreover, 4371 pairs of markers with significant epistatic effects (FDR<0.05) were detected. Most of the significant epistatic effects (95%) occurred between different genomic regions and local epistasis played a minor role. Genomic prediction abilities were estimated with five-fold cross-validation. Contrasting prediction abilities of GBLUP and EGBLUP, we observed a 5% increase in prediction ability by modeling epistasis for grain yield. The benefits were more pronounced for genetically less complex traits such as Fusarium head blight resistance (8.7%), Septoria tritici blotch resistance (10.3%) and plant height (12.4%). Our results clearly underline the benefits in genome-wide prediction when considering also how genes talk to each other.

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Topic: Applying Novel Tools to Practical Wheat Improvement

Genomic assisted selection for enhancing line breeding

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Key message: Early generation genomic selection is superior to conventional phenotypic selection in line breeding and can be strongly improved by including additional information from preliminary yield trials.

The selection of lines that enter resource-demanding multi-environment trials is a crucial decision in every line breeding program as a large amount of resources are allocated for thoroughly testing these potential varietal candidates. We compared conventional phenotypic selection with various genomic selection approaches across multiple years as well as the merit of integrating phenotypic information from preliminary yield trials into the genomic selection framework (Michel et al. 2016). The prediction accuracy using only phenotypic data was rather low ($r = 0.21$) for grain yield but could be improved by modelling genetic relationships in unreplicated preliminary yield trials ($r = 0.33$). Genomic selection models were nevertheless found to be superior to conventional phenotypic selection for predicting grain yield performance of lines across years ($r = 0.39$). We subsequently simplified the problem of predicting untested lines in untested years to predicting tested lines in untested years by combining breeding values from preliminary yield trials and predictions from genomic selection models by an heritability index. This genomic assisted selection led to a 20% increase in prediction accuracy, which could be further enhanced by an appropriate marker selection for both grain yield ($r = 0.48$) and protein content ($r = 0.63$) (Michel et al. 2017). The easy to implement and robust genomic assisted selection gave thus a higher prediction accuracy than either conventional phenotypic or genomic selection alone. The proposed method took the complex inheritance of both low and high heritable traits into account and appears capable to support breeders in their selection decisions in order to develop enhanced varieties more efficiently.

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Topic: Applying Novel Tools to Practical Wheat Improvement

Modelling genotype by environment interaction for genomic selection in wheat

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Key message: A hierarchical multi-trait model to handle multi-environment trials data is described. It allowed to: increase prediction accuracy, better describe genomic covariances between environmental factors, and map genotype by environment effects.

In plant breeding, to assess genotype by environment interactions (GEI), multi-environment trials (MET) are planned where the same genotypes are evaluated in different geographical locations and years. A widely used approach to analyze MET dataset is to consider each environment performance as a separate trait. Such a framework, called multi-traits model (MT), allows estimating variance components for each environment and genetic covariances between them. Although this is the most flexible var/covariance structure, a high number of environments may hamper the estimation of such a large number of parameters. A common solution is to use a simplified structure of the covariance matrix which is flexible enough to accommodate heterogeneity of variances and genetic correlations differences, and parsimonious enough to allow estimation of the parameters. One approach is to objectively define a simplified structure from the data, for instance, by utilizing the leading principal components of a var/covariance matrix (e.g. reduced rank and factor-analytic models). Here, we present a Bayesian hierarchical MT in which the genetic var/covariance between environments is defined by using latent variables. Latent variables are subjectively defined by the user to correspond to specific environmental effects, such as location and year effects. Such a model estimates genomic values that represent different covariance structures while reducing the number of parameters to estimate. Approximately 1300 advanced wheat lines from Nordic Seed A/S breeding program, originating four different breeding cycles were phenotyped in three different locations for four consecutive years and genotyped with the Illumina 15K wheat chip. Accuracy of genomic prediction (GP) for yield and protein content were estimated by using different cross validation schemes reflecting different possible strategies to implement GP in wheat breeding schemes. Single trait models (ST) were compared with a reduction rank MT (RRMT) and a hierarchical MT (HMT). MT always performed better than ST providing an increase of accuracy up to 0.10 and 0.06 for yield and protein content, respectively. Advantages of the HMT over the RRMT were: (i) a moderate increase in GP accuracy; (ii) the possibility of describing genetic covariances between environmental factors (e.g. between years and location instead of only between each year/location combinations); (iii) the possibility of dividing SNP effects in main (independent from the environment) and location and year dependent effects. We concluded that HMT is a suitable way to handle MET for GP. Moreover, it provides some advantages over traditional MT analysis in describing GEI and mapping GEI related SNP effect.



Topic: Applying Novel Tools to Practical Wheat Improvement

Perspective using molecular marker-assisted breeding for dwarf and common bunt resistance in winter wheat

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Key words: Marker-assisted breeding, QTL, *Tilletia*, resistance, *Triticum aestivum*

Dwarf bunt caused by *Tilletia contraversa* J.G. Kühn and common bunt caused by *T. caries* and *T. foetida* are two destructive diseases of wheat (*Triticum aestivum* L.) that reduce grain yield and quality. Phenotyping of the two diseases is extremely difficult and expensive. Very few molecular markers were identified that can be used in selection of bunt resistance. This project used two bi-parental populations and identified two major QTL for resistance to dwarf bunt. One novel QTL *Q.DB.ui-7DS* was identified in a recombinant inbred line population derived from the bunt resistant germplasm Idaho 444 (IDO444) and the susceptible cultivar Rio Blanco which explained 32 to 56% of phenotypic variation among the four field trials. Another QTL *Q.DB.ui-6DL* was identified in a doubled haploid population derived from resistant germplasm IDO835 and susceptible cultivar Moreland. Molecular markers of the two QTL were used to characterize a set of differential lines, known resistance sources and their derived resistant cultivars. This study suggests that molecular markers associated with the two QTL will accelerate new variety development for dwarf and common bunt resistance in winter wheat.

Acknowledgements

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Topic: Applying Novel Tools to Practical Wheat Improvement

Which wheat for smallholder Ethiopian farmers? The quantitative genetics of traditional knowledge

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Key message: Smallholder farmers' traditional knowledge can be used in a genome wide association study (GWAS) to identify genomic loci responsible for farmers' choice of durum wheat.

Ethiopia is one of the biggest and most populous countries in Africa, counting 100 millions of inhabitants, three-quarters of whom employed in subsistence agriculture. The Ethiopian plateau is a center of diversity for tetraploid wheat, a staple food for smallholder farmers and a high-value crop used for bread, pasta, and semolina preparations worldwide. Subsistence farming systems in Ethiopia face highly variable climatic conditions that threaten locally-adapted, low-input agriculture. The benefits of modern breeding may fail to reach such farming communities, as broadly adapted material is inherently unable to address their very specific local requirements. To date, participatory variety selection has only scratched the surface of the exploitability of farmers' knowledge in breeding. We involved 60 smallholder farmers in two locations to evaluate traits of their interest in 400 wheat accessions representing Ethiopian wheat diversity, producing 230 400 data points (Figure 1). We couple this information with metric measurements of 10 agronomic traits, breaking down farmers' preferences on quantitative phenotypes. We describe high heritabilities for farmers' evaluations, matching and sometimes surpassing those of metric phenotypes. We found that the relative importance of wheat traits is gender- and locality- dependent. We produce a ranking of the 400 varieties identifying the combination of traits most desired by farmers. Concurrently, we characterize the Ethiopian wheat molecular diversity for more than 80K SNP loci, the state of the art of wheat genotyping. We found that the genetic makeup of Ethiopian wheat is completely different from the international allele pool, confirming its uniqueness. We use the molecular information in a genome-wide association study (GWAS) considering at once metric traits and farmer preferences. To do so, we employ mixed linear models accounting for genetic relatedness in the wheat panel iterating with different sets of covariates as fixed effects. We screen GWAS outcomes identifying the best covariate combinations and describe significant marker-trait associations (MTA) corresponding to agronomic phenotypes and farmer scores. We discuss the overlap, and lack thereof, of MTA emerging from the two dataset. Our findings demonstrate that farmers' traditional knowledge has a clear quantitative basis that can be explored with the methods of quantitative sciences. We argue that farmers' knowledge may be exploited to identify breeding targets useful to produce wheat addressing the local needs of smallholder farming.



Figure 1: A group of female smallholder farmers evaluated durum wheat genotypes.



Topic: Applying Novel Tools to Practical Wheat Improvement

Utilizing genomics and phenomics in CIMMYT wheat breeding

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Key message: Efficiency of pedigree, genomic and phenomic prediction based selection approaches are being evaluated in CIMMYT wheat breeding program. Our results show the models integrating these approaches improve the prediction accuracies

Wheat, a key cereal for global food security continues to be threatened by multiple challenges due to climate change and evolving pathogen and pests. Based on various predictions an estimated 1.6-2.2% annual growth in productivity is required to meet the demands in the next decade. While traditional breeding approaches have been successful, the rate of genetic gain in grain yields as estimated by different studies is below 1% annually. Genomic selection (GS) and high-throughput phenotyping (HTP) are promising technologies for screening and selection of large number of progenies. Efforts are ongoing in evaluation and integration of genomics and phenomics in the CIMMYT's spring wheat breeding strategy to accelerate the genetic gains in breeding for climate resilient disease resistant wheat varieties. Since, 2014 nearly 40 000 advanced lines in the breeding program have been genotyped using genotyping-by-sequencing markers. These advanced lines were evaluated each year for grain yield (GY) and other traits in Norman E. Borlaug research station at Ciudad Obregon, Sonora, Mexico. Ariel HTP using thermal and hyperspectral camera was conducted at various time points across the crop season for estimating canopy temperatures, normalized difference vegetation index (NDVI) and other spectral indices. Genomic, pedigree and phenomic data were utilized for prediction of grain yield under different environments. It was observed that use of CT and NDVI as predictor traits in pedigree and GS models increased GY predictions accuracies (Rutkoski et al. 2016). While some spectral indices show good predictive ability for GY, prediction accuracies improved considerably using all 250 hyperspectral bands simultaneously. The spectral indices estimated at specific growth stages such as grain filling had higher prediction accuracies than the mean of spectral indices across crop season. Similar results were observed while using the 250 hyperspectral bands as a predictor trait for GY. Pedigree and GS models were also used for GY predictions of the advanced lines in 1st year yield trial in 2015 and 2016. Results from both years show that GY predictions can add additional value to field estimated GY. Selections of lines based on the predictions have been included for evaluation in multi-environment yield trials. Work is also ongoing for GS model development to account for genotype by environment effects and use of GS and HTP for early generation screening.

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Topic: Applying Novel Tools to Practical Wheat Improvement

Optimization of trial networks for genomic selection based on crop models

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Key message: We propose a statistical criterion to optimize trial networks in order to predict genotype × environment interactions more efficiently, by combining crop growth models and genomic selection models.

Genotype × environment interactions (GEI) are common in plant trial networks. In this context, models used for genomic selection (GS), that refers to the use of genome-wide information for predicting breeding values of selection candidates, need to be adapted. One promising way to increase prediction accuracy in various environments is to combine ecophysiological and genetic modelling thanks to crop growth models (CGM) incorporating genetic parameters. The efficiency of this approach relies on the quality of the parameter estimates, which depends on the trial network used for calibration. The objective of this study was to determine a method to design optimal trial networks for estimating genetic parameters in this context. A criterion called OptiNet was defined to this aim, and was evaluated on simulated and real data, with the example of wheat phenology. The trial networks defined with OptiNet allowed estimating the genetic parameters with lower error, leading to higher QTL detection power and higher prediction accuracies. Trial networks defined with OptiNet were on average more efficient than random networks composed of twice as many environments, in terms of quality of the parameter estimates. OptiNet is thus a valuable tool to determine optimal experimental conditions to best exploit trial networks and the phenotyping tools that are currently developed.



Topic: Applying Novel Tools to Practical Wheat Improvement

Crop modelling as a phenotyping tool for enhancing wheat adaptation to abiotic stresses

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Key message: Process-based crop models can serve as quantitative phenotyping tools for improving wheat adaptation to abiotic stresses.

Progress in improving crop adaptation to abiotic stresses will be greatly accelerated by the ability to simultaneously identify favourable combinations of genotypes and management practices in a target population of environments (TPE). The infeasibility of empirical exploration of all possible G×M×E combinations necessitates the development of novel technologies and approaches for a more effective exploration of the G×M×E interactions. Process-based crop growth models offer a great potential for a more effective exploration of the G×M×E interactions and their phenotypic consequences for crop growth and yield formation (Hammer et al. 2006). There are three major areas, where whole-plant physiological modelling could assist in enhancing the breeding efficiency: (i) characterising crop environments to define the intensity and frequency of abiotic stresses in TPE, (ii) understanding and dissecting the physiology and genetics of complex multi-genic traits, and (iii) predicting phenotypes of G×M combinations in the TPE. Here we present two specific examples focusing on drought adaptation in wheat (*Triticum aestivum* L.) to explore the value of this approach. The first study examined the likely effects of drought-adaptive root architectural traits on wheat growth and yield formation across a wide range of environments in Australia with a summer-dominant rainfall pattern (Manschadi et al. 2006). Simulations were performed with the cropping systems model APSIM linked with long-term daily weather data from three locations contrasting in the soil type and the maximum amount of plant available water content in the profile. Results of this simulation analysis indicated a mean relative yield benefit of 14.5% in water-deficit seasons for the root-modified genotype. Only in a very few seasons changes in the root traits resulted in a yield penalty. The second study reports on the observed genotypic variations in traits determining leaf canopy development (e.g. phyllochron and leaf size) among two wheat varieties in Austria and their implications for growth and yield of wheat grown under various nitrogen fertilisation levels in a set of TPE. These case studies highlight the crucial role of crop modelling in characterisation of TPEs and predicting phenotypes of complex G×M×E interactions.

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Topic: Applying Novel Tools to Practical Wheat Improvement

Use of a non-destructive estimate to improve understanding of the genetics of biomass production in wheat

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Key message: Measuring biomass production in wheat populations using LiDAR (Light Detection And Reflection) is non-destructive approach producing more robust estimates than traditional methods with potential to increase grain yield in breeding.

Genetic gains in wheat yields globally have reportedly been driven by improved partitioning of biomass to grain (increased harvest index), though theoretical maximal limits of harvest index are being reached. Increasing biomass production while maintain high harvest index is seen as an opportunity to further increase wheat yields. Measuring biomass production in large wheat populations is difficult due to the immense labour costs associated with traditional methods of cutting, drying, and weighing quadrats sampled at different stages of development. Further, heritabilities associated with these techniques tend to be low reflecting a major issue with size of the harvested sample and the resulting large sampling variance. We are investigating the use of alternative high throughput approaches to increase the ease of biomass sampling as well as the accuracy to which the trait is estimated. To this end we are focussing on use of a LiDAR (Light Detection And Reflection) unit installed on a light-weight ground platform. Manual estimates of biomass production from a large multi-parent wheat mapping population were taken at several stages of crop development in plots in the field and compared with data obtained on the same plots using LiDAR. Heritabilities of manual estimates of biomass at jointing and flowering ranged from low (0.09) to moderate (0.51) on a single run basis. Heritabilities obtained using LiDAR-based estimates were larger, indicating greater robustness and increased confidence of these estimates. Genetic correlations between manual and LiDAR-based estimates were strong, though dependent upon stages of sampling. Several significant QTL were identified. The relationship between manual and LiDAR-based biomass estimates for use in breeding and selection of increased grain yield will be discussed.



Topic: Applying Novel Tools to Practical Wheat Improvement

Exploring genetic diversity in bread wheat using nested association mapping (NAM)

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Key message: A bread wheat nested association mapping (NAM) population is being developed. The population will be genotyped and phenotyped to identify loci associated with grain yield under field conditions.

Crop improvement relies upon genetic diversity. The rate of genetic gain in breeding programs can be increased by extending the amount of variation available for selection using land races and wild relatives. However, exotic germplasm carries a range of undesirable traits such as grain shattering, tall plant type, lodging and low yield potential, that limit their suitability for modern agriculture. Back-crossing to locally adapted varieties and pre-selection for traits is therefore required to ensure meaningful data is generated in field trials. Multi-parental schemes such as nested association mapping (NAM) populations improve the use of exotic germplasm as a resource for the discovery of novel traits and QTL/genes. NAM combines the power of linkage analysis and the precision of association mapping (Yu et al. 2008). When jointly analysed, NAM populations can provide higher power to detect QTL than any of the constituent biparental families separately. We selected 75 highly diverse hexaploid spring-type wheat accessions from regions of the world that are affected by heat and drought stress (Figure 1). These accessions were crossed with two Australian elite varieties as founder parents, and BC₁F₄ populations are being generated (Figure 2). Genotyping of the parents with the Illumina 90K iSelect SNP array identified almost 35 000 polymorphic markers that were mapped against the NRGene assembly. These were used to develop a targeted GBS assay comprising about 12 000 polymorphic markers, well distributed across the genome and in higher numbers at the end of the chromosomes for genotyping the NAM population. In the first instance, we aim to genotype 20 individuals from each NAM sub-population and use simulation to identify the most effective approach for continued genotyping and phenotyping. The simulation will test the effect population number and size of each population on the power for detecting QTL having different effects and allele frequencies. The NAM will be phenotyped in the field across different environments in Australia and Genome Wide Association Analysis (GWAS) performed to identify loci associated with grain yield.

Reference

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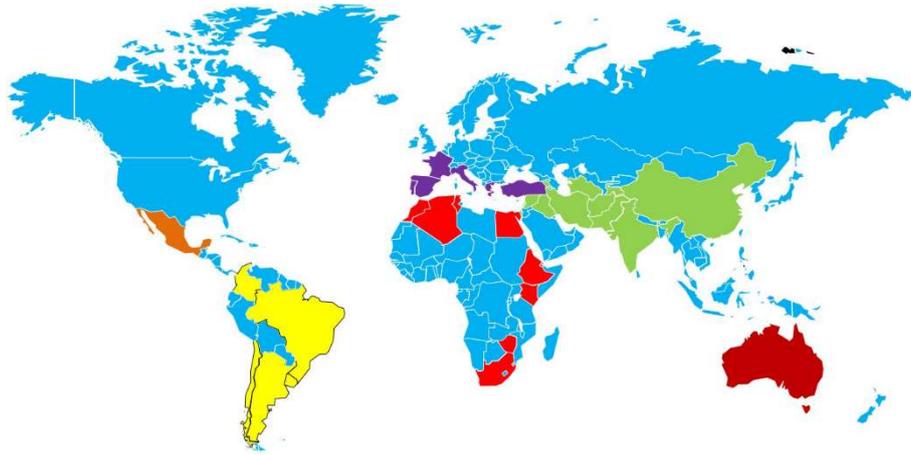


Figure 1: Geographic distribution of the wheat NAM (Nested Association Mapping) parents.

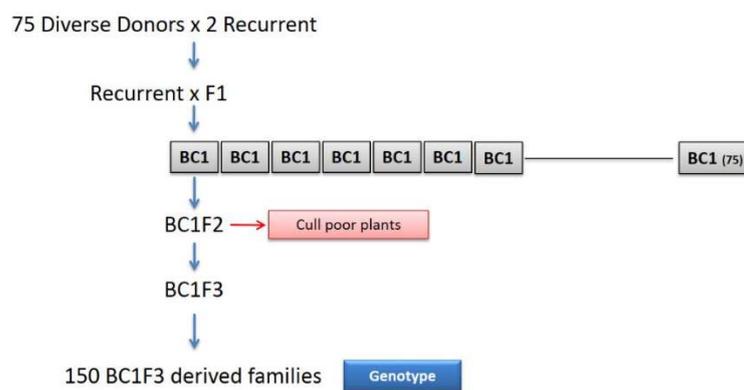


Figure 2: Design of the NAM (Nested Association Mapping) population.



Topic: Applying Novel Tools to Practical Wheat Improvement

Rapid gene isolation in barley and wheat by mutant chromosome sequencing

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Key message: We propose MutChromSeq, a gene cloning method regardless of recombination or fine-mapping, to pinpoint specific genes in wheat, barley and their relatives that might be used in breeding programs

As staple crops across the globe, wheat and barley play a crucial role in global food security. Consequently, the identification and manipulation of genes controlling agronomic traits of interest is of utmost importance to develop new and improved crop varieties. However, isolation of such genes based on traditional map-based cloning strategies is expensive and time-consuming hampered by the large genomes of barley (5.5 Gbs) and wheat (17 Gbs), their low recombination rates and highly repetitive nature. On the other hand, sequencing whole genomes of parents differing for a trait of interest is simply not practical in barley and wheat due to the economic costs and the colossal amount of data which makes the analysis very challenging. To overcome these obstacles, we developed Mutant Chromosome Sequencing (MutChromSeq) (Sánchez-Martín et al. 2016), a complexity reduction approach which combines chromosome flow sorting with classical mutagenesis to identify genes of interest in wheat and barley. As a proof of concept, we applied MutChromSeq to six mutants of the recently cloned barley wax synthesis *Eceriferum-q* gene (Schneider et al. 2016). To further test MutChromSeq out, we targeted a plant resistant gene in wheat, the powdery mildew resistance gene *Pm2*. The comparison of six EMS-derived *pm2* mutant chromosomes flow sorted and sequenced with the wild type chromosome allowed unambiguous identification of a single candidate gene, further verified by Sanger sequencing of six additional mutants. MutChromSeq is reference-free and does not require fine-mapping and construction of a contiguous physical sequence across a map interval. MutChromSeq enables forward genetics in wheat and barley that can be defined by loss-of-function mutagenesis, regardless of recombination, gene structure or the availability of a reference sequence. The method can be readily applied to mutant sets of wheat and barley and adapted to other commercially relevant crops and relatives amenable to mutagenesis and chromosome flow sorting, for example, oat, rice, maize, pea and bean.

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Topic: Applying Novel Tools to Practical Wheat Improvement

Genetic mapping of flavor loci in wheat

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Key message: QTL related to the flavor and/or aroma of wheat grain were mapped in two bi-parental wheat populations. QTL were located on 2D/4BL, 3D, 4BS, 4DL, 5A, 5AS, 6B, 6DL, and group 7.

Flavor is an essential aspect of consumer acceptance, especially with whole-wheat foods. However, little if any selection is performed during breeding of new wheat cultivars for flavor, and little is known regarding the genetics of flavor. Our research is aimed at identifying genes that impart either a desirable (*Yummy*) or undesirable (*yucky*) flavor to wheat grain. We have developed a mouse model system to test the consumption preference of grain (Fuerst et al. 2013). Grain of two varieties is mixed and provided to 10 replicate mice over two sequential 24 h periods. Consumption data are analyzed using Student's *t* statistic. Initial studies identified kernel hardness and bran color as significantly influencing consumption preference. However, dramatic differences were observed within texture and color classes. Initial experiments tested 'single elimination' and 'round robin' tournament designs; both were effective but not well adapted to genetic mapping. More recently, a system of comparing 'experimental' lines to a common check was developed wherein the lines could be derived from doubled haploids (DHs), recombinant inbred lines (RILs) or collections of varieties. A major hurdle was overcome by utilizing the actual *t* statistic as a consumption phenotype (Kiszonas et al. 2015). Most recently, this model system has been used in two mapping studies: Clarks Cream×NY6432-18 (CC×NY, hard by soft white winter, 78 RILs) (Kiszonas & Morris 2016) and Louise×Yumai 34 (L×Y, soft white springs, 373 DHs). CC×NY employed an existing marker dataset with 102 RFLPs; L×Y used a genotyping-by-sequencing markers. For CC×NY, using *Pinb* and hardness as covariates, QTL were identified on 3B, 3D, 5A, 6B, and group 7. For L×Y, dominant markers from Louise (*Yummy* parent) were on 4DL and 6DL; SNP markers were on 5AS, 4BS, and 2D/4BL.

Acknowledgements

E. Patrick Fuerst, Derick McLean, N. Dasgupta, Olivia Lottes, Derick Jiwan, Dan Skinner, Deven See, and Eden Stout. Partial funding was provided by ConAgra Foods and Ardent Mills LLC.

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Topic: Applying Novel Tools to Practical Wheat Improvement

Unfertilized ovary pushes wheat flower open for cross-pollination

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Key message: When self-pollination is impaired in wheat through emasculation or male sterility, the ovary swells and separates the palea and lemma, therefore opening floret. This process provides an opportunity for cross-pollination.

Bread wheat (*Triticum aestivum* L.) is typically cleistogamous (closed flower), resulting in seed produced predominantly by self-pollination. In order to facilitate hybrid breeding in wheat, it is prerequisite to understand details of floral structure and the complexities of flower opening process. At anthesis, the lodicule is the primary floral organ involved in the first phase of flower opening, whereas the ovary plays a role in latter opening phase at post anthesis, in the absence of self-pollination (Hoshikawa 1960). However, there is limited knowledge of the ovary's role in the latter phase of flower opening. Therefore, we carried out a detailed physiological and anatomical investigation into wheat flower opening to understand the underlying mechanisms at play. We identified that the wheat flower opens when the ovary remains unfertilized either by the process of emasculation or male sterility (Figure 1A). Our observations revealed that unfertilized ovaries significantly increase in size, especially depth, in the horizontal orientation after anthesis (Figure 1B). Swollen ovaries exceed the size of the lemma, particularly close to the connection point with the central rachis, separating the lemma from the palea and therefore, stigma is exposed. Mesocarp cells located at the top section of unfertilized ovaries are intact and enlarged, contributing to increased radial ovary size. In contrast, fertilized ovaries enlarge vertically and have mesocarp cells that degrade via program cell death (Radchuk et al. 2011), resulting in the developing grain being retained within the floret which remains closed. Ovary swelling could represent a survival mechanism for self-pollinating grasses like wheat, as it forces floret opening in the absence of fertilization and therefore setting seed by cross-pollination.

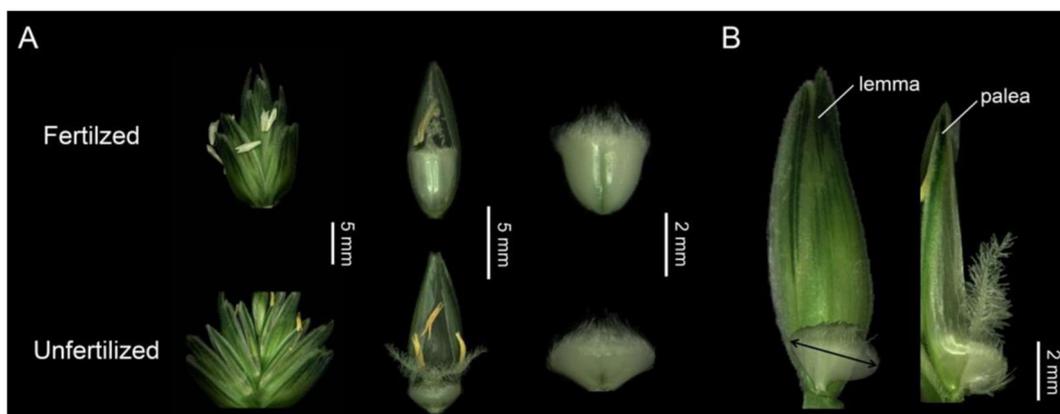


Figure 1: Wheat flower: (A) spikelet, floret and ovary images of fertilized (top) and unfertilized (bottom) samples at 7 days after full heading (= 4 days after anthesis); (B) side view of floret and swollen ovary at 5 days after full heading; a merged image of floret and ovary (left) and a floret with removed lemma removed (right); arrow indicates depth of ovary.



Acknowledgement

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Topic: Applying Novel Tools to Practical Wheat Improvement

CRISPR/Cas9-mediated genome editing in wheat genetic improvement

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Key message: Wheat improvement via genome editing

CRISPR/Cas9 technology enables precise modifications of DNA sequences *in vivo* and offers a great promise for harnessing plant genes for crop genetic improvement. Whereas error-prone non-homologous end joining (NHEJ) repair of a double stranded break (DSB) at specific locations generated by CRISPR/Cas9 often generate mutations and gene knock-outs, homology-directed repair (HDR) potentially can achieve gene replacement. However, it is still challenging to either create mutations or achieve gene replacement in some transformation-recalcitrant species including wheat. Here, we successfully generated multiple mutants through CRISPR/Cas9-mediated genome editing of several genes such as *SBEIIa* related to the synthesis of amylopectin, *JAV1* orthologs related to jasmonic acid synthesis pathway and biotic stresses, and *DERF1* orthologs related to drought tolerance in wheat. Functional and genetic stability analyses of these mutant lines are under way. Besides, simultaneous substitutions of two amino acid residues of acetolactate synthase (ALS) in wheat through gene targeting or replacement are under way. Taken together, these results demonstrate that we can not only generate multiple mutant wheat plants through NHEJ for either functional analysis or genetic improvement, but also precisely substitute amino acid residues in proteins through CRISPR/Cas9-mediated HDR, greatly expanding the ability to modify genes that confer agriculturally important traits in wheat.



Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Combined mutations in *Sbell* genes affect grain yield and bread-making quality in hexaploid wheat

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Key message: Common wheat with mutated *Sbell* genes presented high resistant starch content, reduced grain yield and altered grain, flour and bread quality parameters that generally varied with the cultivar and environment.

Starch of common wheat (*Triticum aestivum*) with high proportion of amylose is correlated with high content of resistant starch, a dietary fiber that can bring human health benefits. Mutations in the *Starch Branching Enzyme II (Sbell)* genes were combined in the *Sbella/b* paralogs of the A and B genomes and in the *Sbella* of the D genome and incorporated into the hexaploid wheat cultivars Lassik and Patwin-515. The aim of this study was to assess the impact of the *Sbell* mutations on common wheat grain yield, as well as on grain, flour, starch and bread quality properties. Results from two replicated field trials suggest the combined mutations were effective in reducing the overall activity of the SBEII proteins, as amylose increased $\approx 60\%$ and resistant starch content increased $\approx 1000\%$ in mutant lines compared to isogenic controls with wild-type *Sbell* alleles. The mutations were also associated with significant decreases in total starch (6%), kernel weight (3%), flour extraction rate (21%) and grain yield (6%). Altered quality parameters were detected, such as significantly increased grain hardness, starch damage, water absorption and flour protein, as well as decreased farinograph development and stability times, starch viscosity and loaf volume (Figure 1). The loaves of *Sbell*-mutated lines presented coarse crumb structure and texture, with thick cell walls and very little oven spring. These characteristics, however, were improved in blending tests with the control flours. Although results from the falling number test suggested lower enzymatic activity on the high amylose flours, addition of fungal α -amylase to the baking tests did not improve loaf volume. A longer proofing time or the addition of fermentable sugars might be necessary for the proper development of the dough. Most of the quality properties presented significant interactions between variety and/or environment, suggesting part of the negative effects of the mutations could be ameliorated by selection of the variety and location for deployment of the trait. The use of common wheat varieties with increased resistant starch and the associated health benefits will likely require economic incentives to compensate growers for the detected reductions in grain yield.

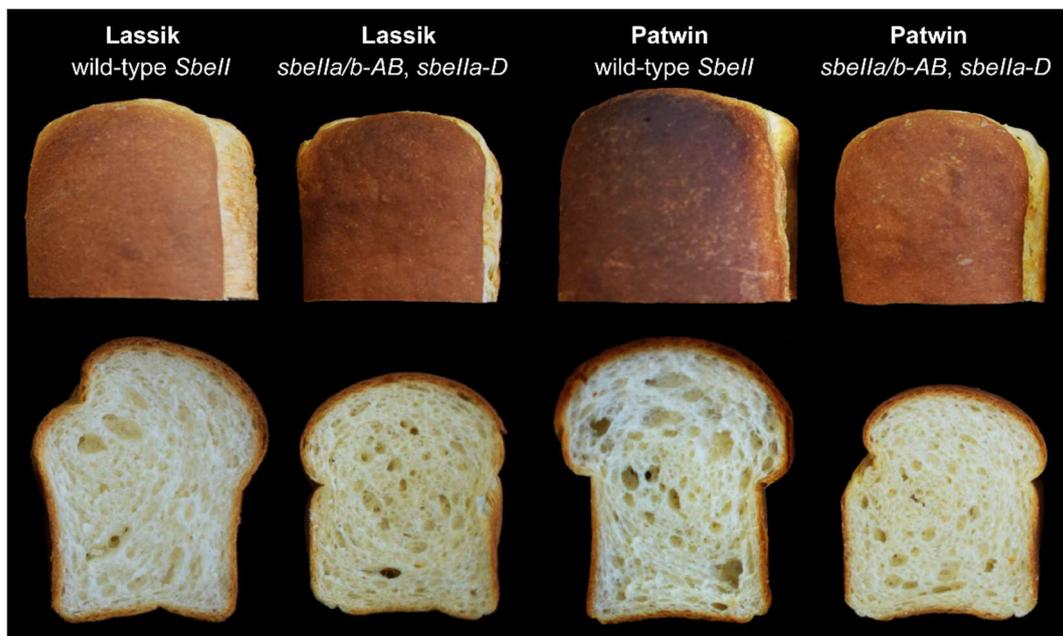


Figure 1: Loaves of wild-type *Sbell* controls and *sbella/b-AB, sbella-D* mutant lines baked using approved method 10-10.03 (AACC International 1999).

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Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

The ancestral origin of Australian wheat

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Key message: Tracking the ancestral origin of Australian wheat since its introduction revealed a complex mosaic structure and a large recent shrinkage in genetic diversity.

Wheat is the most important grain crop grown in Australia. Since its introduction by the first fleet settlers, efforts to breed wheat varieties adapted to Australia's climate have ensued. Initial efforts focused on importing varieties from countries with comparable growing environments; latter efforts involved breeding practices using wheat imported from specific geographical regions. Understanding the ancestral origin of Australian wheat germplasm is important for maintaining long term selection gains in breeding programs, as this knowledge can be used to minimize the loss of genetic diversity over time. This research utilized almost 500 varieties representing the breeding history of bread wheat in Australia since 1840 to define the geographical ancestral background of Australian wheat germplasm by comparing it with a global collection using *in silico* painting. This approach tests the admixture of populations by considering recipient individuals (Australian germplasm) as a mosaic of the donor populations (worldwide germplasm). Our analysis revealed that Australian wheat history can be divided into three main eras. Before 1920, breeders collected varieties from around the world with extensive dependence on European wheat to select potential cultivars that could tolerate Australian growing conditions. Between 1920 and 1970, a dependence on African wheat germplasm became evident but the total germplasm continued to have large diversity. A heavy reliance on CIMMYT germplasm has persisted since 1970 to date. The reduction in genetic diversity since 1970 was further confirmed by estimating the effective population size (N_e) and the linkage disequilibrium (LD) decay for the three time eras. Between 1921 and 1970, N_e was equal to 23 but shrink to 9.4 for cultivars released after 1970. Moreover, third era cultivars had the larger LD blocks comparing to the first two eras. Our study of the ancestral background of Australian wheat documents the evolutionary history of wheat breeding in Australia and provides an understanding for how the wheat genome has been adapted to local growing conditions. This information will be useful for identifying new sources of genetic variation to assist the development of improved wheat varieties. It will also provide a guide for industry to assist with maintain genetic diversity for long term selection gains and plan future crosses.



Topic: Applying Novel Tools to Practical Wheat Improvement

Wheat-bacteria positive interactions: would old wheat genotypes be our hope for the future of agriculture?

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Key words: Fluorescent tagging, plant health & development, *Pseudomonas fluorescens*, screening, sustainable agriculture, *Triticum aestivum*

To sustain the exponential human population growth, current agriculture focuses on productivity. This modern agriculture, democratized in the 1950s, is notably based on the use of chemical inputs (mineral fertilizers, pesticides) and plant genotypes (varieties) able to benefit from these inputs and presenting the highest yields. However, global warming and detrimental environmental issues caused by the use of fertilizers and pesticides make it necessary to seek complementary ways for a more sustainable agriculture. Taking advantage of plant-beneficial bacteria referred to as PGPR (plant growth-promoting rhizobacteria) from the rhizosphere, defined as the soil in contact with the roots, may be one solution. These bacteria, naturally present in arable soils, can colonize the roots of crops, express genes encoding plant-beneficial functions, and improve plant growth and health. Increase in crop productivity occurring with modern plant breeding has been obtained without considering the interactions between PGPR and roots. In addition, the use of chemical inputs could have made dispensable the interactions of modern varieties with PGPR. Thus, our hypothesis is that the ability of these varieties to positively interact with PGPR is lower than that of older varieties (before 1950s). To address this issue, we compared old and modern wheat varieties for their ability to favor root colonization by the model PGPR strain *Pseudomonas fluorescens* F113 and its expression of the bacterial operon *phl*, involved in the biosynthesis of 2,4-diacetylphloroglucinol (DAPG), an antimicrobial compound with root-branching properties. To quantify the interaction between strain F113 and wheat genotypes, a double fluorescent labelling - constitutive (red) for root colonization and inducible (green) for gene expression - has been used. There was a trend for better interactions with the old varieties, in terms of higher colonization and/or *phl* expression, in comparison with modern varieties, but several modern varieties performed as well as older ones. This work shows that the ability to interact with PGPR has been maintained in certain modern crop varieties, and this type of trait is worth considering in breeding strategies to make a better use of symbiotic microorganisms.



Topic: Applying Novel Tools to Practical Wheat Improvement

Testing non-transgenic CRISPR technology for wheat improvement

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Key message: CRISPR-Cas9 is fast becoming the gene modification method of choice for researchers in many disciplines. Here we present a ‘proof-of-concept’ experiment to test its use in a wheat breeding program.

CRISPR-Cas9 is fast becoming the gene modification method of choice for researchers in many disciplines because it is easy, relatively cheap, requires little expertise, and does not currently fall under the United States Department of Agriculture's (USDA) regulation of genetically modified plant material. It utilizes a guide RNA (gRNA) designed to target a specific sequence and a non-specific Cas9 endonuclease to create insertions/deletions (indels), delete strings of DNA, or add strings of DNA using double strand breakage and non-homologous end joining (NHEJ). This opens up a world of crop improvement possibilities that were previously infeasible due to a lack of traits in existing germplasm, tightly-linked undesirable traits, and/or extensive breeding times. Many important traits in wheat are attainable with simple indels, which do not currently exist in usable germplasm, but would be possible to incorporate using CRISPR technology. The caveat is the need to achieve editing with no off-target effects and no incorporation of foreign DNA. Here we present a ‘proof-of-concept’ experiment to test the use of CRISPR in a wheat breeding program. The first step is testing the efficacy and accuracy of CRISPR on a single major gene with a known sequence and an easily observable phenotype. Puroindoline b (*PinB*) is a grain hardness gene located on chromosome 5D, in which the wild type allele produces a soft endosperm, while a truncated mutant allele produces a hard endosperm. Single guide RNAs (sgRNA) will be designed to target the *PinB* gene, the Cas9/sgRNA construct will cleave the DNA, the ends will be repaired by NHEJ, and this will result in indels and thereby truncated genes. The main difficulty is the introduction of the Cas9/sgRNA construct in a way that does not result in any incorporation of foreign DNA. To achieve this, the Cas9/sgRNA construct will be delivered to the wheat plants in two ways: (i) *In vitro* transcripts of Cas9 and the sgRNA will be delivered to wheat scutellar tissue and/or callus using particle bombardment, and (ii) virus induced gene editing (VIGE) using RNA viral constructs containing Cas9 and sgRNA will be delivered to young seedlings through leaf abrasion. Successful gene modifications will be confirmed using PCR and Sanger sequencing in the transformed plants, their seeds, and their progeny.



Topic: Applying Novel Tools to Practical Wheat Improvement

Wheat genome editing with the CRISPR-Cas system

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Key message: We established a procedure for identifying highly active gRNAs, and tested two different methods for making precise edits via homology-directed repair.

The CRISPR-Cas system is a powerful and flexible tool for genome editing in wheat (Zhang et al. 2016). However, editing efficiencies tend to be lower in wheat compared with other crops. Furthermore, although gene knockout via error-prone non-homologous end joining (NHEJ) has been demonstrated, precise editing of the wheat genome via homology-directed repair (HDR) has not. To address the issue of low editing efficiencies in wheat, we established a procedure for identifying highly active gRNAs based on protoplast transfection and TIDE analysis of Sanger sequence reads. In doing so, we identified several active gRNAs targeting *Epsp* synthase (Figure 1). We also detected off-target mutations at low frequency. Next, we tested two different methods for making precise edits via HDR. The first method, which has been shown to be highly effective in human cells, uses an asymmetric single-stranded oligo donor template (Richardson et al. 2016). The second method, which has been shown to be highly effective in dicots, uses a donor template that replicates as part of a Geminivirus replicon (Baltes et al. 2014). HDR was detected by PCR using primers that specifically amplify gDNA containing the desired edit. We are now assessing the efficiency and fidelity of HDR.

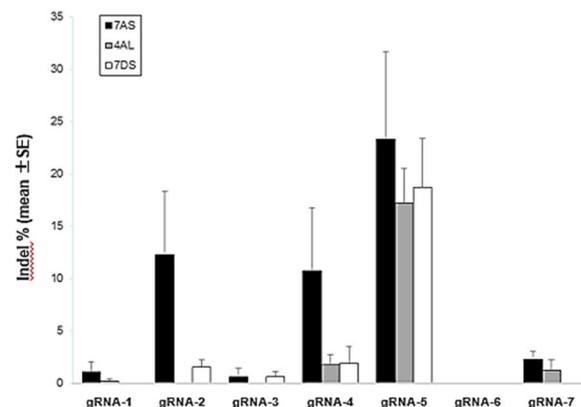


Figure 1: Activity of seven gRNAs targeting homoeologous copies of *Epsp* synthase in wheat protoplasts, based on TIDE analysis of forward and reverse Sanger sequence reads for three biological replicates.

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Topic: Future of Wheat Improvement in Different Parts of the World

Building on the Borlaug Global Rust Initiative: delivering genetic gain in wheat

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Key message: The BGRI is expanding its reach to battle more threats to wheat and is championing genomic selection and high throughput phenotyping to accelerate the improvement and delivery of wheat varieties.

The discovery of stem rust Ug99 in East Africa in 1998 threatened to wipe out over 80% of the world's wheat. Flat funding and decades of complacency meant that wheat stem rust knowledge was held by too few scientists. Realizing that wheat farmers globally were at risk, Nobel Laureate Norman Borlaug rallied world leaders to support wheat rust research in labs and universities all over the world. He established the Borlaug Global Rust Initiative (BGRI) in 2005 to meet the threat head-on, and was instrumental in launching the Durable Rust Resistance in Wheat (DRRW) project (2008-2016). The DRRW coordinated the global effort to fight Ug99 and related races, increasing capacity in national agricultural institutes and delivering improved seeds to wheat farmers. The DRRW developed surveillance networks, screening facilities and knowledge-based platforms in Africa and South Asia to track the pathogen, identify and test underutilized stem rust resistant genes, and breed new high-yielding stem rust resistant wheat varieties. As a result of the DRRW, 40 Ug99 resistant genes were identified, 30 genetic markers for stem rust resistance genes are available and over 80 stem rust resistant wheat varieties were bred and are currently being grown in targeted regions. Additionally, over 120 scientists from East Africa and SAARC region received formal training in wheat breeding, survey methodologies, and the genetics of resistance in addition to hands-on lab and field experience in resistance screening. Global wheat production faces many challenges beyond rust diseases. It is for this reason that the DRRW evolved into a new phase called Delivering Genetic Gain in Wheat, (DGGW) in 2016. Within the DGGW, partner institutions will collaborate globally to build on the successes of the DRRW. In the DGGW, increased effort will be focused on implementation of Genomic Selection and high throughput phenotyping in CIMMYT wheat breeding programs, with the goal of using cutting-edge technologies and methods to shorten breeding cycles, improve genetic resistance, and ultimately develop and deploy varieties of wheat that incorporate climate resiliency and multiple disease resistance for smallholder farmers in politically vulnerable regions of the world. With the idea that the way forward in wheat research is collaboration, the BGRI aims to engage the broader wheat community beyond the DGGW to foster collaboration among other research projects, including enabling data and knowledge exchange, implementing training sessions targeted to specific groups and creating and maintaining online learning resources.



Topic: Future of Wheat Improvement in Different Parts of the World

Genetic gains for grain yield, disease resistance and other traits in CIMMYT spring wheat germplasm targeted for adaptation in diverse environments of Asia, Middle East, Africa and Latin America

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Key message: CIMMYT spring wheat improvement continues to deliver new germplasm with about 1% annual genetic gains for grain yield combined with durable disease resistance, stress tolerance, end-use and nutritional quality.

Improved and diverse wheat germplasm from CGIAR, distributed annually through targeted international yield trials and nurseries to Wheat Improvement Network partners worldwide, has been the source of over 70% new varieties either as direct releases or through utilization as parents by partners in the targeted regions of Africa, Middle East, Africa and Latin America in the last decade. An up scaled breeding, utilizing conventional and modern strategies and tools, has enabled progress of about 1% annual genetic gains in grain yield potential simultaneously with resistance to rusts and other major diseases, tolerance to drought and heat stresses and end-use and nutritional (high Zn) qualities. Two generations per year shuttle breeding at field sites in Mexico and Kenya are used for selecting in segregating populations, and managed phenotyping conducted to determine performance of advanced lines for grain yield, agronomic traits, disease resistance, stress tolerance and quality traits. Genomic selection and high throughput phenotyping strategies are under testing and implementation in the breeding program to enhance the rates of genetic gains. Utilization of slow rusting, minor but additive genes based adult plant resistance (APR) to leaf rust has been mainstreamed in the breeding program, which has resulted in a rapid decline of leaf rust where CIMMYT-derived varieties are grown. About 10-15% germplasm distributed internationally in recent years possesses near-immune APR to races belonging to the Ug99 lineage and being promoted for release in East Africa where high stem rust pressures are common and resistance durability is crucial to protect crop losses. In addition, 40-50% germplasm carries moderate but adequate levels of APR and 20-30 % possesses diverse race-specific resistance genes (R-genes). The worldwide spread and fast evolution of aggressive and temperature tolerant lineages, *Pst1* and *Pst2*, of yellow rust fungus has posed a new challenge to breeding APR especially for areas where yellow rust infection initiates in seedling growth stages under prolonged cool and humid conditions. A strategy that allows field based selection of APR genes with small to intermediate effects R-genes to achieve high resistance levels is being followed. We conclude that new wheat germplasm should continue to result in releases of new popular varieties that have combined genetic gains for yield potential, climate resilience, disease resistance, processing and nutritional quality attributes, and therefore productivity and profitability are enhanced in targeted regions and beyond.



Topic: Future of Wheat Improvement in Different Parts of the World

Wheat breeding at ICARDA: strategies, achievements and prospects

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Key message: The presentation summarizes the challenges and constraints of wheat production in CWANA and SSA regions; ICARDA's breeding strategies and approaches; research results and future recommendations.

Wheat is the most important and strategic food crop in Central and West Asia and North Africa (CWANA) region with average consumption of 200 kg per capita and year. The productivity of wheat in the region is generally very low (2.5 t/ha) principally due to prevalence of major abiotic and biotic stresses. The magnitude and intensity of these stresses are increasing dramatically due to climate change causing recurrent droughts, increased temperature and emergence of aggressive strains of pests and diseases. The ICARDA wheat breeding program applies both conventional and molecular approaches including targeting mega environments, FIGS, shuttle breeding, doubled haploids, marker assisted selection, and key location phenotyping to develop high yielding and widely adapted wheat genotypes with increased water-use efficiency, heat tolerance and resistance to major diseases and pests. In the last 5 years, more than 2000 bread wheat genotypes have been distributed through IN and more than 30 varieties of ICARDA origin have been released by the national programs in the CWANA and SSA regions. Some of the current elite genotypes showed a yield level of up to 2.5 t/ha under drought (250-300 mm) and 11 t/ha under irrigated/optimum (550 mm) conditions. The protein level ranges from 12 to 16% with high frequency of the 5+10 (*Glu-D1*), 7+8 (*Glu-B1*) and 2* (*Glu-A1*) alleles in the elite set of genotypes. Molecular markers linked to drought, heat tolerance and yellow rust resistances have been identified. DArT markers wPt731910 (3B), wPt4680 (4A), wPt3509 (5A), wPt8183 (6B), and wPt0298 (2D) were significantly associated with yield under rain-fed conditions. Under irrigated condition, tPt4125 on chromosome 2B was significantly associated with yield explaining about 13% of the variation. Markers wPt2607 and wPt1482 on 5B were highly associated with protein content and alveograph dough strength (W) explaining 16 and 14% of the variations, respectively. More than 10 SNP markers on chromosome 5A which are significantly associated with wheat yield under heat stress at Wadmedani, Sudan have been identified. Similarly, SNP markers associated significantly with yellow rust resistance have been identified. Pedigree analysis showed that resistance sources for heat and drought in such elite germplasm were introgressed from synthetic wheats and wild relatives mainly *Triticum dicoccoides*. Currently, pre-breeding activities are underway to add novel diversity for complex traits such as drought, heat and salinity tolerance from wild species of different wheat gene pools. The elite genotypes are recommended for direct release/parentage purposes by the respective NARSs.



Topic: Future of Wheat Improvement in Different Parts of the World

Russia - wheat grain exporter no. 1: contribution of breeding, genomics and genetic resources

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Key message: In 2016 the Russian Federation has become the leading wheat exporting country. Contribution of public wheat breeding and genetics research, genetic resources to reaching this goal is substantial.

In 2016 the Russian Federation is expected to become the leading wheat grain exporting country with the estimate of 30 million t (<http://www.indexmundi.com/agriculture/?commodity=wheat&graph=exports>). Total wheat area in Russia in 2016 was 27.7 million ha with the average yield of 2.8 t/ha and overall production 75.8 million t, an increase of 18.7% compared to 2015 (<http://ab-centre.ru>). The Southern Federal Region (Adygeya, Kalmykia, Krasnodar, Rostov, Stavropol and Volgograd) is growing mainly winter wheat (5.5 million ha) and contributes ≈30% of total production. The Central Federal Region is growing both winter and spring wheat (4.1 million ha) and contributes ≈20%, followed by the Volga region (spring and winter wheat with 6.8 million ha) with 20% and Siberia (6.6 million ha of spring wheat) and 15%. For bread wheat, 304 winter wheat and 211 spring wheat varieties were registered by 2015. For durum wheat there are 26 winter and 45 spring varieties registered. The majority of the varieties cultivated in Russia originate from local public breeding programs. The key winter wheat breeding institutions are the Agricultural Research Institutes in Krasnodar, Rostov and Moscow. For spring wheat the leading breeding programs are located in Saratov, Samara, Chelyabinsk, Omsk and Barnaul. Leaf rust represents a major disease across all wheat production areas. Resistance in winter wheat is based on diverse genes including APR. In spring wheat, major genes dominate including introgressions from wild species. Stem rust recently affected the wheat crop in Western Siberia though numerous sources of resistance including Ug99 are available. Studies on the genetic gain in spring wheat demonstrated a yield progress of 0.7-0.8% without incorporation of *Rht* genes. The frequency of the 1B.1R translocation is relatively low in spring wheat, whereas in winter wheat its frequency reaches 35-40%. The utilization of genetic resources has been an important component of wheat breeding led by the Vavilov Institute. Spring wheat breeding programs are united through two networks: EKADA in the European part of Russia and KASIB in Northern Kazakhstan and Western Siberia. Basic research on wheat is led by the institutes in Moscow, St. Petersburg and Novosibirsk, with emphasis on the development of genomic applications for practical breeding. The government policies supporting agricultural production including wheat cannot be underestimated in making Russia a leading wheat exporting country. However, there is great contribution of breeding and research programs to this achievement. The challenges of sustainable production gains are related to climate change and associated biotic and abiotic stresses.



Topic: Future of Wheat Improvement in Different Parts of the World

Genetics-based breeding in winter wheat has led to modern elite varieties with broad resistance to major diseases

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Key message: Breeding progress in European wheat during recent decades is reflected in an extensively improved disease resistance. This partially explains the yield enhancement and is a major reason for the better yield stability of modern wheat varieties.

Various field and statistical studies have documented a substantial improvement of modern versus former wheat varieties in Europe and elsewhere. The breeding progress is particularly relevant in European winter wheat and amounts to an average annual grain yield increase of ≈ 100 kg per ha on farm level. This improvement is considered to be partially due to the breeding efforts in the last half-century. During the last decade, repeated multi-locational exact field trials have been conducted in Germany, including the current collaborative BRIWECS project with a large set of winter wheat cultivars released and cultivated in Germany since the 1960s until today. The results of disease and yield assessments demonstrate a significant genetic improvement of resistance against major fungal diseases as well as grain yield. Particular improvements have been achieved for resistance against powdery mildew (*Blumeria graminis* f. sp. *tritici*) and rust diseases such as brown or leaf rust (*Puccinia triticina*) and stripe or yellow rust (*P. striiformis* f. sp. *tritici*). In the case of such diseases, major resistance genes have been identified and used for breeding and wheat improvement. However, the recent occurrence of virulent rust pathogen races (e.g. Warrior race) has overcome existing resistance (genes). On the contrary, the progress has been weaker in case of more complex host-pathogen interactions such as wheat-*Fusarium* (FHB) or wheat-*Septoria*. In such cases, partial resistance is obviously controlled polygenically and, therefore, more difficult to improve. Genes involved are being gradually identified. Beyond, new breeding methods such as genomic selection (GS) are expected to enable future progress and the development of elite material with quantitative, broad-spectrum disease resistance.

Acknowledgement

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P 1 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Structural variability of *VRN-1* vernalization genes during evolution of wheat: impact to origin of the spring growth habit

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Key message: Variability of the regulatory sites of *VRN-1* gene during evolution and the origin of the spring growth habit in emmer and Timopheev' wheats.

Most of the wild Triticeae species have a winter growth habit, suggesting that the recessive *VRN-1* allele is the ancestral form. By contrast, there are many cultivated polyploid wheat varieties with a spring growth habit and with at least one dominant *VRN-1* allele. Polyploid wheat is divided into two evolutionary lineages, emmer (BBAA) and Timopheev's wheat (GGAA), which formed through hybridization between *Triticum urartu* and *Aegilops speltoides*, and divergence of the first wild tetraploids *T. dicoccoides* (BBAA) and *T. araraticum* (GGAA) occurred over the course of several tens of thousands years. The appearance of spring wheat forms is presumably associated with mutations in the promoter or 1st intron of *VRN-1* locus, mainly, deletions of different lengths. We focused on variability of the regulatory sites of *VRN-1* gene during evolution and on origin of the spring growth habit in emmer and Timopheev's wheats. The promoter and 1st intron of *VRN-1* locus were analyzed for 40 and 23 accessions of diploid progenitors, *T. urartu* and *Ae. speltoides*, for 79 and 45 accessions of wild tetraploids *T. dicoccoides* and *T. araraticum*, respectively (Table 1). The analysis of cultivated wheats included more than 200 accessions, representing tetraploid wheat species, and more than 400 of hexaploid wheat species. No significant intraspecific variation was found at the *VRN-1* genes of *T. urartu* and *Ae. speltoides*. There were only minor changes in the studied *Ae. speltoides* accessions comparing with wheat species. Different indels were revealed in both the promoter and 1st intron of *VRN-1* providing specific identification of *T. urartu*. During the first round of allopolyploidization a 8 bp insertion appeared in the promoter *VRN-1* region of wild tetraploid *T. dicoccoides* and then was inherited by other emmer wheats. The other events, namely, a 50 bp deletion in the promoter region took place during evolution of *T. timopheevii*. These cases of insertion and deletion appeared to be not associated with spring growth habit. Other mutations, which may influence on appearance of the spring wheats were revealed both in the promoter region and 1st intron of *VRN-1* genes of *T. dicoccoides* and in the 1st intron of *VRN-1* genes of *T. timopheevii* (Table 1). Impact of *VRN-1* mutations, including those in such regulatory sites of the promoter region as CArG-box, VRN-box and ACGT-motif, on origin of spring growth habit in cultivated polyploid wheats is discussed.

Acknowledgement

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Table 1: Variability in the promotor region and 1st intron of *VRN-1* genes.

Alleles	Species (haploid genome)	Status of Promoter/ changes	Status of Intron1/ changes	References
A - genome				
<i>vrn-A1</i>	<i>T. aestivum</i> (BAD) <i>T. durum</i> (BA) <i>T. dicoccum</i> (BA) <i>T. dicoccoides</i> (BA)	<i>vrn-A1</i> / 8 bp insertion vs. <i>vrn-A1</i> or <i>VRN-A1</i> of diploids	<i>vrn-A1</i> / no changes	[1-6]
<i>Vrn-A1a</i>	<i>T. aestivum</i> (BAD) <i>T. durum</i> (BA) <i>T. dicoccum</i> (BA)	<i>Vrn-A1a.1-a.3</i> / 231 or 211 or 52 bp insertions	<i>vrn-A1</i> / no changes	[1,2, 5,6]
<i>Vrn-A1d</i>	<i>T. dicoccoides</i> (BA)	<i>Vrn-A1d</i> / 19 and 32 bp deletions	<i>vrn-A1</i> / no changes	[1-3]
<i>VRN-A1b*</i>	<i>T. aestivum</i> (BAD) <i>T. durum</i> (BA) <i>T. dicoccum</i> (BA) <i>T. dicoccoides</i> (BA)	<i>VRN-A1b</i> / 19 bp deletion <i>VRN-A1b.1-b.7</i> / +2 bp+SNP (2-5)	<i>vrn-A1</i> / no changes	[1-3, 5,6]
<i>Vrn-A1e</i>	<i>T. durum</i> (BA) <i>T. dicoccum</i> (BA)	<i>Vrn-A1e</i> / 54 bp deletion	<i>vrn-A1</i> / no changes	[1,2,5]
<i>Vrn-A1i</i>	<i>T. durum</i> (BA)	<i>Vrn-A1i</i> / SNP	<i>vrn-A1</i> / no changes	5-6
<i>Vrn-A1k</i>	<i>T. dicoccum</i> (BA)	<i>Vrn-A1k</i> / 42 bp insertion	<i>vrn-A1</i> / no changes	KX874608
<i>Vrn-A1c</i>	<i>T. aestivum</i> (BAD)	<i>vrn-A1</i> / no changes	<i>Vrn-A1c</i> / 5.5 kbp deletion	[1]
<i>Vrn-A1L</i>	<i>T. dicoccoides</i> (BA) <i>T. durum</i> (BA)	<i>vrn-A1</i> / no changes	<i>Vrn-A1L</i> / 7.2 kbp deletion	[3,4]
<i>vrn-A1u</i>	<i>T. urartu</i> (AA)	<i>vrn-A1u</i> / see <i>vrn-A1</i> of polyploids	<i>vrn-A1</i> / no changes	[3]
<i>vrn-A1f**</i>	<i>T. aestivum</i> (BAD) <i>T. araraticum</i> (GA) <i>T. timopheevii</i> (GA)	<i>vrn-A1f</i> / 50 bp deletion	<i>vrn-A1</i> ?/ no data, in some cases a large mutations were found (see below)	[2,7]
<i>vrn-A1f-del</i>	<i>T. araraticum</i> (GA)	<i>vrn-A1f</i> / 50 bp deletion	<i>vrn-A1del</i> / 2.7 kbp deletion	[7]
<i>Vrn-A1f-ins</i>	<i>T. timopheevii</i> (GA)	<i>vrn-A1f</i> / 50 bp deletion	<i>Vrn-A1ins</i> / 0.4 kbp insertion	[7]
<i>Vrn-A1f-del/ins</i>	<i>T. timopheevii</i> (GA)	<i>vrn-A1f</i> / 50 bp deletion	<i>Vrn-A1del/ins</i> / 0.4 kbp insertion, 2.7 kbp deletion	[7]
B - genome				
<i>Vrn-B1a</i>	<i>T. aestivum</i> (BAD) <i>T. durum</i> (BA)	<i>vrn-B1</i> / no changes	<i>Vrn-B1a</i> / 6.8 kbp deletion	[4-6]
<i>VRN-B1b*</i>	<i>T. aestivum</i> (BAD)	<i>vrn-B1</i> / no changes	<i>VRN-B1b</i> / 36 bp deletion	[8]
<i>Vrn-B1c</i>	<i>T. aestivum</i> (BAD) <i>T. durum</i> (BA) <i>T. dicoccum</i> (BA)	<i>vrn-B1</i> / no changes	<i>Vrn-B1c</i> / 7.6 kbp deletion, 0.4 kbp duplication	[5,6,9,10]
<i>vrn-B1sp</i>	<i>Ae. speltoides</i> (B/G)	<i>vrn-B1sp</i> / minor changes (substitutions) vs. <i>vrn-B1</i>	<i>vrn-B1</i> / no changes	[7]
<i>vrn-B1dic</i>	<i>T. dicoccoides</i> (BA)	<i>vrn-B1dic</i> / 2 bp insertion vs. <i>vrn-B1</i>	<i>vrn-B1</i> / no changes	[3]
<i>vrn-B1</i>	<i>T. aestivum</i> (BAD) <i>T. durum</i> (BA) <i>T. dicoccum</i> (BA) <i>T. dicoccoides</i> (BA)	<i>vrn-B1</i> / no changes	<i>vrn-B1</i> / no changes	[1-6]
<i>vrn-G1a</i>	<i>T. araraticum</i> (GA) <i>T. timopheevii</i> (GA)	<i>vrn-G1a</i> / 0.2 kbp insertion	<i>vrn-G1</i> / no changes	[2,7]

*Some accessions carrying these alleles are associated with winter growth habit. Six variants of this allele were identified (Milec et al. 2012, Shcherban et al. 2012). The *Vrn-A1b.1/2/5/6* allelic variants are associated with spring growth habit, while the *vrn-A1b.3* and *vrn-A1b.4* alleles were found exclusively in winter wheat.

** This allele was firstly described as dominant (Golovkina et al. 2010), however, research by Muterko et al. (2016) revealed no association with spring growth type.



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Number in squared brackets at the end of the references correspond to the reference numbers in Table 1.



P 3 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Genotyping by sequencing (GBS) approaches to verification of wheat precise genetic stocks in germplasm collections

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Key message: Use of genome by sequencing (GBS) data is a useful tool in verifying the presence of the crop wild relative (CWR) chromosome in accessions of wheat-CWR addition and substitution lines.

The Germplasm Resources Unit (GRU) at the John Innes Centre, Norwich, UK, holds a substantial set of material we term as 'Wheat Precise Genetic Stocks' (WPGS), comprising sets of wheat aneuploids, wheat amphiploids, and wheat -alien chromosomal addition and substitution lines, assembled (and in many cases created by) the cytogenetics team originally at the Plant Breeding Institute, Cambridge, UK, and continued at the John Innes Centre. The addition and substitution line class in particular is a largely untapped resource which can be used to examine the phenotypic effect of exotic alleles within a hexaploid wheat background. Such alleles are likely to be required to meet the 'grand challenges' (drought, salinity, climate change and emergent pathogen genotypes) facing wheat breeding in the 21st century. Whilst valuable, the collection presents a challenge for regeneration as it is often meiotically unstable and requires analysis after multiplication to verify the presence of the alien chromosome in the progeny. This poster presents the GBS-based approaches I am trialling to try and verify our multiplied WPGS stocks going forward.



P 5 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Identification of genes on the midget chromosome in a common wheat with rye cytoplasm

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In a common wheat with rye cytoplasm, (*cereale*)-Chinese Spring (CS), an extremely small chromosome, named 'midget' is observed (Nakata et al. 1988). This midget chromosome is thought to carry the genes essential for functioning rye cytoplasm, because no transmission of the midget chromosome results in endosperm abortion, and most of the resultant shriveled seeds are not germinable. To date, some repetitive DNA sequence families have been identified to be localized on the midget chromosome (Murata et al. 1992) but no functional genes identified. In this study, PCR-markers that have been mapped on 1R chromosome were investigated to know their linkage to the midget chromosome. As a result, one marker gene was identified to be linked to the plump seeds of (*cereale*)-CS, and some other genes encoding mitochondria-targeting proteins were detected in the flanking regions of the marker when the barley chromosome 1H map was used as a reference. In addition, RNA-seq analysis was also applied to identify genes on the midget chromosome. Reads putatively encoding genes on the midget chromosome were selected based on the DNA sequence homologies to rye 1R chromosome and to long arms of wheat homeologous 1-group chromosomes, and on whether the proteins deduced from reads contain the mitochondria-targeting signal. Out of approx. 650 reads selected, two protein-encoding genes deduced from reads were found to exist on the midget chromosome. Those two genes seem to be located at pericentromeric region of the midget chromosome, because their barley orthologs have been mapped at the similar region of chromosome 1H. Candidates for *Rcs* (*rye cytoplasm-specific*), a gene(s) that is essential for functioning rye cytoplasm and developing endosperm, are further pared down by crossing with 1R chromosome addition lines of CS, in which long arms of 1R chromosome are rearranged by the gametocidal system (Tsuchida et al. 2008). Finally, a couple of genes remained as possible candidates for *Rcs*.

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P 7 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Breeding and cytogenetic analysis of perennial wheat in the cold regions of Northeastern China

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Key words: cytogenetic, genomic *in situ* hybridization, perennial wheat, *Thinopyrum intermedium*

Cold-hardy perennial wheat plays an important role in the use of barren land for farming, in soil and water conservation, and also for increasing wheat and grass yield. Since 2006, we have been working to develop perennial wheat by crossing the octoploid tritelytrigia 'Ganmai 8' and 'Ganmai 9' with *Thinopyrum intermedium*. The traits of F₁ hybrids were intermediate between the parents; plants were perennial, with luxuriant growth, but had no seed in their first year. At subsequent 2-3 years, a small amount of seeds were produced, and the chromosome number was 49; F₂-F₃ generation plants were mostly of the intermediate types, perennial, and the chromosome numbers varied between 48 and 56. From F₄ to F₆, the separations of three types were observed, including grass types, tritelytrigia types, and common wheat types. Using morphological and molecular cytogenetic methods, perennial wheat was bred that is hardy to -30°C in the field during the winter in Harbin, and exhibited post sexual cycle regrowth, perenniality, seed weight, winter hardiness, and vigor. The chromosome number was 44-56. We performed genomic *in situ* hybridization (GISH) on root tip cells from these perennial lines using digoxigenin-labeled genomic DNA from *Th. intermedium* as a probe. Signal detection was accomplished using anti-digoxin rhodamine. Post-harvest regrowth plants of the perennial Line 4-25-29 (Figure 1A-C) can be survived after the cold winter (between -15°C and -30°C) and regrow at next year in April. Using GISH test, fluorescent signals clearly indicate that 14 of the 46 chromosomes of perennial line 4-25-29 (Figure 2A,B) are of alien origin, and the efficacy of probe binding indicates a member of the *Th. intermedium* as the alien donor. We also observed some perennial lines that the chromosome constitutions are 56 chromosomes, but the alien chromosomes from *Thinopyrum* species are more than 14 and the most one is 24. The further analysis with gDNA as a probe from *Pseudoroegneria spicata* (2n = 14, StSt) for GISH and molecular marker of St chromosome indicates that the alien chromosomes are mainly from *P. spicata*. These chromosomes closely control the traits of perennial wheat, such as post-harvest regrowth, winter hardiness, and perenniality. The genomic origin of the additional alien chromosomes will lead to the naming of a new species of wheat. This new species and improved varieties within this species will have value in perennial wheat breeding programs.

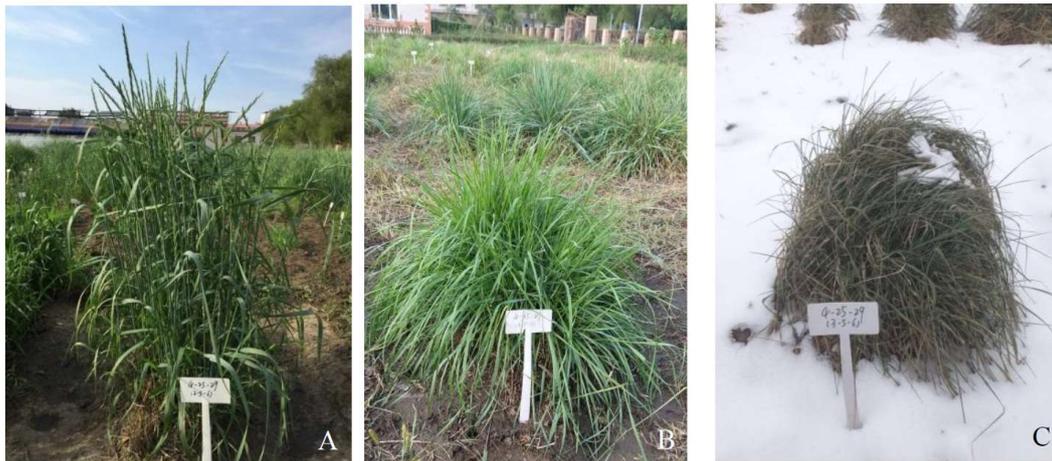


Figure 1: Plant of perennial line 4-25-29 in the field: (A) in June; (B) post-harvest regrowth in September; (C) plant in November.

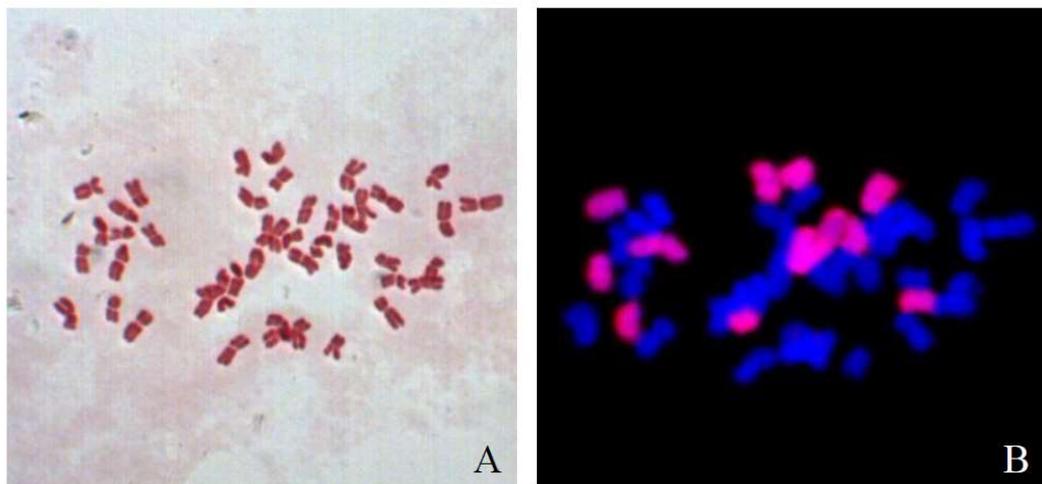


Figure 2: Chromosomes of perennial line 4-25-29: (A) Chromosomes of the root tip cell; 46 chromosomes; (B) GISH reveals 14 chromosomes from *Thinopyrum intermedium* (pink) and 32 chromosomes from common wheat (blue).



P 9 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Molecular cytogenetic tools in characterization of pre-breeding materials produced with *Thinopyrum* species

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Key message: Pre-breeding materials produced with different *Thinopyrum* species were analyzed using molecular cytogenetic methods. To increase the frequency of selection process, marker-assisted selection was applied.

Wild relatives of cultivated wheat represent a rich potential source of genetic variation for many agriculturally significant characteristics. Perennial Triticeae species, genotypes of the *Thinopyrum* genus, are important as tertiary gene pools for wheat improvement. Understanding the organization of the genomes in the *Thinopyrum* genus and their phylogenetic relationships with other related species will greatly facilitate the utilization of these species for transferring agronomically useful genes into bread wheat. Detailed FISH-based karyotype of three diploid wheatgrass species, *Agropyron cristatum* [(L.) Beauv.] v. *Ag. cristatum* (L.) Gaertn., *Thinopyrum bessarabicum* [(Savul.&Rayss) A. Löve], *Pseudoroegneria spicata* [(Pursh) A. Löve], the supposed ancestors of hexaploid *Th. intermedium* [(Host) Barkworth & D.R.Dewey] compiled using DNA repeats and microsatellite markers. Fluorescence *in situ* hybridization (FISH) with repetitive DNA probes was suitable for the identification of individual chromosomes of the diploid JJ, SS and PP genomes. Among seven tested microsatellite markers only (GAA)_n trinucleotide sequence is appropriate to use as single chromosome marker for the *P. spicata* 1S chromosome. Based on COS marker analysis, phylogenetic relationship between diploid wheatgrasses and the hexaploid bread wheat genome was established. One of these findings supports that J and E genomes are in the neighbouring clusters. A *Th. intermedium* × *Th. ponticum* synthetic hybrid wheatgrass is an excellent source of leaf and stem rust resistance. Pre-breeding materials have been developed in Martonvásár and wheat line Mv9kr1 was crossed with this hybrid (*Agropyron glael*) in order to transfer its advantageous agronomic traits into wheat. Progenies were screened by *in situ* hybridization and disomic translocations were selected.

Acknowledgement

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P 11 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

A new cryptic alien introgression from *Haynaldia villosa* with powdery mildew resistance gene *Pm21*

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Key message: Development of a new cryptic alien introgression line harboring the *Pm21* gene and construction of a high density map with a large number of molecular marker covering the *Pm21* locus.

The wild species usually confer high resistance to the serious diseases and tolerance to the frequent abiotic stresses, so they provide rich gene resources for the improvement of the cultivated crop. Chromosome engineering is the widely used strategy for transferring the elite genes from the wild species to the domesticate crop, and GISH is the frequently used technique to identify the germplams with the alien chromosomes, but large chromosome segments transferred to the crop usually contained the disadvantageous redundant genes along with the target gene. Identification of the germplasm containing the target gene but with the smallest introgression alien chromosome segments is important for the cytogenetists. So, development of the specific markers distributed along the alien chromosomes with high density combined with the phenotype evaluation is a feasible approach to develop new germplasm valuable in the breeding. *Haynaldia villosa* ($2n = 2x = 14, VV$), a wild species of common wheat, confers high resistance to the powdery mildew mediated by *Pm21* located on the short arm of chromosome 6V. In the previous study, the substitution line DA6V, translocation line 6VS/6AL and large introgression line with *Pm21* were identified by GISH. In our study, a cryptic alien introgression line with very small introgression segment of *Haynaldia villosa* with powdery mildew resistance gene *Pm21* was identified by molecular markers analysis and powdery mildew resistance evaluation, and this line showed no fluorescent signal by GISH. This introgression line was crossed with different cultivated wheat to develop the population for evaluation the genetic effects of the introduced small segments, and the results indicated *Pm21* containing small segments had no other disadvantageous partner effects on the agronomic traits. The cryptic alien introgression line is a valuable material for the candidate gene screening especially for those target genes located on the alien chromosomes which cannot recombine with the chromosomes of the common wheat. So, a high density map with a large number of molecular marker was constructed covering the *Pm21* locus using the cryptic alien introgression line, and the comparative genetics using other model species were performed and the functional genes in the *Pm21* locus were screened. The following functional study of the candidate genes is being carried out.

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P 13 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Molecular cytogenetics analysis of *Thinopyrum intermedium* and its possible diploid progenitors *Th. bessarabicum*, *Pseudoroegneria spicata* and *Dasypyrum villosum*

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Key message: We studied organization of genomes *Thinopyrum intermedium* and its possible diploid progenitors *Th. bessarabicum*, *Pseudoroegneria spicata* and *Dasypyrum villosum* by use fluorescence *in situ* hybridization and genomic *in situ* hybridization approaches.

The close relative of wheat is intermediate wheatgrass *Thinopyrum intermedium* ($2n = 42$), an important forage crop and a valuable source of genes used for wheat improvement through wide hybridization. *Th. intermedium* is a segmental allohexaploid ($2n = 42$, J^JV^SSt) with complex genomic constitution and its possible diploid progenitors *Th. bessarabicum* (J^b), *Pseudoroegneria spicata* (St) and *Dasypyrum villosum* (V). We used fluorescence *in situ* hybridization (FISH) and genomic *in situ* hybridization approaches (GISH) to study organization of genomes of the species in study. We showed that repetitive DNA may be useful for FISH analysis of the four studied species. The localization of repetitive DNA may reflect the evolutionary changes occurred through the evolution of the genome donors and allohexaploid intermediate wheatgrass. Also we demonstrated that the reverse transcriptase part of Ty3/gypsy centromeric retrotransposon is highly conservative in *Th. intermedium* and its possible diploid progenitors *Th. bessarabicum*, *P. spicata* and *D. villosum* but the abundance of the repeats varied to a large extent. In addition, to study the evolution of repetitive fraction to more extent we carried out Illumina DNA sequencing and reconstructed the main repetitive DNA sequences of *Th. intermedium*, *Th. bessarabicum*, *P. spicata* and *D. villosum*. Data of comparative analyses of repetitive fraction of this species will be presented and discussed.

Acknowledgement

This study supported by Government Program supporting of the Leading Scientific Schools of Russian Federation (grant SS-8315.2016.11) and by grant #16-16-00097 of Russian Scientific Foundation.



P 15 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Karyotype diversity of emmer wheat helps reconstructing possible migration routes of the crop

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Key message: Our analysis revealed that chromosomal characteristics of emmer wheat can be associated with the geographical origin. Bases on karyotypic data we hypothesized possible migration routes of emmer from domestication centers to Europe, Asia and Asia.

Emmer wheat, *Triticum dicoccon* Schrank (syn. *T. dicoccum* (Schrank) Schübl.), is one of the oldest domesticated crops. Along with einkorn wheat, barley, lentil, pea, and flax, it constituted the 'founder crop' assemblage of the Old World. Based on C-banding analyses of a comprehensive collection of emmer wheat (527 lines from 43 countries) we showed that chromosomal characteristics can be associated with the geographical origin. In addition to C-banding polymorphisms, a total of 47 variants of chromosomal rearrangements were identified in 129 lines of *T. dicoccon* (19%), among them six variants were paracentric and pericentric inversions, 32 single translocations, seven double, three triple and one quadruple translocation (Figure 1). The T7A:5B translocation was most abundant in Western Europe and the Mediterranean, being found alone in 51 lines from 22 countries and in combination with secondary rearrangements in six other lines from different countries. Five major karyotypic groups were identified within *T. dicoccon*, each showing characteristic C-banding patterns and translocation spectra: the Balkan, Asian, European, Moroccan, and Ethiopian groups (Badaeva et al. 2015). The Balkan and Asian groups were cytogenetically most similar to wild emmer lines from southeast Turkey and may represent an early diffusion out of the Fertile Crescent (Figure 2). A second diffusion via the Mediterranean to Western Europe gave rise to the European group and probably started in southern Levant. Domestic emmer sampled in North Africa may have been introduced from Spain and was later hybridized with more recently introduced durum wheat or with *T. aethiopicum*. The Ethiopian group could have originated in the eastern Fertile Crescent possibly resulting from a secondary hybridization with wild emmer. This population then spread to Yemen, Oman and Ethiopia possibly via Arabian trading route and to India by other maritime route. Our data suggest that emmer found in the Volga region in Russia may have been introduced from the Balkans and Transcaucasia. The dates of these crop movements remain elusive.

Acknowledgements

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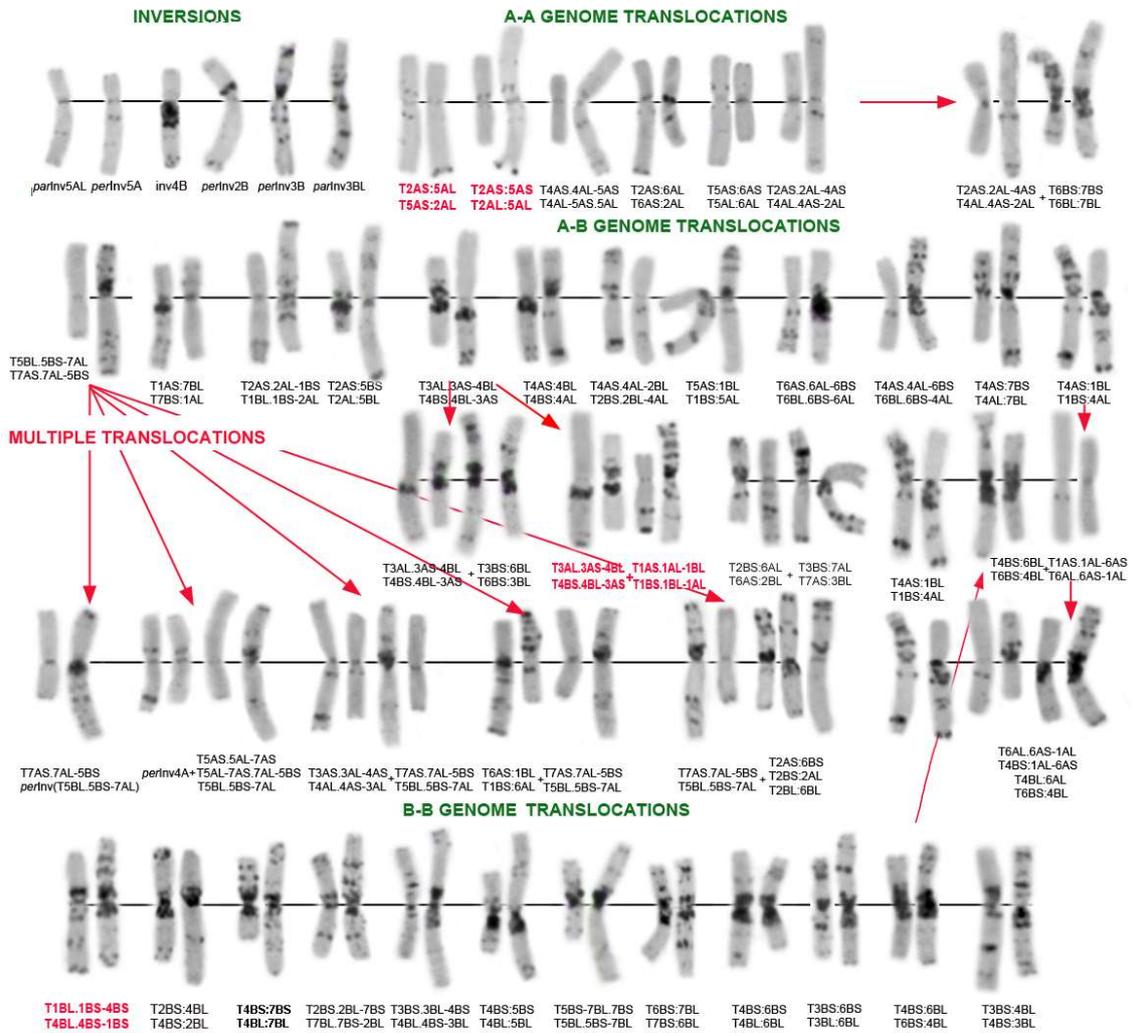


Figure 1: Chromosomal rearrangements and translocation lineages identified in domesticated emmer. The structures of translocated chromosomes are indicated below the respective variants. Translocation variants described earlier (Badaeva et al. 2015) are shown in black, novel translocation variants found in this study are indicated in red. Red arrows indicate translocation lineages, i.e. a series of related translocations occurring one after another.

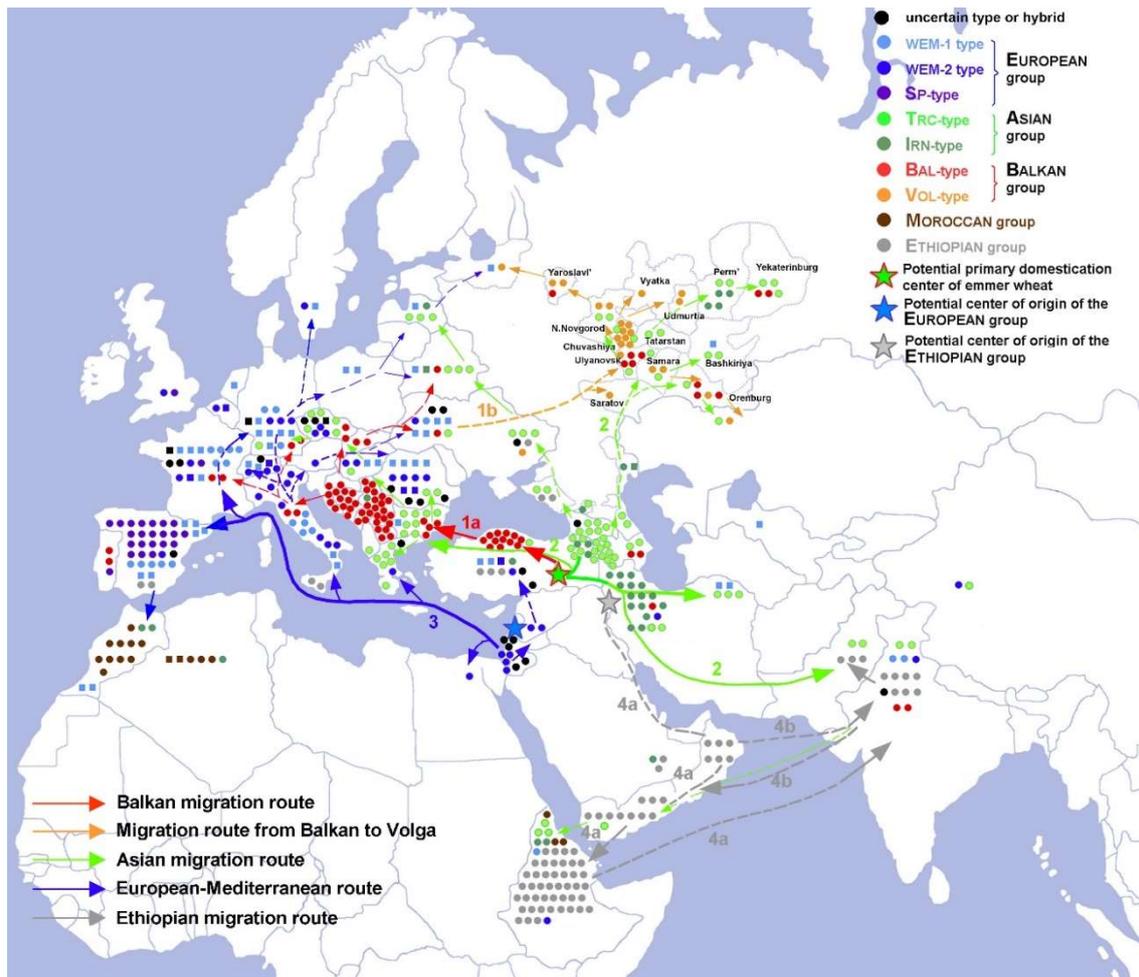


Figure 2: Distribution of chromosomal types and possible migration routes of domesticated emmer. One dot corresponds to one line studied; the dots are colored according to chromosomal groups estimated by visual comparison. Square dots designate lines carrying the 7A:5B translocation; round dots designate lines lacking the 7A:5B translocation. Solid lines designate migration routes supported by our data; dashed lines designate hypothetical migration routes, which were not confirmed in our study. Thick lines correspond to major migration routes, thin lines to secondary migration routes; 1a: possible migration route of BALKAN emmer from Anatolia to the Balkans; 1b: possible migration route of VOL-BALKAN emmer from the Balkans to the Volga region; 3: migration route from the Middle East to Mediterranean; 4a: possible migration route of ETHIOPIAN emmer from Zagros to Ethiopia via the Arabian trading route and to India by maritime route; 4b: possible migration route of ETHIOPIAN emmer from Zagros through India and Oman to Ethiopia.



P 17 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Evaluation of agronomic and quality characteristic in semi-dwarf Korean landrace wheat 'Anzunbaengimil'

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Key words: agronomy, Anzunbaengimil, flour, Korean-landrace, quality, semi-dwarf

The objective of this study was to evaluate the agronomic and quality characteristic in Korean landrace Anzunbaengimil which had been cultivated in Gyeongsangnam-do province in the Republic of Korea. The landrace was in Miryang, located in the South-East of the Korean peninsula, and investigated for its the agronomic characteristics. Grains of Anzunbaengimil were milled into flour with a Bühler experimental mill. Heading and maturity of Anzunbaengimil were earlier compared to Geumgang, the most popular variety in Korea. Plant height with 65 cm was significantly shorter than that of Geumgang. Spike-length was short (4.8 cm) but number of spikes per m² reached 950. Thousand grain weight is low (i.e. 29.2 g) and the whole meal and refined flour of Anzunbaengimil showed lower ash and protein content than that of Geumgang. Anzunbaengimil showed also a darker flour color, lower SDS-sedimentation value and dry gluten content. The average particle size of wheat flour was 44.75 µm (Heo et al. 2013). The evaluation of noodle properties revealed lower hardness and chewiness, but similar adhesive, elastic, gumness cohere to Geumgang. Anzunbaengimil exhibits soft grain texture similar to Geumgang.

Reference

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P 19 - Topic: Cytogenetics; Diversity and Evolution of the Triticeae

Development of wheat diversity map, Hap map, recombination map, selection map and trait map

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Key message: Genome-wide variations revealed by high density SNP array could tell what happened during wheat polyploidization, domestication and improvement.

Wheat is one of the most important food crops in the world. To accelerate the wheat germplasm exploitation, functional genomics and genomic breeding, total 1033 accessions including wild, landrace and commercial varieties were genotyped by W660K SNP array and resequencing. Phenotyping were also carried out in 21 environments. These accessions were primarily classified into three populations: wild population (Wpop) is composed of accessions from *Triticum uratu*, *Aegilops speltoides*, *Ae. tauscii*, *T. dicoccoides* and synthetic hexaploid wheat; landrace population (Lpop) and commercial variety population (Cpop). Distribution of diversity, haplotype block and recombination in each population were computed and compared in order to find genome regions affecting wheat evolution. Selection signals during wheat domestication and improvement were also screened by comparing the changes in allele frequency and diversity between Wpop and Lpop and between Lpop and Cpop. Genome-wide association study (GWAS) were calculated by using a mixed linear model (MLM) with the software GAPIT package. Besides the well-known genes such as *Ppd*, *Rht-D1* and *Vrn*, novel candidates associated with grain number, spike number and yield were also identified. Consistent genomic regions responsible for important agricultural and adaptive traits were obtained.



P 2 - Topic: Harnessing Diversity for Triticeae Improvement

Wheat landrace genome diversity

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Key message: The genetic diversity present in the A.E. Watkins bread wheat landrace collection was used to explore the genomic diversity and complexity of wheat.

The genomic complexity of bread wheat (*Triticum aestivum* L.), being a hexaploid species, has most likely been an important factor in the processes of domestication and the following adaptation of wheat to a wide variety of environments across the globe. Understanding this complexity will also be useful for future improvement of the crop, particularly in the light of changing environments. The landrace pillar of the WISP wheat breeding programme (www.wheatisp.org/) exploited the genetic diversity present in the A.E. Watkins landrace collection to explore this genomic diversity. A nested association mapping (NAM) panel of segregating bi-parental populations were developed from the 119 accession strong core set of the whole collection, selected to cover the majority of genetic diversity (Wingen et al. 2014). The modern spring elite variety 'Paragon', widely used in UK research, was used as common reference parent. Genetic maps were constructed following identical rules to make maps comparable. In total, more than 1600 linkage groups were identified in 60 maps, based on recombination from estimated 126 300 crossover events. A consensus map constructed from these maps contained nearly 2500 genetic loci. These newly developed genetics tools were used to investigate the rules underlying genome fluidity, e.g. by looking at the conservation of marker distances and marker orders. In general, marker order was highly correlated, which provides support for strong synteny between accessions. However, exceptional cases of incongruent linkage groups and evidence for many translocations were found. These translocations fell in general into many different translocation classes, but a few translocation classes were found in several accessions, the most frequent one being the well known T5B:7B translocation. Loci involved in recombination rate, were identified by QTL analyses using the crossover counts as a trait. More than a hundred significant QTL were detected, nearly half of them with increasing effect from the landrace parents. All developed genetic resources, core collection and populations of the NAM panel with genotype informations and maps, are freely available from the WISP landrace pillar (<http://wisplandrappillar.jic.ac.uk/>). Many of the populations have also been trialled under field conditions. Future research will pool results in order to identify useful genetic diversity for breeding.

Reference

Wingen LU, Orford S, Goram R, Leverington-Waite M, Bilham L, Patsiou T, Ambrose M, Dicks J, Griffiths S (2014) Establishing the A.E. Watkins landrace cultivar collection as a resource for systematic gene discovery in bread wheat. *Theor Appl Genet* 127: 1831-1842.



P 4 - Topic: Harnessing Diversity for Triticeae Improvement

Employing winter wheat diversity stored in genebanks to sustainably improve grain yield

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Key message: Genebanks harbor phenotypic and genetic diversity that can be used for future sustainable breeding progress in wheat and to support grain yield improvement.

During the last decades wheat (*Triticum aestivum*) grain yield constantly increased in Europe due to progress in genetics and agriculture. But today only a slow progression can be observed, given the increase in abiotic and biotic stress situations. Generally, best breeding material is repeatedly crossed to identify new varieties with enhanced breeding traits resulting in no broadening of genetic diversity in elite wheat germplasm pool. In contrast, wide genetic diversity is stored in genebanks in terms of plant genetic resources (PGR) that have hardly been used in crop improvement. Hence, the aims of the project are: (1) to characterize promising wheat PGR, (2) to identify new alleles or allele combinations positively associated with grain yield and its components, (3) to utilize the knowledge gained for future sustainable breeding progress in wheat and thus support grain yield improvement under changing growing conditions. For these reasons, wheat PGR of the *ex-situ* genebank at IPK (Gatersleben, Germany) were screened. Using sparse legacy data for plant height, flowering time and thousand grain weight and an automated bioinformatics pipeline, eight sets of contrasting genotypes including in total 209 PGR were selected. In addition, 81 elite breeder lines were also added allowing for appropriate genotypic and phenotypic characterization. This collection was grown in field trials at two locations in two replications. Phenological and morphological traits were assessed as well as grain yield and its components. Furthermore, genotyping by sequencing analysis was performed to determine the genetic diversity. Based on the field trials, significant phenotypic differences were detected for plant height, flowering time and thousand grain weight confirming that the selection of contrasting genotypes was successful. In addition, phenotypic differences for grain yield components were detected, whereby the phenotypic diversity within the PGR was greater than within the elite breeder lines. Genotyping the 290 genotypes resulted in 37 186 informative, high-quality, biallelic SNPs. About 10% of them are specific to the PGR despite excluding rare variants. Genome wide association studies were conducted and QTL regions could be identified which are associated with grain yield and its components. Summarizing, the collection analyzed shows phenotypic and genotypic diversity for grain yield components. That might be beneficial for future breeding programs to constantly increase grain yield under changing growing conditions in the decades to come.



P 6 - Topic: Harnessing Diversity for Triticeae Improvement

From landraces to modern lines: molecular characterization and association mapping in an Italian bread wheat collection

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Key message: Through a genome-wide association study, marker-trait associations have been identified for twelve traits in a panel of 162 bread wheat accessions summarizing the history of Italian bread wheat breeding.

Genome-wide association studies (GWASs) based on linkage disequilibrium provide a promising tool for the detection and mapping of quantitative trait loci governing complex agronomic and qualitative traits. In the present study the genetic basis of variation for several traits related to plant morphology, crop agronomic performance and grain quality in bread wheat was investigated. To this aim, a *Triticum aestivum* collection summarizing the process of wheat breeding in Italy during the latest hundred years was arranged. The collection was used to study the temporal trends of a series of morphological, agronomic and grain quality traits and represents a unique resource to study the molecular bases of traits associated to bread wheat improvement. The collection was phenotypically screened during three growing seasons over two locations in Northern Italy. The genetic characterization was performed with the Infinium 90K array (Illumina), resulting in 23 166 successful single nucleotide polymorphisms (SNPs). On the full set of SNPs, the average failure rate and heterozygosity per accession were 0.17 and 0.002 respectively, with a correlation of 0.31 ($p < 0.001$). 19 430 SNPs mapped at univocal position on the hexaploid wheat consensus map (Wang et al. 2014); the D genome was the least represented, with 1808 markers against the 7890 on A genome and 9734 on B genome. To understand the relationships among the accessions of the entire collection, the population structure was investigated. The first two principal components, deriving from the SNP-based matrix of Euclidean distances, account for 8% and 5% of the variance, respectively. In order to identify a fitting criteria to describe the population structure, successive rounds K-means clustering in a DAPC analysis were run; the most likely number of clusters was identified to be seven. The 19 430 univocal SNPs were used in a GWAS detecting 360 marker-trait associations for twelve traits. A first annotation of the associations, coupling homology based analysis and synteny searches, allowed relating the significant marker-trait associations for six traits to known genes involved in the provision of such traits in wheat, rice or *Brachypodium*.

Acknowledgement

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P 8 - Topic: Harnessing Diversity for Triticeae Improvement

Widening the genetic base of durum wheat

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Key message: The genetic diversity is lacking in modern wheat cultivars. The wheat progenitors and its wild relatives are reservoirs of many useful genes which must be utilised through systematic pre-breeding.

Durum wheat (*Triticum durum*; AABB) is an allotetraploid species, derived from the domestication of wild emmer wheat (*T. dicoccoides*) which arose from a spontaneous cross between *T. urartu* (AA genome) and an ancient relative of *Aegilops speltoides* (BB genome). Durum wheat is cultivated on about 17 million hectares worldwide and is an important cereal in many areas being consumed in different forms, e.g. pasta. Inbreeding and continuous selection after domestication has left little genetic variation in many crops, including wheat, for breeders to exploit. Wheat progenitors and its wild crop relatives are major reservoirs of many agronomically useful genes. For example, *T. timopheevii*, *Ae. speltoides* and *Thinopyrum ponticum* have been reported to carry disease resistance genes. Similarly, *Th. elongatum* and *T. urartu* are known to have yield related genes. In the current study, we are trying to bring novel and potentially useful alleles from two wheat progenitors and eight distant relatives into five elite CIMMYT lines and six ICARDA bred durum varieties. A large number of introgression lines carrying relatively small segments from wheat progenitors and its wild relatives have been developed in bread wheat at the BBSRC/Nottingham Wheat Research Centre (King et al. 2017, <http://www.nottingham.ac.uk/wisp/>). Using these lines as a source of introgressions, a total of 630 F₁ seeds were generated. Some of these progeny carrying introgressions have been backcrossed to the 11 durum lines. To date, 1280 BC₁ seeds have been generated and approximately 40 of them are currently being advanced to BC₂. In order to identify and track the introgressions in the backcross progenies, GISH, KASP markers and phenotyping techniques are being employed. The results of this study will be discussed in the symposium.

Acknowledgement

The authors would like to thank Global Crop Diversity Trust for financial assistance.

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P 10 - Topic: Harnessing Diversity for Triticeae Improvement

Characterization and utilization of currently grown Turkish wheat landraces

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Key message: Recently collected Turkish wheat landraces represent valuable genetic resource for grain yield and other traits. Modern phenotyping and genomic tools greatly assist in identification and utilization of superior landrace selections.

From 2009 to 2014 a nationwide effort was made to document, collect, conserve, characterize and utilize wheat landraces grown by Turkish farmers. Altogether 95 morphotypes were identified representing three species and six subspecies: *Triticum monococcum*, *T. turgidum* ssp. *dicoccon*, *T. turgidum* ssp. *turgidum*, *T. turgidum* ssp. *durum*, *T. aestivum* ssp. *aestivum* and *T. aestivum* ssp. *compactum* (Morgounov et al. 2016). Spike samples were collected from more than 1600 farmers from 59 provinces, planted as single-spike progenies and, consequently, evaluated for diseases, agronomic traits and grain yield in replicated trials at multiple sites within the country. Overall 2000 lines originating from landraces were evaluated in yield trials from 2012 till 2016. Superior landrace selections have been identified for yield potential, drought tolerance, resistance to rusts, grain quality and many other traits. Simple selections from the landraces, even morphologically uniform, allows achieving substantial genetic gains for the traits valued by farmers. Bread wheat lines were characterized for genetic diversity using 63 KASP-SNP markers resulting in 28 clusters. The clusters differed in geographic origin, morphology and agronomic traits and served as a basis for selection of the core set for future detailed characterization. A set of 153 landrace selections tested at 3 sites in 2013 was subjected to genome wide association analyses (Sehgal et al. 2016) which confirmed some known associations (previously reported QTL) as well as identified new candidate genomic regions for grain yield, spike productivity components and stripe rust resistance. New candidate genomic regions reflect the potential of Turkish landraces to further increase the genetic diversity in elite germplasm. The crossing program with landraces targets: (a) improvement of modern winter wheat germplasm by utilizing drought tolerant and high-yielding landraces and back- or top-crossing F₁ to modern varieties; (b) improvement of the landraces by incorporation of disease resistance from other landraces or from modern varieties with back- or top-crosses to landraces. Improvement of the landraces and their return to the farming communities is considered an important component of maintaining on-farm genetic diversity.

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P 12 - Topic: Harnessing Diversity for Triticeae Improvement

Evaluation of hexaploid spring wheat cultivars from Kazakhstan based on adaptation related genes

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Key words: *Eps* genes, genetic variation, *Ppd* genes, *Rht* genes, *Vrn* genes

Plant adaptation to various environmental niches is very important for the improvement of productivity. In wheat, there are major traits associated with plant adaptation, which include vernalization, photoperiod length, earliness *per se* and plant height. In this study we genotyped 96 spring wheat accessions (*Triticum aestivum* L.) from Kazakhstan using *Vrn*, *Ppd*, *Eps* and *Rht* genes. KASP markers were used to genotype *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-A1*, *Ppd-B1*, *TaELF3-D1*, *TAFT3-A1*, *TaFT3-B1*, *TaFT3-D1*, *Rht-B1* and *Rht-D1*. It was observed that the tested germplasm has little or no genetic variation with respect to *Ppd* and *Rht* genes. All spring wheat accessions from Kazakhstan are photoperiod sensitive genotypes.

Acknowledgement

The study was supported by the FP7 EU project ADAPTAWHEAT (<https://www.jic.ac.uk/adaptawheat/>) and grant N1784/GF4 provided by the Ministry of Education and Sciences of the Republic of Kazakhstan.



P 14 - Topic: Harnessing Diversity for Triticeae Improvement

High-throughput genotyping of Swiss bread and spelt wheat identifies unused gene pools for breeding

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Key message: High-throughput genotyping of bread and spelt wheat landraces and modern cultivars with a 15K wheat SNP array reveals differences in their gene pools.

Modern cultivars of cereals carry only a fraction of the genetic diversity present in their wild progenitors and old landraces. The reduction of genetic diversity was caused by the use of only a limited number of plants during domestication and breeding. Genebanks are an important resource to preserve the genetic diversity of old landraces and wild progenitors. In addition, the accessions from genebanks may harbor agriculturally important genes that were missed during domestication and modern breeding. The direct use of landraces in breeding is limited, because they often also show undesired traits. Therefore, a detailed genetic characterization is necessary to make genebank material more accessible for modern breeding and to facilitate the separation of desired from unwanted traits.

We genotyped 502 bread wheat and 294 spelt wheat accessions from the Swiss National database with a 15K wheat SNP array. The bread wheat and spelt wheat are both hexaploid (AABBDD) and close relatives that can be interbred with each other. The genotyped accessions included landraces, mainly from Switzerland, as well as modern cultivars. The data revealed that bread wheat accessions are genetically different from spelt wheat accessions. This clear separation was also visible in all three sub-genomes. In addition, a set of bread wheat landraces that were diverse from modern cultivars was identified. Those accessions are promising targets for identification of novel genes, since they were likely missed in the Swiss wheat breeding program. The genotypic data facilitates the transfer of novel genes from landraces into modern cultivars.



P 16 - Topic: Harnessing Diversity for Triticeae Improvement

Intergenic SNPs reveal spontaneous interchanges between chromosomes 7A and 7D of wheat

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Key message: Genotyping-by-sequencing of cytogenetic stocks provided markers to distinguish between chromosomes 7A and 7D. These markers were used to screen for 7A-7D recombinants in durum wheat.

Durum wheat (AABB) can be readily crossed with bread wheat (AABBDD). This allows for introgression of chromatin from A- and B-genome chromosomes of bread wheat into their durum counterparts. Introgression of D-genome chromatin into durum is more challenging, but can be achieved by using *ph1* deletion mutants. There are also a few reports of apparent spontaneous transfer of D-genome chromatin into durum A or B chromosomes (Martin et al. 2011, Han et al. 2013). Here, genotyping-by-sequencing was applied to Chinese Spring bread wheat, several cultivars of durum wheat and Langdon 7D(7A) and Langdon 7D(7B) disomic substitution lines (Joppa & Williams 1988). Single nucleotide polymorphisms (SNPs) that distinguish between chromosomes 7A and 7D were selected. The positions of these markers were determined by anchoring the SNP-bearing sequences on the International Wheat Genome Sequencing Consortium's 7D sequence assembly. KASPTM assays were designed and applied to 861 F₂, F₃ and BC1-F₂ progeny of crosses between Langdon 7D(7A) and the durum cultivar DBA-Aurora. As expected, almost all of the progeny plants exhibited complete linkage disequilibrium: with the same genotype at all markers: 7A only, 7D only or both 7A and 7D. There were, however, 38 plants (4.4%) with heterozygous results (both 7A and 7D) for part of the chromosome and homozygous results (7A or 7D only) for the rest of the chromosome. For 26 of these, the transition was in the centromeric region and might be due to Robertsonian translocation. For the others, the transition was within either the short arm (2 plants) or the long arm (10 plants). One of these plants carries a gene of interest from chromosome 7D, apparently on only a short distal translocation. This provides new material for backcrossing that gene into durum wheat. The markers developed here could be useful for other applications that require differentiation between chromosomes 7A and 7D. The approach used here could be followed to design sets of markers for other homoeologues.

Acknowledgements

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P 18 - Topic: Harnessing Diversity for Triticeae Improvement

Yield stability variation within Mediterranean durum wheats

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Key message: Mediterranean durum landraces are less responsive than modern cultivars to improved environmental conditions, but variability exists regarding their adaptability and stability and is related to their genetic structure.

Durum wheat (*Triticum turgidum* L. var. *durum*) originated in the Fertile Crescent and spread over the Mediterranean Basin developing into local landraces specifically adapted to their growing regions, with high stress tolerance and yield stability from the static point of view. This implies response maintenance across environmental variations, usually linked to low yields. However, breeding seeks dynamic stability, which refers to a predictable cultivar response in a particular environment (Becker & León 1988), implying performance maintenance with good adaptability. This work aimed for ascertaining differences in the adaptability of Mediterranean durum wheat. Ninety-five Mediterranean landraces and modern durum cultivars classified in 5 genetic subpopulations (Soriano et al. 2016) were tested during 2007-2008-2009 in northern and southern Spain under rainfed conditions. Yield variability was explained in a 42.1% by genotype × environment interaction (G×E). AMMI biplot evidenced differences in subpopulations adaptation pattern. According to the AMMI analysis, modern cultivars, the highest yielding subpopulation, proved to be the best adapted to warm environments. However they also had the highest slope in the Finlay-Wilkinson analysis ($b=1.50$) and the lowest superiority measure ($P_i=746124$) indicating their high dynamic stability and adaptability to different environments. East Mediterranean landraces showed specific adaptation to dry and warm environments during grain filling. Their low b (0.78) and high P_i (2214793) indicated low adaptation capacity. Contrary, East Balkan and Turkey landraces were the closest to the origin of AMMI axes ($v_i=8.77$). This, together with a $b=0.98$ indicated low G×E and wide adaptation. West Balkan landraces showed the lowest yield and the highest v_i (19.2) and P_i (3649512), indicating specific adaptation to cooler environments and general low performance. Despite belonging to the same subpopulation, Egyptian showed a wider adaptation ($v_i=9.55$) than West Balkan ones, however the lowest b (0.59) and the second highest P_i (3016450) indicated a strong static stability. Landraces from West Mediterranean were the highest yielding and the most adaptable according to the AMMI biplot, their b close to 1 ($b=1.02$) and low P_i (2077919).

Acknowledgements

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P 20 - Topic: Harnessing Diversity for Triticeae Improvement

Genetic diversity in populations of wild emmer wheat (*Triticum dicoccoides*) in Palestine

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Key words: genetic distance, geographic distance, microsatellite marker

Wild emmer (*Triticum dicoccoides*) grows under different ecological conditions in the area of the Fertile Crescent in the Middle East which also includes Palestine. In comparison with the domesticated emmer the wild form has a higher genetic diversity and many beneficial properties like the high protein quality and quantity and the biotic and abiotic stress resistance. Because of these advantages wild emmer can be used for the improvement of wheat in breeding programs. The aim of this study was to analyze the genetic diversity of wild emmer from different sites in Palestine by microsatellite markers. Using 22 microsatellite markers on 217 samples of wild emmer from 11 different sites resulted in 460 different alleles in 30 loci with an average of 15.3 alleles/locus (range: 2-59). The number of the rare alleles (occurrence <5%) was 344. *Xgwm443* marker and wild emmer population from Yarza site showed the highest genetic diversity, *He*, with 0.9629 and 0.6347, respectively. Genetic diversity, *He*, for microsatellite and sites averaged 0.6428 and 0.3982, respectively. By comparing the populations of all regions, with the sites Abufalah and Ain Noon provided constant high genetic differences. The matrix of genetic distances for all combinations of sites ranged between 0.012 and 0.191. The genetic diversity within a region behaved contrary to the genetic diversity between regions. It was shown that the plants from the subpopulations from Tubas (a), Yarza and Anin Noon were more likely to be originated from plants that are genetically very similar than the plants from the subpopulations from Tubas (b) and Tubas (c). No correlation between the genetic and geographical distance was found. But it was demonstrated that there was a significant correlation between the genetic distance and the altitude difference of the sites with a significance level of 5%. This work showed that there was a high genetic diversity within a collection site and a little lower genetic diversity between the collection sites. As well, this work showed that microsatellites could be used as an efficient tool for genetic diversity estimation and consequently wheat breeding programs.



P 22 - Topic: Harnessing Diversity for Triticeae Improvement

Towards understanding the genetic regulation of differences in floret number per spikelet within Triticeae, barley vs. wheat.

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Key message: *Multiflorus2* - a rachilla developmental mutant may offer insights into floret developmental differences within Triticeae.

Spike inflorescence is a characteristic feature of tribe Triticeae which includes economically relevant crops like wheat (*Triticum* spp.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.). Within the spike, spikelets (grain bearing structures) are borne on the lower half nodes of a phytomeric structure called 'rachilla'. In wheat, the rachilla extension is indeterminate and up to 10 florets are produced per spikelet in a distichous pattern. Whereas in barley the rachilla extension is determinate and the floret number is restricted to one per spikelet. However, in a barley mutant called *multiflorus2.b* (*mul2.b*), the rachilla becomes indeterminate and produces more than one floret per spikelet distichously. The rachilla indeterminacy in *mul2.b* is restricted to lateral spikelets, whereas the central spikelets have determinate rachilla. With the aim of identifying the gene underlying barley *mul2.b* locus, we have developed two F₂ mapping populations, Morex × *mul2.b* and *mul2.b* × Montcalm. From our phenotypic analysis, we identified that *mul2.b* shows a semi-dominant inheritance pattern. By conducting exome capture experiment using wild type and mutant bulks (25 and 27 individuals respectively) from the Morex × MC(*mul2.b*) population we could localize the *mul2.b* genetic interval to the centromeric region on chromosome 2H. Currently we are in the process of narrowing down the *mul2.b* genetic interval by screening large F₂ mapping population from Morex × MC(*mul2.b*). Identification of *mul2.b* gene may provide important insights into the differences in floret developmental programs in barley, wheat, and other Triticeae species. *mul2.b* also offers potential to improve barley yields since there is a chance to increase the yield/spike.



P 24 - Topic: Harnessing Diversity for Triticeae Improvement

Emmer as a source of variation for bread wheat

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Key message: A number of tetraploid AABB emmer lines were used as sources of variation for grain protein content, water-use efficiency, grain size, and yield under rainfed and irrigated conditions.

Genetic materials studied were made by crossing 9 bread wheats of Indian, CIMMYT and Australian origin with 11 different emmer accessions to produce F₁ that were then backcrossed once with their bread wheat parent. From selected BC₁F₁ plants, doubled haploid lines were produced by crossing with maize as pollen parent, embryo rescue, and chromosome doubling. The DH lines were evaluated in three seasons in replicated field plot trials; one rain-fed trial in 2013 (480 lines), and in both rain-fed and irrigated trials in the 2014 and 2015 seasons (187 lines) at Narrabri in northern New South Wales, Australia. The plots, plants, or grain were evaluated for water use efficiency, developmental and physiological traits, leaf gas exchange traits, grain protein content, test weight, TKW, screenings, water content, and canopy temperature. The parents and DH lines were SNP genotyped on a 90k chip. GWAS analysis and introgression analysis has revealed chromosome segments with beneficial alleles for the traits measured that have been contributed by the both hexaploid wheat, and emmer wheat parents. The emmer-derived segments are new sources of useful traits for wheat breeding transferred into adapted, high yielding, hexaploid bread wheat backgrounds.



P 26 - Topic: Harnessing Diversity for Triticeae Improvement

The dicot *Medicago* gene *STF* increased leaf width in monocot wheat

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Key message: Understanding of the *STF* gene function and regulatory mechanisms in transgenic wheat provides new opportunities for increasing leaf width to generate more biomass and harvestable grains.

Plant leaves are the primary tissues where photosynthesis is performed to convert sunlight energy and carbon dioxide into carbohydrates that are used for the development, growth, and reproduction of the plant. Larger leaves in dual purpose wheat could be used not only to produce more biomass for grazing but also to minimize the loss of grain yield following the removal of leaves during grazing. However, genes controlling wheat leaf development are little known. In this study, we demonstrate that when the gene *STENOFOLIA* (*STF*) from the model legume *Medicago truncatula* is expressed in wheat, the transgenic wheat plants have significantly wider leaves. Furthermore, the *STF* protein binds directly to the DNA *cis* element in numerous wheat genes, which is confirmed using an *in vitro* electrophoretic mobility shift assay (EMSA) analysis. In addition, the full length *STF* protein is used as a probe to screen the Y2H library to test for its interactions in wheat, and *STF* interacts with a variety of proteins in wheat, which is confirmed by performing bimolecular fluorescence complementation (BiFC) assays. When the *STF* DNA sequence is used to search in the genome databases recently released by the International Wheat Genome Sequencing Consortium (IWGSC) or NT/EST databases in GenBank and even though the threshold is reduced from normally used 0.0001 to 0.1, no similar gene is gained, suggesting that wheat might have no orthologue of *STF*. The use of exogenous *STF* in transgenic wheat has opened a starting point to enter into multiple regulatory pathways for leaf development in wheat.



P 28 - Topic: Harnessing Diversity for Triticeae Improvement

Wide recovery ability by Afghan wheat landraces against severe osmotic stress: Its proline playing key role in reviving?

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Key message: Some of the Afghan wheat landraces exhibited reviving potentials during severe osmotic stress, proline accumulation during osmotic stress might play the key role in the resilient genotypes.

Water stress is one of the major crops limiting environmental stress. Afghan wheat landraces (*Triticum aestivum* L.) collected by Dr. Hitoshi Kihara and co-workers (KAWLR) from 17 provinces of Afghanistan during 1956 to 1979, are considered as potential genetic resources for drought tolerance. We screened the KAWLR for their tolerance against severe osmotic stress (polyethylene glycol-PEG induced) in a novel semi hydroponic doubled-cup system (Figure 1A). To optimize PEG concentration for early stage of wheat culture in our double cup system, PEG 6000 concentrations 28%, 45% and 56% were examined for one week, and 30%, 35% and 40% concentrations for two weeks. Two check varieties, Lalmi-2 and Chonti-1, completely died after 15 and 23 days, respectively, at 30% PEG (equal to -1.2 MPa) and the leaves stage which was consequently defined as severe osmotic stress. Screening under this system for 23 days, only 6 accessions out of 37 KAWLRs (16%) survived. The surviving landraces were transferred to normal conditions for four weeks and then physiological parameters including normalized difference vegetation index (NDVI), canopy temperature and stomatal conductance were measured over four weeks to study the progress of recovery. The 6 KAWLRs revived (Figure 1B). Using these resilient KAWLRs, we are measuring proline accumulation and reactive oxygen species (ROS) pathways-related enzymes to know the biochemical basis. We will also apply some bioactive compounds such as KODA (9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid) in this severe osmotic cup culture system to know the feedback of KODA on the reviving potential of plants via proline or ROS signaling pathway. KODA, a stress-inducing substance derived from duckweeds (*Lemna paucicostata*), was found to be involved in enhanced root growth and plant development against alkaline and dry soil (Haque et al. 2016).

Acknowledgement

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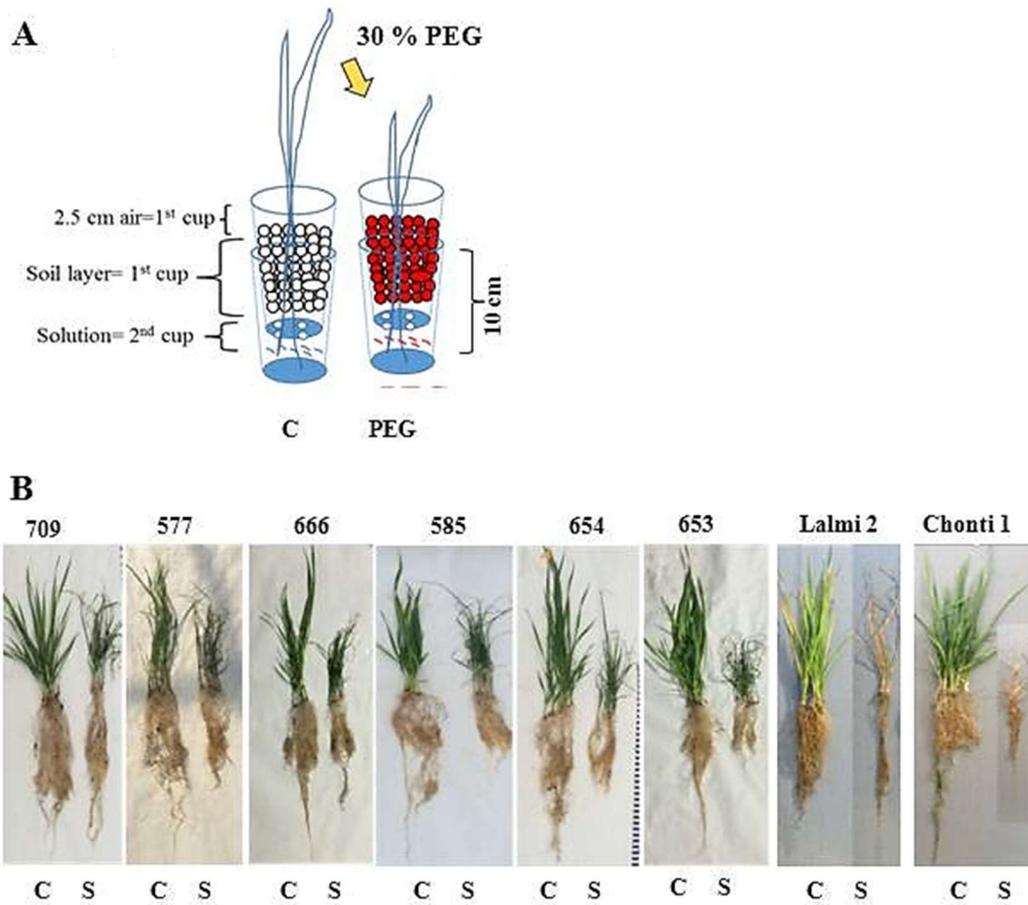


Figure 1: Scheme of the doubled-cup system with severe osmotic stress (A) and six selected KAWLARs with high tolerance and two check cultivars under control (c) and stress (s) conditions (B).



P 30 - Topic: Harnessing Diversity for Triticeae Improvement

Increased stem rust susceptibility in synthetic hexaploids created between *Triticum turgidum* ssp. and *Aegilops tauschii*

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Key message: Stem rust resistance of tetraploids is commonly not expressed in synthetics, hindering the use of these resistances in common wheat.

In search for new sources of stem rust resistance in wheat and its relatives, we found relative high frequencies of resistance in cultivated tetraploids (*Triticum turgidum* ssp.). To facilitate the transfer of stem rust resistance to common wheat, resistant tetraploids and selected accessions of *Aegilops tauschii* were used to create hexaploid synthetics. Stem rust reactions of synthetics and resistant parents were evaluated at seedling and adult plant stages. Although a lack of resistance expression in the synthetics at seedling stage was observed in some combinations of lines and races of *Puccinia graminis* f. sp. *tritici*, the lack of resistance expression were most prominent and common at adult plant stage (Table 1). Of the 93 synthetic lines derived between a resistant tetraploid and a susceptible *Ae. tauschii* (CI 26), 82% were moderate to highly susceptible, comparable to that of the susceptible check (Rusty). Synthetics of a resistant PI 268210 instead of a susceptible *Ae. tauschii* parent showed a reduction in disease in some cases, but the level of resistance did not reach that of the tetraploid resistant parent. A few synthetic lines derived from several emmer accessions of maintained the levels of parental resistance. The resistance in these emmer accessions appeared to be *Sr2*-like. We are exploring methodologies to investigate the underline mechanism(s) that hinders the expression of stem rust resistance in the synthetics, and to re-evaluate whether transferring stem rust resistance from tetraploids to common wheat via synthetics is a viable approach.

Table 1: Lack of stem rust resistance in syntetic wheat lines.

Subsp. parent of synthetic	Line or Pedigree	Seedling reaction to stem rust races used in seedling evaluation			Field adult plant response to a composite of US races	
		RCRSC	SCCSC	TTKSK	2015	2016
<i>T. durum</i>	lumillo	;1-	;1-	;11+	0	5 R
	lumillo /CI26	3+	1+1	3	60 MS	60 S
<i>T. dicoccum</i>	PI94675-1	;	1;	23;	20 R	20 MRMS
	PI94675/CI26	;2-	2-	2+	70 S	50 S
	PI94675/PI268210	;1-	2-	2-	60 MSS	50 MS
<i>T. carthlicum</i>	PI94751	2+3	2-	3	30 MR	25 RMR
	PI94751/CI26	3+	3	2+	70 S	50 S
<i>T. polonicum</i>	PI254215	3+	1+1	11-	30 MS	50 SMS
	PI254215/CI26	4	1+3	2+	70 S	60 S
<i>T. turanicum</i>	CI11390	3+	3	11+	30 MS	30 SMS
	CI11390/CI26	3+	3	3+	50 S	50 S
<i>T. dicoccum</i>	PI94616-1	;	11+3;	2+3	T R	5 R
	PI94616/CI26	;1-	31	3 M	10 R	T R
<i>T. durum</i>	Rusty-susc ck.	3+	3	3+	30 S	60 S



P 32 - Topic: Harnessing Diversity for Triticeae Improvement

Characterization of bread wheat (*Triticum aestivum* L.) germplasm stored in Albanian genebank based on quantitative agronomical traits

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Key words: genetic diversity, wheat genotypes, quantitative traits

The genetic diversity present in the bread wheat (*Triticum aestivum*) germplasm stored in Albanian genebank was studied at the Experimental Field of Agriculture Institute of Peja (Kosovo) during the 2015-2016 season. Hundred bread wheat genotypes of local origin were evaluated for 12 quantitative traits (6 morphological and 6 biochemical). The objective was to characterize and select those genotypes with favorable characteristics for their use in breeding programs and for accomplishment of farmer requests. Statistical analyses revealed significant variability between accessions and associations between some quantitative traits. Principal component analysis and cluster analysis divided the nursery into five clusters. A higher number of accessions was included into one two groups, i.e. 45 and 26 genotypes, respectively. High and significant positive correlations were found between days to flowering, plant height, spikelet time, and protein content and gluten traits (correlation coefficients ranged from 0.31 to 0.96). Six traits contributed to PC1 which accounted for 40.9% of the total variance. The study identified traits of agronomic interest that account for genetic diversity and which will facilitate the maintenance and agronomic evaluation of the wheat collections.



P 34 - Topic: Harnessing Diversity for Triticeae Improvement

Introgression lines of *Triticum timopheevii* in cultivated wheat background and mapping of useful genes using SNP markers

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Key message: Introgression lines from the A and G genomes of *Triticum timopheevii* have been developed in wheat through a marker-assisted backcrossing scheme, generating a putative linkage map of *T. timopheevii*.

Achieving a sustainable supply of food in light of the inexorable rise in the global population is a major challenge for plant breeders. Crop improvement through breeding is only possible where genetic variation is present in the breeder's germplasm, but decades of intensive selection in major crops has inevitably narrowed their genetic diversity. The efficient use of biodiversity of wild relatives of wheat is key for the enhancement of genetic variability. Many wild relatives of wheat possess a vast potential reservoir of genetic variation for abiotic and biotic stresses e.g. drought tolerance, resistance to insects and fungal diseases, biomass, yield and photosynthetic potential. *Triticum timopheevii* is a tetraploid wheat ($2n=28, A^1A^1GG$), which is characterized by complex resistance to many diseases. Genes for resistance to stem rust (*Sr36*), powdery mildew (*Pm2*, *Pm6*, *Pm27*) and leaf rust (*Lr18*) have been exploited. Hence, it is being used in this programme with the prime objective of developing chromosome segmental introgression lines for the A and G genomes of *T. timopheevii*, carrying target genes but lacking deleterious genes, into wheat quickly and efficiently. Characterization of wheat/*T. timopheevii* introgression lines is being carried out via an Axiom® 35K SNP alien array. A putative linkage map of *T. timopheevii* has been successfully developed and confirmed by exploitation of fluorescence *in situ* hybridization (FISH). Phenotyping of the introgression lines is underway, for example, mineral analysis and screening for resistance against Fusarium head blight.



P 36 - Topic: Harnessing Diversity for Triticeae Improvement

Differential SNP polymorphism of hexaploid wheat in relation F₁ heterotic response

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The genetic diversity within 77 accessions of hexaploid wheat growing in Belarus under breeding program was assayed for polymorphism in comparison with 17 cultivars, represented variety across 7 European countries. We used high-throughput array to evaluate 384 gene-associated SNPs from published Cavanagh et al. (2013). A total 331 SNPs were genotyped, representing variability of 97.6% loci (Table 1). Genetic structure of 94 wheat accessions under research was evaluated by neighbor-joining method (Figure 1). We used this information to assess the genetic distances (GD) and differential polymorphism (DP) to examine yield heterosis in 48 testcross F₁ hybrids so as to develop tools for selecting the best pair-wise combinations. Significant positive correlations were found between GD, mid-parent (MPH) and high-parent (HPH) heterosis for grain yield, but proportion of explained variance (R²) obtained from GDs did not exceed 12% (Table 2). For other traits the correlations were not reliable. Based on data SNP allelic composition of wheat we evaluated differential polymorphism (DP) from number and ratio polymorphic (PL) and monomorphic (ML) loci in every pair-wise cross-combination. Further, the impact of DP into F₁ heterotic response was assessed. The total number of polymorphic loci, and the ratio polymorphic/monomorphic loci were significantly correlated with F₁ hybrid performance in tilling capacity at the level of 0.36 and 0.32, respectively, while number of monomorphic loci was associated with this trait in inverse manner (Table 2). From our data, score DP has higher prognostic potential compared to GD. Reliable impact the number of PL (9-16%) into MPH an HPH was found for traits - grain yield, number grain per plant, number spikelets per spike and tilling capacity. Ratio PL/ML was also positively associated with these traits. Our result indicate, that random set of SNP markers and corresponding GD and DP measures have low predictable ability for hybrid performance, mid-parent and high-parent heterosis, but it would be improved by the selection of positive markers.

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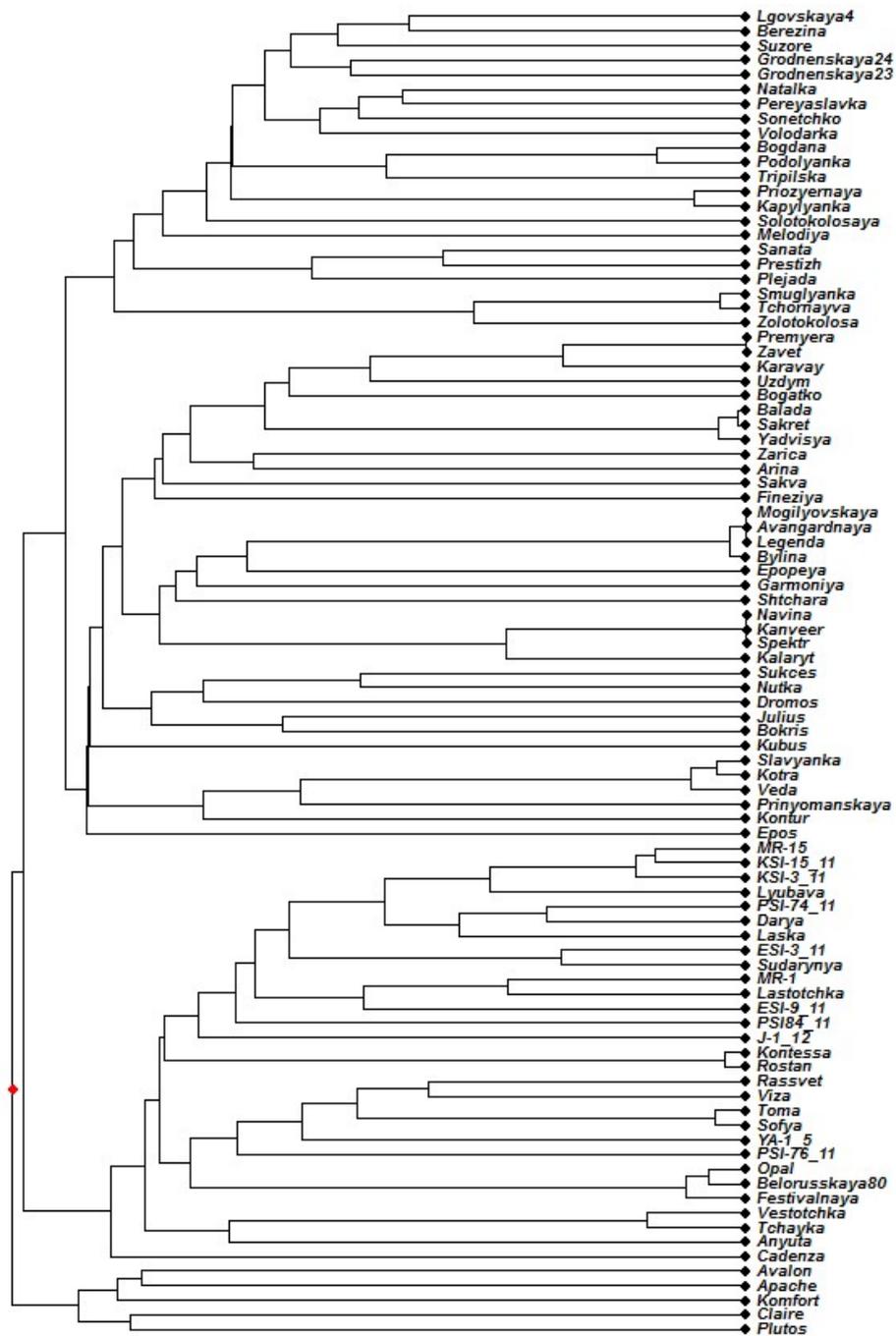


Figure 1: Dendrogram illustrating genetic relatedness of 94 wheat accessions.



Table 1: SNPs genomic distribution among chromosomes and genomes.

Chromosomes	SNP number	% over total number	Spring wheat		Winter wheat		overall	
			Poly-morphic SNPs, %	Mono-morphic SNPs, %	Poly-morphic SNPs, %	Mono-morphic SNPs, %		
A-genome	1	20	6.0	90	10	100	0	100
	2	14	4.2	71.4	28.6	100	0	100
	3	19	5.7	100	0	100	0	100
	4	15	4.5	100	0	100	0	100
	5	21	6.3	90.5	9.5	100	0	100
	6	17	5.1	88.2	11.8	100	0	100
	7	23	6.9	82.6	17.4	86.9	13	95.7
In total	129	39.0	89.0	11.0	98.1	1.9	99.4	
B-genome	1	21	6.3	90.5	9.5	100	0	100
	2	25	7.6	84	16	96	4	96
	3	25	7.6	92	8	96	4	96
	4	11	3.3	100	0	100	0	100
	5	26	7.9	92.3	7.7	92.3	7.7	92.3
	6	17	5.1	94.1	5.9	100	0	100
	7	13	3.9	92.3	7.7	100	100	100
In total	138	41.7	92.2	7.8	97.8	16.5	97.8	
D-genome	1	7	2.1	85.7	14.3	85.7	14.3	85.7
	2	6	1.8	83.3	16.7	100	0	100
	3	3	0.9	66.7	33.3	100	0	100
	4	1	0.3	100	0	100	0	100
	5	2	0.6	50	50	100	0	100
	6	4	1.2	50	50	75	25	75
	7	5	1.5	80	20	100	100	100
In total	28	8.5	73.7	26.3	94.4	19.9	94.4	
NL*	36	10.9	77.8	22.2	97.2	2.8	97.2	
Average	331	100.0	83.2	16.9	96.9	10.3	97.2	

*NL – non-localised markers

Table 2: Correlations between genetic distances (GD), differential allelic SNP polymorphism (DP) and F₁ heterosis in wheat (*T. aestivum* L.)

	Index	GY	GW	GNS	TGW	PL	UIL	SL	TC	SNS
F ₁ hybrid performance										
DP	NPL	0,24	-0,02	-0,02	0,00	-0,13	-0,23	-0,03	0,36**	-0,11
	NML	-0,22	-0,01	-0,05	0,05	0,22	0,32*	0,01	-0,32*	0,12
	K (NPL/NML)	0,22	-0,04	-0,01	-0,03	-0,15	-0,26	-0,04	0,36**	-0,11
GD		0,22	0,04	0,07	-0,03	-0,21	-0,33*	0,02	0,28	-0,10
Mid-parent heterosis										
DP	NPL	0,40**	0,25	0,13	0,30*	0,27	0,35*	0,08	0,31*	0,33*
	NML	-0,37**	-0,26	-0,17	-0,25	-0,26	-0,32*	-0,09	-0,28*	-0,32*
	K (NPL/NML)	0,39**	0,23	0,12	0,29*	0,28*	0,35*	0,06	0,29*	0,35*
GD		0,34*	0,24	0,17	0,21	0,24	0,27	0,11	0,27	0,28*
High-parent heterosis										
DP	NPL	0,31*	0,24	0,30*	0,13	0,15	0,02	0,02	0,29*	0,27
	NML	-0,28*	-0,24	-0,26	-0,07	-0,16	0,01	0,02	-0,28*	0,30*
	K (NPL/NML)	0,29*	0,25	0,28	0,12	0,14	0,02	-0,01	0,28*	0,27
GD		0,26	0,20	0,24	0,04	0,17	-0,05	0,00	0,27	0,29*

NPL: number of polymorphic loci; NML: number of monomorphic loci; K ratio NPL/NML; GY: grain yield per plant; GWS: grain weight per spike; GNS: grain number per spike; TGWP: thousand grain weight per plant; PL: plant length; UIL: upper internode length; SL: spike length; TC: tillage capacity; SNS: spikelet number per spike



P 38 - Topic: Harnessing Diversity for Triticeae Improvement

Introgression of small segments of *Secale cereale* chromatin into *Triticum aestivum* via homoeologous recombination

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Key message: The exploitation of *ph1* mutant wheat to generate introgressions between hexaploid wheat and *Secale cereale* coupled with the identification and characterisation of subsequent introgression lines via SNP markers and GISH analysis.

Due to its evolution, there is relatively little genetic variation in modern day wheat varieties. In contrast, wheat's wild and distant relatives provide a vast reservoir of genetic variation for potentially most, if not all, traits of agronomic importance. Here we describe the initiation of a research programme aimed at transferring valuable genetic variation from *Secale cereale* into wheat. In wheat pairing and recombination is restricted to homologous chromosomes due to the presence of the *Ph1* pairing control gene on the long arm of chromosome 5B. However, in lines where the *Ph1* locus has been deleted, i.e. *ph1* mutant, pairing between homoeologous chromosomes from different genomes can occur. In this work, we have crossed *S. cereale* with the *ph1* mutant to produce an interspecific F₁ hybrid and backcrossed this F₁ hybrid recurrently to wild type wheat (*Ph1*). Thus in the absence of *Ph1* we predicted that recombination would occur between homoeologous wheat and rye chromosomes. In order to identify subsequent introgressions, backcross progeny derived from the wheat/*S. cereale* F₁ hybrid were screened with molecular markers (SNPs) and also analysed with genomic *in situ* hybridisation (GISH). In addition, to facilitate high throughput, cost effective screening of large numbers of plants, large numbers of KASP markers are also currently being developed. Although this programme is at an early stage, we have recently identified two new introgression lines and screening is presently underway to identify further wheat/rye recombinant events.



P 40 - Topic: Harnessing Diversity for Triticeae Improvement

Comparative mapping and targeted-capture sequencing of the gametocidal loci in *Aegilops sharonensis*

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Key message: Generation of 54 SNP markers in an *Aegilops sharonensis* gametocidal (Gc) introgression segment in wheat and identification of 18 candidate genes in *Ae. sharonensis* for the Gc 'breaker' element, invaluable useful for further mapping the Gc locus.

Gametocidal (Gc) chromosomes or elements in species such as *Aegilops sharonensis* are preferentially transmitted to the next generation through both the male and female gametes when introduced into wheat. Furthermore, any genes, e.g. genes that control agronomically important traits, showing complete linkage with gametocidal elements, are also transmitted preferentially to the next generation without the need for selection. The mechanism for the preferential transmission of the gametocidal elements appears to occur by the induction of extensive chromosome damage in any gametes that lack the gametocidal chromosome in question. Previous studies on the mechanism of the gametocidal action in *Ae. sharonensis* indicates that at least two-linked elements are involved. The first, the 'breaker' element, induces chromosome breakage in gametes, which have lost the gametocidal elements while the second, the 'inhibitor' element, prevents the chromosome breakage action of the 'breaker' element in gametes, which carry the Gc elements. In this study, we have used comparative genomic studies to map 54 single nucleotide polymorphism (SNP) markers in an *Ae. sharonensis* 4S^{shL} introgression segment in wheat and have also identified 18 candidate genes in *Ae. sharonensis* for the 'breaker' element through targeted sequencing of this 4S^{shL} introgression segment. This valuable genomic resource will aid in further mapping the Gc locus that could be exploited in wheat breeding to produce new, superior varieties of wheat.



P 42 - Topic: Harnessing Diversity for Triticeae Improvement

Characterisation of *Thinopyrum bessarabicum* chromosomes through genome wide introgressions into wheat

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Key message: Spontaneous recombination resulted in novel wheat-*Thinopyrum bessarabicum* translocation lines. SNP marker genotyping and cytological investigation of these lines allowed for the further characterisation and generation of a putative physical map.

Due to modern breeding practises relatively little genetic variation is available in modern wheat varieties for breeders to develop superior adapted genotypes with increased yield potential and tolerance to abiotic and biotic stresses. The wild relatives of wheat provide a vast and largely untapped reservoir of genetic variation (for traits such as tolerance to abiotic and biotic stresses, biomass, yield and photosynthetic potential). *Thinopyrum bessarabicum* ($2n=2x=14$, JJ) is a valuable source of genes for bread wheat ($2n=6x=42$) improvement because of its salinity tolerance and disease resistance. Development of wheat-*Th. bessarabicum* translocation lines by backcrossing the F_1 in the absence of the *Ph1* gene (allowing intergenomic recombination) can assist its utilization in wheat improvement. In this study, novel wheat-*Th. bessarabicum* translocation lines involving different chromosome segments along with whole/truncated arm additions were identified and characterized using genomic *in situ* hybridization (GISH) and fluorescent *in situ* hybridization (FISH). The detection and characterisation of introgressions generated was also facilitated via the new Affymetrix wild relative SNP array (King et al. 2017), specifically designed to detect introgressions in wheat from its wild relatives. A map of 433 SNP markers spanning all 7 J chromosomes from *Th. bessarabicum* was generated. After combining marker results with cytological detection in each line, the SNP marker map was divided into segmental blocks for each chromosome and markers within each block were ordered according to the wheat POPSEQ data (Chapman et al. 2015) resulting in a putative physical map that characterised all *Th. bessarabicum* J chromosomes. Comparative genome analysis indicates that *Th. bessarabicum* chromosomes maintain macro-synteny with wheat A, B, and D genome chromosomes and like wheat it has the 4/5 translocation. The novel wheat-*Th. bessarabicum* recombinant lines and the physical map developed in this research provide useful stocks and tools for introgression of genes on *Th. bessarabicum* chromosomes into wheat. In addition, the approaches we have employed have resulted in a step change in the generation and detection of wheat/wild relative introgressions that can be exploited for the development of superior, high yielding, environmentally adapted wheat varieties.

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P 44 - Topic: Harnessing Diversity for Triticeae Improvement

Extensive molecular, phenotypic and geographic characterization of natural populations of the wild relative *Triticum urartu* occurring throughout the Fertile Crescent

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Key message: We provide a multi-faceted characterization of *Triticum urartu* populations occurring throughout the area of domestication of modern wheat, identifying genomic loci of potential agronomic and adaptive interest for wheat breeding.

With emerging abiotic and biotic threats to wheat cultivation (Jarvis et al. 2008), *Triticum urartu*, the A^u-genome donor of *T. aestivum* and *T. durum*, may represent an important reservoir of genes/alleles for wheat improvement. The genetic diversity of *T. urartu*, not limited by breeding (Haudry et al. 2007), harness alleles that allow its natural populations them to withstand different environmental conditions. The rusticity features of *T. urartu* could be exploited to identify favorable variants useful to enlarge the genetic pool of cultivated wheat. We assembled a collection of 352 accession of *T. urartu* conserved *ex situ* and sampled across the Fertile Crescent in an area spanning more than 1500 km and including sites in Armenia, Iran, Iraq, Jordan, Lebanon, Syria and Turkey. The characterization of this collection was done at three levels. First, the collection was genotyped using a double-digestion RAD sequencing approach, producing 62 541 high-quality genome-wide SNPs. The diversity analyses revealed a broad molecular variation across the sampled populations, suggesting a complex evolutionary history. Second, we measured two phenologic, one disease and four agronomic traits in the collection in two field experiments. We also proceeded with flour protein characterization. The data analyses highlighted a broad phenotypic variation in all traits, including flowering time, plant height, and yellow rust resistance. Phenotypic data were used to perform a genome-wide association study (GWAS) aimed at the identification of genomic loci responsible for those complex phenotypes. Third, we linked the sampled populations to local environmental information using the GPS coordinates of the sampling areas in a geographic information system. We observed a broad climatic variation in the sites where *T. urartu* was originally collected. We conducted a landscape genome study to identify genomic loci under directional selection and alleles associated to pedoclimatic variation. The genomic loci emerging from our analyses might be relevant for wheat breeding, either through targeting homologous loci, through biotechnology approaches or through compatible crosses. The same collection was used to select 12 diverging founders to produce a nested association mapping (NAM) population. This multiparental mapping resource, now at the F₄ stage, will increase QTL mapping efficiency in wild relatives of wheat.

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P 46 - Topic: Harnessing Diversity for Triticeae Improvement

Wheat multiple synthetic derivatives population (MSD): a new resource for breeding, QTL mapping and genes isolation from *Aegilops tauschii*

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Key message: The MSD population described in this study will provide a good opportunity for effective utilization of *Aegilops tauschii* traits and genes for wheat breeding.

Wheat breeding is becoming increasingly difficult due to the limited genetic variation within the wheat gene pool. Introducing genes from wild relatives is the best option to increase the genetic diversity and discover new alleles for important qualitative and quantitative agronomic traits. We developed a population harboring the intraspecific variation of the diploid wheat progenitor *Aegilops tauschii* in the background of the common wheat *Triticum aestivum* by crossing and backcrossing 43 synthetic wheat lines with the common wheat cultivar Norin 61. This population was named multiple synthetic derivatives population (MSD) (Tsujimoto et al. 2015). To validate the suitability of this population for wheat breeding and genetic studies we randomly selected 400 lines from the basic population and genotyped these lines by using DArT-Seq markers. We scored the black glume and heading time as qualitative and quantitative traits, respectively. Our results showed high genetic diversity and less recombination which is expected from the nature of the population. Out of 397 MSD individuals, 61 MSD individuals exhibited black glumes (15.3% of MSD population). GWA analysis showed one QTL at 22.6 cM of the short arm of chromosome 1D in line with the findings of Khlestkina et al. (2009). By translating the linkage distance into physical distance according to the wheat reference genome sequence we identified the black glume QTL in chromosome 1D in the range from 0.3 to 2.28 Mbps which harbors 64 protein-coding genes. For the heading time, GWA analysis revealed a single QTL in the short arm of chromosome 2D from both genetic and physical map-based analyses. The QTL ranged from 47.5 cM to 84.6 cM which stands for a physical range of 12.0 Mb to 13.3 Mb which harbors 55 protein-coding genes. This position is accordant to the known locus of *Ppd-1D*, a pentatricopeptide repeat (PPR) protein-coding gene. Based on the results we conclude that the MSD population could be a good resource for wheat improvement and genetic studies. Currently, this population is being tested for several breeding objectives.

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P 48 - Topic: Harnessing Diversity for Triticeae Improvement

Genetic marker development from RNA sequencing of *Aegilops tauschii* accessions and its application to fine mapping in synthetic hexaploid wheat

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Key message: Genome-wide polymorphisms from RNA sequencing analysis of ten diverse *Aegilops tauschii* accessions was useful to develop molecular markers linked to the target locus in the D genome of synthetic wheat.

The wild species in the Triticeae tribe possess wide natural variation and have been utilized as genetic resources for crop improvement. However, due to their large and complex genomes, the reference genomes and physical maps are not perfected yet and genetic map construction of high resolution is still challenging. To fully harness their agronomically important traits, an efficient system is needed for molecular marker development in the Triticeae wild species. Here, we assembled RNA sequences of ten representative accessions of *Aegilops tauschii*, the D-genome progenitor of bread wheat, and called polymorphisms (Nishijima et al. 2016). On average, 28 594 SNPs and 1522 indels were obtained in each pairwise combination. The *de novo* assembled transcripts were anchored to the chromosomes of *Ae. tauschii* and barley. The SNPs on the anchored transcripts were distributed throughout the chromosomes, ideal for linkage map construction, even between the genetically closest accessions. The resolution of SNP distribution on barley chromosomes was as high as on *Ae. tauschii* chromosomes. Since the synteny within the Triticeae tribe is well conserved, our strategy allows the genetic markers line up in order based on barley chromosomes and should be applicable to marker development for these species, which have no draft sequences. To assess the usefulness of the SNP dataset, CAPS markers were developed for linkage analysis in three mapping populations; two *Ae. tauschii* populations, one from the cross between the accessions used for RNA-seq and the other from accessions not sequenced, and one synthetic hexaploid wheat population derived from the parental *Ae. tauschii* accessions with RNA-seq data. In the *Ae. tauschii* populations, most of the CAPS markers were assigned to the expected chromosomal regions and some were closely linked to the target trait loci. Even in the population without RNA-seq data, markers were developed successfully based on the SNPs between the accessions each genetically close to the parental accessions of this population. In the synthetic wheat population, this dataset, combined with bulked segregant analysis, allowed us to detect more efficiently the SNPs tightly linked to the target gene. These results indicate that the SNP dataset from RNA-seq and the derived molecular markers accelerated linkage map construction even in other *Ae. tauschii* accessions and in hexaploid genetic background.

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P 50 - Topic: Harnessing Diversity for Triticeae Improvement

A wide collection of wild emmer accessions to recover diversity for cultivated wheats

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Key message: A wide collection of wild emmer accessions, equipped with genotypic data, has been established to recover useful genetic diversity for cultivated wheats.

The wild emmer, *Triticum dicoccoides* (AABB), is the progenitor of the domesticated durum wheat. It harbors a wide spectrum of alleles that were lost during domestication and breeding processes, some features being deleterious, but many others possibly useful for genetic improvement of cultivated wheats. Indeed, a number of genes and alleles positively contributing to biotic and abiotic stress tolerance, yield components and quality have been found in wild emmer and introgressed in cultivated wheats. A collection of 450 wild emmer accessions has been established at CREA-Genomics Research Centre. The accessions have originated from all Fertile Crescent countries and grouped in Western and Eastern races, thus representing all environments where wild emmer naturally occurs. After one cycle of single seed descent, 285 wild emmer accessions have been genotyped using the Axiom 35k Breeders' Array, obtaining about 10 000 poly-high resolution markers. Based on genotyping data, the genetic diversity levels and population structure within the collection have been assessed. Using principal coordinate analysis and structure analysis, several group and sub-group were identified. This collection is being exploited for genome wide association scan (GWAS) for a number of traits including rust resistance and spike fertility.



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Comparative molecular cytogenetic and epigenetic characterization of wheat alien hybrids and introgression lines

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Key message: Transfer and expression of alien chromatin in wheat is critical to access more genetic variability. We report on meiotic and epigenetic behaviour of alien chromatin enabling exploitation of novel variation.

Exploitation of the Triticeae gene pool through chromosome engineering has been the most effective way to introduce novel traits to wheat breeding lines. Many varieties carrying rye or *Thinopyrum* chromatin have been released or are in trials with increased resistance to biotic and abiotic stresses. Genomic *in situ* hybridization (Schwarzacher et al. 1992) identifies alien chromatin in hybrid and introgression lines and, combined with repetitive DNA probes, we have identified novel translocations, chromatin segments and multiple alien introgressions. Sister lines of the wheat variety 'Mace' with wheat streak mosaic virus resistance and *Th. intermedium* chromatin have additionally small segments of rye on the short arm of 1B (Ali et al. 2016). Other lines investigated carry various intercalary *Th. bessarabicum* translocations involving different recipient wheat chromosomes, wheat-barley addition lines and different rye translocations. Alien chromatin transfer uses wheat x alien hybrids followed by back crossing and loss of most alien chromatin. Loss processes are not controllable, but large numbers of lines can be screened by phenotype, markers, and/or chromosomal constitution. It is important to introduce small segments of the alien chromatin so only small numbers of genes carrying desirable traits are transferred. We studied mitotic and meiotic processes to understand stable inheritance of chromosomes, pairing, recombination and segregation. Centromere behaviour is critical and we have investigated epigenetic modification of centromeres using immuno-staining with the centromere specific histone variant CENH3 (Sepsi et al. 2017). We studied DNA methylation and histone modifications to understand the behaviour and epigenetic interactions of alien chromatin in a wheat background. To exploit the diversity of alien species in wheat breeding and super-domestication for sustainable agriculture (Figure 1), we need to identify alien chromatin segments and specific genes, but also control chromosome stability, meiotic behaviour and gene expression. For further details see www.molcyt.com.

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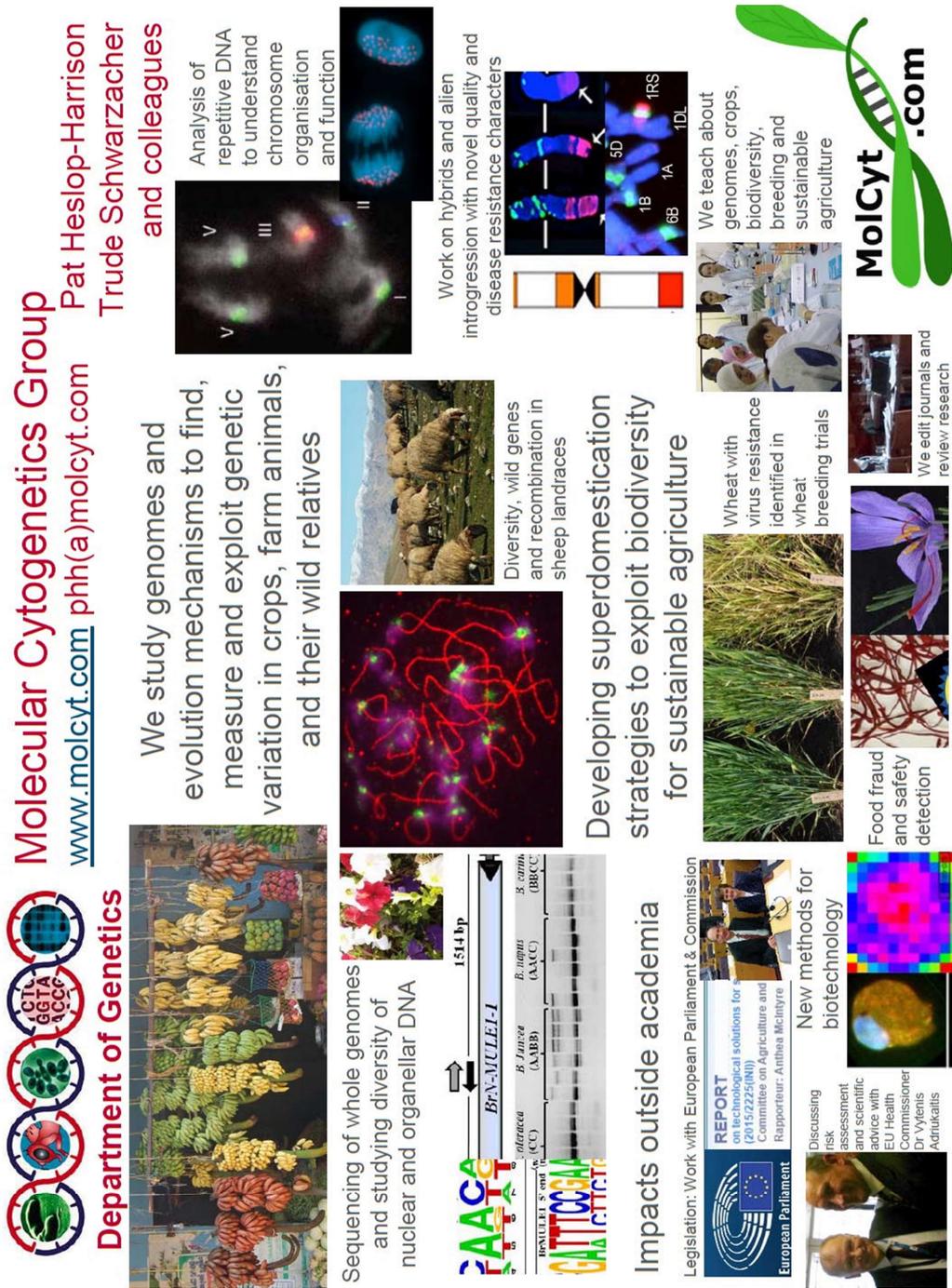


Figure 1: Infographic representation of the research in the Molecular Cytogenetics Group at the University of Leicester. Among many different plant species groups, wheat is one of the major crops we study and our aim is developing super-domestication strategies to exploit biodiversity for sustainable agriculture.



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High-resolution mapping of a QTL for resistance to Fusarium head blight located on chromosome 2A of *Triticum monococcum*

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Key message: The high-resolution mapping allows the identification of co-segregating markers and candidate genes involved in Fusarium head blight resistance and therefore new sources for introgression work and applied breeding.

Fusarium head blight (FHB) is one of the most important diseases in wheat and other small grain cereals worldwide, predominantly caused by the fungal pathogens *Fusarium graminearum* and *F. culmorum*. The infection leads to sterility of spikelets and reduced kernel weight and finally to yield losses. Moreover seed quality is decreased due to the production of mycotoxins during the infection cycle. The best control strategy is resistance breeding, because fungicide applications and agronomic measurements are only partially effective against FHB. By analyzing a DH population derived from the cross *Triticum monococcum* mon10-1 × Sinskaya two neighboring QTL for resistance against FHB were mapped on chromosome 2A of *T. monococcum* based on two year's field trials and a DArT map comprising 1987.5 cM. In order to detect closer linked markers and to get information on genes involved in this resistance, a map based cloning approach is conducted. For this purpose flanking DArT markers were converted to KASP markers in order to identify F₂ plants carrying a recombination event in the target region. Out of these recombinant plants, homozygous recombinant inbred lines (RILs) for the target interval are developed for marker saturation and phenotyping with *F. culmorum* Fc46. Up to now, 1991 F₂-plants, providing a genetic resolution of 0.025% rec. were analyzed and 110 segmental RILs were developed out of 686 identified recombinant F₂-plants. The genetic distance between flanking markers was determined at 11.37 cM between KASP-Marker 1 and KASP-Marker 2 flanking QTL-1 and at 6.43 cM between KASP-Marker 2 and KASP-Marker 3 flanking QTL-2, resulting in an interval for both QTL of around 18 cM. Up to now, 17 markers were developed from the genetic map of *T. monococcum* and used for the analysis of RILs. Additional markers will be selected from the sequences of chromosome 2A of *T. aestivum* and *T. urartu* and from genotyping-by-sequencing. By this approach and increasing the resolution to 0.01% rec. and phenotyping of RILs with *F. culmorum* (Fc46) in greenhouse and in field experiments the QTL interval will be considerably shortened and candidate genes will be identified.



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Reducing the size of a chromosomal segment carrying leaf rust and stripe rust resistance that was introgressed from *Aegilops sharonensis* Eig. into bread wheat

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Key message: We reduced the size of an alien introgressed segment conferring rust resistance by induction of homoeologous pairing, sequencing and mapping the segment and using SNPs to select desired genotypes.

A segment conferring resistance to both leaf rust and stripe rust pathogens was transferred by homoeologous recombination from chromosome 6S^{sh} of Sharon goatgrass (*Ae. sharonensis* Eig) to chromosome 6B of bread wheat (*Triticum aestivum* L.) (Millet et al. 2014). Mapping of this chromosome by DArT markers in a number of different resistant lines revealed a relatively long 6S^{sh} segment substituting for its wheat homoeologous segment. Such long substitution commonly leads to various types of unwanted effects, including yield reduction. Our goal in this project was to produce rust resistant wheat genotypes with a shorter alien segment. As a first step, recombinant lines were hybridized and backcrossed with Sears' high pairing mutant. Homozygous *ph1b* progenies that were heterozygous for the wheat and its homoeologous alien segments were selected and crossed with the elite spring wheat cv. Galil. DNA from 100 resistant and susceptible progenies was sequenced and analyzed by NRGene, a leading genomic big data company, Israel. A genetic map of chromosome 6B was generated based on frequency of recombination events and using the genome sequence of wild emmer wheat as reference. Based on the sequencing results we identified within the introgressed segment (140Mb of recombinant chromosome 6B) SNPs between wheat and Sharon goatgrass. These SNPs were used to develop nine PCR probes, which distinguished between the *Ae. sharonensis* and wheat along this region. We screened over 500 resistant secondary recombinants with the PCR probes, and identified 12 lines that had significantly reduced size of the 6S^{sh} introgression, ranging between 50 Mb to 120 Mb. Consequently we could narrow down the putative location of the resistance loci to a region of approximately 17 Mbp. Finally, in order to produce an elite wheat cultivar with novel leaf and stripe rust resistances we cross and backcross the secondary recombinants with the shorter introgressions to cv. Galil and use the PCR markers to select desired plants among their progenies.

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New productive primary hexaploid synthetics resistant to diseases and pests

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Key message: Characterization of new hexaploid synthetics from CIMMYT and Japan, wide crosses from Kazakhstan in contrasting environments identified unique germplasm with multiple resistance to diseases and pests, good spike productivity traits.

We studied 4 groups of germplasm: (1) CIMMYT primary synthetics originating from winter durum wheat germplasm from Ukraine and Romania crossed to *Aegilops tauschii* accessions from the Caspian Sea basin; (2) Primary Japanese synthetics derived from crosses between Langdon durum wheat and diversity of *Ae. tauschii* accessions across its natural distribution range; (3) Spring and winter wheat lines derived from crosses of wheat varieties from Kazakhstan and wild wheat (*Triticum kihare*, *T. timopheevii*, *T. militinae*, *T. zhukovskyi*); (4) Lines originating from group 1 synthetics crossed to bread wheat varieties. The germplasm was studied in 2014-2016 across several sites in Azerbaijan, Kazakhstan, Russia and Turkey for grain yield, spike productivity traits, diseases and pest resistance. Primary synthetics (groups 1 and 2) were generally characterized by tall stature (100-110 cm) and number of days to heading slightly longer than the checks. Lines originating from wide crosses (group 3) and synthetics crossed to bread wheat (group 4) were closer to check varieties for many traits. High frequency of lines with resistance to leaf, stripe and stem rust, common bunt and soil borne pathogens were identified across all groups. Molecular screening of group 1 synthetics for *Lr* genes identified *Lr10*, *Lr21*, *Lr24*, *Lr34* and *Lr39* (*Lr41*). All these genes reduced leaf rust severity compared to susceptible checks in Western Siberia. Three highly resistant synthetics (Ukr.Od1530.94/*Ae. tauschii*) with unknown major genes and several synthetics possessing adult plant resistance genes were identified. Seedlings evaluation of groups 1-3 synthetics against *Septoria tritici* and *S. nodorum* identified 35 genotypes resistant to both pathogens. Spikes of most of synthetic lines are longer than the checks. The number of grains per spike was variable though many exceed checks. The grain size expressed by 1000 kernel weight in many lines exceeded 50 g while checks grown under the same conditions barely reached 40 gr. Threshability of the synthetic lines from group 1 varied from 0 to 95% demonstrating genetic variation for this important domestication trait. Preliminary screening for wheat blast (*Magnaporthe oryzae*) under controlled conditions and artificial inoculation identified three synthetic lines with low infection. Screening of synthetic lines against Hessian fly, sunny pest and Russian wheat aphid allowed selection of several resistant lines (El-Bouhssini, pers. commun.) There was clear effect of both durum and *Aegilopes* parents on synthetic wheat traits. Superior wide crosses line originating from crosses between Kazakhstanskaya 10 and Kaz. 25 varieties with *T. timopheevii* were identified.



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Simple inheritance of nonhost resistance mechanisms to wheat stripe rust in *Brachypodium distachyon*

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Key message: Components of nonhost resistance to wheat stripe rust in *Brachypodium* are genetically simple and may provide a potential resource for host resistance in wheat.

Nonhost resistance (NHR) provides plant immunity to most potential pathogens, thereby greatly limiting the number of phytopathogens capable of parasitizing each plant species. NHR has often been difficult to characterize due to its mechanistic redundancy and polygenic inheritance (da Cunha et al. 2006, Jafary et al. 2008, Bettgenhaeuser et al. 2014). In this study, *Brachypodium distachyon*, a model grass related to wheat, was used to investigate the genetic complexity of NHR to the wheat stripe rust pathogen, *Puccinia striiformis* f. sp. *tritici*. Screening of *Brachypodium* accessions with a panel of Australian and UK *P. striiformis* isolates identified variation in disease phenotypes ranging from complete immunity to partial susceptibility. Three mapping families (BdTR10H × TEK4, BdTR13K × Bd21, ABR6 × Bd21) were established by crossing *Brachypodium* accessions that showed contrasting stripe rust infection phenotypes. In each family, macroscopic immunity to rust infection was dominantly inherited with one to three independent stripe rust NHR genes segregating. These resistances were mapped to three loci in the *Brachypodium* genome and designated *Yrr1*, *Yrr2* and *Yrr3*, respectively. *Yrr1*, which is common in all three families, was effective against all rust pathogen isolates tested and fine mapped to a 100kb region containing six candidate genes, none of which appear similar to known plant resistance genes. *Yrr2*, which is present in the BdTR13K × Bd21 and ABR6 × Bd21 families, was shown to be race-specific and mapped to a 300kb region encoding a family of NLR resistance genes. *Yrr3*, present in the ABR6 × Bd21 family, is effective against all stripe pathogen isolates tested and has been refined to a 74kb region encoding two polymorphic NLR genes. Candidate resistance genes from each locus are being transformed into *Brachypodium* for complementation studies and also into wheat to test for functional interspecies transfer of these NHR genes. The relatively simple inheritance of some components of nonhost resistance to wheat stripe rust in *Brachypodium* demonstrates the potential of this species as a genetic resource for host resistance in wheat.

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Physical mapping of the stripe rust resistance gene *YrG303* derived from wild emmer wheat

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Key message: The stripe rust resistance gene *YrG303*, derived from wild emmer wheat, was mapped to the same location as *Yr15*, and therefore we assume that it is allelic to *Yr15*

Stripe rust, caused by the fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a serious disease of wheat occurring in most wheat areas with cool and moist weather conditions during the growing season. In case of susceptible varieties of wheat, yield losses can reach up to 100%. Breeding of resistant varieties is the most sustainable and cost-effective method to control this disease. Depletion of effective resistance to *Pst* in cultivated wheat has led to search for new genes among wild progenitors of wheat. Recently, we have identified a major dominant gene, designated *YrG303*, derived from wild emmer wheat, *Triticum dicoccoides*, which we have mapped to chromosome arm 1BS. The main objective of our project was to clone *YrG303* using the positional cloning approach. Fine mapping of *YrG303* was achieved based on graphical genotyping of a large hexaploid mapping population produced by composite crosses of the donor *T. dicoccoides* accession G303, first to bread wheat variety Vaskar and then to Avocet. This mapping population is segregating for a *T. dicoccoides* chromosome segment carrying *YrG303*. We have developed a physical map of the *YrG303* gene region by anchoring the genetic markers to the physical map of chromosome 1BS constructed by our group (Raats et al. 2013) and various wheat genomic sequences available in the public databases. The results of high density mapping showed that *YrG303* was assigned to the same location as *Yr15*, derived from *T. dicoccoides* accession G25. Therefore, we hypothesized that *YrG303* is allelic to the well-known dominant gene *Yr15*, which confers resistance to a broad spectrum of stripe rust races. Further work is under way to validate the function of this gene.

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P 64 - Topic: Harnessing Diversity for Triticeae Improvement

Identification of wheat dwarf virus (WDV) resistance/tolerance in wheat

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Key message: The identification of QTL for WDV resistance and development of molecular markers allows the replacement of laborious resistance tests with WDV-bearing leafhoppers and facilitates applied breeding for WDV resistance.

Wheat dwarf virus (WDV) causes high yield losses in wheat and other cereals. WDV is transmitted by the leafhopper *Psammotettix alienus*. Symptoms include yellowing and dwarfing of infected plants along with high yield loss. Due to global warming, insect-transmitted viruses will become more important. Growing WDV-resistant/tolerant varieties is the most effective and environmentally friendly way to control WDV. Hence, the aim of our project is to identify WDV resistant/tolerant genotypes by screening gene bank accessions and breeding lines for WDV tolerance/resistance and to identify quantitative trait loci (QTL) by genome-wide association studies. A set of 500 genotypes, comprising different wheat species and wild relatives (e.g. *Aegilops* accessions), was tested by artificial infection in gauze houses and under natural infection in field trials in Žabčice, Czech Republic, during the last two growing seasons. The majority of the tested genotypes turned out to be highly susceptible. The susceptible standard cultivar 'Hybnos' revealed an average infection rate of 75% indicating a high infection pressure. Six accessions were identified with a very low infection rate (average 0-12%) in two year trials. Relative yield per plant (compared to a non-infected control variant) ranged from 0% to 100%. Among these, one accession showed no yield reduction after virus infection during the test in 2014/15. Crosses with this cultivar were conducted by the breeding partners and SSD/DH populations will be developed. The most resistant genotypes (42) are tested again this year. A subset of 250 hexaploid genotypes expressing different WDV resistance levels was selected and genotyped by the 15k iSelect chip. The identification of QTL for WDV resistance and development of molecular markers are the prerequisite to replace the laborious and time consuming resistance tests with WDV-bearing leafhoppers. This will facilitate the integration of breeding for WDV tolerance/resistance into applied wheat breeding.



P 66 - Topic: Harnessing Diversity for Triticeae Improvement

Wild and cultivated barley chromosomes can recognise and associate in pairs during early meiosis in the wheat background

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Key message: Related chromosomes from two barley species can associate but not recombine during early meiosis in the wheat background. Subtelomeres might play a major role on this interspecific chromosome association.

The success of meiosis strongly depends on regular pairing of homologous (identical) chromosomes, which is mainly controlled in wheat by the *Ph1* locus. This means that chromosome pairing and recombination of related chromosomes rarely occur in the presence of this locus, making difficult wheat breeding through the incorporation of genetic variability from related species. In this work we show that chromosome pairing between barley related chromosomes can be possible in the wheat background in the presence of the *Ph1* locus. We have made genetic crosses between wild and cultivated barley addition lines for the equivalent and different homoeology chromosome group in the wheat background. Genetic *in situ* hybridisation revealed that chromosomes from both barley species for the equivalent homoeology group can recognise and pair during early meiosis in wheat. However, although these related barley chromosomes for the same homoeology group can associate in pairs, recombination does not occur at any time between them and they remained always as univalents during meiosis metaphase I. Our results suggest that the *Ph1* locus does not prevent chromosome recognition and pairing between related chromosomes in the wheat background and might be implicated in a later meiotic stage (recombination). Subtelomeres might play a major role allowing chromosome recognition and pairing between related barley chromosomes since similar subtelomeric sequences are present in both equivalent chromosomes. Related barley chromosomes from different homoeology group do not associate at any time during meiosis in wheat. The results as well as their biological implications are discussed in this work.

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Altered expression of *TaRSL4* gene by genome interplay shapes root hair length in allopolyploid wheat

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Key words: Polyploidy, Wheat, Root hair, *TaRSL4*

Polyploidy is a major driving force in plant evolution and speciation. Phenotypic changes often arise with the formation, natural selection and domestication of polyploid plants. However, little is known about the consequence of hybridization and polyploidization on root hair development. Here, we report that root hair length of synthetic and natural allopolyploid wheats is significantly longer than those of their diploid progenitors, whereas no difference is observed between allohexaploid and allotetraploid wheats. The expression of wheat gene *TaRSL4*, an orthologue of *AtRSL4* controlling the root hair development in *Arabidopsis*, was positively correlated with the root hair length in diploid and allotetraploid wheats. Moreover, transcript abundance of *TaRSL4* homoeologue from A genome (*TaRSL4-A*) was much higher than those of other genomes in natural allopolyploid wheat. Notably, increased root hair length by overexpression of the *TaRSL4-A* in wheat led to enhanced shoot fresh biomass under nutrient-poor conditions. Our observations indicate that increased root hair length in allohexaploid wheat originated in the allotetraploid progenitors and altered expression of *TaRSL4* gene by genome interplay shapes root hair length in allopolyploid wheat.



P 70 - Topic: Harnessing Diversity for Triticeae Improvement

Analysis of 1RS.1BL translocations in the lines of bread wheat of Ukrainian selection using molecular markers specific to rye and wheat chromatin

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Key message: This research reveals 3% recombination and 9.5% retranslocations of 1RS.1BL chromosome among the 63 lines developed from crossing *ErythrospERMUM* 125/03 (contains 1RS.1BL translocation) × Chinese Spring *ph1b*-mutant.

The rye (*Secale cereale* L.) 1RS chromosome is used in hybridization with wheat as a source of resistance genes to leaf rust (*Lr26*), stem rust (*St31*), yellow rust (*Yr9*) and powdery mildew (*Pm8*) (Merker & Forsström 2000, Graybosch 2001). The presence of 1RS.1BL translocations can also increase the yield of wheat and tolerance to adverse weather conditions. Despite a number of positive effects, the 1RS rye chromosome brings also flour quality deterioration. This effect is associated with the introduction of the *Sec-1* gene that encodes the expression of rye storage proteins, i.e. ω-secalins, and the loss of genes encoding gliadins and glutenins located on wheat chromosome 1BS (Dhaliwal & MacRitchie 1990). Therefore, it is important to create lines of wheat, which carry homeological recombinations between the short arm of rye chromosome 1R and wheat chromosome 1BS, with the replacement of the *Sec-1* gene by *Glu* and *Gli* loci of 1BS. The aim of our study is to determine the presence of 1RS.1BL translocation and recombination between chromosomes 1BS and 1RS in the original breeding material. Sixty three lines (BC₁F₃) derived from the cross between *ErythrospERMUM* 125/03, which contains the 1RS translocation, with Chinese Spring *ph1b*-mutant were investigated. Nine markers specific to the 1R chromosome (*Sec1Gene*, *Sec1Pro*, *AF1/AF4*, *IB-267*, *NOR*, *PAW161*, *Rye F3/R3*, *RIS*), three SSR markers specific to 1BS (*XTaglgap*, *Xgwm 18*) and 1AS (*Xgwm 33-1D*) were used to detect the 1RS.1BL translocation and recombinations. There were found 27 wheat lines that contain the 1RS.1BL translocation, 28 lines with 1B chromosome of wheat, 6 lines with 1RS retranslocation to another chromosomes of A or D genomes and 2 lines that have a recombinant 1RS.1BL translocation. PCR-analysis with marker to 1AS chromosome confirmed the retranslocation 1RS on 1A in the two wheat lines. Future research will involve more SSR markers to reveal more precise information about recombinant translocations and retranslocations.

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P 72 - Topic: Harnessing Diversity for Triticeae Improvement

Identification and characterization of a new *Vrn-A1f-like* allele responsible for flowering lateness in wheat

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Key message: *Vrn-A1f-like* allele caused flowering lateness in two bread wheat mapping populations. The new allele originated from *Triticum militinae* and was identified in 80 % tetraploid spring wheat varieties with AAGG genome.

Flowering is a crucial process in plant life, which impacts crop yield. Here we identify tetraploid wheat *Triticum millitinae* ($2n=4x=28$, AAGG) as a resource for mining new genes/alleles influencing flowering time. We observed flowering time variation in a mapping population of doubled haploid (DH) lines. It was developed from a cross between introgressive line 8.1 and elite bread wheat cv. Tähti. Line 8.1 carries introgressions from *T. militinae*, in Tähti background. The analysis of DH mapping population revealed presence of seven loci influencing flowering time. The most significant QTL for the flowering time variation was identified within the introgressed region on chromosome 5A and its largest effect was associated with the *VRN-A1* locus, covering up to 67 % of phenotypic variation. The analysis of F₂ mapping population developed from cross DH81 × Mooni confirmed the effect of this locus on flowering time. DNA sequence analysis revealed the origin from *T. militinae* and the allele was designated as *VRN-A1f-like* (KT696537). The allele incurred a delay of 1.9-18.6 days in flowering in different growing conditions. Comparison *VRN-A1f-like* allele to *VRN-A1a* sequences from the common wheat parental lines of the mapping populations revealed major mutations in the promoter region as well as in the first intron, including a MITE insertion and a large deletion. Moreover, the allele was identified to be responsible for spring habit emergence in 80% of the analyzed tetraploid wheat varieties with AAGG genome. Finally, analysis of difference in the relative expression level between parental lines of two mapping populations (DH81, cvs. Tähti and Mooni) was carried out. The identification and quantification of the effect of the *VRN-A1f-like* allele from *T. militinae* provides a valuable source of new alleles suitable for wheat improvement as well as for studying fine regulation of flowering pathways in wheat.



P 74 - Topic: Harnessing Diversity for Triticeae Improvement

Control of specific developmental stages as tool to wheat improvement

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Key message: The genetic control of timing and duration for phases of wheat inflorescence development, and its interaction with the environment, is an important tool to maximize harvest index.

The control of timing and duration for phases of wheat inflorescence development, and its interaction with the environment, is an important tool to maximize harvest index. For this purpose, we have selected and crossed wheat varieties with different origin and traits to generate populations with a high degree of diversity. In particular, varieties characterized by earliness, including CIMCOG15, MISR1, Super 152, Pfau, Waxing, Baj, and high yielding lines, such as CIMCOG49, CIMCOG47 and GARCIA, were crossed with UK cv. Paragon. F₄ and F₅ generations were grown both in the UK and Mexico where they have been scored for several phenotypic traits, namely days until GS31, heading, bolting, anthesis, stem height, grain yield, grain dimension and thousand grain weight. Plants were genotyped using the 35k Wheat Breeders Axiom array and the results elaborated through MST mapping. With the obtained phenotypic and genotypic data, we performed QTL analysis to associate genomic regions to specific traits. As a validation of reliability for data collection and analysis methods, genes known to be involved in the regulation of the flowering time pathway, such as *VRN1*, *Rht1*, *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, *Ppd-D1* showed strong correlation with traits of time to heading, bolting and anthesis in different parental crosses. We then searched for genomic regions, not comprising any known gene that correlated with a specific developmental stage. Among all the candidate locus identified, a genomic regions located on chromosome 7D of plants coming from a Paragon × Baj cross was selected because of its strong correlation with time of heading in both Mexico and UK dataset. This genomic region comprises the wheat homologue of the important plant florigen *FT*, thus constituting a strong and promising candidate for future analyses. Characterization of the *FT-D1* gene in the Baj, Paragon and Pfau populations is currently underway with the aim to investigate whether the phenotype differences among these varieties is specifically due to a defect in the FT protein activity and/or in its transcriptional regulation. In parallel to this analyses, confirmation of the phenotype segregation will be performed using markers closely located to the *FT-D1* locus and by crossing the selected FT-D1 line with wheat plants known to be wild-type for the 7D locus. The confirmation of the involvement of the *FT-D1* gene of wheat in the control of the flowering time and, consequently, of the plant yield, will certainly have far reaching commercial and agronomical implications.



P 76 - Topic: Harnessing Diversity for Triticeae Improvement

Development of a genetic basis for the comprehensive study of the inheritance and physiological role of leaf pubescence in bread wheat

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Key message: The near-isogenic lines with different genes for leaf pubescence have been developed for further investigation of the inheritance and physiological role of this trait

Leaf pubescence of different density and morphology is typical for bread wheat and its close relatives (Pshenichnikova et al. 2017). Its diversity and adaptation value in various environmental conditions are poorly understood. For such investigations, the genetic material is necessary in the form of isogenic lines - carriers of certain genes for leaf pubescence that will help to explore the inheritance, mode of expression and the physiological significance of this trait. During the last years we have created such kind of genetic material using the genes for leaf pubescence from the lines with introgressions from wheat relatives. The recipient genotype was a densely pubescent cultivar Saratovskaya 29 (S29) which carries 2 genes for leaf pubescence (Doroshkov et al. 2011) and is characterized by a high resistance to drought. Two near-isogenic lines were developed on this genetic background. One of them was created by backcrossing to a glabrous cultivar Rodina in order to obtain the near-isogenic line of S29 without leaf pubescence. Another line was created by the introduction of the additional *HL2^{oesp}* gene from the line with the introgression of this trait from *Aegilops speltoides*. Quantitative characteristics of these lines (trichome density and length) have been characterized in detail by means of the computer image processing program LHDetect2. These two lines differ significantly from the recipient S29 for physiological parameters, such as gas exchange in leaves and leaf chlorophyll fluorescence. Also, the isogenic lines with *HL2^{oesp}* gene have been obtained on the genetic background of poorly haired or glabrous cultivars Diamant 2 and Rodina. The development of these lines was accompanied by analysis of the progeny for the microsatellite marker *Xgwm400* closely linked to *HL2^{oesp}* on chromosome 7B. Another gene for a specific, very long pubescence was introgressed into S29 from the endemic tetraploid species *Triticum timopheevii*. The monosomic analysis has showed that the gene is located on chromosome 5AL and is not allelic to bread wheat genes previously mapped to chromosomes 4BL and 7AS. The development of near-isogenic lines is accompanied by monitoring with the parental alleles of microsatellite markers. This genetic material will be used to identify the genes for leaf pubescence in wheat genome, to establish there belong to gene families, to study their structure and expression. It will also allow associating the different types of pubescence with physiological reactions of the leaf under adapting to environmental factors.

Acknowledgement

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P 78 - Topic: Harnessing Diversity for Triticeae Improvement

Association genetics and validation strategies in European winter wheat varieties

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Key message: A comprehensive GWAS analysis was conducted for agronomic traits including yield and resistance to fungal pathogens and validation strategies were implemented.

An overview of two long term studies (WHEAT, 2008-2011 and VALID, 2011-2015) regarding genome-wide association mapping in European winter wheat will be presented. A comprehensive dataset based on multi-environmental field data was developed for yield, other agronomic traits including resistance to biotic and abiotic stresses and baking quality. The phenotypic data were generated in replicated field trials at different locations and over several years respectively, using a panel of 358 European winter wheat and 14 spring wheat varieties mainly released between 2000 and 2005. The genotypic data consisted of microsatellite markers and sets of mapped Illumina iSelect 90K Infinium and 35K Affymetrix SNP markers that reflect the genome-wide haplotype diversity. Furthermore, specific markers for major phenology traits (such as *Rht* and *Ppd*) were included. The analysis of marker-trait associations was carried out by using a mixed linear model and a kinship matrix based on microsatellite markers to correct for population stratification. For validation of identified marker-trait associations (MTAs), a second wheat panel consisting of 133 more recent (mainly released in 2005 to 2010) winter wheat varieties was established and tested in two years of field trials. DH-populations and, via marker-assisted selection, BC2S2/3-lines have been developed for the validation of selected QTL for yield and resistance to *Fusarium* and *Septoria*.

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P 80 - Topic: Harnessing Diversity for Triticeae Improvement

Candidate gene based association analysis of frost tolerance in bread wheat (*Triticum aestivum* L.)

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Key message: Sequence analysis of locus specific PCR fragments of seven candidate genes in 276 bread wheat genotypes revealed association to frost tolerance.

One of the most important limiting factors in wheat production in North America, North and Eastern Europe and Russia is low temperature. In order to minimize yield losses due to frost in these areas, it is necessary to identify genes and alleles, respectively involved in this response and introduce most efficient alleles into elite cultivars. Understanding the genetic basis of frost tolerance (FT) in wheat (*Triticum aestivum* L.) is essential for preventing yield losses caused by frost due to cellular damage, dehydration and reduced metabolism. FT is a complex trait regulated by a number of genes and several gene families. Here we report on sequence analysis of 19 candidate genes. Out of these, the tandem duplicated C-repeat binding factors (*CBF*) *CBF-A3*, *CBF-A10*, *CBF-A13*, *CBF-A14*, *CBF-A15*, the vernalisation response gene *VRN-A1* and the photoperiod response gene *PPD-B1* in 276 wheat cultivars revealed association to FT. Within four genes (*CBF-A3*, *CBF-A15*, *VRN-A1* and *PPD-B1*) amino acid substitutions in important protein domains were identified. The amino acid (AA) substitution effect in *VRN-A1* on FT was confirmed and new AA substitutions for *CBF-A3*, *CBF-A15* and *PPD-B1* located at highly conserved sites were detected. Since these results rely on phenotypic data obtained at five locations in two years, the detected significant associations of FT to AA changes in *CBF-A3*, *CBF-A15*, *VRN-A1* and *PPD-B1* may be exploited in marker assisted breeding for frost tolerance in winter wheat.



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The main areas of genetics and breeding research of wheat in the All-Ukrainian Scientific Institute of Breeding

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Key words: All-Ukrainian Scientific Institute of Breeding (AUSIB), interspecific hybridization, molecular markers, official variety testing, rare species, valuable traits, wheat breeding, wheat varieties

The All-Ukrainian Scientific Institute of Breeding (AUSIB) is one of the first private academic institutions in Ukraine, which is focused on breeding activities. The main priorities of our work are studying the diversity of wheat and creating new varieties. Today, our company has four varieties of wheat which are registered in the State Register of Plant Varieties in Ukraine. AUSIB is the only company in Ukraine which has its own registered varieties of *Triticum spelta*. Our breeding work focuses on the creation of new varieties of winter and spring wheat, common and durum wheat, emmer and spelt wheat. We are also interested in other species of rare wheat which have economically valuable features and can improve known wheat varieties. For our work we cooperate with other plant breeding institutions, farmers and genebanks not only in Ukraine but also abroad. The main aim of our breeding work is the creation of high-yielding and widely adapted wheat varieties which are resistant to biotic and abiotic environmental factors. We are looking for varieties with high grain quality which may be used in different growing areas. Our collection demonstrates a high yield potential under various climatic conditions of Ukraine. In genetic breeding studies we use also underutilized wheat species such as *T. monococcum*, *T. dicoccum*, *T. aethiopicum*, *T. persicum*, *T. ispahanicum*, *T. polonicum*, *T. compactum*, *T. spelta*, *T. spaerococcum* and *T. petropavlovskiyi*, as well as interspecific hybrids and artificially created new amphidiploids (Tverdokhleba 2012, Rozhkov et al. 2014). For our research we use different scientific methods such as evaluation by molecular markers and physiological studies of the samples on artificial backgrounds (Oboznyi 2013). This approach increases the effectiveness of the breeding process and reduces the time of variety development.

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P 84 - Topic: Harnessing Diversity for Triticeae Improvement

Genetic associations in the detection of QTL for important agronomic and quality traits in durum wheat (*Triticum durum* L.)

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Key message: Analysis of molecular variance revealed that there was variation within and among the groups of the genotypes. The weighted neighbor-joining tree confirmed the groups identified in the PCoA, showing high and wide diversity in the durum genotypes.

Agro-morphological, agronomical and quality characteristics are the important traits for durum wheat. Understanding the genetic control of those traits can help breeders to develop varieties with improved characteristics. Fourteen of the above-mentioned characteristics were investigated in 130 durum wheat genotypes, which were divided into four groups, showing a wide range and significant variation. Variance analysis showed significant differences for most of the studied traits. Significant correlations were also observed between those traits. Analysis of molecular variance (AMOVA) revealed that there was variation within and among the groups of the genotypes studied. The weighted neighbor-joining tree confirmed the groups identified in the PCoA, showing high and wide diversity in the durum genotypes. Structure analysis revealed that the studied genotypes were divided into five groups with respect to the number of tree clusters. The mixed linear model used for accurate marker trait associations revealed that 92 of the 144 MTAs (marker-trait associations) were major MTAs, of which two were significantly associated with plant height (PH) and (vitreous kernel count) VKC. Pleiotropic effects were found in the MTAs. Taken together with the published genetic results, the MTAs determined in this study could be the targets of marker-assisted selection to improve many traits in durum wheat.



P 86 - Topic: Harnessing Diversity for Triticeae Improvement

Genetic diversity and association mapping for agromorphological and grain quality traits of a structured collection of spanish durum wheat

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Key message: Genetic characterisation and association study of a collection of Spanish durum wheat germplasm

Durum wheat (*Triticum turgidum* L. subsp. *durum*) is a crop of strategic importance in the Mediterranean area. However, the massive introduction of homogeneous and more productive modern cultivars have contributed to greatly reduce its genetic diversity. Wheat landrace collections contain a wide genetic diversity and constitute an easily transferable and valuable source of genetic variation for agronomical, morphological, adaptive and quality traits. A durum wheat collection of Spanish landraces comprising accessions of the three main inter-fertile subspecies (*durum*, *turgidum* and *diccocon*) was evaluated in a previous work using several molecular marker systems. The genetic diversity and agromorphological traits were correlated with geographic and climatic features. The broad variation found for all the evaluated traits underlined the large genetic variability of the collection (Ruiz et al. 2012). These study allowed the creation of the Spanish durum wheat core collection (Ruiz et al. 2013). Association mapping was performed for 18 agromorphological and grain quality traits in a collection of 183 Spanish landraces. A total of 85 stable MTAs (marker–trait associations) were identified for agromorphological and quality parameters, some of them common among subspecies and others subspecies-specific. For all the traits, MTAs explaining more than 10 % of the phenotypic variation were found in any of the three subspecies. The validation of several adaptive and quality trait MTAs by combining the association mapping with an analysis of the signature of selection, identifying the putative gene function of the marker, or by coincidences with previous reports, showed that this approach was successful for the detection of MTAs and the high potential of the collection to identify marker–trait associations. Novel MTAs not previously reported, some of them subspecies specific, have been described and provide new information about the genetic control of complex traits (Giraldo et al. 2016). The genetic characterisation and association studies of different wheat germplasm sets will provide researchers comprehensive information of the tetraploid wheat gene pool and the tools to improve the characterisation and utilisation of this germplasm.

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P 88 - Topic: Harnessing Diversity for Triticeae Improvement

Enrichment the genetic background of a wheat breeding project

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Key message: A possible breakthrough in significant grain yield increase of wheat could be gained by the large-scale exploiting of the genetic diversity of gene banks.

From the very beginning of the acknowledged wheat breeding project of Cereal Research Nonprofit Company we sustain a genotype (seed) collection, which contains all the successfully grown cereal genotypes of the Southern Great Plains of Hungary. This repository was broadened by foreign exotic breeding materials and in 1993 the Cereal Gene Bank of CRI came into existence. During the variety production which were based on prebreeding lines (resistant to biotic and abiotic stresses), local successful varieties and breeding materials - a novel, locally typical genetic background was generated by which we gained appreciable successes in variety production. Fungal disease resistance genes (*Sr36*, *Pm6*, originated in variety Arthur 71) were widely used in cultivar GK Kincső and its successful progenies (GK Békés, GK Csillag), while new genetic resources were involved to increase their tolerance to biotic and abiotic stresses. Parallel to conventional phenotyping in our program we concentrate on genotypic/marker assisted selection for involving the most potent resistance genes against leaf diseases and/or to improving the gluten composition for higher quality. Due to prebreeding activity our genotype collection increased by new highly adaptable lines with tolerance to biotic and abiotic stresses. During prebreeding procedure exotic genetic resources and synthetic hexaploid lines play significant role. These genotypes are produced by crossing our own durum wheat lines with locally adapted *Aegilops tauschii* biotypes. To define genetic diversity and population structure among a collection of wheat cultivars and lines of mainly European origin, Kompetitive Allele Specific PCR technology was used to characterize a population of 95 bread wheat genotypes. All these genotypes were bred in Hungary or widely used in Hungarian breeding programs. In total, 860 of 960 tested markers were polymorphic and could be used for further analysis. Four subgroups of wheat genotypes were identified using neighbor joining (NJ) cluster analysis. Two of this subgroups comprised mainly varieties from Hungarian breeding programs (GrI, GrII); one subgroup contained varieties from Western Europe (GrIII) and one contained varieties with various origin (GrIV). GrI mainly contained genotypes originated from crosses including GK Kincső (Arthur 71/Sava) as one of the parents, or derivatives of this genotype. The results of this study should provide valuable information for future association mapping studies using this wheat collection.

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P 90 - Topic: Harnessing Diversity for Triticeae Improvement

QTL for leaf nitrogen status in a bread wheat mapping population

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Key message: N dressing conditions and phenological phases reveal different QTL for chlorophyll content and N Balance Index

The leaf nitrogen (N) content positively correlates with crop biomass and yield, as well as with the N grain content. The chlorophyll/ flavonoids ratio (Chl/Flav), called N Balance Index (NBI), has been proposed as an indicator of leaf N content, more sensitive than chlorophyll content alone because of the Chl and Flav inverse dependence on the crop N nutrition status. Indeed, optimal growth conditions support an elevated primary metabolism and protein synthesis thus pushing toward N-based compounds, while carbon-based secondary compounds increase under N deficiency. Therefore, the NBI is an indicator of the nitrogen/carbon allocation balance. The genetic determinants of the leaf N status have been investigated by QTL analysis in a bread wheat RIL population (Victo × Spada), for which a high-resolution genetic map, based on 90k Infinium Illumina SNPs, is available. The phenotyping had been performed through the Dualex 4 Scientific (Dx4), a chlorophyll meter for the simultaneous estimation of leaf chlorophyll concentration and epidermal flavonoids. Measurements were recorded in several environments (three years and three locations), at two phenological stages (stem elongation and heading), and under different N dressing conditions (standard and deficiency) at heading phase. At stem elongation phase, the QTL analysis has revealed regions associated to chlorophyll content and NBI on chromosomes 7A and 4B. At heading stage, two major QTL, stable across environments, have been found on chromosomes 2B and 5B for chlorophyll content and NBI in standard N dressing, explaining a moderate level of phenotypic variance. Under N deficiency, two different QTL, located on chromosomes 5A and 7A, resulted significant. No one of these regions is coincident with the two QTL of the stem elongation phase. A very robust QTL for flavonoid content, conserved across environments, N dressing conditions and phenological stages, has been mapped on 3B. Co-localization of these QTL with known QTL and/or genes related to relevant metabolisms has been assessed.



P 21 - Topic: Structural and Functional Wheat Genomics

Validation of hexaploid wheat chromosome-scale assemblies by GBS and POPSEQ

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Key message: GBS and POPSEQ linkage maps were used to compare and help validate 10 hexaploid whole genome assemblies.

The genome of the allopolyploid bread wheat (*Triticum aestivum* L.) is large and highly repetitive. There is an ongoing collaborative project to sequence and assemble 10 hexaploid wheat genomes using the Illumina short read and NRGene DeNovoMAGIC 2.0 technologies. This approach is delivering the largest and most contiguous assemblies to date. Here, we use genotyping-by-sequencing (GBS) and POPSEQ to validate the assemblies. Two reference populations and three independent designs were used for GBS: a population of double haploids (SynOpDH) of 90 individuals, and two subsets of the population of recombinant inbred lines (SynOpRIL) of 179 and 1026 individuals, respectively. GBS-SNP markers that passed the quality filters for each design were used for linkage analysis. High-quality GBS-SNP marker maps were constructed with strict tests for linkage. For POPSEQ, to genotype the SynOpDH mapping population, the 90 individuals were lightly shotgun sequenced and reads mapped against all 21-pseudomolecule wheat genomes. POPSEQ-SNPs were clustered into bins for framework construction, and consensus genotypes computed across markers in a bin. The GBS and POPSEQ maps will be used to compare and help validate the assemblies. In addition, the GBS-SNP markers and genetic maps will be used to study recombination patterns and relationships between centiMorgan distances and physical distances across the hexaploid wheat 21 chromosomes.



P 23 - Topic: Structural and Functional Wheat Genomics

The reference genomes for *Aegilops tauschii* (AL8/78)

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Wheat is a most important food crop in the world. Wheat had been a leading crop in research area for a long period of history before the genomics era. However, it has lagged behind rice, maize, soybean and many other crops for lack of genome sequence information due to its large and complicated genome. The rapid development of sequencing and assembling techniques provides an opportunity to solve this issue. We integrated the most recent sequencing and assembling techniques such as high-throughput next generation and third generation sequencing, 10× library construction, NRgene assembling, Hi-C, and high density genetic map and provide an high-quality reference genome for *Aegilops tauschii*, which was the progenitor of wheat D genome. In addition, we will mainly focus on TE analysis, including their components, distribution and their association with genome shaping, genome evolution, gene expression, pseudogenes, methylation and genetic recombination. The detailed results will be presented on the workshop.



P 25 - Topic: Structural and Functional Wheat Genomics

Whole genome shotgun sequencing and de novo assembly of the German wheat cultivar 'Julius' using NRGene DeNovoMAGIC2.0TM assembly

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Key message: This study will provide a close to reference quality whole genome assembly of the Germany winter wheat cultivar 'Julius'. It will contribute to a better understanding of haplotype diversity and the extent of structural variation in bread wheat.

Wheat is one of the most important food crops in the world. Unavailability of a gold standard, reference genome sequence for wheat makes it difficult for geneticists and breeders to develop cultivars with superior traits in a short span of time. With the effort of many research groups and IWGSC, a chromosome based survey sequence of the 'Chinese Spring' genome was made available recently (IWGSC 2014). The study from Chapman et al. (2015) provided an additional draft whole genome assembly of a synthetic hexaploid wheat. In the meantime, IWGSC has obtained a gold standard reference genome assembly of the genotype 'Chinese Spring' integrating physical maps of individual chromosomes with a whole genome assembly derived by Illumina short read sequencing and assembly with NRGene's proprietary computational tool *DeNovoMAGIC2.0*TM. Here we report the status of producing a whole genome *de novo* assembly of a German winter wheat cultivar 'Julius'. Status of assembly and annotation will be presented at the conference. Generation and comparison of more *de novo* assemblies of wheat cultivars can help with identification of large-scale structural variations in genomes and in turn help identify the dispensable and variable genomes in wheat. The project runs in collaboration with the '10 wheat genomes' project addressing the wheat pan-genome.

Acknowledgement

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P 27 - Topic: Structural and Functional Wheat Genomics

Reference genome of a modern Chinese leading wheat variety

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Key message: We report here a high quality genome sequence of the modern wheat cultivar Aikang 58.

Wheat is the most important food crop in the world. Wheat genome sequencing will bring wheat research into the genomic age and accelerate wheat breeding. The reference genome of wild ancestors and landraces are available in recent years which is a milestone in wheat research. However, no modern reference genome was reported until now. Here, we report a reference genome of a modern Chinese wheat variety, i.e. Aikang 58. Aikang 58 (AK58), bred in 2003, is an epochal modern commercial wheat variety in China, which was cultivated on accumulated 17 M hectares and has become one of the famous wheat varieties in China because of its good overall agronomic traits such as good environmental adaptation, high yield and good biotic and abiotic resistance. We employed the advanced sequencing and assembling technology to establish the AK58 reference genome, of which the scaffold N50 length was as big as 28 Mb, and more than 95% of the genome was anchored to chromosomes. The gene distribution, TE distribution, recombination distribution, methylation distribution and gene expression distribution on chromosomes were analyzed. The comparison between hexaploid wheat accessions including modern variety and landraces and its diploid ancestors and three subgenomes were carried out and the results will be reported in this meeting.



P 29 - Topic: Structural and Functional Wheat Genomics

Lost part of the wheat genome: tandem repeats

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Key message: Tandem repeats, including rRNA loci, pose a major challenge to genome assemblers and are under-represented in whole genome assemblies. They can be tackled by bioinformatics tools or optical mapping.

Tandemly organized DNA repeats pose a major challenge to de novo genome assemblies and are the major source of gaps (Chaisson et al. 2015). Short arrays of simpler repeats can be resolved by long-read technologies but arrays with units of several kb in length spanning over tens to hundreds kilobases are intractable for any of the current technologies. In such cases, alternative approaches such as optical mapping or devoted bioinformatics tools complemented by fluorescence *in situ* hybridization (FISH) have to be applied to identify, characterize and position long regular arrays of tandem repeats. In our project focusing on bread wheat chromosome arm 7DS, we conducted a graph-based clustering of 3.6 mil randomly selected 7DS Illumina reads (Berkman et al. 2011) using RepeatExplorer pipeline (Novak et al. 2013), which yielded four tandem repeats with monomers ranging from 1.1 to 2.7 kb. FISH provided distinct 7DS-specific signals for three of them. While we found short arrays (8 and 19 units) for the shorter monomers (1242 and 1390 bp) in the IWGSC v0.5.4 assembly of the 7DS, the longest repeat with monomer of 2726 bp providing a strong FISH signal in (sub)telomeric region of the 7DS arm was missing. Another approach to analysing long arrays of tandem repeats relies on optical mapping using BioNano Genomics Irys[®] System, which visualizes a short sequence motif on DNA molecules of several hundred kilobases in length. Analysis of optical maps generated from flow-sorted 7DS chromosome arm revealed an array of tandem repeats with unit of ≈ 9 kb, which spans over several hundred kb (Staňková et al. 2016). This array, corresponding to a minor 18S-26S rRNA locus harboured by the 7DS arm (Mukai et al. 1995), could not be identified in the IWGSC v0.5.4 assembly, just as the major rRNA loci carried by 1BS and 6BS chromosome arms. So the optical mapping provides the missing tool to tackle these challenging regions of the genome.

Acknowledgement

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P 31 - Topic: Structural and Functional Wheat Genomics

Structure and function of *Ph1* (*Pairing homoeologous 1*) genes in wheat and other cereals

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Key message: The 5B copy variant of *Ph1* gene evolved the novel function of chromosome pairing regulation via novel expression pattern, insertions/deletions and alternate splicing.

More than 50% of all higher plants, including some of the most important crop plants, are polyploid or ancient polyploid. Recovery of a sexually reproducing, meiotically and hence, reproductively stable polyploid, requires a precise diploidization mechanism in such a way that only bivalents involving true homologs are formed during meiosis. The chromosome pairing control genes regulating diploidization have been reported in sexually propagated polyploid species such as wheat, oats, cotton, tobacco, *Lolium*, and tall fescue. The *Ph1* gene in wheat regulating chromosome pairing by differentiating homologous from homoeologous/orthologous pairing was discovered in 1958 but so far not been cloned. In our previous report (Bhullar et al. 2014), we reported the identification of a novel gene (*C-Ph1* gene) present in the *Ph1* locus, transient as well as stable silencing of which resulted in a phenotype like that of *Ph1* gene mutations including homoeologous chromosome pairing, multivalent formation and disrupted chromosome alignment on the metaphase I plate. Genomic and cDNA cloning along with detailed bioinformatics analyses revealed three structural copies of the *C-Ph1* gene coding five transcripts with only the 5B copy showing alternate splicing. 5B copy of the gene evolved the novel function of differentiating homologs from homoeologs via unique changes in promoter leading to 457-fold higher expression during prophase I, acquisition of novel motifs, specific insertions/deletions and tissue-specific retention of introns. The three gene copies with different expression patterns putatively translating at least seven protein variants possibly controlling many different functions associated with *Ph1* locus. One of these putative proteins (5B⁸⁰) structurally resembles the RuvA-RuvB motor protein complex required for the Holliday junction branch migration and other similar processes, and showed the highest expression during the stages where such motor protein complexes are required. *Ph1* locus of wheat was successfully used to induce homoeologous recombination to transfer genes from distant wheat wild relatives and silencing of *C-Ph1* gene also resulted in alien/homoeologous recombination. Genes with a function like that of chromosome pairing regulators of polyploid species are present in diploid species as well. Stable RNAi silencing of the gene in *Arabidopsis* also showed phenotype like that of the wheat RNAi silenced plants and expression of true orthologue of *C-Ph1* gene during meiosis in ancient polyploid such as maize and oats strengthens the above-mentioned fact. Thus, suggesting that *C-Ph1* gene evolved via neofunctionalization in hexaploid wheat to regulate chromosome pairing and recombination.

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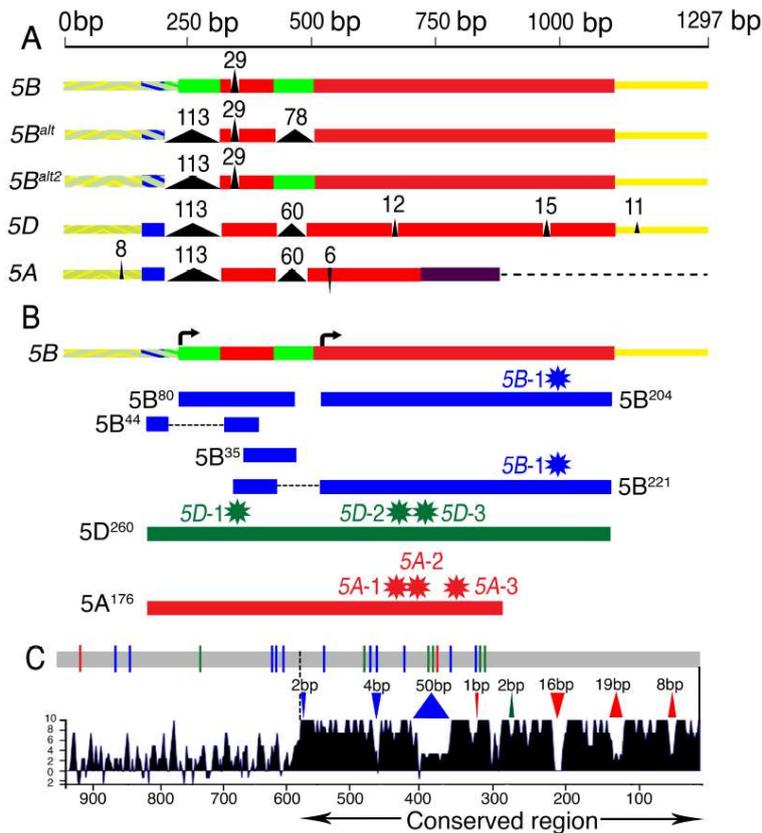


Figure 1: Comparison of the *C-Ph1* gene copies. (A) Comparison of the cDNA of *C-Ph1* gene homoeologs and their alternate transcripts. (B) Various putative proteins of the *C-Ph1* gene copies. The 5A protein is given in red, the 5D as dark green, and the 5B proteins in blue. All proteins are drawn to scale. (C) Comparison of the promoter regions of *C-Ph1* gene copies.

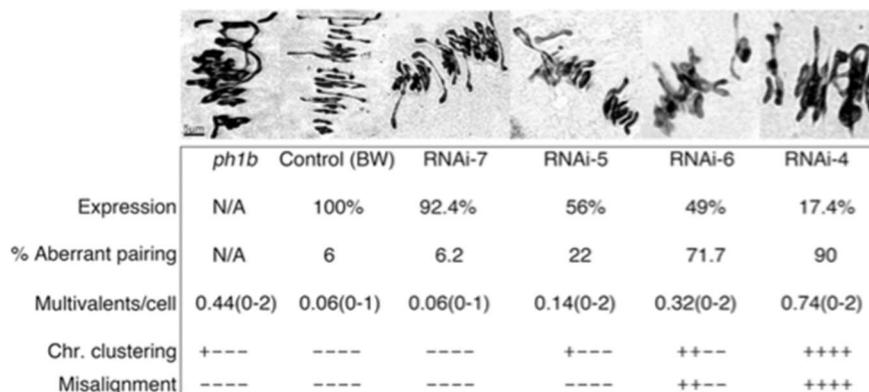


Figure 2: Cytogenetic analysis showing different levels of *C-Ph1* gene silencing in RNAi plants compared with *ph1b*.

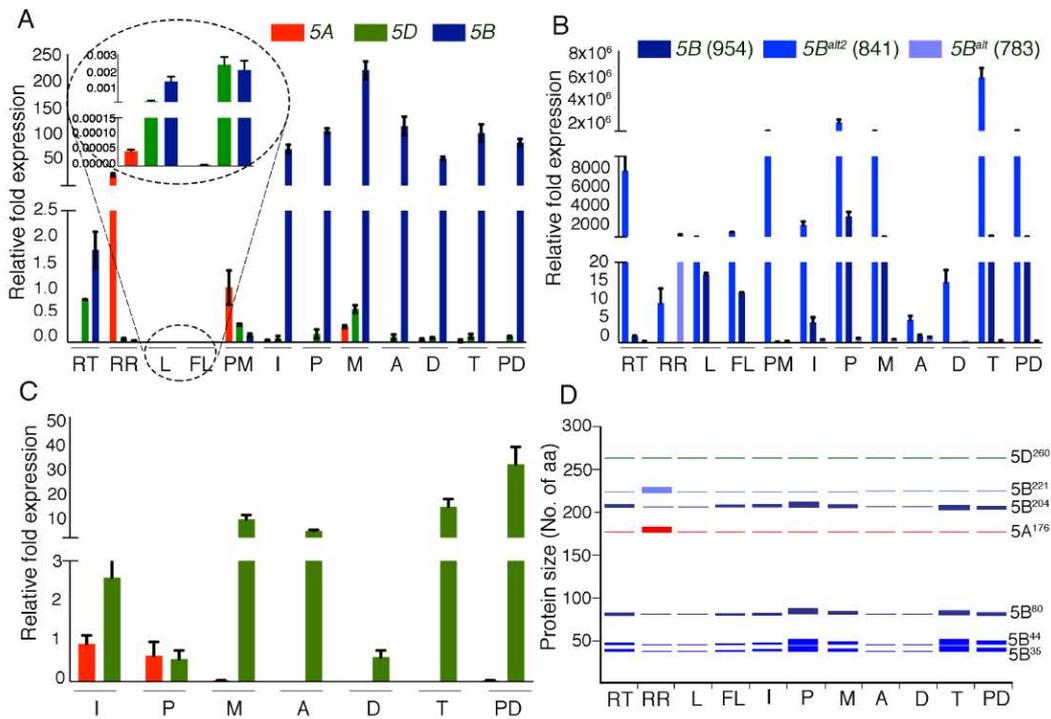


Figure 3: Real-time gene expression analysis of the *C-Ph1* gene copies. (A) Quantitative expression analysis of the *C-Ph1* homoeologs using copy-specific primers in the root-tip cells (RT) and the remaining of the 2-3 cm root (RR), leaf (L), flag leaf (FL), meiotic anthers at various sub-stages of meiosis I viz. interphase (I), prophase (P), metaphase (M), anaphase (A), dyad (D), tetrad (T) and pollen development (PD) of CS. (B) Quantitative expression analysis of 5B, 5Balt, 5Balt2 transcripts using transcript-specific primers in the stages mentioned above. (C) Quantitative expression analysis in the meiotic anthers at various sub-stages of meiosis I of the *ph1b* mutant, containing $\approx 1 \mu\text{m}$ interstitial deletion. (D) Relative transcript abundance of all of the gene copies in various tissues mentioned above. The relative abundance of the predicted proteins were estimated on the basis of relative transcript abundance of the *C-Ph1* gene copies (mentioned in A, B) encoding the specific proteins. Different colored bars represents the predicted 5A, 5B and 5D-specific proteins from the respective gene copies. The thicknesses of the bars that are drawn to scale show varying expression levels. The Y-axis indicates the protein sizes. The red bars correspond to 5A, green = 5D, blue= 5B (common), dark blue = 5B (954 bp), fluorescent blue = 5Balt2 (841 bp), and purple = 5Balt (783 bp). The Y-axis in (A), (B) and (C) denotes the relative transcript abundance normalized to Actin using delta Ct method of quantitative real-time PCR analysis.



P 33 - Topic: Structural and Functional Wheat Genomics

Evolution of *Ph1* gene in polyploid wheat

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Key message: Cloning of major chromosome pairing control gene in wheat paved the way to understand the underlying basis of variable chromosome pairing phenotypes observed in wheat diploid and tetraploid wild relatives.

Wheat is an allohexaploid ($2n=6x=42$) that originated after two independent events of polyploidization with three sub-genomes i.e. A, B and D that can pair with each other during meiosis. To maintain integrity of the nucleus and reduce abnormal meiotic behavior a precise mechanism to regulate chromosome pairing is required that not only differentiates homologous from non-homologous chromosomes but also differentiates homoeologous from homologous chromosomes for normal bivalent formation and accurate recombination. In our previous study, we reported the cloning and functional characterization of major chromosome pairing control gene, *Ph1* that regulates diploid-like pairing behavior of wheat (Bhullar et al. 2014). Expression and structural analyses of the gene clearly indicated significant differences amongst the (*C-Ph1*) homoeologs with 5B copy primarily regulating the *Ph1* gene function (Bhullar et al. 2014). We have also shown that the novel function of the 5B copy has evolved via neofunctionalization of the *C-Ph1* gene that happened due to (i) 29bp deletion and (ii) 60bp insertion leading to the acquisition of unique motifs (iii) Alternate splicing and (iv) early PI to MI specific expression. To understand the evolution *C-Ph1* gene and chromosome pairing control in wheat, cloning and expression analyses from diploid progenitors (*Triticum urartu*, *Aegilops speltoides* and *Ae. tauschii*) and tetraploids wheat was undertaken. Structural comparison of gene revealed that 29bp deletion in the 5B copy is polyploidization specific. *TuC-Ph1* gene showed 97% sequence identity with *CS-C-Ph1-5A* copy. Similarly, *AsC-Ph1* and *AtC-Ph1* showed 93 and 98% sequence identity with *CS-C-Ph1-5B* and *CS-C-Ph1-5D* copy respectively. 5A of B-genome and G-genome lineage tetraploids showed higher sequence identity with *CS-C-Ph1-5D* copy. 5B copy of B-genome tetraploids showed 97% sequence identity with *CS-C-Ph1-5B* copy. Whereas, 5B copy of G-genome tetraploids showed 95% sequence identity with *CS-C-Ph1-5D* copy. Further expression analysis of *C-Ph1* gene in diploid progenitor species revealed that ancestral version of *C-Ph1* gene has highest expression during pollen developmental stages in comparison to hexaploid wheat, where maximum transcript of *C-Ph1-5B* copy is present during early meiotic stages. Cloning of *C-Ph1-5B* copy from *T. dicoccoides* with varying level of chromosome pairing control (Ozkan et al. 2001) showed that structurally there are no differences, whereas high pairing lines show weak expression during early meiotic stages in comparison to low pairing lines (Figure 1). Thus, suggesting that *C-Ph1* gene evolved its function of chromosome pairing control via polyploidization specific changes in gene structure and expression.

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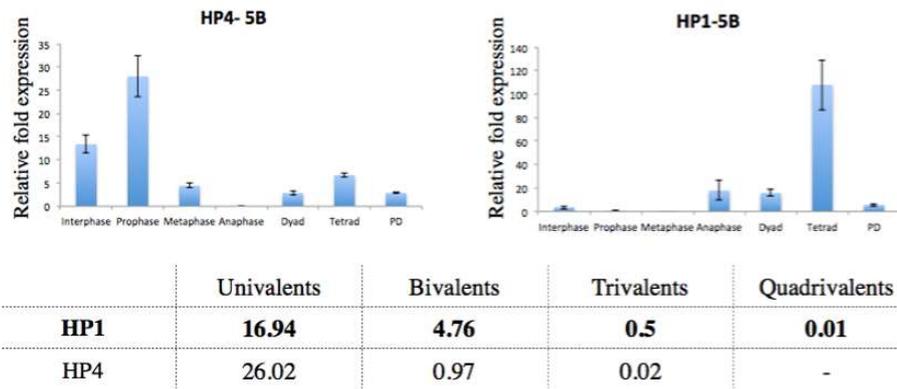


Figure 1: Expression variation for *Ph1* gene in *Triticum dicoccoides* correlates with variation for chromosome pairing. Chromosome pairing data from Ozkan & Feldman (2001).



P 35 - Topic: Structural and Functional Wheat Genomics

Development of deletion lines for physical mapping of *Ph2* gene in bread wheat

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Key message: We developed and characterised deletion lines for bread wheat chromosome arm 3DS to physically delimit region of *Ph2* gene.

Bread wheat (*Triticum aestivum* L.) is an allohexaploid species. Its genetic information consists of 3 subgenomes, formed by hybridisation of three progenitors. Hybridisation between 3 close-related species caused a coexistence of highly similar homoelogenous chromosomes. Mechanisms of precise chromosome pairing had to be developed, so diploid-like behavior is secured. Homologous pairing of chromosomes in wheat is primarily controlled genetically by *Ph* genes (Martinez et al. 2001). *Ph2* gene was located on a short arm of chromosome 3D. Mutant plants of this gene *ph2a* and *ph2b* were observed and only small effect on homoelogenous pairing suppression was witnessed. On the other hand, removal of this gene caused pairing of wheat and alien chromosomes in hybrids with close-related species (Sutton et al. 2003). These findings suggest much potential of *Ph2* gene for introgression of alien genes into wheat genome. Certain alien chromosomes introduced into wheat are inherited preferably by causing sterility in gametes, in which it were absent, therefore chromosomes like these are named 'gametocidal'. The mechanism of causing sterility is by inducing genomic rearrangements. Gametes carrying only semi-lethal genomic rearrangements can be used to transfer aberrations into progeny. By using monosomic addition of 2C gametocidal chromosome derived from *Aegilops cylindrica* into 'Chinese Spring' cultivar, it is possible to create deletion lines of wheat. We have been continuously extending a set of deletion lines for a short arm of chromosome 3D. The obtained deletion lines are being characterised by a set of molecular markers up to average resolution of 5 Mbp, focusing on distal 80 Mbp of short arm of 3D chromosome, which is the identified area of *Ph2* gene presence. The aim of this project is to narrow down region of *Ph2* gene through deletion mapping. Eighteen novel lines with terminal deletion of short arm of chromosome 3D have been developed so far. In the frame of the project we would like to map the *Ph2* gene physically to a region smaller than 5 Mb, followed by more precise mapping using a set of radiation deletion lines.

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P 37 - Topic: Structural and Functional Wheat Genomics

The *Ph1* locus

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Key message: The *Ph1* locus is a duplicated region of chromosome 3B encompassing a heterochromatin segment and the *ZIP4* gene (originally termed *Hyp3*) inserted into a Cdk2-like and methyltransferase gene cluster

The *Ph1* locus on chromosome 5B arose during wheat's polyploidisation. It is responsible for ensuring that true homologues pair and crossover rather than related chromosomes (homoeologues) during meiosis, thereby assuring wheat's polyploid genome stability and fertility. A 3B chromosome region encompassing a heterochromatin segment and the *ZIP4* gene duplicated and then inserted into a Cdk2-like and methyl transferase cluster on chromosome 5B creating the *Ph1* locus. *ZIP4* is a major meiotic gene controlling interfering crossovers. The *ZIP4* copy (*ZIP4* 5B) within the locus is the dominant expressing allele and regularises crossovers in wheat ensuring that homologues are linked by one crossover per arm at metaphase I. *ZIP4* 5B does not appear to affect chromosome pairing consistent with *Arabidopsis* and rice studies. *Ph1*'s pairing effect must therefore be controlled by the Cdk2-like/methyl transferase gene cluster as deletion mapping defined this effect (as well as the crossover effect) to the Cdk/methyl transferase locus containing *ZIP4* (originally termed *Hyp3*). How is *Ph1* affecting chromosome pairing and crossover? At the start of meiosis, telomeres cluster as a bouquet at one pole, and the centromeres pair at the other pole. This clustering of telomeres facilitates the correct pairing. Homologues pair during the telomere bouquet, while homoeologues can only pair after the telomere bouquet disperses, whether *Ph1* is present or absent. So why does a level of homoeologous pairing take place during wheat meiosis without *Ph1*, when it should be prevented by early homologue pairing? Homologue pairing is slower in the absence of *Ph1*, and is completed after the telomere bouquet stage when homoeologous pairing can occur. Thus *Ph1* promotes more efficient homologue pairing early in meiosis during the telomere bouquet, reducing the chance of homoeologous pairing. Homologues undergo a more synchronised conformation change prior to pairing in the presence of *Ph1*, than its absence. Wheat-rye hybrids contain only homoeologues, which pair to the same level after the telomere bouquet disperses, with or without *Ph1*. Thus *Ph1* can't prevent homoeologous pairing. The recombinational machinery loads onto these paired homoeologues with or without *Ph1*, including the MLH1 complex. In most species studies, all MLH1 sites on paired homologues become crossovers. However, MLH1 sites on paired homoeologues only become crossovers in the absence of *Ph1*, with the level achieved being affected by environmental factors. It may be that crossovers between homoeologues require a lower dose of *ZIP4*, than homologues. We are assessing this using *Arabidopsis* and wheat.



P 39 - Topic: Structural and Functional Wheat Genomics

Structural and evolutive analysis of an ancestral chromosomes fusion point within the hexaploid wheat genome

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Key words: BAC library, chromosome, evolution, fusion, hexaploid, sequencing, wheat

The explosion of genomes sequencing has highlighted whole genome duplications (WGDs) as a major evolutionary driving force shared by multiples branches of the tree of life. However, the genome sizes and chromosome numbers have not increased exponentially over time because WGDs have been balanced by chromosome number reduction (CNR) and gene losses. In plants these two mechanisms have been found through genomes sequences comparisons and ancestral karyotype reconstruction. For monocots models have been proposed explaining CNR by nested chromosome fusions (NCFs). However, molecular mechanisms driving ancestral chromosome fusions that have led to the present day monocots karyotypes are still largely unknown. Based on this ascertainment, we aimed at characterizing a wheat genomic locus corresponding to ancestral chromosomes fusion point (FP). To obtain a high quality sequence of a FP we implemented a strategy based on the reconstruction of the ancestral grass karyotype, the definition of the synteny relationships between wheat and rice (as the modern representative of the Ancestral 12 chromosomes grass karyotype) and the screening of wheat chromosome specific BAC libraries and genomic sequences newly available in the databases. We focused on the FP located on the long arm of chromosomal group 1. We investigated this region on chromosomes 1AL, 1BL, 1DL, obtaining sequences for each of them. The annotation of genes and transposable elements allowed us to established orthology relations chromosome 5 and 10 of rice (images of ancestral chromosomes A5/A10). The origin and the order of the genes neighboring the FP were confronted to models of chromosomes fusion of Triticeae proposed in the literature.



P 41 - Topic: Structural and Functional Wheat Genomics

RdDM pathway is associated with pre-harvest sprouting in small grain cereals

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Key message: Pre-harvest sprouting and seed dormancy is epigenetically regulated in cereal grains.

Seed dormancy, a quiescent state of the seed that prevents germination under favorable conditions is primarily an adaptive trait of many plant species to unfavorable weather conditions. For many agricultural crops it is an important goal to breed for the right balance of resistance to pre-harvest sprouting (PHS) on one hand and reduced seed dormancy for rapid and uniform germination on the other. Basic mechanism known for seed dormancy includes antagonistic action of phytohormones abscisic acid (ABA) and gibberellic acid (GA) in promoting dormancy and germination respectively. There is also emerging evidence for role of epigenetic mechanisms in seed dormancy which could be an alternate genetic mechanism for seed dormancy (Zhang et al. 2012). We are employing transposon- and gene expression- based approaches to investigate seed dormancy in small grain cereals. A key gene of RdDM (RNA dependant DNA methylation) pathway, *ARGONAUTE4_9* has been found to be associated with pre-harvest sprouting/dormancy in barley and wheat. Significant variation in the expression of *AGO4_9* class genes in dormant and non-dormant barley (Singh & Singh 2012) and wheat genotypes (Singh et al. 2013) was observed. This data along with DNA methylation changes suggest that *AGO4_9* class may be acting as an epigenetic switch during seed dormancy.

Acknowledgement

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P 43 - Topic: Structural and Functional Wheat Genomics

Identification of Dof transcription factor in genomic survey sequences of *Triticum aestivum* and structural insight into Dof domain-DNA interaction

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Key message: This study involves computational approach to identify chromosome-wise Dof transcription factor and understand the detail molecular mechanism and dynamics underlying DOF domain-DNA interactions that modulate of Dof TF activity in wheat.

Wheat crop faces a number of abiotic and biotic stresses which reduces its production and productivity. Dof is well characterized plant-specific transcription factor, play significant roles as transcriptional regulators in plant growth, development, and responses to biotic and abiotic stresses. With the availability of genomic survey sequences data of wheat, large-scale identification of transcription factor families by bioinformatic approaches is more promising and necessary. In the present study GSS data of chromosome 2 was subjected to the computational pipeline, eighteen Dof protein have been identified, distributed on chromosome 2A, 2B and 2D using BLAST sequence similarity search against the local database (Figure 1). The motif analysis confirms the presence of the DNA-binding domain containing C2C2-type zinc finger motif at N-terminal and a transcriptional regulation domain at C-terminal in the identified sequence. The information regarding the Dof protein is limited in wheat and its interaction with DNA is not unveiled yet in cereal crop. The three-dimensional model of the Dof protein was predicted using Iterative threading assembly refinement method (I-TASSER) whereas 5 base pair 5'-(T/A) AAAG-3' nucleotide sequence was modeled using 3D-DART server. The active site information based protein-DNA complex was performed using HADDOCK web server. To study the structural characterization, a molecular dynamics approach was undertaken to get a better understanding of the changes in the arrangement of secondary structure and residue interaction. The statistical tool was used to study the mechanical properties of the domain which suggested that, wild Dof protein was more stable than the mutant type. The Protein-DNA interaction was highly stabilized by various hydrophobic interactions especially Tyr20, Lys34, and Arg38 amino acid residues. The alpha helix of the DNA binding domain interacts with the major groove of DNA. Lys34 was mutated with Arg34 and docked again with the DNA (Figure 2). The mutant Lys34→Arg34 showed complete structural damage of β strands and Lys34-ADE2 and ADE3 interaction, indicating the importance of Lys34 in complex stability. We have first time reported the interaction and dynamics study of Dof domain with DNA in wheat. Our study will not only provide a functional analysis of Dof transcription factor but also enhance mechanism of interaction between protein and DNA and crucial amino acid involved that will genetic engineering and crop improvement.

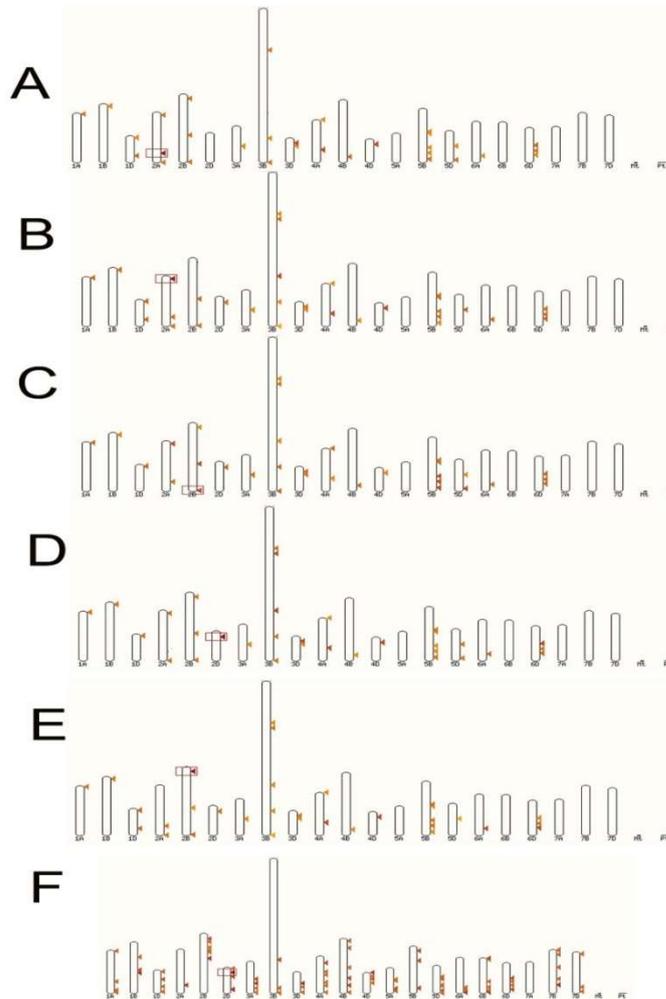


Figure 1: Chromosomal locations for predicted wheat dof genes; (A) 2AL, (B) 2AS, (C) 2BL, (D) 2BS, (E) 2DL, (F) 2DS. Boxes represent the maximum probability of occurrence of gene on chromosomes based on the E-value.

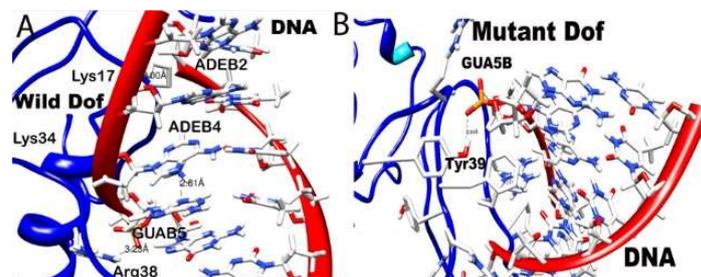


Figure 2: Schematic drawing showing hydrogen bonding between the Dof domain of (A) wild and (B) mutant Dof domain with DNA molecule.



P 45 - Topic: Structural and Functional Wheat Genomics

Expression and variation of *Glo-2* locus in bread wheat (*Triticum aestivum* L.)

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Key message: The position and the similarity in sequences between the globulin and HMW glutenin genes indicates the presence of these two genes as part of original duplication creating the paralogous x- and y-type HMW glutenin genes, and a paralogous *Glo-2* gene.

Wheat is the most widely grown cereal crop in the world and the wheat seed is the single greatest source of protein in the human diet. Wheat prolamins are subdivided into gliadins and glutenins according to their polymerisation properties and represent about 80% of the total protein in the wheat grain. *Glo-2* genes are closely linked to the HMW glutenin genes at the *Glu-1* locus. Analysis of their sequence raises the possibility that the product of these genes are incorporated into the gluten matrix. Accumulation of the putative product of the *Glo-2* genes was investigated using purified antisera raised against the encoded polypeptide. The aim of this work was to investigate the accumulation in the endosperm of protein corresponding to the EST-deduced sequence. The polypeptide could not be detected at 10 DAF but could be detected from 25 DAF in the soluble phase and was present in the mature grain. Overloading of protein samples enabled the polypeptide to be detected in the insoluble phase as well. PCR was used to amplify globulin genes from the *Glo-2* locus of hexaploid wheat cultivars containing different *Glu-D1* alleles. The complete coding sequence of *Glo-2* gene was obtained for each of the eight bread wheat cultivars. Chromosome assignment of PCR products using Chinese Spring nulli-tetrasomic lines confirmed the amplified products were copied from sequences present on chromosome 1D. Comparison of globulin gene sequences obtained from the different wheat cultivars showed one single nucleotide polymorphism (SNP). All varieties with 2+12 high-molecular-weight glutenin-subunits (HMW-GS) encoded at *Glu-D1* locus had an adenine replaced by a guanine in all varieties with the subunits 5+10. These results indicated that SNP markers could be produced from *Glo-2* sequences which could be used for tracing HMW glutenin alleles or to follow attributes determined by the *Glo-2* proteins themselves.



P 47 - Topic: Structural and Functional Wheat Genomics

Characterization of wheat photoperiod insensitive *Ppd-B1a* allele

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Key message: We characterized structural and functional specifications of *Ppd-B1a* photoperiod insensitive allele and discussed about its interaction with other photoperiod genes.

Heading date is an important agronomic trait, critical for wheat development and adaptability. Detection and description of genes, determining flowering time and its interactions with each other, is significant for cereal improvement. *Ppd-1* genes are one of the major regulators of heading date in common wheat. Increased copy number of *Ppd-B1* is one of the reasons for its photoperiod insensitivity. Using two pairs of near-isogenic lines contrasting in their photoperiod sensitivity we studied distinct copies of *Ppd-B1* and identified the indel in promoter region distinguished the investigated lines from other alleles with copy number variation, but revealed no polymorphisms between *Ppd-B1* gene copies. *In silico* analysis of promoter regions of *Ppd-1* genes, we identified cis-regulatory elements, associated with flowering, and divided them into three groups according to their input signal nature: circadian-clock regulated, light regulated and phytochrome regulated groups. Further improvement of its association is required. However, these transcription factors are to complement current scheme of wheat flowering transaction. Some *cis*-elements, specific to *Ppd-B1* promoter, were detected. Among them *MADS* genes are likely to be transcription factors, involved in expression of *Ppd-B1a* with increased copy number in night period. The majority of *MADS* genes with binding sites in *Ppd-B1* promoter are known to be involved in flowering time repression in other plant species. To investigate *Ppd-B1a* photoperiod insensitive allele interactions with other genes, we applied diurnal expression study (Figure 1). Correlation analysis of flowering genes expression revealed some relations between expression patterns. *PhyC* and *Ppd-B1a* expression patterns correlated significantly in dark period suggesting about positive regulation of *PhyC* by the photoperiod insensitive *Ppd-B1a* allele. We also discuss a role of genes, located in 5B chromosome pericentromeric region, in *Ppd-B1* regulation.

Acknowledgement

This study was supported by the Russian Scientific Foundation (Project No. 14-14-00161).

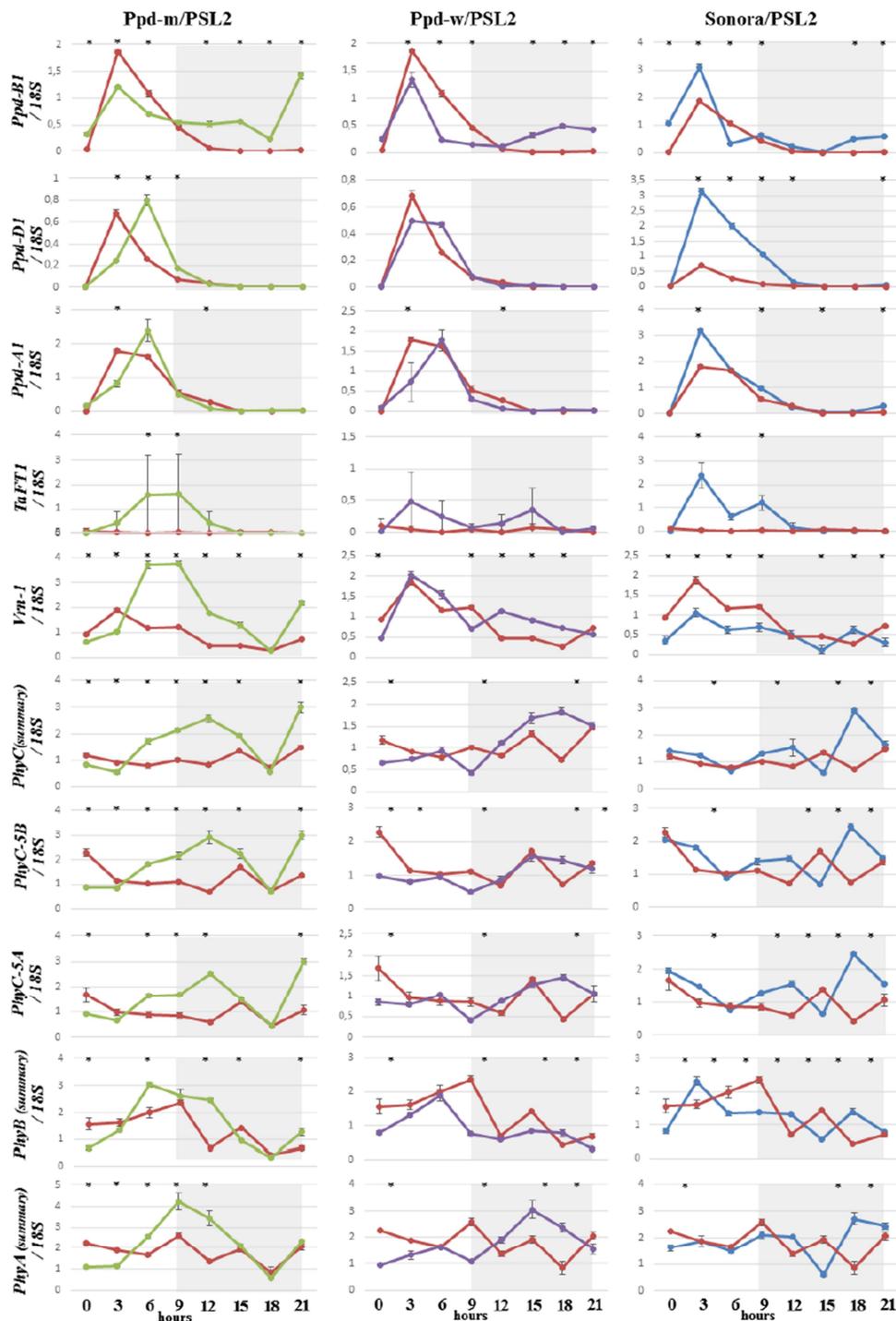


Figure 1: Patterns of the diurnal gene expression. Quantitative gene expression data from plants grown in short days (0–9 h light period) in climatic chamber. Grey shadowing indicates dark period (9–24 h). The graphs compare expression between photoperiod sensitive parental line PSL2 (red) and photoperiod insensitive NILs (*Ppd-m* (green), *Ppd-w* (purple)) and parent Sonora (blue). Values are expressed as relative levels normalized against 18S ribosomal RNA. Error bars indicate SE of means. Asterisks indicate significant ($p < 0.05$) differences in one-way ANOVA with a post-hoc Tukey test comparing photoperiod insensitive NILs (*Ppd-m*, *Ppd-w*) and parent Sonora with photoperiod sensitive parental line PSL2 in each time point.



P 49 - Topic: Structural and Functional Wheat Genomics

Same number of *Ppd-B1* copies, different heading date

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Key message: Heading date variation within wheat lines with same number of *Ppd-B1* copies is associated with decreased *Ppd-B1* expression and possibly with methylation status.

Optimal flowering time is for annual plants the most crucial for ensuring seed production and viability. The main environmental clues affecting the flowering time are ambient temperature and day length. *PPD* (photoperiod) genes are the major integrators of signals about the day length in cereals. Due to allohexaploid nature of its genome, bread wheat carries three *PPD-1* genes: *Ppd-A1*, *Ppd-B1* and *Ppd-D1*. While variation of photoperiod response contributed by *Ppd-A1* and *Ppd-D1* is based on changes in the gene sequence, the impact of *Ppd-B1* allele on flowering time was reported to be associated with variation in gene copy number and/or methylation status. Here we report that plants with same copy number of *Ppd-B1* may differ in flowering time. Some of SSD F₇ plants derived from crossing of two spring hexaploid wheat varieties Kärntner Früher (KF) and Paragon (P) differed in flowering time. Most of these lines vary in *Ppd-B1* copies where KF like lines with three *Ppd-B1* copies are an early while P like are late variety with one copy. However, some F₇ lines carrying three copies of *Ppd-B1* headed later more than 16 days later compared with other lines with three copies. The effect was associated with decreased expression level and methylation status of *Ppd-B1* allele. The acquired results and tools will be used to further study role of *Ppd-B1* alleles in the flowering time pathway.

Acknowledgement

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P 51 - Topic: Structural and Functional Wheat Genomics

Evolutionary dynamics of wheat prolamin genes revealed by comparative genomics approach

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Key message: Wheat prolamins are encoded by multiple families of genes with great genetic complexity. Our study reveals that multiple evolutionary mechanisms have contributed to the rapid expansion of prolamin gene families.

Prolamins are an important cereal protein source for human beings and determine the bread-making quality of wheat flour. Bread wheat is a hexaploid containing three subgenomes (A, B, D). The wheat prolamin loci (*Glu-1*, *Gli-2*, *Glu-3*, *Gli-1*) are located in three complex genomic regions, each locus carrying multiple members of one or more gene families that exhibit great genetic diversity among varieties. Due to the large size and polyploidy nature of the wheat genome, the genomic organization and evolution of wheat prolamin genes have been difficult to determine. We previously determined the complete sequences of the *Glu-1* regions containing the HMW-GS genes from the three subgenomes, compared them in detail, and revealed their origin from a duplication of an ancestral globulin gene. Recently, we sequenced a 2.8-Mb genomic region, representing an 8.8 cM genetic interval and spanning both the *Glu-3* and *Gli-1* loci that encode LMW-GS and γ/ω – gliadins, respectively, from the diploid grass *Aegilops tauschii*. Comparison with orthologous regions from rice, *Brachypodium* and sorghum showed that the *Ae. tauschii* region has undergone dramatic changes since the common ancestor of these species; it has acquired more than 80 non-syntenic genes including prolamin and resistance-like genes. These non-syntenic genes originated from various genomic regions and likely moved to their present locations via gene duplication and translocation. Local duplication of non-syntenic genes contributed to the significant expansion of the gene families. Our analysis indicated that the insertion of prolamin-related genes occurred prior to the separation of the Brachypodieae and Triticeae lineages, but in the latter, the inserted prolamin genes have rapidly evolved and expanded to encode different classes of major prolamins in Triticeae species. We have also compared the *Gli-2* regions from *Ae. tauschii* and hexaploid wheat and found that while gene synteny surrounding the locus is well maintained, the α -gliadin genes within the locus have undergone rapid changes in the A, B, and D genomes. Multiple and differential sequence duplication events have contributed to the recent expansion of α -gliadin genes in the different subgenomes. In addition to homologous unequal crossover recombination, we propose a mechanism involving sequence rolling-circle replication to explain the rapid copy number increase of α -gliadin genes. Sequence alignment of expressed prolamin genes along the large contiguous prolamin regions further elucidated the structure, expression, evolution, and function of wheat prolamin genes. Taken together, our results reveal the complex evolutionary dynamics of prolamin genes in Triticeae species.



P 53 - Topic: Structural and Functional Wheat Genomics

Target enrichment sequencing of wheat pentatricopeptide repeat (PPR) gene family -- Searching wheat fertility restorer gene candidates

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Key message: The study focus on the high potential *Rf* gene candidates: Fertility Restorer like Pentatricopeptide repeats genes in wheat by using target enrichment sequencing. Aim to provide the *Rf* candidate gene pool and the molecular marker for breeding.

Bread wheat, *Triticum aestivum* (AABBDD, 2n = 42), is the third most-produced cereal crops in the world. As the main human staple food crop, it is high in carbohydrates, protein and vitamins B and E. Hybrid breeding is the most efficient strategy to obtain the hybrid vigor, especially yield improvement of wheat. Among the different hybrid breeding technologies or facilities, cytoplasmic male sterility-fertility restoration (CMS-*Rf*) system is illustrious with the advantages of low cost, stable and no impact to the environment etc. However, currently there is no efficient fertility restoration (*Rf*) gene available in wheat that would enable commercial scale hybrid wheat breeding due to the weak and unstable performance of male fertility restoration. Based on the previous studies and published literatures, almost all of the cloned *Rf* genes of plants are come from a same gene family: *PPR* gene family. Fertility restorer like (*RFL*) gene class is one of the subclade of *PPR* gene family. In almost all cases, cloned *Rf* genes are cluster together with *RFL* genes. In the protein function level, *RFL-PPR* proteins are targeted to mitochondria, where they prevent accumulation of the CMS specific gene products. Our aim of this study is to mine the fertility restorer gene resource of wheat. Since we could confirm that *PPR* gene family is the *Rf* gene candidate pool, we applied target enrichment sequencing technology to enable discovery and annotation of *PPR* gene family members in different wheat *Rf* gene present and absent genotypes. The identified *PPR* genes in all genotypes were applied to detect SNP markers for the restorer gene mapping and later on the hybrid wheat breeding.



P 55 - Topic: Structural and Functional Wheat Genomics

Understanding the structure and function of alpha amylase gene family with special reference to wheat

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Key message: Alpha amylase gene length variation among dicots and monocots is mainly due to the differences in the number and size of the introns. This gene is present in multiple copies in wheat genome.

Alpha-amylase is responsible for degradation of starch in plants and provides energy to the germinating seedling by hydrolysis of endosperm reserves. The present study was conducted to reveal structural and functional evolution of this gene among higher plants with special reference to wheat. The best-characterized rice α -amylase gene (*OsAmy*) was used as a reference gene to identify its true orthologs in wheat, barley, maize, sorghum, *Arabidopsis*, soybean and *Medicago* (Neill et al. 1990) following the criteria described by Dhaliwal et al. (2014). The size variation in gene length was observed due to differences in the number and size of introns. However, the intron phase distribution and insertion sites were mostly conserved despite this size variation. The predicted protein size ranged from 414 amino acid (aa) in soybean to 449 aa in *Brachypodium*. Comparison of protein sequence showed 56.4 to 97.4% protein sequence similarity among different orthologs. Functional signature sequences along with their relative distances were conserved among plants. The domain length of the glycosyl hydrolase superfamily varied from 342 aa in soybean to 384 aa in maize and, while length of the C-terminal β -sheet domain was highly conserved with 61 aa in all monocots and *Arabidopsis* but was 59 aa in soybean and *Medicago*. *In silico* prediction and superimposition of alpha amylase 3D structures for all orthologs have shown similar spatial arrangements of domains and motifs and this similarity is higher in monocots than dicots. Copy number analysis in wheat revealed that alpha amylase belongs to large gene family that went through gene duplication. These copies were present mainly on chromosome 4, 5, 6 and 7. Gene structure analysis revealed the presence of variable gene structure for duplicated copies. Further expression analysis of the gene showed that these duplicated as well as homoeologous copies of alpha amylase gene show differential gene expression.

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P 57 - Topic: Structural and Functional Wheat Genomics

Computational modeling of spatial structure of gibberellin response RHT-1 *Triticum aestivum* protein from DELLA-GRAS proteins family

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Key message: The spatial structure of the protein Ta-RHT1 has been modelled, presented DELLA-(section Met1-Pro113) and GRAS-domain (site Cys314-Pro623), the connecting domain Pro114-Ser313 with high structural disorder is under development.

The aim of the work was modeling the 3D structure of wheat RHT-1 protein (reduced height protein 1, *Ta*-RHT1) which is a modulator of plant growth hormone gibberellin response and belongs to the DELLA subfamily of GRAS family of plant protein repressors of transcription. BLAST-analysis, 3D structure modeling by Robetta, I-TASSER and Swiss-Model web-servers were applied. For *Ta*-RHT1 protein the secondary and subdomain structure and regions of high structural disorder are predicted. Crystal structures of SAM-dependent methyltransferases containing β -layer with seven β -strands were used as structural templates for modeling. Conservative tyrosine Y594 residue in SAW-domain can probably be phosphorylated and ensure the regulation of activity of *Ta*-RHT1 protein. N-terminal deletion M1-E64 in *Ta*-RHT1 is associated with altered response to growth hormone gibberellin and therefore shorter stalk of wheat and its resistance to lodging, leads to loss of specific 38DELLA42 and 60LExLE64 motifs. Since 38DELLA42 and 60LExLE64 motif is required for binding to gibberellin receptor, apparently these mutations resulted in violations of specific binding of *Ta*-RHT1 with GID1 and violation of the subsequent proteolysis and ubiquitination of DELLA-protein.



P 59 - Topic: Structural and Functional Wheat Genomics

The iSelect 9K SNP analysis revealed polyploidization induced revolutionary changes and intense human selection causing strong haplotype blocks in wheat

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Key words: 9K SNP, association, breeding, domestication, haplotype block, *Triticum*

Based on re-sequencing strategy or high-density SNP array, dissections of genomic regions (haplotype blocks) related to important agronomic traits have already become the central issue for applied genomics in crops. A Chinese wheat mini core collection was genotyped using the wheat 9K iSelect SNP array. Total 5156 polymorphic SNPs with single chromosome location were detected, of which 2420 and 2396 were distributed on the A and the B genome chromosomes. These SNPs formed 878 haplotype blocks in the two genomes, with a mean genetic distance 1.16 cM in size, ranging from 0 to 13.61 cM. Haplotype-based association can improve detection power compared to single SNP markers. There were more blocks in the B genome, but the average block size was significantly ($p < 0.05$) smaller than those in the A genome. Between the two homoeologous chromosome pairs, intense selection (domestication and breeding) had a stronger effect on the A than on the B genome chromosomes. We also genotyped the likely diploid ancestors of the A and the B genomes as well as tetraploid wheat collections. Though the haplotype blocks were identified in the tetraploid wheats, they were mostly prominent in the landraces and modern cultivars of common wheat with the block size extending further in modern cultivars. Based on the genetic pedigrees, many blocks can be traced back to a well-known Strampelli cross, which was made one century ago. Our data further indicated that polyploidization of wheat (both tetraploidization and hexaploidization) induced revolutionary changes in both the A and the B genomes, with a greater increase of gene diversity compared to their diploid ancestors. Modern breeding has dramatically increased diversity in the gene coding regions, though obvious blocks were formed on most of the chromosomes in both tetraploid and hexaploid wheats. In common wheat, the breeding process has shortened the block size at the distal regions on most of the chromosome arms, but not for the other parts of the chromosomes. Tag-SNP markers identified in this study can be used for marker assisted selection using haplotype blocks as a wheat breeding strategy. This strategy can also be employed to facilitate genome selection in other self-pollinating crop species.

P 61 - Topic: Structural and Functional Wheat Genomics

A gene nomenclature for the *Triticeae* tribe

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Key message: We present a gene nomenclature scheme for the *Triticeae* tribe. The scheme is focused on the orthologous and syntenic relationships that are highly conserved within the cereals.

A number of high-quality genome sequences have been completed in recent years for *Triticeae* species including a whole-genome reference for the Chinese Spring cultivar of the large hexaploid genome. The availability of these resources, which only few years ago were thought to be technically inaccessible, have revolutionised our approach to research and breeding in cereal crops. This follows the generation of genomic sequences for other related *Triticeae* species including the wheat progenitors, the first durum wheat genome and barley. Most of these genomes have been annotated using high-throughput automatic methods adding to the wealth of information to the research and breeding communities. Since 1968 the Catalogue of Gene Symbols for Wheat (McIntosh et al. 2013) has curated and compiled names and symbols for the wheat community becoming a recognised authority by researchers and breeders. There is, however, limited use of these gene symbols and names in the annotation of the current genomics sequences restricting the adoption of these valuable resources. The correct identification of gene structures in annotated genome references using a coherent and standard nomenclature across the *Triticeae* species will support the wider adoption of these resources as well as facilitate the integration of data from different sources and diverse related species. We will present a scheme that emerged from the 'Triticeae Gene Nomenclature Workshop'. This meeting was organised with the sponsorship of the Wheat Initiative on 11-13 October 2016 at the Helmholtz Centrum in Munich (Germany) as a collaboration between the 'Wheat Information System' and 'Improving Wheat Quality for Processing and Health' Expert Working Groups. The scheme is focused on the orthologous and syntenic relationship that is highly conserved in genes across the *Triticeae* tribe (Figure 1). The proposed notation uses the hexaploid bread wheat genes as the basis for the assignation of names to genes in other related species making explicit the orthologous relationship between them. In the coming months, the priority is to designate symbols to gene structures that are already annotated and currently characterised in the literature and that have an established name in the Catalogue but we have also set the principles for the assignment of new symbols and descriptions.

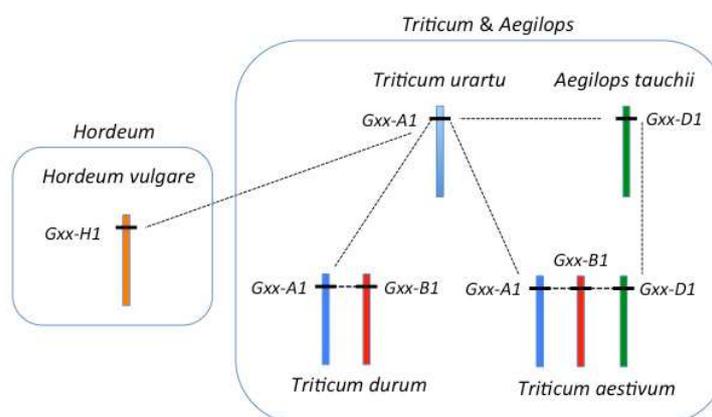


Figure 1: Example for annotation of a hypothetical gene called Gxx.



P 63 - Topic: Structural and Functional Wheat Genomics

Cloning of the *BC1* gene affecting culm and leaf resilience of diploid wheat

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Key message: Using a EMS-induced brittle culm and leaf mutant of *Triticum monococcum* L., the gene affecting culm and leaf resilience was mapped and cloned.

Plant mechanical strength is an important agronomic trait. Up to now, few reports have documented the mechanical strength-related genes in wheat. In this study, we investigated a brittle culm mutant (*br1*), identified from an ethyl methane sulfonate (EMS)-induced mutant library of diploid wheat (*Triticum monococcum* L.). This mutant phenotypically displayed brittle culm and leaf, but no other changes. The mutant leaves had a thinner sclerenchyma cell wall than the wild type and the proportion of big vascular bundles/the total vascular bundles was significantly reduced. Moreover, the leaf cellulose content of the mutant was significantly reduced. We found the mutant phenotype was controlled by a recessive gene near the centromere region of chromosome 5A. Through fine mapping, the *br1* gene was confined to an 143Mb interval. Annotation of this interval revealed one gene homologous to *OsBC1* that has similar phenotypic effects. The wheat *br1* gene is different from the wild type allele by a single SNP causing premature termination of the protein translation. Further characterization of the mutant is still under way.

Acknowledgments

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P 65 - Topic: Structural and Functional Wheat Genomics

Towards marker assisted selection for a major QTL *Rht24* controlling plant height in wheat

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Key words: CAPS marker, genome mining, MAS, *Rht24*, SNP chip, *Triticum aestivum*

Plant height is an important trait related to plant architecture and yield potential in bread wheat (*Triticum aestivum* L.). We previously identified a major quantitative trait locus (QTL) QPH.caas-6A flanked by simple sequence repeat (SSR) markers Xbarc103 and Xwmc256 on chromosome 6AL, reducing plant height by 8.0-10.4%. Here QPH.caas-6A, designated as *Rht24*, was confirmed using recombinant inbred lines (RILs) derived from the Jingdong 8/Aikang 58 cross. Subsequently the target sequences of *Xbarc103* and *Xwmc256* were used as queries to BLAST against International Wheat Genome Sequence Consortium database and hit a super scaffold with approximately 208 Mb. Based on gene annotation of the scaffold, three gene-specific markers were developed to genotype the RILs, and *Rht24* was narrowed within a 1.85 cM interval between TaAP2 and TaFAR. In addition, three single nucleotide polymorphism (SNP) markers linked to *Rht24* were identified from SNP chip-based screening in combination with bulked segregant analysis. The allelic efficacy of *Rht24* was validated in 242 elite wheat varieties using TaAP2 and TaFAR markers, indicating a significant association between genotypes and plant height as well as thousand grain weight (TGW). Additive effect analyses showed *Rht24* could reduce 6.0–7.9 cm in plant height and increase TGW by 2.0–3.4 g. These findings showed that *Rht24* was an important major QTL in wheat breeding, and TaAP2 and TaFAR could be efficiently used for its marker-assisted selection.



P 67 - Topic: Structural and Functional Wheat Genomics

Mapping of the heading date gene *HdAey2280* in *Aegilops tauschii*

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Key message: Using public and new developed SSR markers, the heading date gene *HdAey2280* was mapped onto *Aegilops tauschii* chromosome 7DS at distance of 1.9 cM to the closet marker.

An optimum heading date is essential for sustainable crop productivity and to ensure high yields. In the present study, $F_{2:3}$ populations were generated by crossing an early-heading accession, Y2280, with a late-heading accession, Y2282. The heading dates of the F_2 and F_3 populations were investigated in a field study. Using publicly available simple sequence repeat (SSR) markers, the early heading date gene *HdAey2280* was mapped onto *Aegilops tauschii* chromosome 7DS between the flanking markers Xwmc438 and Xbarc126 at distances of 15 cM and 9.1 cM, respectively. Further analysis indicated that *HdAey2280* is a novel heading date gene. New SSR markers were developed based on the *Ae. tauschii* draft genome sequence, resulting in four new markers that were linked to the heading date gene *HdAey2280*. The closest of these markers was 1.9 cM away from the gene. The results collected in this study will serve as a framework for map-based cloning and marker-assisted selection in wheat breeding programs in the future.



P 69 - Topic: Structural and Functional Wheat Genomics

Identification of a novel QTL for early flowering on 7A chromosome in a variety of emmer wheat

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Key message: We tried to identify early flowering genes in a variety of emmer wheat. From this study we identified a novel QTL for on 7A chromosome causing early flowering phenotype.

Flowering time is important trait for yields and grain quality. We investigated flowering trait of two tetraploid wheat varieties (AABB), TN26 (*Triticum turgidum* L. ssp. *dicoccum*) and TN28 (*T. turgidum* L. ssp. *turgidum* conv. *pyramidale*) that do not require vernalization (Table1). Although TN26 harbors photoperiod-sensitive *Ppd-A1b* allele (late flowering allele), it comes into flower earlier than TN28 harboring photoperiod-insensitive *Ppd-A1a* allele (early flowering allele) (Nakazaki et al. 2011). This supposes that TN26 harbors genes that surpass effect of photoperiod-insensitive *Ppd-A1a* allele. We performed QTL analysis for identifying early flowering genes TN26 harbors. TN26, TN28 and TN26×TN28 F_{5:6} 173 RILs (TN RILs) are subjected for the QTL analysis. The RILs were grown in the field in the experimental farm of Kyoto University. Heading date of these lines were investigated. Linkage map of these RILs were constructed by using 98 SSR makers and the genetic maker identifying *Ppd-A1*. QTL analysis was performed by Windows QTL cartographer ver. 2.5. TN26 and TN28 were subjected for sequence analysis of *Vrn-A3*. We identified two QTL on 2A and 7A chromosome (which were named *qHde1* and *qHde2*, respectively) by using the TN RILs (Figure 1). Closest maker of *qHde1* was the genetic maker of *Ppd-A1*. The *qHde1* of TN28 accelerated heading date with about 8 days. This supposed that *qHde1* corresponds to *Ppd-A1*. Closest maker of *qHde2* was *barc128* on short arm of 7A chromosome. The *qHde2* of TN26 accelerated heading date with about 5 days. Since it is reported that *Vrn3* is on short arm of homologous group of 7 in wheat (Yan et al. 2006), sequence analysis for *Vrn-A3* locus of TN26 and TN28 were performed. We detected insertion/deletion polymorphisms (7bp and 25bp) on promoter region of *Vrn-A3* (Figure 2). The allele of TN26 was insertion allele, and 7bp insertion site included sequence of a deduced cis-element. This lead to our presumption that *qHde2* correspond to *Vrn-A3* locus. The novel flowering QTL (*qHde2*) was identified on 7A chromosome near *Vrn-A3* locus in tetraploid wheat and allele of TN26 accelerated heading date with about 5 days. This suggests that *qHde2* is a major gene that cause early flowering of TN26 without *Ppd-A1a*.

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Table 1: Flowering trait and major flowering loci of TN26 and TN28

Varieties	Allele of major flowering loci				Days from germination to heading
	<i>Vrn-A1</i>	<i>Vrn-B1</i>	<i>Ppd-A1</i>	<i>Ppd-B1</i>	
TN26	Spring type	Winter type	Sensitive	Sensitive	159
TN28	Spring type	Winter type	Insensitive	Sensitive	165

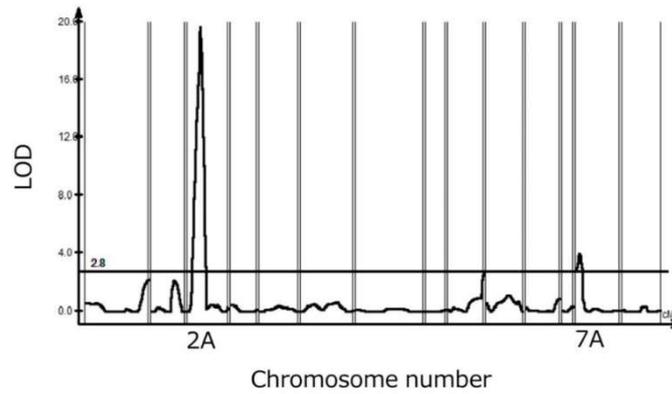


Figure 1: LOD score based on QTL analysis of heading date.

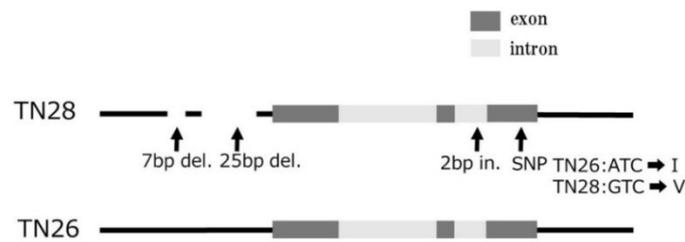


Figure 2: Structures of *Vrn-A3* locus in TN26 and TN28.



P 71 - Topic: Structural and Functional Wheat Genomics

Genome-wide QTL mapping reveals genetic architecture of grain size in einkorn wheat

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Key message: A high-density genetic map was developed in einkorn wheat, and 46 consistent QTL for grain size were detected across multi-environments, including regions harboring *AGPL* and *NAL1* genes.

High-density genetic map is an important tool to locate biologically or agronomically important traits along the chromosomes and to assist genome research. Genetic architecture of grain size as an important component of yield is less understood in wheat. In this study, we constructed a high-density genetic map of einkorn wheat, conducted whole genome-wide QTL mapping of grain size related traits and examined the candidate genes underlying QTL. A high-density genetic map was developed using restriction-associated DNA sequencing (RAD-seq) of 109 recombinant inbred lines (RILs) derived from an inter sub-specific cross, KT1-1 × KT3-5 (*Triticum monococcum* ssp. *boeoticum* × ssp. *monococcum*). The map contained ≈10K single nucleotide polymorphism (SNP) markers and 936 other types of molecular markers assigned to 1551 bins on seven linkage groups, and covered the 1873-cM with average marker interval of 0.2 cM and bin length of 1.2 cM (Figure 1). We examined seven agronomic traits in four to five location-year environments, including thousand grain weight (TGW), grain length (GL), grain width (GW), grain length/width (GLW), grain circumference (GC), grain area (GA), and grain roundness (GR) that related with grain size. These traits showed broad-sense heredity larger than 80% and approximately normal distribution, demonstrating that they were mainly under genetic control. In total, 46 consistent QTL, detected along six chromosomes except 4A, explained phenotypic variations ranging from 45.7% (GR) to 66.7% (TGW) (Figure 1). Two QTL regions, 200.8-224.3 cM on chromosome 1 and 162.2-190.1 cM on chromosome 2, were focused, which contained QTL *QGlw.igdb-1A.2*, *QGr.igdb-1A.1*, *QTgw.igdb-1A.2*, *QGl.igdb-2A.1*, *QGa.igdb-2A.1*, *QGc.igdb-2A.1* and *QTgw.igdb-2A.1*. Based on the RAD-seq tags, we aligned the two QTL regions with barley and Chinese Spring A genome physical map, and found that two reported genes, *AGPL* and *NAL1* were located on respective regions, which were further confirmed by mapping these two genes at this RIL population. Therefore, the genes or gene pathways underlying QTL, *AGPL*-starch syntheses and *NAL1*-hormone transport pathways, might be the genetic factors contributing grain size of einkorn wheat. However, RT-PCR and RNA-sequencing experiments were needed to conduct and validate the formation of grain shape in einkorn wheat. Furthermore, the genetic map revealed that 4AL/5AL translocation and pericentric inversion 4A on hexaploid wheat (Figure 1). Thus, this high-density genetic map and QTL data provide valuable genetic information to dissect genetic architecture of grain shape associated traits in einkorn wheat and contribute to the genome research.



P 92 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Genomic tool for fine mapping and marker development of wheat resistance genes

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Key message: Resistance gene analogs (RGAs) based wheat genomic resources were developed for marker development of wheat resistance breeding.

Resistance gene analogs (RGAs) are a class of potential resistance (*R*) genes, including three major types, NBS-encoding proteins, receptor-like protein kinases (RLKs) and receptor-like proteins (RLPs) (Kumar et al. 2015). To date, most of cloned *R*-genes can be grouped into one of them. RGAs based marker development has been an effective approach for fine mapping and cloning wheat *R* genes. Owing to their conserved domain and motif features, RGAs can be predicted using bioinformatics tools. An integrative pipeline of both command-line and web-based version, named RGAugury (<https://bitbucket.org/yaanlpc/rgaugury>), was developed to automate genome-wide RGA prediction (Li et al. 2016). Using this tool, 4848 (2.2% of total genes), 1591 (4.6%), 1803 (2.2%) RGAs from hexaploid common wheat (AABBDD), and its diploid progenitors *Triticum uratu* (AA) and *Aegilops tauschii* (DD) were identified, respectively. Of these in the common wheat genome, 1424 (29.3% of total RGAs), 1722 (35.5%) and 1430 (29.5%) RGAs were distributed in A, B and D sub-genomes, respectively, and the remaining 272 (5.6%) were not aligned to chromosomes. RGAs were unevenly distributed and most of them were clustered on chromosomes. A total of 14 299 genome-specific SNPs on 1727 out of 4848 RGAs (35.6%) were identified from exome sequencing data of 89 wheat cultivars and breeding lines in Canada and other countries. These RGAs and RGA-specific SNPs have been successfully used for fine mapping high impact wheat resistance genes such as Ug99 stem rust resistance gene (*SrCad*), leaf rust resistance gene (*Lr16*), and orange wheat blossom midge resistance gene (*Sm1*). Cost effective, breeder friendly and diagnostic SNP markers that are suitable for high throughput marker assisted selection (MAS) have been developed for *Lr16* (Kassa et al. 2017) and *SrCad* (Kassa et al. 2016a). Moreover, marker haplotypes were identified to determine the presence and absence of wheat midge resistance gene *Sm1* (Kassa et al. 2016b). An RGA database integrating the developed genomic data has been developed. All these data provides genomic resources to locate specific *R* gene candidates and find gene-specific markers for fine-mapping and cloning of wheat *R* genes, and resistance breeding.

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P 94 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Using wild relatives for creating disease-resistant spring wheat varieties

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A nursery for the evaluation of leaf and brown rust resistance of spring common wheat originated from wild wheat relatives and spelt wheat varieties was established from 2013 to 2016 in Northern (Karabalyk) and Southern (Almaty) Kazakhstan and in Izmir, Turkey. Within the climatic conditions of Karabalyk Experimental Station in 2016 resistance to both brown and leaf rust was observed in the Pharaoh spelt variety 'Temirbekova S.' and wheat varieties Diva and Umai (0-5%). Among the spring wheat varieties created from crossing with wild relatives (Kozhakhmetov K.K.) the following crosses have shown resistance: 6625 × *Triticum timopheevii* and Kazakhstanskaya 10 × *T. dicoccum* (5-10%). Check varieties were 100% affected. Low degree of resistance correlated with lower yields: Diva (4.44 t/ha) > Umai = Gremme; Kazakhstanskaya 10 × *T. kiharae* > 6625 × *T. timopheevii* (3.8-4 t/ha). Within the range of introgressive forms of the control nursery the following genotypes were distinguished by minimal rust lesions: 6631 × *T. timopheevii* (0-5%), 6628 × *T. timopheevii* and 6569 × *T. militinae-1* (5-10%); 6625 × *T. timopheevi-1* (10-15%). Minimally affected by mildew were genotypes of Kazakhstanskaya 10 × *T. timopheevii*, 6631 × *T. timopheevii* (0-5%), 6625 × *T. timopheevi-2* (10-15%). Genotypes in advanced nurseries were almost free from brown rust, except Kazakhstanskaya 10 (10%) and 6628 × *T. timopheevii* (50%). These varieties were attacked by stem rust at a rate of 5%, except the above mentioned genotypes (75% and 10% respectively). The results of the CIMMYT-Turkey trials were as following: (i) resistant wild relatives: *T. kiharae* 0/20 MS; *T. timopheevii* -0/20 MS and MS *T. dicoccum* 5; (ii) original forms: Ilyinskaya × *T. timopheevii* (0-10 MS); Kazakhstanskaya early ripening × *T. timopheevii*; 6683 × *T. timopheevii* (0-10 MS) and Kazakhstanskaya 10 × *T. dicoccum* 10 (0%). On the basis of long-term data (2013-2016) the following lines have been selected: (a) lines that act as sources of resistance to disease (on a natural background) and require confirmation on an artificial background and genetic analysis; (b) lines with high NDVI potential and high yield (4.4-5.4 t/ha) in senior nurseries of the breeding process with simultaneous reproduction of siblings; (c) genotypes for registration as new varieties (high yielding and stable) based on novelty, distinctness and uniformity: Tim-biday and Guntikum (patent applications RK №20548 and №20549 from June 15, 2016).



P 96 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Development of a new pre-breeding scheme combining marker-assisted selection and genomic selection for improving wheat disease resistance

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Key message: Development of a pre-breeding strategy in two main steps combining marker-assisted selection (MAS) for major resistance genes coming from exotic materials, and genomic selection (GS) to optimize the elite genetic background.

Genomic selection (GS) is a valuable approach for plant breeding, mainly for complex traits controlled by many loci. To achieve high GS accuracy, the relationship between the training population and the test population is essential as genome-wide markers needs to be in the same linkage disequilibrium in both populations to be good predictors. In pre-breeding programs, exotic plant materials are often introduced for their notable behaviour for a specific trait, for example disease resistances, but are not well-characterized for complex traits. Applying GS directly on this exotic material would not be powerful because of the poor relationship between this exotic material and training populations, mostly composed of breeding elite material. We developed a pre-breeding strategy in two main steps combining marker-assisted selection (MAS) for major genes coming from exotic materials, and genomic selection (GS) to optimize the genetic elite background. The first step was based on several crosses, controlled by MAS, in order to combine all the desirable alleles coming from exotic lines into the same genotypes with a significant elite genetic background. Hybrids cumulating these major genes were then used to produce a large doubled haploid population. Finally, the DH individuals were selected by genome-wide predictions to optimize their elite background (Figure 1). Two real and separate pre-breeding schemes are described in bread wheat (*Triticum aestivum* L.) for the introgression of disease resistance genes into optimized elite backgrounds. The first one focused on the construction of genitors with improved yellow rust and leaf rust resistances. Four major genes were followed across the different steps. The rust resistance genes *Lr42* and *Lr57/Yr40* were introgressed in the exotic part of the scheme, the eyespot resistance gene *Pch1* and the fusarium head blight resistance QTL *QTL5A* were followed from the elite parents. The second pre-breeding scheme was based on the creation of fusarium head blight (FHB) resistant genitors with improved genetic background. Two major genes of FHB resistance (*Fhb1* and *Fhb5*) were followed in this pre-breeding scheme. The two DH populations that were produced were then genotyped with the Axiom® Wheat Genotyping Arrays (<http://www.affymetrix.com>) and will be selected based on prediction algorithm trained on a large breeding population.

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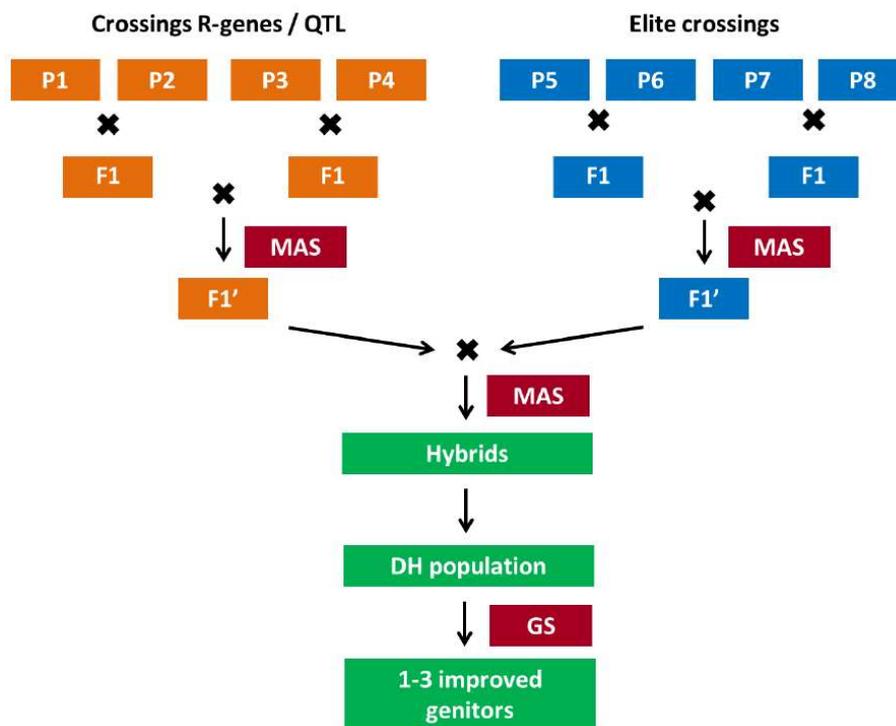


Figure 1: The overall pre-breeding scheme combining marker-assisted selection (MAS) and genomic selection (GS).



P 98 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Evolution and adaptation of wild emmer populations to wheat pathogens

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Key message: Wild emmer wheat gene pool is a highly promising source for improvement of the resistance of durum and bread wheats by exploitation of genes that were lost during domestication.

Wild emmer wheat, *Triticum dicoccoides*, the tetraploid progenitor of domesticated wheat, distributed along a wide range of eco-geographical conditions in the Fertile Crescent, has valuable 'left behind' adaptive diversity to multiple diseases and environmental stresses. Segregating mapping populations, developed by crossing of selected *T. dicoccoides* genotypes with *T. durum* cultivars, revealed numerous loci associated with disease resistance, drought tolerance, high grain protein content, and yield. Furthermore, wild emmer is a promising source of resistance to stripe rust. For example, *Yr15* is a dominant genes that confer particularly high resistance, while *Yr36* confers slow rusting quantitative resistance. Comparative genomics approaches were used to develop high resolution physical maps for *Yr15*, and for cloning of *Yr36*. *Yr36* has a unique architecture with a kinase and a START lipid-binding domains, designated *WKS* hereafter. The distribution and sequence conservation of *WKS* R-genes were compared with those of NBS-LRR R-genes (e.g. *Lr10* and *Pm3*) among wild emmer natural populations. The sequence diversity of *WKS1* was much lower than that of *Lr10* and *Pm3*, indicating that these R-genes, representing different resistance mechanisms, are shaped by different evolutionary processes. Further work is underway to clone *Yr15* located on chromosome arm 1BS, using the complete 1BS physical map, constructed by our group, as well as the recently assembled wild emmer reference genome. These studies demonstrate the potential of wild emmer wheat gene pool for improvement of durum and bread wheats by exploitation of genes that were lost during domestication.



P 100 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Analysis of breeding progress for resistance against fungal pathogens in winter wheat (*Triticum aestivum* L.)

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Key message: Resistances against fungal pathogens differ in relation to N-supply but were significantly improved by wheat breeding in Germany during the last 50 years. 339 significant marker-trait associations related to disease severity have been revealed.

Resistances against important fungal pathogens have been major goals of winter wheat breeding in Germany during the last 50 years. A genotyped set of 220 winter wheat varieties differing by year of release has been tested for resistance against *Puccinia striiformis* (*PS*), *P. triticina* (*PT*), *Blumeria graminis* (*BGT*), *Fusarium culmorum* (*FC*), and *Zymoseptoria tritici* (*ZT*) in two year field trials under four different growing systems (low nitrogen, low nitrogen + fungicide, high nitrogen, high nitrogen + fungicide). Stripe rust (caused by *PS*) was the most prevalent disease, followed by leaf rust (*PT*), powdery mildew (*BGT*), Septoria tritici blotch (*ZT*), and Fusarium head blight (*FC*). Varieties revealed highly significant differences ($p < 0.001$) in resistance to fungal pathogens. N fertilization significantly increased the susceptibility to biotrophic pathogens (*PS*, *PT*, *BGT*) as well as to the hemibiotrophic *FC*, but not to *ZT*. Infection levels of stripe rust, powdery mildew and Fusarium head blight revealed significant negative correlations to yield. Resistance against *PS*, *PT*, *BGT* and *FC* was significantly improved over the last 50 years, but not resistance against *ZT*. The yield increase over time was higher in the production systems without fungicides and highest in the treatment with high nitrogen input without fungicides indicating a two-way success of resistance breeding. Genome-wide association studies (GWAS) were performed using a mixed linear model (QK) on a set of 9248 polymorphic SNP markers and resistance data. In total 339 (*PS*: 73, *PT*: 91, *BGT*: 109, *FC*: 35, *ZT*: 31) significant marker-trait associations ($p < 0.001$) were detected on all chromosomes. A number of genomic regions identified were previously detected in biparental populations, but some may be novel.



P 102 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Plant morphology plays a large role in resistance to Fusarium head blight in wheat: focus on plant height and flowering morphology, implications for resistance breeding

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Key message: Plant height and extent of extruded anthers modulate resistance to initial infection with *Fusarium* spp. on wheat heads under field conditions. Variation for both traits is large in the wheat gene pool and can be exploited for resistance breeding.

During the past years we focused in our Fusarium head blight (FHB) resistance research also on morphological characters of the plant. Increasing evidence accumulated that plant height and flowering morphology play a significant role particularly in establishment of the disease and thus in resistance to initial infection, also called type 1 resistance. In mapping studies QTL for height and for the rate extruded anthers after flowering frequently overlapped with QTL for FHB severity assessed under epidemic conditions in field experiments both with bread wheat (Buerstmayr & Buerstmayr 2015, 2016) and with durum wheat (Prat et al. 2017). Typically tall plants and plants with a high proportion or even complete extrusion of anthers after flowering were more resistant to FHB. In addition height reducing alleles (*Rht-B1b*, *Rht-D1b*) do not only modulate stem length but also the extent of anther extrusion (Buerstmayr & Buerstmayr 2016). A role of morphology on modulating FHB infection severity is not surprising because *Fusarium* spp. is an opportunistic pathogen on wheat heads with a quite narrow infectious window around flowering. For both traits genetic variation in the wheat gene pool is large, and heritability is particularly high. While for plant height, tall plants with lower susceptibility may be undesirable due to increased lodging susceptibility, the trait anther-extrusion appears an attractive indirect selection criterion for improving FHB resistance in wheat. The genetic control of these traits and their relevance for FHB resistance breeding are discussed.

Acknowledgements

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P 104 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Detoxification of mycotoxins as a source of resistance to *Fusarium* head blight in cereals: an innovative translational biology approach between *Brachypodium distachyon* and bread wheat

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Key message: This work will contribute to increase the knowledge concerning the functional relationship between DON glucosylation and FHB resistance in wheat and it will provide candidate genes to include in selection processes.

Fusarium head blight (FHB) caused by fungi of the *Fusarium* genus is a widespread disease of wheat (*Triticum aestivum*) and other small-grain cereal crops. The main causal agent of FHB, *F. graminearum*, can produce mycotoxins mainly belonging to type B trichothecenes, such as deoxynivalenol (DON), that can negatively affect humans, animals and plants. Several QTLs for resistance to FHB have been identified some of which have been correlated with efficient DON detoxification, mainly through the ability to conjugate DON into DON-3-*O*-glucose (D3G) *via* UDP-glucosyltransferases (UGTs). Nevertheless, only few studies have conducted functional analyses to directly correlate DON glucosylation and resistance *in planta* and none were performed on wheat detoxification gene(s). In order to develop efficient strategies of FHB resistance in wheat, there is a need for more detailed functional analyses of the relationship between detoxification of mycotoxins and resistance to FHB. Our team, using the model cereal species *Brachypodium distachyon*, has recently demonstrated that the *Bradi5g03300* UGT is able to confer tolerance to DON following glucosylation of DON into DON 3-*O*-glucose and is involved in early establishment of quantitative resistance to FHB. In the present work, we aim at transferring the functional analyses conducted on the model species *B. distachyon* to bread wheat. In a first approach the *B. distachyon Bradi5g03300* gene has been introduced through biolistic-mediated transformation in the wheat variety Apogee, susceptible to FHB. The phenotypic analyses conducted on homozygous transgenic wheat constitutively expressing the *Bradi5g03300* gene show that they exhibit higher resistance to FHB as well as increased root tolerance to DON compared to the control line. In parallel, using a synteny approach between *B. distachyon* and bread wheat genomes we identified wheat candidate genes orthologous to the *B. distachyon Bradi5g03300* gene. The selected wheat best candidate gene orthologous to *Bradi5g03300* has been validated for its expression pattern during wheat infection. Transformation of this wheat candidate gene into *B. distachyon* was performed to rapidly determine its ability to conjugate DON into D3G *in planta* and its relationship with FHB resistance. Production of the corresponding recombinant protein in *Escherichia coli* was achieved and enzyme activity assays using DON as substrate coupled with LC-MS analyses was conducted to determine its ability to conjugate DON *in vitro*. In conclusion, this project will contribute to increase the knowledge concerning the functional relationship between DON glucosylation and FHB resistance in wheat and provide candidate genes to include in selection processes.



P 106 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Potential involvement of antioxidant activity and fluorescence on *Fusarium* resistance and mycotoxin accumulation

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Key message: These results have the potential to give a new insight into the physiological cost of host resistance to *Fusarium* head blight.

Fusarium head blight, mainly caused by *Fusarium graminearum* and *F. culmorum*, is a major disease in European cereals. Due to their ability to produce mycotoxins, including deoxynivalenol (DON) and zearalenone (ZEA), which can be harmful to human and animal health, these *Fusarium* species pose a serious threat to food safety. The best approach to control FHB and to reduce mycotoxin contamination is to create wheat genotypes which are carrying effective resistance genes. Effects on the physiological parameters in plants with different FHB resistance remain unclear. The objective of this study was to evaluate the activity of guaiacol peroxidase (POD) and ascorbate peroxidase (APX), catalase (CAT) and polyphenol oxidase (PPO), malondialdehyde (MDA) content and hydrogen peroxide concentration. Also we wanted to evaluate the applicability of the parameters derived from the fast chlorophyll a fluorescence kinetics in order to evaluate biotic stress response of wheat and distinguish disease tolerance among the tested wheat varieties, as well as to measure some most important agronomical and technological properties of wheat. In susceptible variety *Fusarium* infection caused yield losses more than 35%, very high disease intensity (>100 AUDPC units), as well as *Fusarium* colonized kernels and initial infection. Concentrations of DON and 3-acetyldeoxynivalenol (3-ADON) in *Fusarium*-infected kernels in three winter varieties ranged from 68.55 to 3246.52 µg/kg and 30.87 to 501.39 µg/kg, respectively. After malting, varieties contained DON and 3-ADON (184 to 967 µg/kg and <20 to 110 µg/kg, respectively). The deacetylation of 3-ADON into DON may appear during malting process. Amount of ZEA was only detected in low amounts in infected malt and in control malt of tolerant variety. More tolerant variety was less affected by FHB infection in terms of photosynthetic function as indicated in a small reduction in performance index (PI) and maximum quantum yield of PS II (Fv/Fm), together with lower reduction in grain yield and test weight under *Fusarium* infection, but decrease in 1000 kernel weight occurred. In contrary, protein quality, particularly HMW-GS were the highest affected in more tolerant variety, where glutenins were decreased, but albumins and gliadins increased in infected kernels in comparison to control plants. *Fusarium* tolerance could be enhanced by the APX, a hydrogen peroxide detoxifying enzyme, and PPO, which has ability to oxidize flavonoids and phenolic acids induced by pathogen attack reducing thus a mycotoxin biosynthesis.

Acknowledgement

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P 108 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Fusarium head blight in wheat in the Czech Republic

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Key message: Samples of random collections of wheat cultivars grown in the Czech Republic were analysed for detection of *Fusarium* species using PCR and for determination of mycotoxin content.

Samples from random ear collections of winter wheat cultivars grown in the Czech Republic (2011-2015) were analysed for *Fusarium* species and mycotoxin content using PCR and ultra-high performance liquid chromatography coupled with tandem mass spectrometry, respectively. The study revealed a prevailing occurrence of *F. poae* and *F. graminearum* in the Czech Republic. *F. graminearum* dominated only in 2011 (50.8% of infected samples). *F. poae* increased significantly in 2012 (93.3% of infected samples) in association with relatively warmer and drier weather. Also 2014-2015, *F. poae* dominated. Other species were detected in much lower frequencies. *F. culmorum* was detected in 25.4% of the 2011 samples, while in the following four years its frequency was low (1.7% in 2012, 2.6% in 2013, 5.1% in 2014 and 3.2% in 2015). These findings are in agreement with Xu & Nicholson (2009), who reported that *F. poae* and other minor FHB-causing species increased their presence in years when the presence of *F. graminearum* decreased and vice versa. *F. graminearum* s. str. was detected in all samples with increased DON values (>1250 µg/kg), either alone or in mixed infection together with *F. poae*, *F. avenaceum* and *F. equiseti*. Competition among species could lead to a stronger production of DON, as it is considered to be a virulence factor. The results showed that annual weather conditions, additionally in interaction with local climatic conditions, are important factors influencing the composition of *Fusarium* species and consequently mycotoxin contamination. Considerable changes in *Fusarium* species' composition can lead to a greater occurrence of so-called 'emerging' mycotoxins (BEA and ENNs), especially in combination with the traditional *Fusarium* mycotoxins DON and NIV. Our experiments which were performed under conditions of high infection pressure demonstrated the threat that may be posed to wheat production by growing cultivars susceptible to FHB. Among the currently registered varieties a group of varieties (e.g. Bakfis, Cimrmanova raná, Dagmar) were identified which show the same level of resistance to FHB as check variety Arina, which is widely used as a source of moderate resistance.

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P 110 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Identification of genomic regions associated with resistance to Fusarium head blight, leaf rust and stem rust in winter wheat

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Key message: QTL for FHB resistance were found on chromosomes 2D, 4B and 4D and for leaf and stem rust resistance on 1B, in a doubled-haploid (DH) winter wheat population from Canada.

Fusarium graminearum (Schwabe) (FG) is the principal cause of Fusarium head blight (FHB), one of the most serious diseases of wheat (*Triticum aestivum* L.). Deoxynivalenol (DON) is the most important mycotoxin produced by FG. Since 1996 FHB epidemic, it is mandatory to screen for FHB all candidate cultivars to be registered in Ontario, Canada (Tamburic-Ilincic et al. 2011). Leaf rust and stem rust are also among the most destructive wheat diseases throughout wheat-growing regions. Breeding resistant cultivars is considered the most effective way to control these diseases. In addition to exotic sources of resistance, native resistance sources are required in winter wheat breeding programs. Winter wheat cultivar Vienna is moderately resistant to FHB, while Pioneer 25R47 has good resistance to leaf and stem rust. Therefore, both Canadian winter wheat are good native source. The objective of this study was to map loci associated with FHB, leaf rust and stem rust in a doubled-haploid (DH) population derived from the cross Vienna and Pioneer 25R47. DArT markers were used to generate a genetic map and QTL analysis were performed evaluating 113 DH lines for FHB severity, incidence, index, DON accumulation, leaf rust and stem rust severity and plant height in three trials in Ontario, Canada. Significant QTL for FHB resistance were found on chromosomes 2D, 4B and 4D. The FHB QTL on 4B and 4D were co-localized with QTL for plant height. Significant negative correlation was reported between FHB resistance and plant height across our different winter wheat populations (Tamburic-Ilincic et al. 2007, Tamburic-Ilincic 2012) where taller plants had lower FHB index and DON level compared to shorter ones. QTL for both leaf and stem rust resistance were identified on 1B and on a non-determined chromosome segment. The QTL for FHB, leaf rust and stem rust resistance are important in marker assisted selection for development of new winter wheat cultivars.

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P 112 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Reaction of Iranian wheat landraces to Fusarium head blight (FHB) under field and greenhouse conditions

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Key message: The reaction of 117 Iranian landraces was determined to Fusarium head blight (FHB) under field and greenhouse conditions. By considering the results of field and greenhouse experiments, we conclude that 21 genotypes should have Type I resistance.

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, is one of the destructive diseases of wheat in northern parts of Iran and any other parts of the country with unusual excessive rainfall. The present study was carried out to determine the reactions of 117 wheat landraces from Seed & Plant Improvement Institute (SPII), Karaj, Iran, to FHB under field and greenhouse conditions. Wheat genotypes were from the species *Triticum aestivum*, *T. durum*, *T. polonicum*, *T. compactum* and *T. turgidum*. They were tested in two cropping seasons (2013-14 and 2014-15) under artificial spray inoculations at the Araghi-Mahalleh Agricultural Research Station, Gorgan, Golestan and Moghan Agricultural Research Station, Parsabad, Ardabil to detect so-called total field resistance which is a combination of Type I and Type II resistance (Buerstmayr et al. 2009). The plants were inoculated at 50% anthesis with a mixture of four isolates of *F. graminearum* (5×10^4 spores/ml) collected from the corresponding location and this was repeated 2-3 days later. Three weeks after the first inoculation, disease incidence and severity were recorded to calculate disease index. In the present study, the selected genotypes from the field experiments were also investigated using point inoculation in the greenhouse (Karaj) to detect Type II resistance. A total of 5 spikes were inoculated at anthesis with a mixture of four isolates of the pathogen collected from Moghan (5×10^4 spores/ml) and three weeks after, disease progress (severity) was determined by calculating the proportion of infected spikelets to total spikelets. Based on the results of field evaluations, 31 genotypes with mean disease index of $\leq 10\%$ were detected to be resistant to FHB (Table 1). As 10 genotypes out these seemed to be late-matured in the field, we were not sure whether they had a genetic-based resistance or had escaped from the disease, regardless of our precise inoculations applied. However, resistance present in at least 21 genotypes should be of genetic-based. Of these, 16 accessions were from bread wheat and five from durum wheat. Results of greenhouse experiments showed that all genotypes were susceptible to FHB which is referring to lack of Type II resistance. We conclude that these genotypes should have Type I resistance. Considering the presence of Type I resistance in these genotypes, which may keep them clean from the disease under field conditions, these genotypes can be used as FHB-resistant parents in wheat breeding programs.

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Table 1: Disease severity, disease incidence and disease index of 31 'resistant' wheat accessions evaluated for reaction to Fusarium head blight.

Serial Number	CRD Collection Code	Wheat Species	Origin	Mean Disease Incidence, Severity and Index (Mogan + Gorgan, 2014 + 2015)			Remarks
				Disease Incidence (%)	Disease Severity (%)	Disease Index (%)	
1	370	Triticum compactum	Moghan	11.7	33.3	7.5	Late-matured
2	804	Triticum aestivum	Moghan	22.5	50.0	9.8	
3	806	T. aestivum	Moghan	22.5	45.0	8.3	
4	900	Triticum durum	Gorgan	20.0	45.0	6.5	
5	901	T. durum	Gorgan	15.0	10.0	3.0	Late-matured
6	905	T. aestivum	Gorgan	10.0	10.0	2.0	
7	911	T. durum	Gorgan	17.5	45.0	6.0	
8	912	T. durum	Gorgan	17.5	35.0	4.3	
9	913	T. durum	Gorgan	15.0	40.0	5.0	
10	914	T. durum	Gorgan	12.5	45.0	6.3	
11	915	T. aestivum	Gorgan	0.0	0.0	0.0	
12	918	T. aestivum	Gorgan	2.5	35.0	1.8	
13	919	T. aestivum	Gorgan	7.5	35.0	2.0	
14	920	T. aestivum	Gorgan	2.5	30.0	1.5	Late-matured
15	1663	T. aestivum	Behbahan	11.7	50.0	8.5	Late-matured
16	1668	T. aestivum	Behbahan	6.7	43.3	4.3	Late-matured
17	1673	T. aestivum	Shoushtar	20.0	30.0	7.3	Late-matured
18	1739	T. aestivum	Ahvaz	15.0	33.3	5.0	Late-matured
19	1794	T. aestivum	Ahvaz	25.0	45.0	9.0	
20	1899	T. aestivum	Sari	13.3	33.3	4.7	
21	1900	T. aestivum	Sari	15.0	43.3	4.2	
22	1901	T. aestivum	Sari	10.0	8.3	1.5	
23	1902	T. aestivum	Sari	15.0	31.7	5.0	
24	1903	T. aestivum	Sari	13.3	40.0	5.0	
25	1905	T. aestivum	Sari	6.7	21.7	2.2	
26	1921	T. aestivum	Gorgan	18.3	45.0	6.8	Late-matured
27	1924	T. aestivum	Gorgan	18.3	36.7	9.3	
28	1926	T. aestivum	Gorgan	21.7	40.0	7.3	
29	2669	T. aestivum	Gorgan	26.7	41.7	8.0	Late-matured
30	2671	T. aestivum	Gorgan	2.5	50.0	2.5	Late-matured
31	2690	T. aestivum	Ahvaz	25.0	38.3	8.8	
Resistant check	-	Sumai 3	-	0.0	0.0	0.0	
Susceptible check	-	Falat	-	93.3	83.3	78.0	



P 114 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

A systemic genetic and genomics approach to improve FHB resistance of wheat: walking through bi- and multi-parental QTL mapping to genomics prediction

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Key message: A systemic genetic and genomics approach from bi-parental and joint-multiple family QTL mapping to genomics selection for improving FHB resistance of wheat will be presented and discussed

Fusarium head blight (FHB) resistance is quantitatively inherited, controlled by multiple genes with minor effects, and highly affected by the interaction of G×E. The evaluation of FHB using conventional approaches therefore lacks accuracy, rendering breeding for this trait time consuming and costly. Bi-parental QTL mapping is one approach used to identify FHB QTL, but it can be difficult to identify QTL with minor effect. Joint-multiple family QTL mapping has been proposed as an option that has a high statistical power to identify FHB genes with minor effect, but still relying on the effect size QTL. The advent of cost-effective genotyping systems allows genome-wide marker information to be applied to calculate genomic estimated breeding values (GEBV). With this approach, selection can be made on GEBV without phenotyping and QTL detection, significantly accelerating breeding for FHB resistance. Genomic selection (GS) involves predicting breeding values based on genome-wide markers using a model trained with phenotypic and genotypic data. Recently, three populations were created by crossing FL62R1, an Eastern Canadian spring wheat line with very good FHB resistance, to two CWRS wheat varieties, Stettler and Muchmore, and a winter wheat variety, Emerson, which also possesses effective, novel FHB resistance. These related populations were phenotyped for FHB severity (SEV), incidence (INC), *Fusarium* damaged kernels (FDK), deoxynivalenol levels (DON), and heading date at Carman, MB, and Ottawa, ON in 2015 and 2016. This relatively large dataset was used to perform a comprehensive analysis of the efficiency and effectiveness of bi-parental and joint-multiple family QTL mapping as well as GS for improving FHB resistance. We found that joint-multiple population QTL mapping had more statistical power for common QTL with minor effects, but low power for rare QTL. Genomics selection had high prediction accuracies from 0.4 to 0.6, depending on the trait predicted. When GS prediction incorporated QTL, higher accuracies were achieved most of the time. All our findings indicate that combining these different approaches in a systemic genetic and genomics way will be a more effective approach to enhance FHB resistance in wheat.



P 116 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Characterization of Fusarium head blight resistance in Canadian spring wheat cultivar AAC Tenacious

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Key message: Fusarium head blight resistance was characterized in spring wheat cultivar AAC Tenacious using 90K Infinium iSelect SNP Assay

Wheat suffers severe losses in yield, grade and end-use quality due to Fusarium head blight (FHB) caused by *Fusarium graminearum* and other spp. of genus *Fusarium*. FHB is one of the most devastating diseases of wheat in Canada and losses to the wheat industry ranges from \$50 million to \$300 million annually. Growing FHB resistant cultivars along with other disease management practices can effectively mitigate FHB outbreaks and reduce economic losses. A number of QTL/genes conferring FHB resistance have been identified in wheat. However, most of the known FHB resistance sources do not possess adequate level of FHB resistance and predominantly impart quantitative resistance with minor additive effect on FHB. Identification and characterization of new sources of FHB resistance is necessary to keep pace with emerging risk of FHB outbreaks and their associated economic losses. A recently developed Canadian spring wheat cultivar, AAC Tenacious, possesses excellent resistance to FHB and DON accumulation. In this study, a doubled haploid (DH) population containing 224 DH lines produced from AAC Innova/AAC Tenacious was phenotyped for FHB Type-I (disease incidence; DI), Type-II (disease severity; DS) and Type-III (DON content) resistance for two years. The population was genotyped using the wheat 90K Infinium iSelect SNP Assay to dissect the FHB resistance in AAC Tenacious. FHB visual rating index (VRI) was also estimated using observed DI and DS ratings to identify a common mechanism of type-I and -II resistance. Phenotypic results showed that AAC Tenacious possess a major gene and two minor additive genes which co-segregate with Type-II resistance. Results from QTL analysis to identify the genomic regions associated with these genes/QTL will be presented.



P 118 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

The expression of the DON-detoxifying barley UDP-glucosyl transferase HvUGT13248 enhances resistance to Fusarium head blight disease in durum wheat

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Key message: The expression of a barley UGT that convert DON in D3G is effective in enhancing resistance to FHB disease in durum wheat

Fusarium head blight (FHB) is one of the most severe fungal disease of wheat worldwide, caused mainly by the fungus *Fusarium graminearum*. FHB causes yield reduction and contamination of grains with mycotoxins, particularly deoxynivalenol (DON). DON is a protein synthesis inhibitor, it acts as a virulence factor during pathogenesis and it is essential for the spread in the spike. One of the mechanisms involved in enhancing plant tolerance to DON is the conversion to deoxynivalenol-3-β-D-glucoside (D3G) by the activity of specific UDP-glucosyl transferases (UGTs), often followed by compartmentation of the product. Previous studies demonstrated that the expression of the gene *HvUGT13248* from barley confers resistance to DON in *Arabidopsis thaliana* (Shin et al. 2012) and type II resistance to FHB in bread wheat (Li et al. 2015). Since sources of FHB resistance in durum wheat are lacking, we wanted to verify whether the expression of the *HvUGT13248* could improve FHB resistance also in durum wheat. We produced transgenic lines of *Triticum durum* cv. Svevo constitutively expressing the *HvUGT13248* gene. After confirming the presence of the transcript and the protein, we infected transgenic wheat plants with *F. graminearum* to assess FHB severity, DON content and D3G conversion compared to the wild type plants. Our results showed that this approach was also effective in durum wheat since a significant reduction of FHB symptoms (≈30%; $p < 0.01$), as compared to control plants, was observed from 6 dpi up to 12 dpi. In the late stages of infection, the reduction of FHB symptoms was less evident as compared to control plants. This last result differed from what observed in the transgenic bread wheat plants expressing the same UGT gene where FHB severity never exceeded 20% at 21 dpi (Li et al. 2015). To verify the effectiveness of this approach we are also assaying other pathogens able to produce DON such as *F. culmorum* and *F. pseudograminearum*, main causal agents of Fusarium crown rot disease in wheat.

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P 120 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Genes for wheat resistance and susceptibility to *Fusarium* head blight and *Septoria tritici* blotch disease of wheat

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Key message: Using a variety of approaches we have identified wheat genes that enhance wheat resistance to *Septoria tritici* blotch and *Fusarium* head blight resistance.

For the last ten years, we have searched for wheat and barley genes that enhance cereal disease resistance. Our research is primarily focused on identifying genes that enhance cereal resistance to *Septoria tritici* blotch (STB) disease and *Fusarium* head blight (FHB) disease. Using populations of both wheat and the model monocot *Brachypodium* that segregate for STB resistance, we are conducting bulk segregant analysis to identify genes that enhance disease resistance or susceptibility. Concurrently, we have identified TILLING (Targeted Induced Local Lesions IN Genomes) mutants that are resistant to STB disease. Ongoing studies are validating the role of specific genes in defence against STB disease. For FHB disease, we analysed the wheat transcriptome response to the *Fusarium* disease virulence factor deoxynivalenol (DON) using a bulk segregant analysis of a population segregating for disease resistance, and thus we discriminated genes associated with disease resistance. Our search led us to study the role of specific genes in resistance to FHB disease, including a cytochrome P450s, a kinase, and genes encoding a novel wheat interactome. Using a combination of virus-induced gene silencing and overexpression studies, the role of three genes in disease resistance was validated. During this work, it became apparent that orphan genes (unique to the Pooideae) play a role in FHB resistance. We now enter an era where we expand our work to delineate the genes that improve disease resistance without any negative trade-off effects. Indeed, several FHB resistance genes have positive effects on grain development. A wheat ABC transporter and a cytochrome P450 gene, both of which enhance DON resistance, also enhance grain development.



P 122 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

The transcriptome and metabolome of *Fusarium graminearum* during infection of wheat in presence and absence of the resistant QTL *Fhb1* and *Qfhs.ifa-5A*

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Key message: Analyzing the transcriptome and metabolome of *Fusarium graminearum* during infection of wheat in presence and absence of resistant QTL suggests different growth strategies depending on the specific mechanism of host-plant-resistance.

Toxin contaminations and yield losses render *Fusarium* head blight (FHB) one of the most devastating plant diseases. In order to ensure stable and save supply of nutrition for humans and livestock the development of strategies to fight FHB based on knowledge about this host-pathogen interaction are needed. Host-plant resistance of wheat to FHB is based on many quantitative trait loci (QTL). The mechanism of host-plant resistance controlled by QTL has been target of numerous studies, whereas the response of the pathogen to these QTL is poorly known. We analyzed differences in the transcriptome and metabolome of *Fusarium graminearum* (Fg) during infection in a pair of near-isogenic bread wheat lines (NILs) differing in presence and absence of the two prominent resistance QTL *Fhb1* and *Qfhs.ifa-5A*. Wheat heads were inoculated in the greenhouse with Fg and harvested 0, 3, 6, 12, 24, 36, 48, 72, 96 hours after inoculation (hai), whereby mock inoculation with water served as control. QPCR was used to monitor total Fg biomass and RNA-sequencing was used to measure global gene expression. Additional targeted GC-MS and untargeted LC-HRMS analysis were applied to complement the data with information about primary and secondary metabolites. Fg infection developed fast in both genotypes. The Fg biomass increased equally in both genotypes from 0-48 hai (0.05-2.2%) but was higher (7-14 %) at 96 hai in the susceptible NIL lacking *Fhb1* and *Qfhs.ifa-5A*. The detected number of transcribed genes increased up to 24h (>7000) with no further changes at later time points. Whereas the number of expressed genes were similar, 200 genes showed an alternated expression pattern over time between the genotypes already at early time points. 3% of ≈4000 determined Fg features (m/z and retention time value, ≈1000 metabolites) could be annotated during database search. Most of them were detected as early as 24 hai showing higher abundances in the susceptible NIL at later time points. Taken together the resistance QTL did not alter early (0-48 hai) Fg biomass development but showed differences in gene expression and metabolite composition. This finding suggests different Fg growth strategies depending on the specific mechanism of host-plant resistance. This study provides a detailed insight into the transcriptomic and metabolomic network of Fg during infection of wheat lines with contrasting FHB resistance, and thus strongly broadens the understanding of this host-pathogen interaction.

Acknowledgement

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P 124 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Resistance of winter wheat breeding lines to *Fusarium* head blight and *Fusarium* toxins accumulation in grain

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Key message: Winter wheat lines combining resistance to *Fusarium* head blight (type I+II), to *Fusarium* kernel damage (type III) and the low accumulation of toxins (type V) were identified.

Fusarium head blight (FHB) is a disease of cereals caused by fungi of genus *Fusarium*. These fungi produce toxic metabolites (mycotoxins). There are several types of resistance to FHB: type I (to infection), type II (to the spread of *Fusarium* in the ear), type III (to the kernel damage by *Fusarium*). Another types are the tolerance to FHB or toxins (DON) (type IV) and type V (resistance to the accumulation of toxins (trichothecenes) in the grain through their chemical modification or blocking of synthesis). In order to obtain the forms of winter wheat that combine different types of resistance 71 lines were evaluated in field experiments in two locations. Among them there were five resistant checks: '20828[*Fhb1*-]', 'A40-19-1-2', 'Arina', 'Fregata', 'UNG 136.6.1.1[*Fhb1*+]'; three lines with *Fhb1* gene from crosses of winter wheat cultivars with 'Sumai 3'; four susceptible checks; three lines with high accumulation of trichothecenes. Wheat heads were inoculated with the spore suspension of *F. culmorum* isolates producing DON, NIV and ZEN. FHB index (FHBi) was evaluated. The proportion of *Fusarium* damaged kernels (FDK) was determined visually by dividing the sample on healthy looking kernels and with symptoms of *Fusarium* damage. Using the technique of gas chromatography and immunoenzymatic tests the contents of the DON and acetyl derivatives, NIV and ZEN in the grain were analysed. Average FHBi was 14.1%; at the range of 4.7-40.0%. The least infected were lines 'S30[*Fhb1* +]', 'UNG 136.6.1.1', 'S10[*Fhb1*+]', 'S32[*Fhb1*+]', 'A40-19-1-2', 'POB 679/03', '20828', 'STH 105', 'KBP 05.271' and 'Fregata', the most infected were four susceptible checks. The average value of the FDK amounted to 13.2%; at the range of 3.0-29.0%. The least damaged kernels had lines 'S 10[*Fhb1*+]', 'S32[*Fhb1*+]', 'A40-19-1-2', 'S30[*Fhb1*+]', 'POB 679/03', 'UNG 136.6.1.1', 'SMH 7983', 'STH 9059', 'SMH 7974', 'STH 2041' and 'Fregata', the most two susceptible lines and two lines with low FHBi, i.e. 'SMH 9005', 'AND 260/10'. There was a significant linear relationship between the severity of FHB and the degree of damage of kernels ($r = 0.546$, $p < 0.001$). Chemical analyses showed the presence of *Fusarium* toxins DON, 3AcDON, NIV and ZEN in the grain samples. Lines differed significantly in terms of content of toxins in the grain. It was possible to identify lines combining resistance to FHB (type I + II), to kernel damage (type III) and the low accumulation of toxins (type V).



P 126 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

***Fusarium* toxins and *Fusarium* species occurring in grain of winter wheat in Poland in the years 2014 and 2015**

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Key message: *Fusarium graminearum* and *F. poae* were main species in wheat grain in Poland in 2014 and 2015. Low amounts of deoxynivalenol and zearalenone were detected, especially in dry year 2015.

Fusarium head blight is a disease of wheat caused in Poland mainly by the species *Fusarium graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae*. These species differ in the pathogenicity and profile of produced mycotoxins. In 2014 and 2015 samples of winter wheat grain were collected. *Fusarium* damaged kernels (FDK) was evaluated and the content of *Fusarium* toxins and content of DNA of five *Fusarium* species in the grain were analysed. The average FDK in 2014 was 6.6%. In 2015 FDK was lower and amounted to 3.4%. The greatest value of the FDK was found in 2014 in South-Eastern Poland. In 2015 there were no clear regional differences regarding FDK values. All *Fusarium* species were present in wheat grain in two years. *F. avenaceum*, *F. graminearum* and *F. poae* were detected in all samples. However, the first species was present only in trace amount. Content of DNA of the second species in 2014 amounted to 0.067% (% by mass) and to 0.026% in 2015. Content of DNA of *F. poae* in 2014 was 0.017% and in 2015 was higher at 0.042%. *F. culmorum* occurred in 45% of the samples in 2014 (0.04%, in one sample, traces in remaining) and in 50% in 2015 (only traces). *F. langsethiae* occurred in 45% of the samples in 2014 (0.015%, in one sample, traces in remaining) and in 50% in 2015 (0.01%, in two samples, traces in remaining). Average contents of deoxynivalenol (DON) in the grain from 2014 was 0.254 mg/kg in the range of 0 to 1750 mg/kg. In 39% of the samples DON was not detected. The highest amounts of DON occurred in samples from the South-Eastern Poland. The average content of zearalenone (ZEN) in the samples of grain from 2014 was 0.01 mg/kg with a range of variation from 0 to 0.159 mg/kg. In 87% of the samples ZEN was not detected. The average amount of T-2 toxin and HT-2 toxin in grain from 2014 was 0.121 mg/kg with a range of variation from 0.087 for 0.129 mg/kg. These toxins occurred in all samples of wheat grain. In the samples from 2015 very low content of DON and ZEN was found. First toxin appeared in 2 samples (0.19 and 0.204 mg/kg), the second in two samples (0.031 and 0.035 mg/kg). T-2 toxin and HT-2 toxin were found in all samples (average 0.117 mg/kg) in the range of 0.053 to 0.274 mg/kg.



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Expression markers closely associated with the 2DL QTL locus for Fusarium head blight resistance

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Key message: Identification of 3 candidate genes for the 2DL QTL for FHB resistance.

The 2DL QTL for FHB resistance from Wuhan 1 is a moderate resistance locus for Fusarium head blight (FHB), which has potential to improve the FHB resistance of bread wheat, and confer effective and durable resistance to novel wheat breeding lines. Differentially expressed genes which expression profile associated with the 2DL QTL resistance locus were identified by comparing a line carrying the 2DL QTL with a null sister line. Fourteen differentially expressed genes that are physically located within the interval for the 2DL QTL were further characterized for their expression profile in 78 lines of a double haploid mapping population derived from the cross Wuhan 1xNuyBai, the population where the 2DL QTL was first identified. The expression QTL for genes *2DL_159137_AA0533120* and *2DL_160788_AA0554140* (from the recent TRIAE_CS42_TGACv1 annotation) as well as an unannotated gene (represented by FGA014667 in the EST collection at NCBI) overlapped with the mapping interval for the 2DL QTL; however that of the unannotated gene was centered very close to the peak of the 2DL QTL. Our results suggest that the unannotated gene and possibly all three genes, contribute to the FHB resistance associated with the 2DL QTL.



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Localization of *Fusarium* root rot-induced metabolites related to wheat stem resistance using high-resolution mass spectrometry imaging

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Key message: High-resolution mass spectrometry allows metabolite detection with information on spatial distribution by recording compounds directly from tissue sections.

Fusarium graminearum is a major fungal pathogen of wheat causing the head blight disease (FHB). Its ability to colonize wheat via root infection has been examined recently (Wang et al. 2015). Although FHB and *Fusarium* root rot (FRR) represent different pathosystems, suggesting organ-specific attack strategies, our histological and molecular studies have disclosed that relevant components of the wheat-*F. graminearum* interaction are shared in roots and spikes - including the FRR-induced systemic expression of FHB defense-related genes in the shoot tissues. The second layer of FRR resistance is the inhibition of fungal spread into the stem with the vascular tissues as important sites of fungal ingress and wheat protection (Wang et al. 2015). To localize the topography of metabolites related to *F. graminearum* invasion of stem, atmospheric-pressure scanning microprobe matrix-assisted laser desorption ionization mass spectrometry imaging (AP-SMALDI MSI) system was applied. MS imaging combines metabolite detection with information on spatial distribution by recording compounds directly from tissue sections, enabling reference to histological observations. This tool has been utilized to tissue sections of animals, insects, mammals (Römpp & Spengler 2013), and has recently been established for all major plant organs (Bhandari et al. 2015). MS imaging disclosed metabolic changes over time (10, 14, 21 days after root inoculation) and plant tissue-specific localisations. For example, while the leaf sheath responded by increasing lipid compounds related to stress signalling, the internal stem vasculature revealed the enrichment of antifungal metabolites - also known from FHB resistant spikes. Pathogenesis-related mycotoxin compounds verified fungal ingress via the leaf-sheath-route, as was observed by microscopy.

Acknowledgements

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P 132 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Genetic control of resistance and tolerance to crown rot in wheat

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Key message: Several genomic regions associated with tolerance to crown rot were identified in an association analysis. This is the first report of the genetic control of tolerance to this disease.

Crown rot of wheat is a serious disease caused by the fungus *Fusarium pseudograminearum*, a stubble-borne pathogen common in no-tillage farming systems in semi-arid grain growing regions. Affected plants display characteristic honey-brown discoloration on the crown and lower stem. Premature ripening of developing heads caused by disruption of vascular tissue results in whiteheads under post anthesis drought conditions, directly leading to grain yield loss. While resistance and tolerance to this disease is available, genetic control is complex, and many minor QTL convey only partial control. A genome wide association analysis (GWAS) was undertaken to identify regions controlling the crown rot response in a breeding population combining multiple sources of crown rot resistance and tolerance. The population was phenotyped for both crown rot resistance and tolerance over two years and genotyped using a high-density 90K SNP genotyping array. A mixed linear model with kinship matrix and principal components to account for population relatedness was used for the GWAS. A number of genomic regions/QTL associated with resistance and tolerance traits were identified. These include putative QTL for tolerance on chromosomes 3A, 5A, 3B and 4B, as well as confirmation of a previously reported resistance QTL on chromosome 2B. These results are now being used to combine multiple trait alleles into a single genotype using a marker assisted recurrent selection (MARS) approach, whereby markers are used to combine complimentary pairs of sister lines, in order to pyramid unique alleles.



P 134 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Tolerance of bread wheat to root-lesion nematode (*Pratylenchus thornei*) mapped on chromosome 3B

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Key message: Tolerance to root-lesion nematode (*Pratylenchus thornei*) has been mapped and localised to the chromosome 3B assembly.

Root-lesion nematode (*Pratylenchus thornei*) is a major pathogen of wheat and is estimated to cost the Australian wheat industry up to A\$36M per year in lost production. Control of nematodes in broad acre farming using nematocides or fumigation is impractical and the development of resistant and tolerant cultivars is viewed as the only option. Resistance to nematodes generally indicates that the plant possess a mechanism whereby it prevents the infection process developing while tolerance is the ability of the plant to grow in the presence of the nematode while limiting its effect. Lines that are tolerant differ from non-tolerant lines in their ability to maintain yield compared to the non-tolerant lines in similarly infested conditions; reduced yield loss in response to nematode infestation is generally used as a measure of tolerance in a plant. A doubled haploid mapping population was derived from a cross between two Queensland varieties, EGA Wylie which was reportedly carrying tolerance and the susceptible EGA Hume. Replicated yield trials were run in 2015 at sites known to have a significant *P. thornei* infestation. We report on the localisation of the tolerance gene to chromosome 3B and the positioning of linked markers to the International Wheat Genome Sequencing Consortium (IWGSC) gene assembly.



P 136 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

***PmHo*: a new powdery mildew resistance gene in wheat cultivar Mv Hombár**

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Key message: The aims of our study were to determine the chromosomal location of genetic factors associated with powdery mildew resistance in Mv Hombár wheat cultivar, and to characterize the host×pathogen interaction at microscopic level.

Wheat powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is one of the most important diseases worldwide. The average yield loss due to this disease ranges from 5 to 8% and may exceed 35% in the case of severe infection. Breeding for resistance is the most environmentally sound measure in disease control. A new powdery mildew resistance gene designated as *PmHo* was identified in the winter wheat cultivar 'Mv Hombár', bred in Martonvásár, Hungary. It has exhibited a high level of resistance over the last two decades. Genetic mapping of recombinant inbred lines derived from the cross Ukrainka/Mv Hombár located this gene on the chromosome 2AL. It was detected in all environments, for the 4 phenotypic datasets recorded in the field, and showed a major effect on powdery mildew resistance, indicated by a LOD score of 17.94 (Figure 1). The segregation ratio and consistent effect in all environments indicated that *PmHo* is a major dominant powdery mildew resistance gene. The proportion of phenotypic variance (R^2) explained by this genetic factor was 14.3% in 2012, 18.2% in 2013, and 33% in 2014 indicating that it was the major contributor to powdery mildew resistance in the field, and it was the only locus that was found to be associated with powdery mildew resistance at the seedling stage. The race-specific nature of resistance in 'Mv Hombár' was shown by the emergence of a single virulent pathotype designated as 51-Ho. This pathotype was, to some extent, able to infect 'Mv Hombár', developing visible symptoms with sporulating colonies (Figure 2). Microscopic studies revealed that, in incompatible interactions, post-haustorial hypersensitivity reaction was the most prevalent but not exclusive plant defence response in 'Mv Hombár', and fungal growth was mostly arrested during haustorium formation or in the early stages of colony development (Figure 3). The delayed fungal development of the virulent pathotype 51-Ho may be explained by additional effects of other loci that were also involved in the powdery mildew resistance of 'Mv Hombár'.

Acknowledgements

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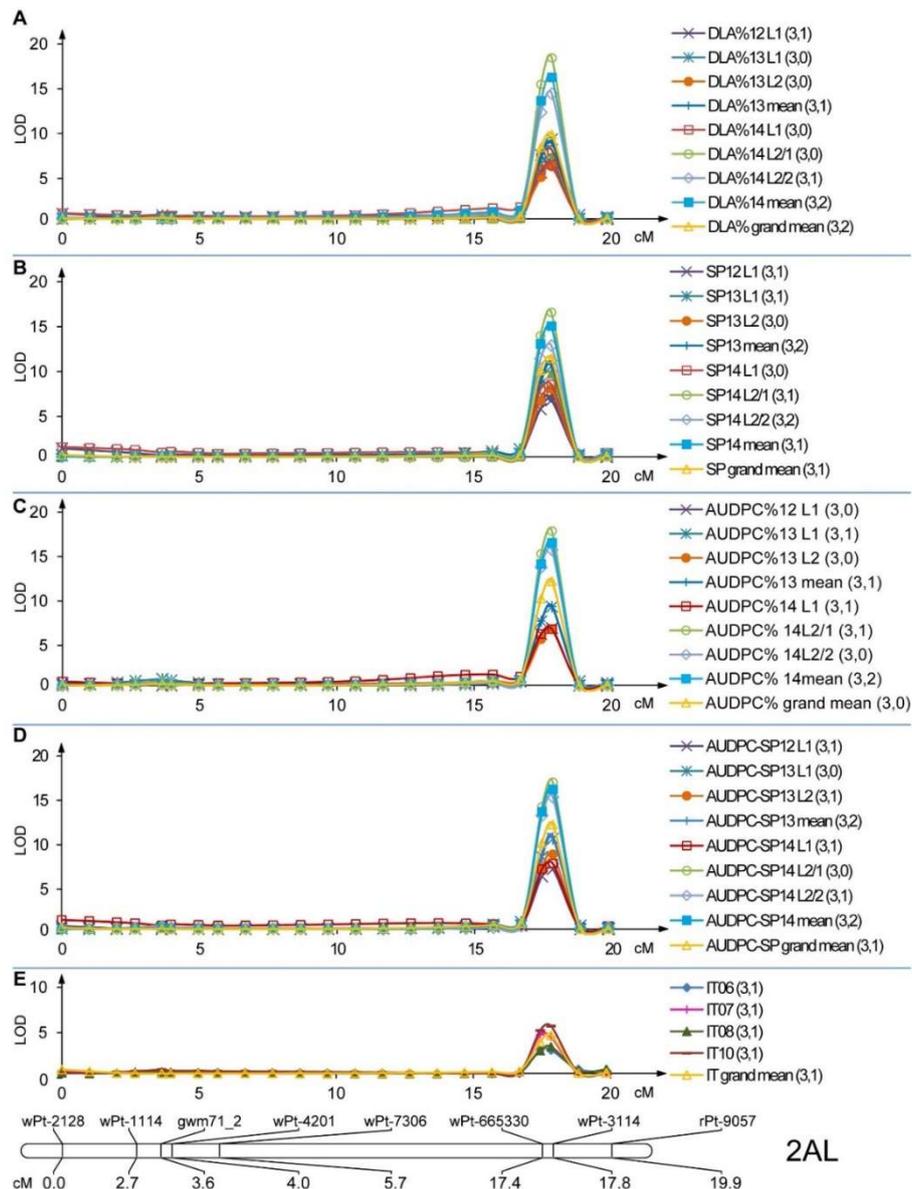


Figure 1: LOD peak values of the major effect QTL for powdery mildew resistance localized on chromosome 2AL and flanked with DaRT markers *XwPt-3114* and *XwPt-665330*. LOD peak values were calculated using the multiple QTL model (MQM) procedure, and represent the effect of the QTL on the resistance expressed by (A) percentage diseased leaf area at the last scoring date (DLA%); (B) infection level based on the Saari-Prescott scale at the last scoring date (SP); (C) area under the disease progress curve (AUDPC) calculated from the percentage diseased leaf area (AUDPC%); (D) AUDPC calculated from the Saari-Prescott data (AUDPC-SP); and (E) infection types in the greenhouse seedling test (IT). The designations of the powdery mildew infection datasets include abbreviations of the scoring methods, years, experimental locations with replications, if any, and the threshold of LOD significance ($p=5\%$) in brackets. Numbers 06, 07, 08, 10, 12, 13, and 14 stand for the years 2006, 2007, 2008, 2010, 2012, 2013, and 2014, respectively. L1 and L2 represent location 1 and location 2, respectively, with L2/1 and L2/2 indicating the two replications at location 2 in 2014. Means and grand means calculated from the datasets obtained from single scoring methods in single years and in all years of the experiment, respectively, are also presented.

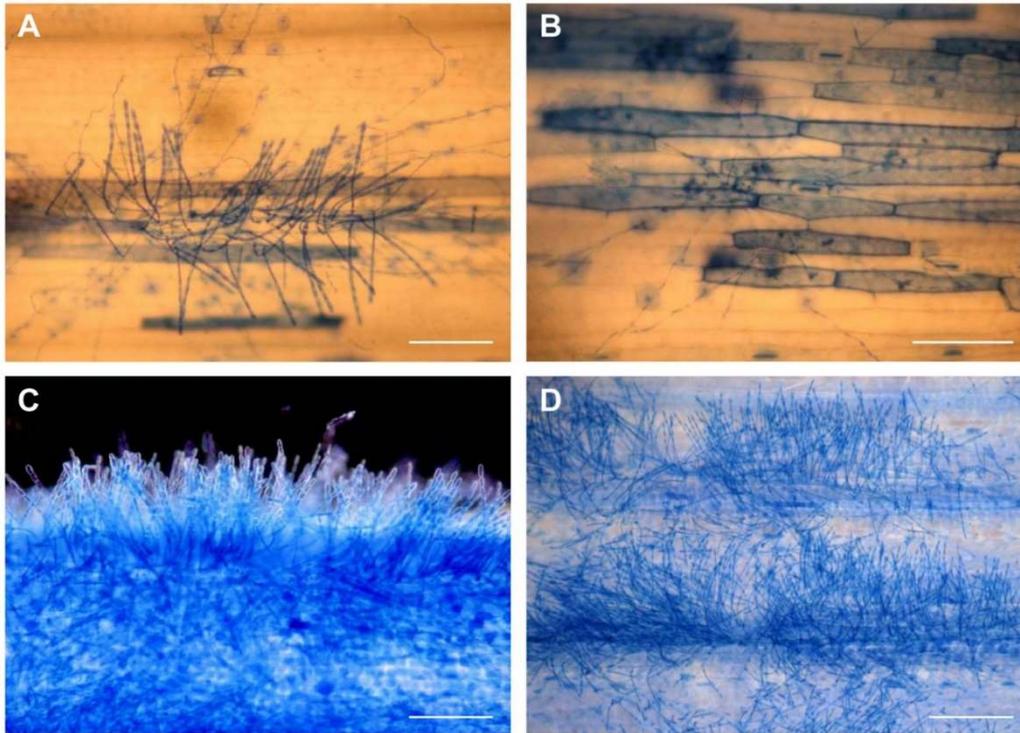


Figure 2: Micrographs of plant-pathogen interactions observed on winter wheat cultivars Mv Hombár (A, B) and Carstens V (used as susceptible control) (C, D) 168 hpi with pathotypes 51-Ho (A, C) and 51 (B, D) of *Blumeria graminis* f. sp. *tritici*. (A) a sporulating powdery mildew colony representing a compatible interaction between Mv Hombár and pathotype 51-Ho. Note the delayed fungal development compared to that on the susceptible control (C) and the hypersensitivity reaction in epidermal cells; (B) incompatible interaction between Mv Hombár and pathotype 51 characterized by arrested fungal development and hypersensitivity reaction in the plant epidermis; (C, D) compatible interactions with no difference in fungal development and plant responses between the 2 pathotypes, 51-Ho and 51, on Carsten V. Scale bar = 200 μ m.

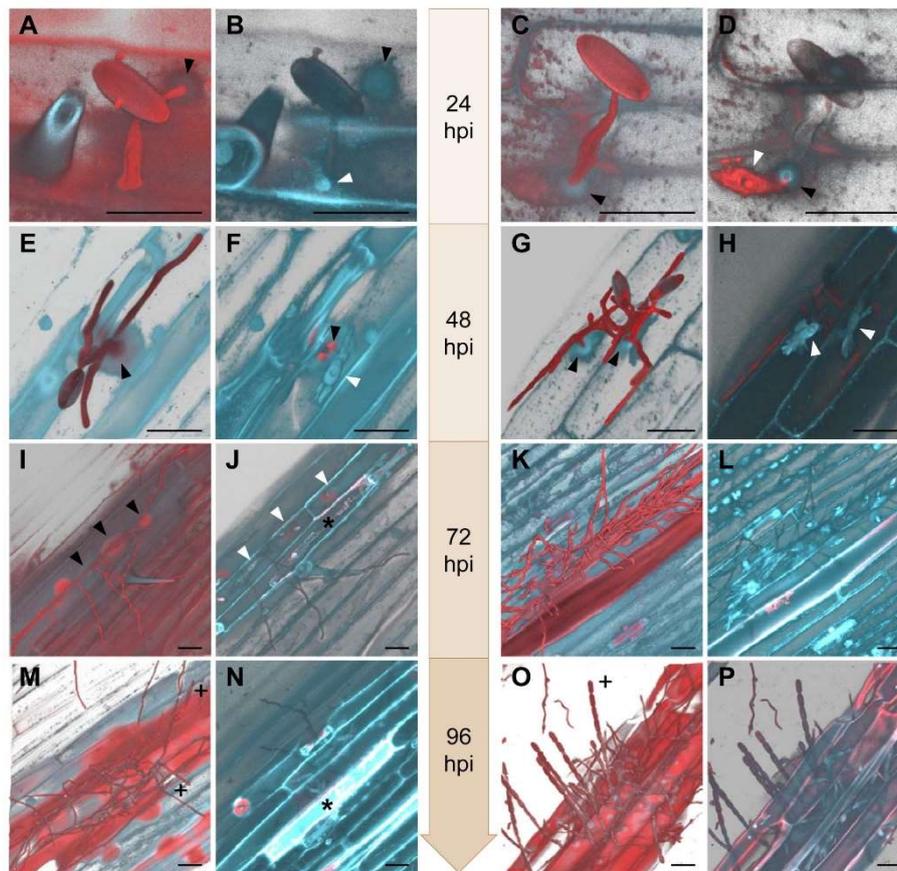


Figure 3: Three-dimensional reconstructions of plant-pathogen interactions on the resistant winter wheat cultivar Mv Hombár carrying *PmHo* following inoculation with pathotype 76 of *Blumeria graminis* f. sp. *tritici* compared to those observed on the susceptible winter wheat cultivar Ukrainka. The time scale in the middle represents the 4 main sampling dates (hpi). Interactions corresponding to each sampling date on cultivar Mv Hombár are presented on the left column of the time-scale, those on cultivar Ukrainka on the right column. Interactions are shown in two adjacent pictures from two viewpoints: the leaf surface and the interior of the host epidermal cell. (A, B) a conidium with 3 short primary germ tubes (PGTs) and 1 appressorial germ tube (AGT). A cell wall apposition (papilla) beneath one of the PGTs is shown by black arrowheads, and a papilla initial is just emerging opposite the AGT prior to penetration (white arrowhead); (C, D) a conidium with an AGT at the post-penetration stage. A papilla is shown by black arrowheads around the penetration peg, which developed into a haustorium initial (white arrowhead); (E, F) a young colony formed a well-developed haustorium with digitate lobes (white arrowhead) and a papilla at the penetration site (black arrowheads); (G, H) two young colonies with well-developed haustoria (white arrowheads) and branching extracellular hyphae. Black arrowheads indicate penetrated papillae; (I, J) a colony consisting of sparse thin hyphae with a few branching points and haustoria at different developmental stages (white arrowheads) near the corresponding penetration sites (black arrowheads). The asterisk shows an epidermal cell with agglutination of the cytoplasm; (K, L) a colony consisting of dense branching hyphae that have penetrated into host epidermal cells at many sites, and haustoria at various developmental stages; (M, N) an undeveloped colony bearing two young conidiophores (plus signs) and retarded by an intensive plant defence response including papilla formation and hypersensitivity reaction (asterisk); (O, P) a sporulating colony with numerous well-developed conidiophores (plus sign). Fungal development on Mv Hombár was in most cases arrested at around the penetration stage (approximately 50%) (A, B) or at the young colony stage (approximately 40%) (E, F), while conidiophores only developed occasionally (approximately 1%) (M, N). Scale bar = 50 μ m.



P 138 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Application of RGAP technique for genotype screening of introgressive wheat lines resistant to powdery mildew

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Key message: RGAP analysis of the resistant introgressive wheat lines indicates presence/absence variation and appearance of new components in electrophoretic spectra that can be associated with lines resistance to powdery mildew disease.

Interspecific hybridization is a widely used and effective way for genetically resistant crops development. However, such hybridization leads to genome stress that complicates investigation of the resistance origination. In the current study, 44 introgressive wheat lines derived from crosses between cultivar Aurora (AABBDD) and its amphidiploids with substituted D genomes (Aurotica with TT genome from *Aegilops mutica*, Aurodes with SS from *Ae. speltoides*, Aurolata with UU from *Ae. umbellulata*, and Aurosis with S^{sh}S^{sh} from *Ae. sharonensis*) were investigated. These introgression lines demonstrate durable resistance to powdery mildew. For identification and investigation of R genes conferring resistance in available introgressive lines the Resistance Gene Analog Polymorphism (RGAP) technique was chosen (Chen et al. 1998). This technique displays a wide range of components in the electrophoretic spectrum that may be associated with a resistance trait. In this work combinations of RGAP primers specific to the conservative NBS (Kinase 1, Kinase 3a) and LRRs regions of *Cre3* (Cheng & Chen 2010), *Rps2*, and *Xa21* (Chen et al. 1998) genes were used. Also, three RGAP primers to the MHD and LRRs regions were developed to corresponding conservative sequences between *Pm8* and *Pm3b* genes. Amplification product of expected length to *Pm8* LRR region was obtained in the genome spectra of almost all resistant lines and susceptible Aurora suggesting that *Pm8* should be present in their genomes. However, in this case introgressive lines resistance may not be provided by this gene. Analysis of different combinations of RGAP primers revealed the following variation in introgressive lines: presence/absence variation (PAV) and the presence of new components differed from parental spectra. Probably, PAV can occur due to the introgressions/deletions events in the plants' genomes whereas the presence of new components indicates genomic rearrangements. Such alterations can be explained by the hybrid origin of wheat lines that undergone genomic stress during their development. According to the resistant phenotypes of introgressive lines and susceptible one of Aurora, all variations observed within RGAP spectra compared to the same in Aurora spectrum potentially can be associated with the powdery mildew resistance. Nonetheless, considering to the RGAP technique specificity, possible amplification of pseudogenes and LRR-containing receptor kinases regions should be taken into account. Consequently, determination of components associated with the resistance trait requires the further screening of F₂ segregating populations.

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P 140 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Fine mapping and validation of a major locus for race non-specific partial resistance to powdery mildew on chromosome arm 1AS in the German spring wheat cv. 'Naxos'

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Key message: SNP markers closely linked to the race non-specific powdery mildew resistance locus on 1AS in Naxos have been identified and validated for use in wheat breeding.

Powdery mildew, caused by *Blumeria graminis* f.sp. *tritici* is a major wheat disease in maritime and temperate climates, and can cause yield losses up to 40% when not controlled properly. Fungicides are of environmental concern and add extra costs to the farmers. Resistance breeding is therefore more sustainable and cost-effective. While race-specific resistance usually confers complete protection, it is often not durable due to fast emergence of new pathogen races with matching virulence. Partial resistance, on the other hand, is controlled by genes that work through different mechanisms to slow down disease development. As it is not based on the recognition of specific effector molecules from the pathogen, partial resistance is considered more durable. The German spring wheat cv. 'Naxos' has shown high levels of partial resistance to powdery mildew in the field, and in a previous study we identified a major QTL on 1AS with resistance from 'Naxos' in the SHA3/CBRD × Naxos population based on QTL mapping with SSR and DArT markers (Lu et al. 2012). In the present study, we further refined the linkage maps with inclusion of SNP markers from the 90K wheat chip and narrowed down the QTL area in the SHA3/CBRD × Naxos population. For validation, the SORU#1 × Naxos population was tested for powdery mildew resistance in four field trials in south-eastern Norway and 131 RILs genotyped with the same 90K wheat chip. Also in this population, the 1AS QTL from Naxos was identified as a major determinant of powdery mildew resistance, explaining from 10 to 19 % of the phenotypic variance. The co-linearity of common SNP markers confirmed that the QTL was the same in the two populations (Figure 1). In order to validate markers for use in wheat breeding, the most closely linked 90K markers were converted to the KASP system and genotyped on 140 F₆ RILs from the cross Avocet × Naxos. This genotyping identified one F₆ family that segregated for the 1AS QTL, and progenies from this segregating family are currently used for further fine mapping of the QTL. Progress from this work will be presented at the meeting.

Reference

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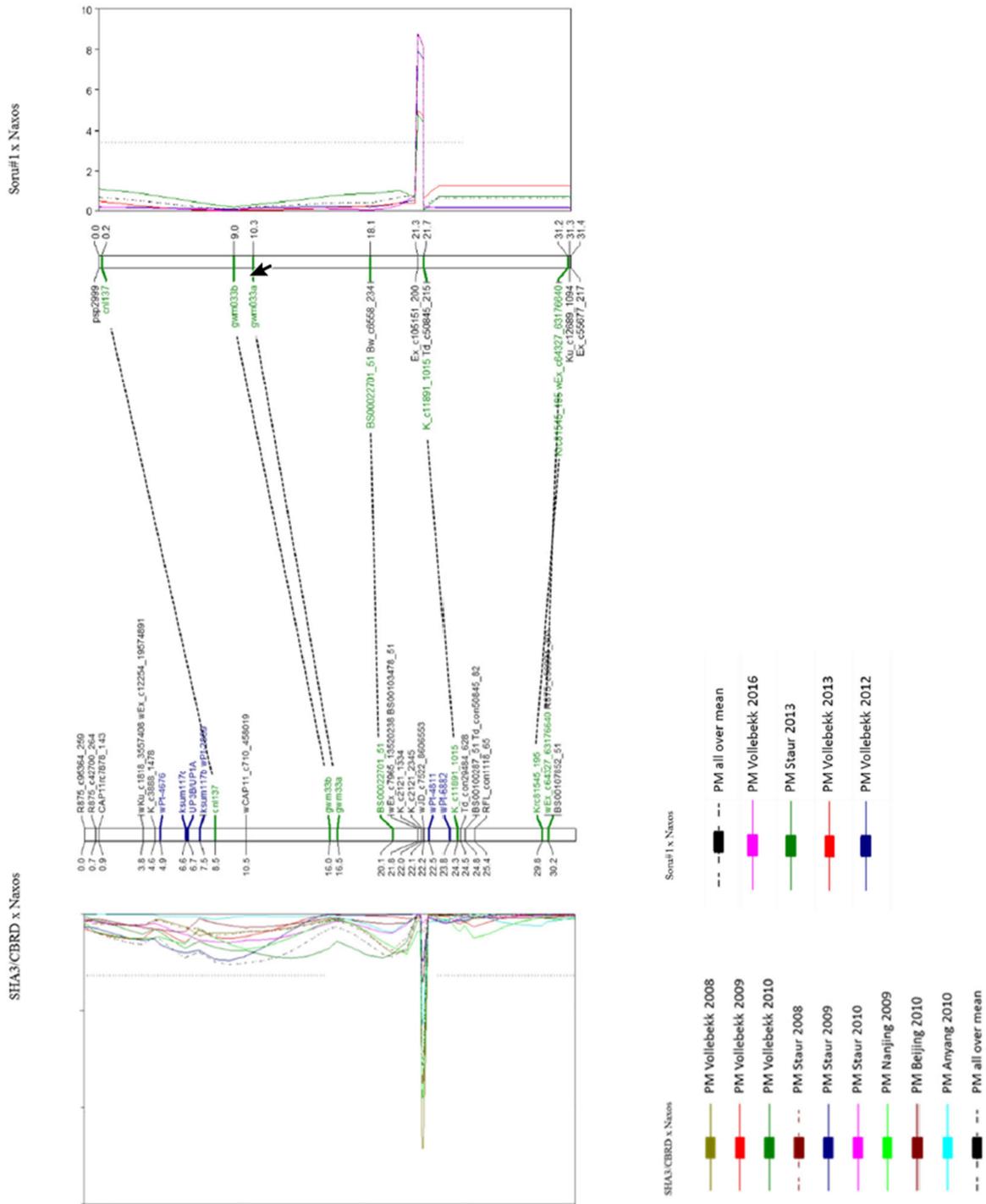


Figure 1: Segment of chromosome 1AS with major QTL with resistance from Naxos in the two RIL populations SHA3/CBRD x Naxos and Soru#1 x Naxos. The corresponding LOD curves were obtained from multiple QTL mapping (MQM) in MapQTL. Genetic distances are shown in centimorgans (cM) to the left of the chromosome in SHA3/CBRD x Naxos and right of the chromosome in Soru#1 x Naxos. A threshold of 3.2 is indicated as a dashed line in the LOD graph of SHA3/CBRD x Naxos and a threshold of 3.4 is indicated as a dashed line in the LOD graph of Soru#1 x Naxos. Common markers between the two populations are marked in green, and markers also mapped in the QTL area by Lu et al. (2012) marked in blue. Dotted lines show the position of the common markers between the two populations.



P 142 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Positional cloning of powdery mildew resistance gene introgressed to bread wheat from *Triticum militinae*

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Key message: This study reports improvement of wheat genome by introgression of *Triticum militinae* gene *QPm.tut-4A* conferring race non-specific powdery mildew resistance and its positional cloning.

Introgressions from related species have frequently been employed in crop improvement programs. Powdery mildew resistance locus *QPm.tut-4A* originating from tetraploid *Triticum militinae* significantly improves seedling and adult plant resistance of hexaploid bread wheat (*T. aestivum*). The resistance locus was mapped to the distal end of 4AL chromosome arm using a mapping population comprising 7500 lines which was created from a cross between resistant introgression line 8.1 and susceptible cv. Chinese Spring (Jakobson et al. 2012) and 2053 lines of a mapping population derived from a cross between line with introgression and Chinese Spring carrying a recessive *Ph1* locus. The gene region was identified by chromosome walking employing 4AL chromosome arm-specific BAC libraries of Chinese Spring and the introgression line 8.1. The *QPm.tut-4A* region (650 kb) shows low sequence conservation between the parental lines as a consequence of insertions of different transposable elements, which resulted in suppression of recombination. The locus comprises about 80 putative protein coding genes in Chinese Spring. To reduce the number of potential candidates, a TILLING population of 975 lines was established and six mutants susceptible to powdery mildew were selected. Chromosome 4A will be flow-sorted from these lines, sequenced and the data obtained will be used to identify a candidate resistance gene.

Acknowledgements

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P 144 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Transfer of rust resistance from perennial rye (*Secale cereanum*) to bread wheat via recombinant 1RS.1BL translocation

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Key message: A leaf and stripe rust resistant wheat line carrying new, recombinant 1RS.1BL translocation was produced from a cross between *Triticum aestivum* and *Secale cereanum* (*S. cereale* × *S. montanum*).

The most intensively used source of rye chromatin in bread wheat has been the 1RS chromosome arm in the form of 1RS.1BL translocation because it provided a source of resistance genes against leaf rust (*Lr26*), stem rust (*Sr31*), stripe or yellow rust (*Yr9*) and powdery mildew (*Pm8*). Nowadays, the proportion of wheat varieties carrying the 1RS.1BL translocation is decreasing due to the fact that resistance genes *Lr26*, *Pm8* and *Yr9* are no longer effective against new biotypes of diseases. Virulence to the *Sr31* resistance gene was first reported from Uganda in 2010. Appearance of the *Sr31*-virulent pathotype in countries where wheat production is based on cultivars carrying the 1RS.1BL chromosome translocation can cause serious problems for agriculture. The genetic vulnerability of 1RS.1BL cultivars is the consequence of the genetic uniformity of the 'Petkus'-derived 1RS arm. Therefore, it is important to involve new rye breeding materials in resistance breeding programmes in order to improve the genetic base of cultivated wheat. In order to widen the genetic diversity of this rye chromosome arm, the winter wheat line Martonvásári 9 *kr1* (Mv9kr1) was crossed with Hungarian *S. cereanum* cv Kriszta. The F₁ hybrids were propagated in tissue culture, the regenerated plants were backcrossed with the parental wheat genotype Mv9kr1 and selfed. Progenies were propagated in the Martonvásár nursery. Selection of disease resistant lines carrying recombinant 1RS.1BL started in 2012. A wheat line designated as line 179 was grown together with the highly susceptible parental wheat line Mv9kr1 genotypes in a pesticide-free nursery and in the Breeders nursery in three consecutive seasons (2013-2014, 2014-2015 and 2015-2016). Mv9kr1 suffered severe attack by stripe rust, while the leaves of the line '179' did not show any symptoms. Disease resistance tests under greenhouse conditions by artificial inoculations with *Puccinia triticina* isolates proved that this line was also resistant to leaf rust. Furthermore, this line has elevated arabinoxylan content. Molecular marker analyses showed that the 1RS arm present in the line '179' was different from that of Petkus rye.

Acknowledgement

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P 146 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Resistance to the Ug99 stem rust race group among landrace wheat accessions from the USDA-ARS National Small Grains Collection

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Key message: Resistance to the Ug99 stem rust race group based on *Sr15*, *Sr9h*, and *Sr28* was found among wheat landraces. Apparently novel adult plant resistance was found on chromosome arm 6AS.

Landrace accessions of common wheat (*Triticum aestivum* L. subsp. *aestivum*) present in the world's germplasm collections represent a potential source of novel traits for plant breeding, especially new sources of disease resistance. We have sought resistance to the Ug99 lineage of the stem rust pathogen (race TTKSK and its variants) among landrace accessions from the USDA-ARS National Small Grains Collection (NSGC). Resistance to Ug99 was assessed in Kenya and Ethiopia field tests and in seedling tests under quarantine conditions in the US. The presences of molecular markers associated with breeding activity, including markers for *Sr* genes introgressed from wild relatives of wheat, were used to eliminate non-landrace accessions. A bulk segregant analysis approach was used on bi-parental populations developed from crosses with selected accessions that showed field resistance in Kenya. *Sr15* was identified in spring habit accession PI 374670 from Bosnia and Herzegovina and, using a KASP assay for a SNP closely linked to *Sr15*, the gene was identified among other accessions originating from the Balkans. The Ug99 resistance in the spring habit accession Ctr 4311, and many other accessions originating from Iran, was due to *Sr9h* on chromosome arm 2BL. Using several mapping populations, KASP assays for SNP markers closely linked to *Sr9h* were developed that will be useful for pyramiding this resistance gene with other *Sr* genes effective against the Ug99 lineage. Based on mapping results, race specificity, and infection types observed for spring habit accession PI 177906 from Turkey, the TTKSK resistance was postulated to include *Sr28* also on chromosome arm 2BL. One KASP assay for the tightly-linked SNP marker *IWB1208* was predictive for the presence of *Sr28* in a diverse set of wheat genotypes. Potentially novel adult plant resistance was identified in accession Ctr 15026 from Afghanistan on chromosome arm 6AS (Babiker et al. 2017). Initial analysis has shown possible novel seedling resistance to the Ug99 race group located on chromosome arms 6DS in a Turkish accession and 7AS in an Iranian accession. Future work will characterize the Ug99 resistance identified in spring landraces and seek new resistance genes among NSGC winter habit landraces.

Reference

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P 148 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Adult plant stem rust resistance in a durum wheat 'Glossy Huguenot': mapping and marker development

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Key message: Mapping and marker development for adult plant stem rust resistance in durum wheat, an alternative to *Sr2*.

The stem rust resistance gene *Sr2* was introgressed from tetraploid emmer wheat (*Triticum turgidum* ssp *dicoccon*) into common wheat cultivar 'Marquis' (*Triticum aestivum* L.) in early 1900s and is the most widely used adult plant non-race specific gene across the globe. The *Sr2* response is characterised by partial resistance which restricts the number and size of uredinia and also shows resistance against leaf rust and powdery mildew. *Sr2* is linked to pseudo black chaff, which is considered to be undesirable in some jurisdictions. With the aim to identify new sources of adult plant stem rust resistance, we report here mapping of stem rust resistance in another durum wheat 'Glossy Huguenot'. Similar to *Sr2*, the stem rust resistance in Glossy Huguenot has been effective for over a century. However, unlike *Sr2*, rust resistance in Glossy Huguenot is not linked with pseudo black chaff. A recombinant inbred family was derived from crossing the resistant Glossy Huguenot and a susceptible selection from a cross between Glossy Huguenot and the susceptible durum wheat Bansi. Phenotyping was done on 192 F₇ and F₈ recombinant inbred lines (RILs) and mapping was done using the 90k Infinium iSelect platform. Two quantitative trait loci (QTL) were identified each contributing 25% of the phenotypic variance. Linked SNP markers have been identified and will be useful in introgressing this resistance in wider germplasm.



P 150 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Molecular characterization of stem rust Ug99 resistance gene *SrA2K* and its allelism with *Sr42*

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Key message: *SrA2K* gene probably allelic to *Sr42*, explaining up to 72% of phenotypic variation, for stem rust resistance race Ug99 was detected on chromosome 6 DS.

Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, has historically, caused significant losses to wheat production. Detection and spread in East Africa of new virulent race TTKSK known as Ug99 has made the situation even worse as about 90 percent of wheat cultivars currently grown were susceptible to this race. Field evaluations in Kenya and greenhouse evaluations at the USDA-ARS Cereal Disease Laboratory have identified at least 6 Georgia lines (GA98401-5E45, GA991209-6E33, GA991371-6E12, GA001435-6E23, GA011636-6E22 and AGS 2020) that were rated resistant or moderately resistant to Ug99. AGS 2000, the common parent for all the 6 lines, was assumed to provide the source of resistance. To characterize the resistance gene, a RIL mapping population developed from 26R61 (susceptible to Ug99) and AGS 2000, was inoculated in the field at Njoro, Kenya with Ug99 during two growing seasons. One major QTL was consistently detected on chromosome 6 DS, at the very similar location of *Sr42* from Norin 40, explaining up to 72% of phenotypic variation. The gene, temporarily named *SrA2K*, was flanked by two DArT markers, *wPt-1519* and *wPt-730835*, and about 3.0 cM from *Xbarc183*, the common marker used to map *Sr42*, *SrCad*, *SrTmp* and the other stem rust resistance genes identified in the similar location. Allelism test was conducted between *SrA2K* and *Sr42* using a 202 F₂ plants from the cross of Norin 40 and AGS 2000, and 210 F_{2:3} families including about 4264 plants. All the F₂ plants and progenies of F_{2:3} families were resistant to race TTKSK (Ug99) at seedling indicating the genes *SrA2K* and *Sr42* are probably allelic. AGS 2000, a check cultivar in Uniform Southern Soft Red Winter Wheat Nursery, combined stem rust resistance with high yield potential, is valuable source for breeding resistant cultivars to mitigate the Ug99 threat.



P 152 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Seedling and adult-plant resistance to stem rust among the 2014 elite wheat genotypes of the north warm and humid zone in Iran

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Key message: The reactions of 20 elite wheat lines from Iranian wheat breeding program in the north warm and humid zone were determined to stem rust at seedling stage and adult-plant stages.

Stem rust, caused by *Puccinia graminis* f.sp. *tritici*, has historically been important for wheat production in Iran. In recent years, due to high frequency observations of the disease and the emergence of Ug99 race in Iran, stem rust has drawn much attention. Having an acceptable level of resistance to diseases including stem rust, is a crucial factor for cultivar release in northern parts of Iran which is conducive for disease development. In this study, the reactions of 20 lines of Elite Regional Wheat Yield Trials from the north warm and humid climate in Iran in 2014 (ERWYT-N-93) were determined to stem rust at seedling and adult-plant stages. To carry out the seedling evaluations, wheat plants were inoculated separately with four pathotypes of the pathogen including PTRTF, TKTTF, TTTTF, and TTKSK in the greenhouse, and two weeks after, they were scored using a 0-4 scale. Adult-plant experiments were conducted under natural infection at Kelardasht, Mazandaran, Iran, for two years of 2015 and 2016. Based on the results of seedling tests, 1, 13, 1, and 2 lines displayed the infection types 3 (moderately susceptible) or 4 (susceptible) to the races PTRTF, TKTTF, TTTTF, and TTKSK, respectively (Table 1). The rest of the genotypes were determined to be resistant or similar. The results of adult-plant experiments showed that the vast majority of the genotypes were resistant or moderately resistant in both years (Table 1). The only genotypes with a higher disease levels were the line 12 in both years and the lines 10, 11, and 13 in 2016 (Table 1). It seems that the possible seedling resistance genes present in the lines 11 or 12 has not given a considerable resistance level to these lines at adult-plant stage in one or both years. Considering the results of pathotype analysis and the results of seedling and adult-plant reactions, we conclude that the genes *Sr11*, *Sr24*, *Sr31*, *Sr36*, and *SrTmp* are not present or not effective in the genotype number 1. The genes *Sr11*, *Sr21*, *Sr24*, *Sr30*, and *Sr31* were not present or not effective in the line 17 as well. While none of the 20 genes used for pathotype analysis were present or were effective in the lines 4, 8, 11, 12, 15, and 18, effectiveness/ineffectiveness of any gene in the line 20 remained unclear. For the rests of the lines, three genes of *Sr11*, *Sr24*, and *Sr31* were not present or not effective.



Table 1: Seedling and adult-plant reaction to stem rust among the 2014 elite wheat genotypes of the north warm and humid zone in Iran

Plot No. 2014-2015	Plot No. 2013-2014	Pedigree	Origin	Seedling reactions to different pathotypes				Adult-plant reactions	
				PTRTF (Kelardasht-2015)	TKTFF (Kelardasht-2016)	TTTTF (Boroujerd-2015)	TKSK (Dezfoul-2009)	2015	2016
ERWYT-N-93-1	-	Morvand	-	1+	3	2	3+	0	10MR
ERWYT-N-93-2	-	Gonbad	-	1	3	2	1	0	10MR
ERWYT-N-93-3	5	(ARWYT) UP23.38*2JKTS*2//YANAC	8EBWYT ¹	2+	3+	2+	1	0	10MR
ERWYT-N-93-4	6	(ARWYT) UP23.38*2JKTS*2//YANAC	9EBWYT ²	1	2+	2C	1	0	10MR
ERWYT-N-93-5	7	(ARWYT) ATILA*2/PBW65*2//BOW/NKT//CBRD/3/CBRD	10EBWYT ³	2+	3	2	2	0	10MR
ERWYT-N-93-6	11	(ARWYT) KAUZ//AL/TAB84/AOS/3/MILAN/KAUZ//HUITES//CAU/NH/H567.71/3/SER/4/CAL/NH/H567.71/5/2*KAUZ6/PASTOR	32ESWYT ⁴	1	3+	2	1+	0	10MR
ERWYT-N-93-7	13	(ARWYT) FRET2*2//SNI/TRAPH/1/3/KAUZ*2//TRAP//KAUZ/5/PARUS/6/FRET2*2//KUKUNA	32ESWYT	2-	3	1+	2+	0	10MR
ERWYT-N-93-8	17	(ARWYT) ATILA*2/PBW65/6/PVN//CAH42/2/ANA/5/BOW/CROW//BUC/PVN/3/YR4/TRAPH/1//JATILA/2*PASTOR	32ESWYT	2-	3-	2+	1	0	20MR
ERWYT-N-93-9	18	(ARWYT) CHAPIO/3/BOR/05/2*EXCAUBUR/EXCAUBUR	19HRWYT ⁵	2-	3+	2+	1+	0	10MR
ERWYT-N-93-10	19	(ARWYT) ATILA*2/PBW65//TNMU	19HRWYT	3-	3	3+	2+	20MR	50MS
ERWYT-N-93-11	20	(ARWYT) ATILA*2/PBW65//TNMU	19HRWYT	1	1+	1	2-	TR	30MS
ERWYT-N-93-12	23	(ARWYT) ATILA*2/PBW65//TNMU	19HRWYT	2-	2+	2	2-	40MS	50MS
ERWYT-N-93-13	-	MUNAL#1	CIMMYT	2-	3+	2C	2+	0	50MS
ERWYT-N-93-14	27	(ARWYT) WBILLI/KUKUNA//TACUPETO F2001/3/UP2338*2//MVT5I	19HRWYT	2	3	2C	2+	0	10MR
ERWYT-N-93-15	34	(ARWYT) KACHU/SAUL	44IBWSN ⁶	0	2-	2-	2+	30R	10MR
ERWYT-N-93-16	35	(ARWYT) SAUAL/3/MILAN/5872.36//BAV92	44IBWSN	1	4	2+	1+	0	10MR
ERWYT-N-93-17	36	(ARWYT) SAUAL/3/MILAN/5872.36//BAV92	44IBWSN	3+	3	2	2+	0	10MR
ERWYT-N-93-18	37	(ARWYT) SAUAL/3/MILAN/5872.36//BAV92	44IBWSN	1	1	2	2	0	20MR
ERWYT-N-93-19	38	(ARWYT) FRET2/KUKUNA//FRET2/3/PASTOR//HXL7573/2*BAU/5/FRET2*2//SNI/TRAPH/1/3/KAUZ*2//TRAP//KAUZ	44IBWSN	1	3	2	1+	0	10MR
ERWYT-N-93-20	39	(ARWYT) FRET2/KUKUNA//FRET2/3/PASTOR//HXL7573/2*BAU/5/FRET2*2//SNI/TRAPH/1/3/KAUZ*2//TRAP//KAUZ	44IBWSN	;	3-	2-	2+, 3	0	10MR
Susceptible check	-	Morocco	-	3+	3+	3+	4	80S	90S

1. 8th Elite Bread Wheat Yield Trial received from International Maize and Wheat Improvement Center (CIMMYT), Mexico.
 2. 9th Elite Bread Wheat Yield Trial received from International Maize and Wheat Improvement Center (CIMMYT), Mexico.
 3. 10th Elite Bread Wheat Yield Trial received from International Maize and Wheat Improvement Center (CIMMYT), Mexico.
 4. 32th Elite Spring Wheat Yield Trial received from International Maize and Wheat Improvement Center (CIMMYT), Mexico.
 5. 19th High Rainfall Wheat Yield Trials received from International Maize and Wheat Improvement Center (CIMMYT), Mexico.
 6. 44th International Bread Wheat Screening Nursery received from International Maize and Wheat Improvement Center (CIMMYT), Mexico.



P 154 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Inducing stem rust resistance gene *Sr59* into adapted wheat cultivars

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Key message: Incorporation of stem rust resistance gene *Sr59* from *Secale cereale* into locally adapted wheat cultivars that will lead to greater sustainability of wheat production and improved food security.

Stem rust of wheat, caused by the fungus *Puccinia graminis* f. sp. *tritici*, is one of the most important threats to the wheat production worldwide. Novel widely virulent group of *P. graminis* f. sp. *tritici* races such as those in the Ug99 lineage and TKTF have emerged and represent a serious threat to food security worldwide due to the susceptibility of extensively grown wheat cultivars. We have identified three Swedish 2R (2D) wheat-rye disomic substitution lines (SLU210, SLU239 and SLU239) that possess resistance effective to these emerging races of the stem rust pathogen (Rahmatov et al. 2016a). A new stem rust resistance gene *Sr59* from 'SLU238' was introgressed into wheat as a 2DS·2RL Robertsonian translocation (line TA5094) that provides an additional asset for wheat improvement. Kompetitive Allele Specific PCR (KASP) markers were developed linked to the *Sr59* resistance gene that facilitates marker-assisted selection and gene pyramiding. *Sr59* conferred a high level of resistance to races TTKSK, TTTSK, TTTTF, TPMKC, RKQQC and RCRSC (Rahmatov et al. 2016b). The transfer of this gene into adapted wheat cultivars may contribute to long-lasting stem rust resistance. Research is currently in progress to transfer *Sr59* into adapted wheat cultivars to assess whether the 2RL introgression is associated with any deleterious effects. In this research, stem rust seedling assays, molecular marker and cytogenetic analyses at multiple generations will be used to validate the presence of *Sr59* from line TA5094. Field tests will be established to assess adult plant resistance in different geographical locations where virulent stem rust races are present. The lines with highest levels of stem rust resistance will be tested for other important traits such as yield, quality, resistance to other diseases (i.e. stripe rust, leaf rust, powdery mildew, tan spot etc.), and tolerance to abiotic stresses such as drought. The expected outcome of this research will be novel genetic resources to improve stem rust resistance in wheat, thereby enhancing sustainable wheat productivity and food security.

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Molecular organization of wheat stem rust resistance locus *Sr26* introgressed from *Thinopyrum ponticum*

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Key message: Molecular characterisation of wheat stem rust resistance locus *Sr26* using comparative genomics and RenSeq (Resistance gene enrichment and sequencing) approaches.

Multiple rust resistance gene combinations are considered as a practical solution for providing durable rust resistance and preventing resistance breakdown arising from single gene deployment. The stem rust resistance locus *Sr26*, originally derived from *Thinopyrum ponticum* and introgressed into wheat as a chromosome translocation, is one of the very few genes conferring resistance for almost 40 years to all known races of stem rust including the highly virulent stem rust race Ug99 (TTKSK) and its derivatives (Dundas et al. 2015). To understand the underlying mechanisms of its unusual long-term effectiveness and to explore allelic diversity in different *Th. ponticum* accessions for other functional alleles that may offer new sources of resistance, we used comparative genomics and gene capture techniques (RenSeq) as complementary strategies for isolating the target gene (Steuernagel et al. 2016). We generated mutagenized population for *Sr26* using EMS (Ethyl methanesulfonate) and grouped the population into deletions and putative point mutants. *Sr26* region was first mapped using NB-LRR (nucleotide-binding site and leucine-rich repeat) sequences from the orthologous gene members located on the long arm of chromosome 6 from *Aegilops tauschii* reference genome (the D-genome donor of wheat). Subsequently, we revealed a cluster of NB-LRR sequences located at the distal end of the *Th. ponticum* introgression segment that was deleted in the smallest interstitial *Sr26* deletion mutant. Based on these findings we substantially narrowed down the genetic interval for *Sr26* and made progress towards identifying potential *Sr26* gene candidates. Along with comparative genomics, we subjected the mutant population to RenSeq pipeline as well. Candidates from both pipelines are currently being screened. In order to broaden the range of gene combinations that enhance durable resistance, genetic stocks of *Sr26* from different backgrounds as well as a panel of *Sr26-APR* gene combinations have been generated to further investigate the resistance response of the *Sr26* gene in combination with different multi-pathogen *APR* (adult plant resistance) genes.

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Use of virulence mutants in identifying wheat stem rust *Avr* genes

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Key message: Comparative genomics of wild-type avirulent stem rust strains and their virulent mutant derivatives enable short listing of *Avr* genes from among effector candidates.

The wheat stem rust fungus *Puccinia graminis* f. sp. *tritici* (Pgt) is one of the most destructive pathogens of wheat. Secreted proteins termed effectors play an important role in fungal pathogen growth by modulating the host cellular environment and suppressing the plant defense response. Resistance or susceptibility of host lines is often governed by recognition or otherwise of fungal effectors (avirulence/virulence proteins) by plant resistance proteins (R proteins). Mutations in *Avr* genes may result in inability of R proteins to recognize the *Avr* gene product leading to loss of resistance (gain of virulence). We have taken a genomics approach to identify candidate *Avr* genes in Pgt. We built a pan genome based on four Australian Pgt strains, including 21-0 (Upadhyaya et al. 2015), and identified 592 haustorially-expressed secreted proteins (HSPs). By genome sequence comparison of wildtype (avirulent) and virulent mutant derivatives we could further short list candidate *Avrs*. Comparison of 21-0 with two presumed clonal field derivatives (collected in 1982 and 1984) that had evolved virulence on four additional resistance genes (*Sr5*, *Sr11*, *Sr27*, *SrSatu*) identified non-synonymous mutations in 13 HSP effector candidates. In addition, five genes showed expression loss of one of the two alleles in the mutants. We have also isolated new spontaneous mutants with virulence for *Sr50*, *Sr5* or *Sr27*. Sequence comparison identified large deletions in each of these mutants that could include the respective *Avr* loci and which contain 25, 12 and 1 HSP gene candidates respectively. These candidate effectors are being assessed for recognition in wheat accessions with the corresponding R genes using a bacterial type three secretion delivery system based on an engineered *Pseudomonas fluorescence* strain (Upadhyaya et al. 2014).

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Mapping and characterization of the adult plant wheat leaf rust resistance gene *Lr77*

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Key message: The hard red winter wheat Santa Fe carries a highly effective adult plant leaf rust resistance gene on chromosome 3BL that has been designated as *Lr77*.

The hard red winter wheat Santa Fe grown in the southern Great Plains of the USA has had effective resistance to leaf rust caused by *Puccinia triticina* since its release in 2003. The objectives of this study were to identify the leaf rust resistance genes in Santa Fe. Santa Fe was crossed with the leaf rust susceptible spring wheat Thatcher (Tc), and F₁ plants were vernalized and crossed with Thatcher. Backcross (BC) F₁ plants were selfed and 90 BC₁F₂ families were developed. The BC₁F₂ families were tested for segregation of leaf rust resistance in seedlings with different leaf rust races. Segregation data indicated the likely presence of *Lr3a* and a gene that had the same race specificity as *Lr17a*. Santa Fe was also determined to carry the VPM1 translocation from *Aegilops ventricosa* based on the 2NS specific markers VENTRIUP-LN2. Plants from BC₁F₂ families that were susceptible in seedlings and lacked the 2NS translocation were tested as adults for leaf rust resistance in greenhouse tests. BC₁F₂ adult plants with resistant infection types of 22⁺ were selfed and BC₁F₃ and BC₁F₄ adult plants were tested for homozygosity of resistance. A resistant BC₁F₄ plant was crossed with Tc to develop a Tc*3/Santa Fe recombinant inbred line (RIL) population. The RIL population and parents were genotyped with the Illumina 90K Infinium iSelect array. The RIL population and parents were evaluated for leaf rust resistance in inoculated field plots in 2014, 2015, and 2016. The resistant Tc*3/Santa Fe parent and the resistant RILs had leaf rust severity and response of 10-30MRMS in the three years of field tests. A major quantitative trait locus (QTL) for resistance was mapped to chromosome 3BL, with logarithm of odds (LOD) scores of 23.05, 37.05, and 6.85 in 2014, 2015, and 2016 respectively. The SNP marker IWB 10344 that mapped at 17.31 cM in the 3BL linkage group was at the top of the LOD peak in all three years, flanked by IWB 73555 at 16.9 cM and IWB 6988 at 21.35 cM. Competitive allele specific (KASP) markers were developed for IWB 9059 (12.08 cM), IWB 73555 (16.9 cM), IWB 12260 (25.39 cM) and IWB 32805 (26.74 cM). The adult plant leaf rust resistance gene on 3BL was designated as *Lr77*. In a similar parallel study the hard red winter wheat Duster was also determined to carry *Lr77*.



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Postulation and mapping of leaf, stem and stripe rust resistance in Option/Potent population

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Key message: Across Europe it is important to find new genotypes with effective resistance genes for new winter varieties.

The wheat rust diseases, stem rust, leaf rust and yellow rust, caused by *Puccinia graminis*, *P. triticina* and *P. striiformis* respectively, are destructive diseases worldwide and sometimes can cause severe losses by reducing grain yield and quality. Wheat breeders often place high priority on ensuring improved resistance to new and old races of these three rust diseases in new varieties. Breeding for durable resistance to rust is important, but also is a challenge due to the complexity of interactions among resistance genes and to the wide diversity and continuous evolution of the pathogen races. In our research we studied rust resistance in mapping population Option/Potent because it is known from previous studies that Potent is a good source of adult-plant resistance to leaf rust. The seedling resistance to leaf rust of 96 lines together with parents was tested in the greenhouse by tests with a range of *P. triticina* pathotypes that included a newly detected one (104-1, 3, 4, 6, 7, 8, 9, 10, 11, 12+Lr37) that combines virulence for three genes (the complementary genes *Lr27+Lr31*, *Lr15* and *Lr28*). Infection types on the seedlings were scored 14 days after inoculation on a 0 to 4 scale. The data obtained from the seedling tests confirmed that the resistance was due to a combination of several genes. We detected that the population carries the completely linked genes *Lr26-Yr9-Sr31*, associated with 1BL/1RS translocation in bread wheat lines and *Lr37-Sr38-Yr17*, which is present in many European wheat varieties. We also concluded that Option and Potent share either *Lr13* or *Lr42*. Quantitative trait loci associated with adult plant resistance to leaf rust, stripe rust and stem rust severity will be mapped using DArT markers, which will give us clear confirmation of potentially novel resistance sources to the three rust diseases. In 2013, an epidemic of yellow rust occurred in Europe. Therefore, across Europe it is important to find new genotypes with effective resistance genes for new winter varieties.

Acknowledgements

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Additional leaf rust resistance genes found in the Thatcher near-isogenic wheat lines for *Lr1* and *Lr20*

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Key message: Additional leaf rust resistance genes were found in the Thatcher near-isogenic wheat lines for *Lr1* and *Lr20*.

The late Dr. Peter Dyck, working at the Cereal Research Centre in Winnipeg, Canada, developed a series of near-isogenic wheat lines in the Thatcher background to study the effects of these genes in isolation and in a susceptible genetic background. These lines have been very useful for studies on the *Puccinia triticina* Eriks.-wheat pathosystem world-wide, and are commonly used as differential sets for virulence surveys. Some isolates tested in virulence surveys in Canada from 2001 onward revealed that both the Tc-*Lr1* line RL6003 (Thatcher*6/Centenario) and the Tc-*Lr20* line RL6092 (Thatcher*6/Timmo) were not homogeneous and could contain additional resistance genes. These lines were analysed genetically by crossing with Thatcher and testing progeny lines with various virulence phenotypes. A second gene in the Tc-*Lr1* line segregated independently of *Lr1* and was temporarily named *LrCen*. Two sub-lines were then established, one with only *Lr1* (RL6003a) and one with only *LrCen* (RL6003b). A doubled haploid population of Tc-*LrCen*(RL6003b) crossed by Thatcher was used to map this gene to the long arm of chromosome 7A, and identify closely linked markers. Analysis of the cross of the Tc-*Lr20* line with Thatcher is ongoing, but it appears to be segregating for two resistance genes in addition to *Lr20*. One of these additional genes has a similar phenotype to *LrCen*, but this gene segregated independently of the markers associated with *LrCen*.



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Bulk segregant analysis (BSA) detects two major loci for leaf rust resistance in *Triticum turgidum* ssp. *durum*

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Key message: Two major QTL for leaf rust resistance in durum wheat have been detected on chromosomes 4B and 2B using a bulked segregant analysis (BSA).

Leaf rust (*Puccinia triticina* Eriks.) is a major foliar disease affecting durum wheat production and causing up to 60% yield losses. Breeding of new leaf rust resistant durum varieties is the most important strategy to control this disease. Although several leaf-rust resistance (*Lr*) genes have been identified in hexaploid wheat germplasm, only *Lr3*, *Lr14a*, *Lr23* and *Lr33*, *Lr61*, *Lr72* were directly detected in the cultivated tetraploid germplasm and are therefore exploitable by breeders. Moreover, some of these *Lr* loci are either partially or completely defeated by recently spread leaf rust races (Loladze et al. 2014). Based on our phenotypic and molecular observations, two recently released elite varieties, Monastir and Saragolla, carry valuable leaf rust resistance genes other than *Lr14a*. Our objective was to map these novel resistance genes with high-throughput SNP marker system and bulk segregant analysis (BSA). F_2 and $F_{2:3}$ lines were produced by crossing Monastir (resistant) × Kofa (susceptible) and Saragolla (resistant) × Mohawk (susceptible). To perform BSA we selected from each population 8 groups of 12 lines each (4 resistant bulks and 4 susceptible bulks) made of independent lines that displayed contrasting phenotypes for leaf rust resistance. DNA samples were then obtained by pooling the DNA of all individuals from each group. The Illumina 15K SNP array and the recently developed wheat consensus map (Maccaferri et al. 2014) were deployed for the BSA. One major QTL was detected in Monastir × Kofa F_3 population on chromosome 4B while in Saragolla × Mohawk a major QTL was detected on chromosome 2B. Several additional minor BSA signals were detected that need further confirmation in single-line screening. The objective is now to develop reliable and diagnostic KASP® markers to allow for marker-assisted selection for this loci and to characterize the newly identified loci.

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Leaf rust resistance on chromosome 7B of common wheat

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Key message: At least two loci identified on chromosome 7B controlling partial resistance to leaf rust are available for use in genetic enhancement of wheat

Puccinia triticina, which causes leaf rust of wheat, is a variable pathogen that regularly overcomes monogenic major-gene resistance resulting in significant yield losses. Stacking minor resistance genes to supplement major gene resistance may offer a more sustainable solution to controlling this disease. The objective of this study was to evaluate multiple biparental populations for quantitative forms of resistance. Doubled haploid populations Carberry/AC Cadillac, Stettler/Red Fife and Vesper/Lillian were grown and assessed for adult plant leaf rust resistance in nurseries near Swift Current, Saskatchewan, in 2011 to 2015, Morden, Manitoba, in 2015, and Lincoln, New Zealand, in 2014. The lines of the populations were genotyped using the 90K Infinium iSelect assay and quantitative trait locus (QTL) analysis was performed. Two QTL contributing to resistance were identified near the telomeric regions of the long and short arms of chromosome 7B with resistance derived from Vesper, AC Cadillac and Red Fife. The QTL, which expressed in multiple environments, should contribute to resistance when stacked with other leaf rust resistance genes during the breeding of commercial cultivars.



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Wheat leaf rust virulence in the Czech Republic

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Key words: Leaf rust, *Lr* genes, resistance, wheat, Czech Republic

In the Czech Republic leaf rust (*Puccinia triticina* Eriks.) on wheat occurs regularly every year and can cause considerable yield losses. Breeding for rust resistance is the most economic way of the control. Knowledge of virulence in the rust population contributes to successful resistance breeding. In the Czech Republic virulence in the rust population has been studied since the sixties of the last century. At present determination of virulence is based on reactions of leaf rust isolates on a set of Thatcher near-isogenic lines possessing genes *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr9*, *Lr10*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr19*, *Lr21*, *Lr23*, *Lr24*, *Lr26* and *Lr28*. In the years 2012 to 2015 no virulence was recorded to *Lr9* and only 0.5% (1 isolate) to *Lr19*. Low virulence was registered to *Lr2a* (11%), *Lr2b* (13%), *Lr2c* (14%), *Lr24* (15%) and *Lr28* (21%). Virulence to *Lr3a* was 74%, to *Lr26* it was 79%, to *Lr1* it was 86% and to the remaining *Lr* NILs it was 90-100%. In the years 2005 to 2015 virulence frequency to *Lr24* increased. It was 1% in the period 2005-2008, 7% in the period 2009 to 2011 and 15% in the period 2012 to 2015. According to molecular marker analysis the following cultivars registered in the Czech Republic possess *Lr24*: Carroll (+*Lr10*), Athlon, Gordian (+*Lr28*). Virulence frequency to *Lr28* varied. It reached 39% and 32% in the years 2013 and 2015, respectively. In 2012 it was only 6%. The following cultivars possess *Lr28*: Tobak, Passport (+*Lr37*), Gordian (+*Lr24*), Frisky (+*Lr10*, *Lr37*). Cultivar Tobak possessing *Lr28* has the largest increase area in 2015 in the Czech Republic. Whereas in the years 2011-2014 cv. Tobak was resistant in field trials in 2015 it was highly resistant susceptible. Genes *Lr9* and *Lr19* remain the most effective leaf rust resistance genes. However, only one of the registered cultivars, i.e. Citrus, has the *Lr19* gene. In the neighbouring Slovakia *Lr19* was recorded in registered cultivars Brejk, Bona Dea and Bona Vita.

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Phenotypic screening and QTL mapping for adult plant resistance to leaf rust in a Popo/Kariega recombinant inbred line population

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Key words: Adult plant resistance, leaf rust, QTL mapping

Leaf rust, caused by *Puccinia triticina* (Pt) is a highly diverse and widespread disease affecting wheat. Considerable progress has been made to control the disease through resistance breeding. However, frequent evolution of new and aggressively virulent pathotypes still presents a major challenge to the achievement of durable resistance. A continuous search for new sources of effective leaf rust resistance genes is necessary to develop improved wheat varieties with stable and durable resistance. The aim of this study was to screen for adult-plant resistance (APR) in a recombinant inbred line (RIL) population of a cross between 'Popo' and 'Kariega', and to identify leaf rust resistant lines that could be used for breeding. A panel of 179 RILs, two parental varieties and three checks 'Gariep', 'SST88' and 'Morocco' were evaluated in the field, across four diverse South African environments, for resistance to leaf rust. Disease response ranged from highly resistant to highly susceptible reactions with severity scores ranging from 0% to 100%. Analysis of variance indicated highly significant ($p < 0.001$) differences among the tested RILs across environments. The broad sense heritability estimate was 0.53. Twenty-six RILs had average severity scores that were better than the parental varieties, and showed higher levels of resistance to leaf rust. Quantitative trait loci (QTL) analysis was performed using inclusive composite interval mapping (ICIM) method with QTL IciMapping 4.0 software based on stepwise-regression linear model. A consistent genomic region designated as *QLr.sgi-5A.1* contributed by Kariega was identified on chromosome 5A controlling leaf rust at the Tygerhoek testing site during 2014 and 2015. Another QTL, *QLr/Sr.sgi-7D.1.3*, also derived from Kariega was detected under two testing sites (Cedara 2015, Tygerhoek 2015), controlling both leaf rust and stem rust and explaining 4.8% and 3.2% of the phenotypic variation, respectively. Due to the moderate heritability estimate for leaf rust, the use of the newly developed RILs in the genetic background of Popo/Kariega could enhance pre-breeding for resistance against the leaf rust. The identified QTL can possibly be explored in a marker-assisted breeding for wheat.



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Mapping genes for and developing wheat germplasm with resistance to stripe rust

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Key message: We have mapped a large number of genes for stripe rust resistance in wheat cultivars/landraces and developed a large number of germplasm lines with resistance to stripe rust

Stripe rust (*Puccinia striiformis* f. sp. *tritici*) is one of the most important diseases of wheat. Growing resistant cultivars is the most easy to use, effective, economic and environmentally friendly approach to control the disease. To identify genes for resistance to stripe rust in wheat cultivars and landraces, we made crosses with hundreds of wheat germplasm lines based on the results of our germplasm screening. Recombinant inbred line populations were phenotyped at adult-plant stage in fields under natural infection and/or at seedling stage in the greenhouse with selected races of the pathogen depending upon the type of resistance in the resistant parent and genotyped with various markers. As listed in Table 1, a total of 21 genes or quantitative trait loci (QTL) conferring all-stage resistance in 14 cultivars or landraces; and a total of 25 genes or QTL conferring high-temperature adult-plant (HTAP) resistance in 11 cultivars or landraces were mapped. Of the 46 genes or QTL, 33 have been published (e.g. Chen 2013, Cheng et al. 2014), and 13 in landraces PI 182103, PI 182126, PI 184597, PI 195097 and W18 have not been published. New germplasm lines were developed from progenies for improved agronomic traits (Wang et al. 2012). For stripe rust resistance genes that were mapped on same chromosomes, crosses were made and progeny lines with two linked genes were selected by phenotyping and marker-assisted selection. So far, we have developed spring wheat lines with *Yr64+Yr65* (14.2 cM) on chromosome 1BS; *Yr5+Yr53* (35.6 cM) on 2BL; *Yr50+Yr62* (27.1 cM) on 4BL; and *Yr39+Yr52* (31.2 cM), *Yr39+Yr59* (41.1 cM), *Yr39+QYr.PI182103.wgp-7BL* (9.6 cM), *Yr52+Yr59* (5.4 cM), *Yr52+YrZh84* (12.2 cM), *Yr52+QYr.PI182103.wgp-7BL* (6.1cM), *Yr59+YrZh84* (6.0 cM), *Yr59+QYr.PI182103.wgp-7BL* (5.8 cM), *YrZh84+QYr.PI182103.wgp-7BL* (6.4 cM) and *Yr67+QYr.PI182103.wgp-7BL* (7.5 cM) on chromosome 7BL and winter wheat lines with *Yr50+Yr62* and *YrZh84+QYr.PI182103.wgp-7BL* on 7BL. The genes, molecular markers and new germplasm lines should be useful in developing wheat cultivars with durable resistance to stripe rust.

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Table 1: Genes or quantitative trait loci (QTL) for resistance to stripe rust mapped in wheat cultivars and landraces.

Yr gene/QTL	Wheat source	Chromosome	Flanking markers
All-stage resistance			
<i>Yr43</i>	IDO377s	2BL	<i>M2, M6</i>
<i>Yr44</i>	Zak	2BL	<i>M2, M6</i>
<i>Yr45</i>	PI 181434	3DL	<i>RLRR-Rev/Ptokin1IN, NLRR-INV2/Ptokin4</i>
<i>Yr53</i>	PI 183627	7BL	<i>X6268, Xbarc182</i>
<i>Yr64</i>	PI 331260	1BS	<i>Xgwm413, Xbarc119</i>
<i>Yr65</i>	PI 480016	1BS	<i>Xgwm18, Xgwm273</i>
<i>YrSP</i>	AvSYrSPNIL	2BL	<i>Xgwm18, Xgwm273</i>
<i>Yr76</i>	Tyee	3AS	<i>Xwmc11, Xwmc532</i>
<i>YrAlp</i>	Alpowa	1BS	<i>Xwgp47, Xwgp48</i>
<i>YrExp1</i>	Express	1BL	<i>Xwgp78, Xwmc631</i>
<i>YrExp2</i>	Express	5BL	<i>Xwgp81, Xwgp82</i>
<i>YrPI195097</i>	PI 195097	3DL	<i>Xgwm341, Xgwm497</i>
<i>YrW18-1BL</i>	W18	1BL	<i>Xgwm498, Xwmc419</i>
<i>YrW18-1BS</i>	W18	1BS	<i>Xgwm153, Xgwm934</i>
<i>YrW18-5DL</i>	W18	5DL	<i>Xwmc357</i>
<i>QYrdr.wgp-5BL.1</i>	Druchamp	5BL	<i>IWA6271, IWA2093</i>
<i>QYrdr.wgp-5DL</i>	Druchamp	5DL	<i>IWA8331, IWA8404</i>
<i>QYrdr.wgp-6BL.1</i>	Druchamp	6BL	<i>IWA3297, IWA3298</i>
<i>Qyr.PI182103.wgp-2AS</i>	PI 182103	2AS	<i>Xgwm312, S10</i>
<i>Qyr.PI182103.wgp-3AL</i>	PI 182103	3AL	<i>Xgwm2, S52</i>
<i>Qyr.PI182103.wgp-5BS</i>	PI 182103	5BS	<i>Xgwm540, Xgwm213</i>
HTAP resistance^a			
<i>Yr39</i>	Alpowa	7BL	<i>Xwgp43, Xwgp45</i>
<i>Yr52</i>	PI 183527	7BL	<i>X5258, Xbarc182</i>
<i>Yr59</i>	PI 178759	7BL	<i>Xwgp5271, Xbarc32</i>
<i>Yr62</i>	PI 192252	4BL	<i>Xgwm251, Xcfd39</i>
<i>Qyrex.wgp-6AS</i>	Express	6AS	<i>Xwgp56, Xgwm334</i>
<i>Qyrex.wgp-3BL</i>	Express	3BL	<i>Xwgp66, Xgwm299</i>
<i>Qyrex.wgp-1BL</i>	Express	1BL	<i>Xwgp78, Xgwm631</i>
<i>Qyrlo.wgp-2BS</i>	Louise	2BS	<i>Xgwm132, Xgdm113</i>
<i>Qyrst.wgp-6BS.1</i>	Stephens	6BS	<i>Xgwm508, Xgwm132</i>
<i>Qyrst.wgp-6BS.2</i>	Stephens	6BS	<i>Xbarc1169, Xbarc136</i>
<i>Qyr.PI182103.wgp-4DL</i>	PI 182103	4DL	<i>Xbarc288, S26</i>
<i>QYr.PI182103.wgp-7BL</i>	PI 182103	7BL	<i>Xwmc335, S38</i>
<i>QYrPI192252.wgp-5BS</i>	PI 192252	5BS	<i>Xgwm335, IWA6910</i>
<i>QYrdr.wgp-1BL.1</i>	Druchamp	1BL	<i>Xgwm131, Xwmc694</i>
<i>QYrdr.wgp-1BL.2</i>	Druchamp	1BL	<i>IWA8581, Xgwm259</i>
<i>QYrdr.wgp-1DS</i>	Druchamp	1DS	<i>IWA2268, IWA7797</i>
<i>QYrdr.wgp-2BL</i>	Druchamp	2BL	<i>IWA7583, IWA4294</i>
<i>QYrdr.wgp-3AL</i>	Druchamp	3AL	<i>IWA6834, IWA602</i>
<i>QYrdr.wgp-5AL</i>	Druchamp	5AL	<i>IWA2558, IWA6988</i>
<i>QYrdr.wgp-5BL.2</i>	Druchamp	5BL	<i>IWA6383, IWA3025</i>
<i>QYrdr.wgp-6BL.2</i>	Druchamp	6BL	<i>IWA6420, Xgwm608</i>
<i>Qyr.PI182126.wgp-5BL</i>	PI 182126	5BL	<i>Xgwm540, Xbarc32</i>
<i>Qyr.PI182126.wgp-7BL</i>	PI 182126	7BL	<i>Xwmc51, Xwmc311</i>
<i>QYrPI184597.wgp-2AL.1</i>	PI 184597	2AL	<i>IWB41389, IWB77561</i>
<i>QYrPI184597.wgp-2AL.2</i>	PI 184597	2AL	<i>Xgwm312, IWB79669</i>

^a HTAP = high-temperature adult-plant



P 176 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Characterisation of the *QYr.sgi-4A.1* region conferring partial stripe rust resistance in 'Kariega'

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Key message: Progress is reported on the isolation and further characterisation of the adult plant slow rusting (APR) *QYr.sgi-4A.1* gene of Kariega.

The hard red spring wheat cultivar Kariega shows high levels of adult plant resistance against stripe rust that is controlled by three major QTL: *Lr34/Yr18/Sr57/Pm38*, *QYr.sgi-2B.1* and *QYr.sgi-4A.1* (Agenbag et al. 2014). Following four backcrosses to the original susceptible mapping population parent Avocet S, a doubled haploid line was developed that contains the target QTL (*QYr.sgi-4A.1*). Field trials in 2016 confirmed its slow rusting nature. Recent advancements in wheat genetics resulted in the development of molecular technologies and tools that allow for more rapid wheat gene cloning compared to the tedious methodologies of the past. To clone *QYr.sgi-4A.1* we will be using two complementary approaches: (i) the novel *MutChromSeq* method which combines chemical mutagenesis, phenotyping of a large mutant population and next generation sequencing (Sanchez-Martin et al. 2016) and (ii) accelerated classical map-based cloning supported by high-throughput molecular marker platforms, genome complexity reduction and high-quality wheat genomic sequence. Following EMS treatment of 5000 Avocet S + *QYr.sgi-4A.1* seeds, ≈2800 seedlings were planted in a greenhouse to produce M1 seed for field screening to identify potential *QYr.sgi-4A.1* mutant lines. The *QYr.sgi-4A.1* region was also targeted for the identification of informative SNPs by comparative analysis on the Illumina iSelect 90K wheat SNP array. A high resolution mapping population is under construction to map these SNP markers. The cloning of *QYr.sgi-4A.1* will broaden our knowledge of an agriculturally important, yet poorly-understood resistance mechanism.

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P 178 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Screening of Central Asian wheat germplasm for stripe rust resistance

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Key words: Resistance genes, stripe rust, *Triticum aestivum* L., virulence

Bread wheat (*Triticum aestivum* L.) is the most important crop in Central Asia and is directly linked to regional food security. Winter wheat, cultivated in all countries of Central Asia, is often affected by stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici* (Pst), and there have been frequent occurrences of stripe rust epidemics in many parts of the region, including four epidemics during 2009-2014. This study was conducted to: (i) examine pathotype variability of Pst and (ii) evaluate stripe rust resistance in cultivars and advanced breeding lines. Analysis of a mixed population of Pst showed 10 distinct pathotypes. Analysis of these pathotypes using 12 stripe rust resistance genes (*Yr*) showed different virulence patterns, with pathotypes 86E16 and 79E187 the most virulent. Seedling evaluation of 62 genotypes using the 10 pathotypes showed variations for resistance. Bynodkor and Barhayot showed resistance to all 10 pathotypes whereas Jaikhun, Hisorok, Shafag-2, and Egana were resistant to 9 of the 10 pathotypes. Murob-2 was resistant to 8 of the 10 pathotypes and KR12-09 and Layagati 80 were resistant to 7 of the 10 pathotypes. The biplot analysis showed that - based on reactions of all 10 pathotypes - genotypes with the most stable resistance were Bynodkor, Barhayot, Shafag-2, Egana, Hisorok, Murob-2, Jaikhun and KR12-09. The wheat genotypes also showed different levels of resistance in adult plant stage under field conditions. Twenty genotypes showed <20% severity in both Kazakhstan and Uzbekistan. The disease severity on several genotypes differed in Kazakhstan and Uzbekistan, suggesting different Pst populations in the two countries. Several stripe rust resistant wheat genotypes were identified, which should be further evaluated for release as new varieties or used in breeding programs. Two resistant lines from this study were identified as new varieties, one each in Georgia and Uzbekistan.

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P 180 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Determination of physiological races of *Puccinia striiformis* f. sp. *tritici* in Iran, 2015

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Key words: Physiological race, resistance, *Triticum aestivum*, yellow rust

The stripe (yellow) rust of wheat is one of the wrecker diseases in Iran. Since 1980 several epidemics have occurred in Iran and causing the breakdown of widely utilized sources of resistance in wheat cultivars. In 1993 wheat yield loss due to stripe rust was estimated 1.5 million tones in Iran. In the spring of 2010 widespread of stripe rust was started from west and north west of our country, and most old cultivated wheat cultivars in this region showed susceptibility reaction. In this study twenty-four isolates of *Pst* were collected from different parts of Iran during 2015. Infection types were assessed on a 0-9 scale 16 and 18 days after inoculation using a scale similar to that described by McNeal et al. (1971). Infection types (ITs) 7 to 9 were regarded as virulent (susceptible) and less than 7 are avirulent. Pathotypes 230E150A+,Yr27+ (from Karaj), 174E150A+,Yr27+ (from Shavoor) and pathotype 166E190A+,Yr27+ from Mashhad were more aggressive during this study. Virulence on plant with gene/s *Yr2*, *Yr6*, *Yr7*, *Yr9*, *Yr25*, *Yr27*, *YrSD* and *YrA* were more common and detected in greenhouse condition, and virulence for plants with the gene/s *Yr1*, *Yr3*, *Yr32*, *YrSU*, *YrND* and *YrCV* was limited. For genes *Yr4*, *Yr5*, *Yr10*, *Yr15* and *YrSP* virulence was not detected during 2015. Use of resistant cultivar is the best method to control the disease. Because of screening of wheat germplasm over last 10 years to pathotype of stripe rust with virulence on *Yr27*, new released cultivars included Mehregan, Parsi, Sirvan, Baharan, Morvareid, Gonbad, Pishgam, Zareh, Urom, Maihan, Haydarei and Shabrang were resistant to the new raising race with virulence on plants with *Yr27* in the field condition. Strategy of our breeding program is using of pathology data to release new resistance cultivars.

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P 182 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Four stripe rust resistance genes at or close to the *Yr5/Yr7* locus in wheat

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Key message: Critical phenotyping and molecular data indicate that *Yr Spaldings Prolific* and *Yr Jubilejna II* are both allelic to *Yr5/Yr7* or very closely linked in repulsion.

Genetic studies enable more effective utilization of genetic resources. Chromosome 2B is rich in rust resistance genes. Four stripe rust resistance genes (*Yr5*, *Yr7*, *Yr Spaldings Prolific* (*YrSp*), and *Yr Jubilejna II* (*YrJubII*)) appear to be allelic; *Yr5* and *Yr7* were shown to be allelic in a previous study (Zhang et al. 2009). Resistance in *Spaldings Prolific*, a European differential, is conferred by *YrSp*. Allelism tests based on F₂ phenotypes indicated that *YrSp* was closely linked to, but not allelic with, *Yr5* or *Yr7* (Feng et al. 2015). However, our allelism tests performed on 256 F₃ lines from AvS+*YrSp*/AvS+*Yr5* and 208 lines from AvS+*YrSp*/AvS+*Yr7*, as well as some progeny tests, found no recombinants. Rather our study provides evidence for allelism or very close linkage of *YrSp* with *Yr5* and *Yr7*. We suggest the recombinants observed by Feng et al. (2015) were caused by erroneous phenotyping of F₂ plants, which were not progeny tested. In our experience 100% phenotyping accuracy for stripe rust response in F₂ plants is not possible. Four resistance genes were identified in cv. *Jubilejna II*, a Chinese differential (Zhao et al. 2004), one of which was either allelic or closely linked to *YrSp*. Our allelism tests involving 261 F₃ lines from AvS+*Yr7*/JubII and 110 F₃ lines from Av+*YrSp*/JubII detected no recombinants. 90K SNP genotyping of AvS, Av+*Yr5*, Av+*Yr7*, Av+*YrSp*, and Av+*YrJubII* showed that the introgressed segments from the donors for *Yr5*, *Yr7* and *YrSp* remained large even after five or six backcrosses. Nevertheless, there was clear overlap between the introgressed regions for *Yr5*, *Yr7* and *YrSp* and they co-localized to a 29-cM (SNP genetic map) region containing 82 SNP markers. These markers will be tested on F₂ populations segregating for each gene to enable fine mapping of each one. In addition, susceptible mutants generated by mutagenesis are being used in RenSeq analysis.

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P 184 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Genome-wide association mapping for stripe rust (*Puccinia striiformis* f. sp. *tritici*) resistance in *Aegilops tauschii*

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Key message: New source of stripe rust resistance genes effective at seedling and adult plant stage have been identified using genome wide association studies in global *Aegilops tauschii* panel.

Stripe rust (yellow rust) caused by *Puccinia striiformis* f. sp. *tritici* (Pst) is a globally important disease of concern in wheat. Use of genetic diversity from wild progenitor species has made a significant contribution to improving wheat productivity and provide new sources of resistance for different wheat diseases. To characterize new genes conferring resistance to stripe rust, we utilized a genome-wide association study (GWAS) with collection of 112 accessions of *Aegilops tauschii* which were genotyped using genotyping by sequencing (GBS) approach. A total of 59 456 SNPs were detected out of which a total of 11 489 SNPs were mapped to seven *Ae. tauschii* chromosomes which were carried forward for linkage disequilibrium analysis and association mapping. Further LD pruning to remove the monomorphic or non-informative SNPs resulted in 5249 association mapping suitable SNPs. *Ae. tauschii* association mapping panel was screened for stripe rust resistance at adult plant stage under field conditions for four consecutive years (2012-2016) in the North India, Punjab Agricultural University, India and seedlings were screened with prevailing mixture of stripe rust races under controlled conditions. A total of 15 SNPs in four genomic regions (1D, 2D, 3D, 6D) were found to be linked with resistance to stripe rust at the adult plant stage while 6 SNPs in three genomic regions (1D, 2D, 7D) were associated with resistance to stripe rust at seedling stage. Further 6D potential genomic region was commonly detected for four tandem years for stripe rust resistance at the adult plant stage. Two genomic regions (1D, 2D) were found to be associated with seedling stage as well as adult plant stage resistance for year 2015-16. Correction for population structure, Kinship and linkage disequilibrium was carried out in association mapping panel by using mixed linear model. For marker-trait association analysis Fixed and random model Circulation Probability Unification (FarmCPU) package was used in R-software. Our study provides comprehensive analysis of wild progenitor species *Ae. tauschii* for identifying new source of resistance to *Pst* at adult plant stage and seedling stage. Closely linked SNP markers to these genes will be used for marker assisted transfer of yellow rust resistance from *Ae. tauschii* to hexaploid wheat.



P 186 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Effect of climate on the expression of adult plant stripe rust resistance genes in wheat

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Key message: Adult plant resistance (APR) expressed at flag leaf growth stage and APR gene *Yr29* provided better resistance at low temperatures whereas *Yr18* was more effective at higher temperature profiles.

Wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), can be managed using APR. The knowledge of expression of APR under different environments is crucial in the integrated management of this disease. Wheat varieties Baxter, GBA Sapphire, Janz, Kennedy, Spitfire, Yitpi and Avocet background near isogenic lines (NILs) carrying *Yr18*, *Yr29* and *Yr18+29*, all carrying adult plant stripe rust resistance, were studied for the expression of APR under controlled environment conditions. Presence of APR genes *Yr18* and or *Yr29* in the selected genotypes was confirmed using molecular markers. Weekly plantings of all genotypes and susceptible control Avocet S at 15/10°C, 20/15°C, 25/20°C, 30/25°C and 35/30°C day/night temperatures, permitted simultaneous comparisons of infection at the seedling, tillering, jointing and flag leaf growth stages. Ten replicates of each genotype growing at four different growth stages were inoculated with *Pst* pathotype 134E16 A*17*27*. Percentages of leaf area affected by stripe rust and the host response were recorded at 14, 21 and 28 days after inoculation. All the genotypes grown at different temperatures showed significantly less amount of infections at flag leaf growth stage when compared to the susceptible control Avocet S. Yitpi remained resistant both at seedling and flag leaf growth stages when grown at all the above said temperatures, which indicates that Yitpi carries a major gene for stripe rust resistance in addition to the APR gene *Yr29*. All the growth stages of Avocet NILs *Yr18*, *Yr29* and *Yr18+29* grown at all the temperatures showed significantly less amount of *Pst* infections in comparison to the susceptible control Avocet S. Interestingly, flag leaf growth stage of Avocet NIL *Yr29* showed significantly less amount of infections as compared to Avocet NIL *Yr18* when grown at 15°C/10°C and 20°C/15°C temperature profiles but Avocet NIL *Yr18+29* was significantly better as compared to both genes *Yr18* and *Yr29*. There was no significant difference between Avocet NIL *Yr18* and Avocet NIL *Yr18+29* when grown at 25/20°C, 30/25°C and 35/30°C day/night temperatures. Results indicated that APR gene *Yr29* can provide better protection at lower temperatures and *Yr18* can be more effective at moderate to high temperatures. Combination of APR genes (*Yr18+Yr29*) can provide better resistance against *Pst*. Information achieved on the interaction of temperature and the expression of APR in selected wheat varieties and NILs can be very useful in making predictions of stripe rust epidemics and in making decisions for chemical intervention against this disease.



P 188 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

MutRenSeq to elucidate the relationship between *Yr7* and *Yr5*

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Key message: MutRenSeq has been proven to be efficient in the cloning of wheat resistance genes. Using MutRenSeq we have identified candidate genes for *Yr7* and are validating their relationship with *Yr5*.

Maintaining wheat yield to meet future global demand is crucial, but remains challenging, partly due to the numerous diseases threatening wheat. Among them, yellow rust (caused by *Puccinia striiformis*) is distributed worldwide and was reported to be one of the most globally damaging cereal rust diseases. Despite over fifty described yellow rust resistance genes (*Yr*) in wheat, only a handful have been cloned to date e.g. *Yr36*. This lack of knowledge hinders the development of perfect markers for marker-assisted breeding, as well as the potential exploitation of novel allelic variation. We recently sequenced the coding regions (exome) from a mutant population of UK cultivar Cadenza, which carries the major gene *Yr7* on chromosome arm 2BL (Ma et al. 2015). Screening 500 mutant individuals with a *Yr7* avirulent, yellow rust isolate identified three mutant lines as susceptible; we hypothesize that they carry mutations in *Yr7*. To test this, resistance gene enrichment sequencing (MutRenSeq, Steuernagel et al. 2016) was carried out on the susceptible lines and the resistant Cadenza parent. Five potential, candidate mutations in NB-LRR genes have been identified on chromosome arm 2BL (Figure 1). We are using SNP markers to assess the linkage between these five candidates and the susceptible phenotype in F₂ populations. With this knowledge, we aim to elucidate the relationship between *Yr7* and *Yr5*, which have been postulated to be allelic (Zhang et al. 2009).

Acknowledgement

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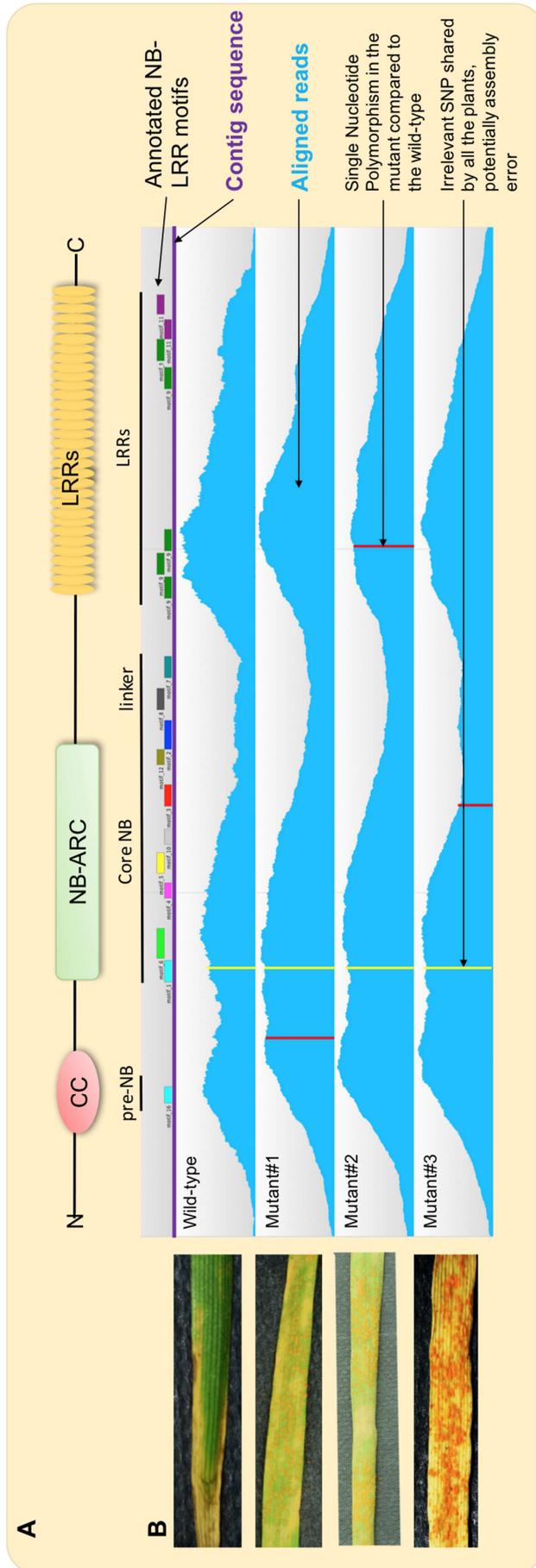


Figure 1: (A) Structure of a NB-LRR protein and example of visual validation of a candidate contig identified with the MutRenSeq pipeline. NB-LRR proteins comport specific domains, which can be targeted for enrichment and sequencing purposes (RenSeq). MutRenSeq (4) is an extended application of RenSeq, which enable the comparison of the NB-LRRome of a wild-type variety and EMS-derived susceptible mutants of the same variety in order to clone dominant resistance genes. A candidate gene should then carry a mutation in most of the independent susceptible mutant lines compared to the wild-type. The figure illustrates a candidate contig (purple line), which has previously been annotated as a NB-LRR (motifs). Wild-type and mutant reads were mapped to this contig (blue) to first check its coverage and then confirm the SNPs identified by the bioinformatics pipeline (MutantHunter). SNPs in the mutants are represented in red and irrelevant SNPs (shared by all the plants and at the same position) in yellow. Here the three susceptible mutants carry a SNP in the illustrated contig compared to the wild-type, thus it is a potential candidate. (B) Phenotype illustration of wheat seedlings inoculated with yellow rust 20 days post-inoculation. The wild-type response is characterized by necrosis and slight chlorosis on the infected leaf whereas the susceptibility is depicted as pustule production on the infected leaf, showing that the pathogen is able to full-fill its entire life-cycle on the plant.



P 190 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Inheritance and mapping of leaf rust and stem rust resistance genes in eight durum wheat genotypes

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Key message: New sources of resistant to leaf rust and stem rust in durum wheat.

Leaf rust caused by *Puccinia triticina* Erikss. (Pt) and stem rust caused by *Puccinia graminis* f. sp. *tritici* Erikss. and E. Henn (Pgt) pose a serious challenge to the production of durum wheat (*Triticum turgidum* L. var. *durum*) worldwide. However, there are only few leaf rust resistance (*Lr*) genes and stem rust resistance (*Sr*) genes characterized and mapped in durum wheat. The objective of this study was to determine the inheritance and genomic locations of *Lr* and *Sr* genes in durum accessions from the USDA-National Small Grain Collection. Eight genotypes (PI 534304, PI 313096, PI 387263, PI 209274, PI 278379, PI 244061, PI 192051, PI 195693) with leaf rust resistance to the durum virulent race BBBQJ were used to develop bi-parental populations. Two of these accessions, PI 192051 and PI 534304 were also resistant to the stem rust pathogen race TTKSK. The resulting F₁, F₂, F₃, and F₆ progenies were phenotyped for leaf rust and stem rust response at the seedling stage. Inheritance study and bulked segregant analysis on bi-parental populations developed from eight leaf rust resistance accessions showed that five of these accessions carry single dominant *Lr* genes on chromosomes 2B, 4A, 6BS, and 6BL. The inheritance of *Lr* genes in the other three accessions (PI 534304, PI 278379, PI 195693) deviated from Mendelian inheritance of a single dominant gene. All eight accessions carry different *Lr* genes than those already mapped in durum cultivars except PI 313096 that carries *Lr61*. Linkage mapping in two of the bi-parental populations carrying single dominant *Lr* genes showed that the gene in PI 209274 (*LrCA*) was mapped to 6BS between SNP markers *IWA3298* and *IWB39456*, while the gene in PI 192051 (*LrPort*) was mapped to 4AL, flanked by *IWA4254* and *IWA8341*. The stem rust resistance to Pgt race TTKSK in PI 534304 was mapped to 6AL and it is most likely conferred by *Sr13*. PI 192051 carries a previously uncatalogued *Sr* gene (*SrPort*) mapped to 7AS and flanked by *IW8390* and *IWA1805*. The genotype PI 192051 has an additional QTL (*Qsr.ndsu-5B*) to Pgt races in a field trial in Ethiopia in 2016. The *Qsr.ndsu-5B* was mapped to 5BL and delimited by *IWA6992* and *IWA2181*. These findings will enrich the genetic basis of resistance to leaf rust and stem rust in durum wheat.



P 192 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Identification of closely linked markers for wheat leaf and stripe rust resistance genes and virulence monitoring

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Key message: Molecular markers closely linked to resistances against leaf rust and stripe rust in wheat derived from 90k Iselect chip analysis allow pyramidizing and breeding of highly resistant cultivars in future.

Leaf rust (*Puccinia triticina*) and stripe rust (*P. striiformis*) are one of the most important fungal pathogens of wheat worldwide leading to yield losses up to 60%. In order to avoid epidemics and respective yield losses, resistance genes have been employed in cultivars. These resistances are race specific and vulnerable to a breakdown by the emergence of virulent rust races. Hence goals of our project are the identification of effective rust resistances in field trials followed by the development of closely linked markers for *Lr* and *Yr* genes based on genotyping near isogenic lines (NILs) with the 90k Illumina iSelect Chip. The virulence/avirulence patterns of leaf rust and stripe rust populations were analyzed at five different locations in Germany and locations of CIMMYT in Mexico. As a result leaf rust (*Lr*-) resistance genes *Lr9*, *Lr19*, *Lr25*, *Lr29*, *Lr34* and *Lr46* and stripe rust (*Yr*-) resistances *Yr5*, *Yr8*, *Yr10*, *Yr26* and *Yr27* turned out to be still effective using 41 Thatcher-NILs (carrying *Lr*-genes) and 15 Avocet NILs (carrying *Yr*-genes) carrying each a different resistance gene. However, the majority of resistance genes used in European wheat breeding e.g. *Lr10*, *Lr13* and *Lr37*, were already overcome. In order to develop closely linked markers for marker based selection and pyramidization respective NILs were genotyped using the 90k iSelect Chip (TraitGenetics, Gatersleben). Taking the marker order known for the iSelect chip SNPs and identifying polymorphisms between the susceptible recurrent parent and the resistance gene carrying NILs resulted in the identification of genomic regions of less than 1cM harbouring effective resistance genes *Yr5*, *Yr10*, *Yr15* and *Lr9*, *Lr25* or *Lr34*. Based on the sequence information KASP markers were derived for efficient marker based selection and pyramidization. Furthermore, sequences of the iSelect markers are aligned to contigs available from publically available databases (e.g. <https://urgi.versailles.inra.fr/>) at the moment for the identification of candidate genes.



P 194 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Mapping of quantitative adult plant resistance to leaf rust and stripe rust reveals co-location of three QTL conferring resistance to both rust pathogens descending from the durably resistant cultivar 'Capo'.

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Key message: We detected several, most likely novel QTL for adult plant resistance to rusts. Notably three QTL improved resistance to leaf rust and stripe rust simultaneously indicating broad spectrum resistance QTL.

The rusts of wheat (*Puccinia* spp.) are the most damaging fungal diseases of the wheat crop. The deployment of resistant cultivars plays a central role in rust disease management. Durability of resistance would be preferred, but is difficult to analyse. The Austrian winter wheat cultivar Capo was released in the 1989 and grown on a large acreage for more than two decades and maintained a good level of quantitative leaf rust and stripe rust resistance. Two bi-parental mapping populations: Capo × Arina and Capo × Furore were tested during several seasons and locations for severity of leaf rust and stripe rust at the adult plant stage in replicated field experiments. Quantitative trait loci associated with leaf rust and stripe rust severity were mapped using DArT and SSR markers. Five QTL were detected in multiple environments associated with resistance to leaf rust designated as *QLr.ifa-2AL*, *QLr.ifa-2BL*, *QLr.ifa-2BS*, *QLr.ifa-3BS*, and *QLr.ifa-5BL*, and five for resistance to stripe rust *QYr.ifa-2AL*, *QYr.ifa-2BL*, *QYr.ifa-3AS*, *QYr.ifa-3BS*, and *QYr.ifa-5A*. For all QTL apart from two (*QYr.ifa-3AS*, *QLr.ifa-5BL*) Capo contributed the resistance improving allele. The leaf rust and stripe rust resistance QTL on 2AL, 2BL and 3BS mapped to the same chromosome positions, indicating either closely linked genes or pleiotropic gene action. All three multiple disease resistance QTL (*QLr.ifa-2AL/QYr.ifa-2AL*, *QLr.ifa.2BL/QYr.ifa-2BL*, *QLr.ifa-3BS/QYr.ifa.3BS*) potentially contribute novel resistance sources for stripe rust and leaf rust. The long-lasting resistance of Capo apparently rests upon a combination of several genes. The described germplasm, QTL and markers are applicable for simultaneous resistance improvement against leaf rust and stripe rust. Details are published by Buerstmayr et al. (2014).

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P 196 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Characterization of introgressed segment linked to *Lr76* and *Yr70* using flow-sorting and sequencing of recombinant chromosome of an introgression line

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Key message: Using high end sequencing technology like chromosome sorting and sequencing, it is possible to characterize the alien introgressed segment and fine map the genes even with reduced recombination.

Rust pathogens are constantly threatening wheat production. The narrow genetic diversity poses great difficulty to fight against the rapidly evolving pathogens. Wild relatives of wheat are rich sources of diversity but deploying this diversity to commercial cultivars entails challenges such as strenuous crossing schemes, linkage drag and suppressed recombination. A non-progenitor species with the UU genome, *Aegilops umbellulata*, was found to be resistant to both diseases. To simplify the transfer of genes in any commercial cultivar, homozygous introgression lines resistant to leaf and stripe rust were developed. One of the introgression lines pau16057 was used to generate linkage map. Inheritance studies depicted that more than 97% of RILs and F₂ population were co-segregating for both the genes with rare recombinants between the two phenotypes. *Lr76* and *Yr70* were previously mapped on chromosome arm 5DS distal to a linked SSR marker *Xgwm190* at 7.6 cM distance and a co segregating STS marker *Lr57/Yr40MAS-CAPS16* (Bansal et al. 2017). Recombination in the alien segment led subsequently to the fine mapping of the genes. New microsatellite markers and NB-LRR based sequence tagged site markers were designed from the IWGSC survey sequence of chromosome 5DS. An SSR marker, *5DS-219*, dominant in nature, was found further close to the genes (Figure 1). Chromosome 5D of pau16057 and its counterpart WL711 were sorted out and sequenced to identify the *Ae. umbellulata* introgression specific SNPs. Based upon high confidence SNPs between the two chromosomes in each POPSEQ bin, size of the introgression linked to *Lr76* and *Yr70* was determined from 0 to 11.94 cM POPSEQ bin and after which the two parental line sequences were observed to be 99.9% similar (Figure 2). SNP genotyping on F₂ critical recombinants showed *KASP227*, *KASP228* and *KASP225* were found to be at 1.4 cM away from *Lr76*. *Yr70* was found to be at distal end with 2.1 cM away from *Lr76*. A co-dominant *KASP1* marker was designed from *Lr57/Yr40_CAPS* wheat contig and mapped on F₂ criticals recombinants. *KASP 1* and *KASP 225* were observed to be located in 4.37 cM and 11.94 cM bin, respectively which depicted that the *Ae. umbellulata* genes are different from *Ae. geniculata* genes (Figure 3).

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Figure 1: Comparison of partial linkage map of short arm of chromosome 5D carrying *Lr76* and *Yr70* from *Aegilops umbellulata* (a) based on SSR markers of wheat consensus map by Somers et al. (2004), amplified on BC-RIL population derived from cross wheat-*Ae. umbellulata* IL pau16057/3*PBW343 (Bansal et al. 2017); (b) fine map of *Lr76* and *Yr70* with newly designed SSR and STS markers from IWGSC survey sequence of chromosome 5DS of Chinese Spring along with previously linked SSR markers.

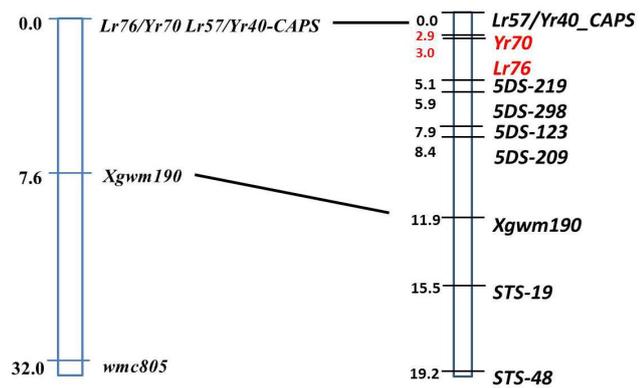
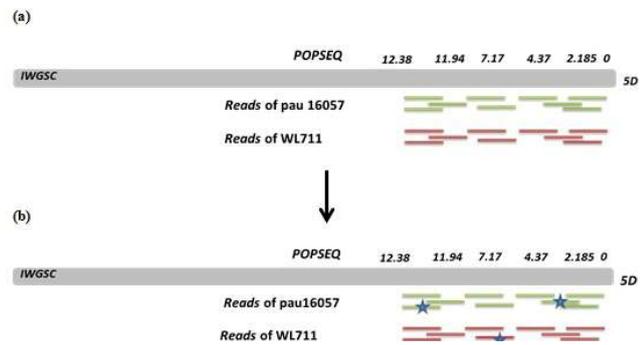


Figure 2: Diagrammatic representation of mapping of chromosome 5D sequences of pau16057 and WL711 with IWGSC sequence assembly using POPSEQ as a roadmap (a) T393-4 and WL711 reads mapped to the reference sequence of IWGSC chromosome 5DS with POPSEQ coordinates assigned to the each contig; (b) after mapping to the reference, SNPs were called between IWGSC/pau16057 and IWGSC/WL711.



IWGSC_POPSEQ Bins	Cluster I										Cluster II										Cluster III			No. of recombinants													
	4.37	4.37	4.37	4.37	4.37	4.37	4.37	4.37	2.19	2.19	4.37	4.37	0	0	0	0	4.37	4.37	4.37	2.19	2.19	7.17	7.17		7.17	7.17	11.94	-	-	11.94							
KASP markers	KASP85	KASP82	KASP22	KASP78	KASP78	KASP169	KASP71	KASP165	KASP81	KASP150	KASP171	KASP97	KASP108	KASP57	KASP24	KASP217	KASP219	KASP221	KASP220	KASP215	KASP153	KASP1	KASP93	KASP102	KASP201	KASP119	KASP117	KASP116	KASP214	KASP227	KASP228	KASP225	Lr76	Yr70			
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	2	
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	1
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	8
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	9
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	1
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	1
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	1
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	12
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	1
	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B/H	A	23

Figure 3: Graphical genotyping representation of high resolution mapping. KASP markers were designed from different contigs of each POPSEQ recombination bin and amplified on 1500 F₂ plants. Each marker was scored as 'A' for having WL711 specific, as 'B' for pau 16057 specific and 'H' for heterozygous amplification. '.' depicted the missing amplification. 'B/H' score of phenotype represents homozygous/heterozygous resistance in F₂ population. Out of 59 recombinants from 1500 plants, no. of recombinants observed for each type of genotype were written against it.



P 198 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Fine mapping of new loci for resistance to leaf rust and powdery mildew derived from *Triticum turgidum* ssp. *dicoccum*

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Key message: Fine mapping of new sources of resistance to leaf rust and to powdery mildew from the *Triticum dicoccum* accession MG5323 is in progress.

The tetraploid wheat relative *Triticum turgidum* ssp. *dicoccum* is particularly promising as a donor of useful genetic variation for several traits, including disease resistances, to be introgressed in cultivated wheats. The accession MG5323 of ssp. *dicoccum*, which showed useful level of resistance to leaf rust and powdery mildew diseases, was crossed with the susceptible durum wheat cultivar Latino. A total of 110 recombinant inbred lines (RILs) were produced and a saturated linkage map was developed based on the 90K Infinium (Illumina). The parents and RIL population were phenotyped using two *Puccinia triticina* and one *Blumeria graminis* isolates. About leaf rust, quantitative trait loci (QTL) analysis led to the identification of one major resistance gene on the short arm of chromosome 1B (named as *Q_{Lr}.gpg-1BS*), explaining a total phenotypic variation ranging from 41.4 to 49.5%. Two additional minor genes located on chromosome 7B (*Q_{Lr}.gpg-7BL-1*, *Q_{Lr}.gpg-7BL-2*) explained a phenotypic variation ranging between 17.8 and 25.8%. For all QTL the resistant allele was provided by MG5323. A significant positive epistatic effect was detected between QTL, indicating that different QTL contribute different degrees of resistance. Moreover, analysis of the leaf rust responses of the RILs demonstrated complementary actions between genes on chromosomes 1B and 7B. Analysis of powdery mildew resistance identified a single dominant gene on the short arm of chromosome 2B (designed as *Pm49*, <http://shigen.nig.ac.jp/wheat/komugi/genes>) explaining 78.7% of total phenotypic variation and MG5323 provided the resistant allele. No obvious positional relationships were observed when the map position of the major genes was compared with those of other previously identified wheat resistance genes, suggesting that new resistance sources to leaf rust and powdery mildew were identified in the tetraploid background. A fine mapping of the major genes for both diseases (*Q_{Lr}.gpg-1BS* and *Pm49*) was undertaken by developing a large F₂-based high resolution mapping population. Flanking and peak markers were used to select a number of recombinant lines that were phenotypically evaluated. The results obtained allowed to narrow down the genetic region and to select a new set of closer markers to be analyzed on the recombinant lines. These data together with mapping of the SNPs sequences on the durum reference genome are defining the physical regions underlying resistance QTL for leaf rust and powdery mildew.



P 200 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Identification of genes for resistance to leaf, stem, yellow rust and powdery mildew in the varieties of winter and spring wheat using molecular markers

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Key words: Powdery mildew, rust diseases, *Triticum aestivum*

The goal was to screen varieties of winter and spring wheat cultivars released in the Republic of Belarus, the presence of resistance genes to leaf, stem, yellow rust and powdery mildew. The study included the 56 varieties of winter and 23 varieties of spring wheat of the State Register of Varieties of the Republic of Belarus for 2015-2016. Varieties of soft winter wheat were studied using molecular markers for resistance genes: *Lr1*, *Lr9*, *Lr10*, *Lr19/Sr25*, *Lr20/Sr15/Pm1*, *Lr21*, *Lr22a*, *Lr24/Sr24*, *Lr25/Pm7*, *Lr26/Sr31/Yr9*, *Lr28*, *Lr29*, *Lr34/Yr18/Pm38*, *Lr35/Sr39*, *Lr37/Sr38/Yr17*, *Lr42*, *Lr47*, *Sr2*, *Sr22*, *Sr26*, *Sr1RS^{Amigo}*, *Sr36*, *Sr40*, *Sr44*, *Sr45*, *Yr10*, *Yr26*, *Pm3* (*Pm3a*, *Pm3b*, *Pm3c*, *Pm3d*, *Pm3e*, *Pm3f*, *Pm3g*), *Pm4* and *Pm8/17*. In winter varieties the identified resistance genes were: *Lr1* in Sanata, Uzdim, Yadvisya, Elegiya, Sakret, Balada, Mroya (all Belarus), Akteur (Germany), Finezja, Muza, Turnia (all Poland), Dorota (France); *Lr10* in Arctis, Skagen (all Germany), Finezja (Poland), Dorota, Olivin (all France), Bohemia (Czech Republic); *Lr26/Sr31/Yr9/Pm8* in Fantaziya, Kapela, Gorodnichanka (all Belarus), Markiza (Poland); *Lr34/Yr18/Pm38* in Fantaziya (Belarus), Dar Zernograda, Don-93 (all Russia), Akteur (Germany); *Lr37/Sr38/Yr17* in Sailor (France), Skagen (Germany); *Sr2* in Balada (Belarus); *Pm4* in Sanata, Grodnenskaya 7, Veda, Fantaziya, Zarica, Mroya (all Belarus), Muza, Natula (all Poland), Lucius, Bockris, Skagen (all Germany). Spring wheats were studied using markers for *Lr1*, *Lr9*, *Lr10*, *Lr19/Sr25*, *Lr24/Sr24*, *Lr25/Pm7*, *Lr26/Sr31/Yr9*, *Lr34/Yr18/Pm38*, *Lr35/Sr39*, *Lr37/Sr38/Yr17*, *Sr2*, *Sr26*, *Sr1RS^{Amigo}*, *Sr36*, *Yr10*, *Yr26*, and *Pm8/17*. The identified resistance genes were: *Lr1* in Koksa, Verbena (all Poland), Venera (Serbia), Septima (Czech Republic), Laska (Belarus); *Lr10* in Venera (Serbia); *Lr24/Sr24* in Kvintus (Germany); *Lr37/Sr38/Yr17* in Septima (Czech Republic). Winter wheat varieties identified with a set of genes for resistance to powdery mildew, leaf, stem and yellow rust: Fantaziya contains *Lr26/Sr31/Yr9/Pm8*, *Lr34/Yr18/Pm38* and *Pm4*; Kapela *Lr26/Sr31/Yr9/Pm8*; Skagen *Lr10*, *Lr37/Sr38/Yr17* and *Pm4*; Akteur *Lr1*, *Lr34/Yr18/Pm38*. These varieties may serve as sources of resistance genes to pathogens of powdery mildew, brown, stem and stripe rust.



P 202 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Resistance of European winter wheat cultivars to *Zymoseptoria tritici* isolate IPO88004

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Key message: Resistance to *Septoria tritici* blotch in European cultivars is contributed mainly by quantitative loci and those with main effects.

Septoria tritici blotch (STB) of wheat (*Triticum aestivum*), caused by the fungal pathogen *Mycosphaerella graminicola* (anamorph: *Zymoseptoria tritici*, syn. *Septoria tritici*), is present in most wheat-growing areas worldwide. Host resistance is the most economical and safest method of controlling the disease and information on resistance loci is crucial for effective breeding for resistance programs. In the study we used a set of 83 wheat cultivars registered in the Descriptive List of Agricultural Plant Varieties (COBORU 2012), 92 cultivars from other European countries and 25 cultivars/lines with identified STB resistance loci. The wheat genotypes were tested on adult plant stage under polytunnel conditions with watering system. Fully expanded flag leaves were sprayed with spore suspension of IPO88004 *Z. tritici* isolate. After incubation period, the percentage leaf area covered by necrosis (NEC) and covered by pycnidia (PYC) were measured on flag leaf of each wheat cultivar/line that were used in agglomerative hierarchical clustering (AHC) analysis with UPGA algorithm (unweighted pair-group average). Six groups of wheat cultivars/lines were identified and the largest group comprised 132 resistant genotypes with average NEC 18.7% and PYC 8.0%. Within this group, set of 43 highly resistant wheat cultivars were identified (NEC min. 1.1% and max. 15.1%, PYC min. 0.4%; max. 8.1%): Tuareg, Salutos, Samurai, Florett, Intro, Capone, Grapeli, Caroll, Praktik, Tabasco, Chilton, RGT Kilimanjaro, Zappa, Belenus, Joker, Butaro, Kalahari, KWS Dacanto, Riband, Fregata, Elixer, Forum, Estivus, Meteor, Glaucus, Bockris, Satyna, RGT Djoko, Jenga, Heros, Olivin, Zobel, Magnus, Kreda, Kepler, Pamier, Pengar, Julius, Desamo, Gordian, Eron, Pionier, Lear, Mandub and Kranich. In addition in the same subgroup were classified three cultivars with identified resistance loci: Florett (*QTL-3B*, *QTL-6D*; *Stb6+Stb15*), Tuareg (*QTL-4B*, *QTL-6B*; *Stb6*) and Riband (*QTL-6B*). This may suggest that resistance to STB in European cultivars is contributed mainly by quantitative loci and those with main effects. Presented work (phenotyping data) is a part of larger project aiming at identification of resistance genes (*Stb*) to *Septoria tritici* blotch in winter wheat and will be used in near future in association mapping approach.

Acknowledgments

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P 204 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Identification and mapping of QTL for *Zymoseptoria tritici* resistance in the winter wheat accession HTRI 1410

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Key message: QTL analyses using a doubled haploid population and based on two year phenotypic data for *Zymoseptoria tritici* resistance revealed QTL on chromosomes 4B and 5A. Additional studies using known STB isolates in detached leaf assays will be conducted.

Zymoseptoria tritici, the causal agent of Septoria tritici blotch (STB) causes yield losses of up to 50% in wheat, globally. Growing of resistant cultivars is the most cost effective and environmentally friendly way to avoid these losses. *Z. tritici* causing leaf blotch can be found worldwide and has gained evident importance due to changes in wheat cultivation. Therefore, there is a need to conducted screening of gene bank accessions for resistance, get information on the genetics of resistance and develop molecular markers for the efficient deployment of new resistances in wheat breeding. In extensive screening programs for resistance, the gene bank accession TRI 1410 turned out to be resistant in field tests and to be a valuable source for improvement of resistance to *Z. tritici*. In order to get information on the genetics of the STB resistance in TRI 1410, a DH-population consisting of 135 lines derived from crosses of TRI 1410 to three susceptible cultivars was generated. Artificial inoculation in field tests was conducted in 2014/2015 and 2015/2016 at three different locations in Germany and the area under the disease progress curve was determined. A quantitative variation for the reaction to a *Zymoseptoria* infection was observed and a significant genotypic effect detected. Heritability was estimated at $h^2=0.72$. In parallel this population was genotyped by the wheat 90k iSelect SNP chip. The genotypic data were used for map construction. About 6100 SNPs turned out to be polymorphic between the resistant cultivar and the three susceptible cultivars. Out of these 1118 SNPs mapped to the A-genome with an average distance of 3.46 cM, 1326 SNPs mapped to the B-genome with an average distance of 2.76 cM and 267 SNPs to the D-genome with an average distance of 5.69 cM. Preliminary QTL analyses based on two year phenotypic data for *Z. tritici* resistance revealed QTL on chromosomes 4B and 5A. Additional studies using known STB isolates in detached leaf assays will be conducted.



P 206 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

The wheat *Stb6* gene for resistance to the *Septoria tritici* blotch disease encodes an evolutionary conserved wall-associated kinase like protein

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Key message: The wheat *Stb6* for resistance to *Septoria tritici* blotch encodes an ancient WAK-like protein. This is the first report demonstrating gene-for-gene disease resistance mediated through this class of proteins in plants.

Evolution of disease resistance genes (R) represents one of the most successful strategies used by plants to resist pathogens. In gene-for-gene relationships, the overwhelming majority of R genes isolated to date encode intracellular nucleotide-binding leucine-rich repeat proteins (NLRs or NB-LRRs) that recognize either directly or indirectly specific secreted pathogen avirulence (Avr) proteins. This often triggers a localized hypersensitive response (HR) at sites of attempted infection, which halts disease development. *Zymoseptoria tritici* (syn. *Septoria tritici*, *Mycosphaerella graminicola*) is a fungus that causes *Septoria tritici* blotch (STB) disease in wheat and represents a significant threat to global food production. Resistance to STB is an important target in wheat breeding and during the past several decades at least 21 major resistance loci, most of which confer an isolate-specific resistance, have been identified and genetically mapped. However, none of these have so far been cloned and the resistance mechanism(s) has not been fully elucidated. *Stb6* is the most well characterized gene for resistance to STB in wheat. This semi-dominant gene provides resistance to fungal isolates possessing the matching avirulence gene *AvrStb6* by an unknown mechanism not involving HR. Here we report map-based isolation of *Stb6* from 'Chinese Spring' wheat. *Stb6* was found to reside in a large cluster of related genes, and encodes a wall-associated kinase (WAK) like transmembrane receptor protein. Allele mining revealed a remarkable sequence conservation of *Stb6*, with a single resistance allele predominating in the bread wheat germplasm. We identified *Stb6* also in several wild and domesticated diploid and tetraploid wheat species containing an A-genome. Alleles from known susceptible hexaploid wheats encoded either of two STB6 protein variants each containing changes in conserved amino acid residues in the intracellular kinase domain. Biochemical assays suggest that disease susceptibility associated with these mutations likely results from a loss of kinase catalytic activity and thus abrogated downstream signalling.

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P 208 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Novel necrotrophic effector and sensitivity gene interaction of *Septoria nodorum* blotch in wheat adult plants

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Key message: This study identified a novel necrotrophic effector and sensitivity gene interaction of *Septoria nodorum* blotch (SNB) in wheat adult plants which could be useful for breeding for SNB resistance.

The fungus *Parastagonospora nodorum* is a causal agent of an important foliar and glume disease *Septoria nodorum* blotch (SNB) on wheat. *P. nodorum* causes significant yield reduction of wheat despite the application of fungicides and a heavy focus over the last 30 years on traditional breeding for resistance. Resistance or susceptibility to SNB in wheat is complex and quantitatively inherited. The discovery of necrotrophic effectors has given breeding for disease resistance new methods and tools. Three effector-host sensitivity gene systems are well characterized in this pathosystem; SnToxA-*Tsn1*, SnTox1-*Snn1* and SnTox3-*Snn3*. Genetic analysis of various mapping populations and pathogen isolates has shown that different effectors have varying impacts, are effective at different development stages of wheat and that epistatic interactions also occur. As a result of these factors the deployment of these effectors for SNB resistance breeding is difficult. In this study, we have deleted genes that code for *SnTox 1* and *SnToxA 1* and *3* in a *P. nodorum* isolate to develop *tox1* and *toxa13* isolates, respectively. The wild type (SN15) *tox1* and *toxa13* were used for quantitative trait loci (QTL) analysis in seedling as well as adult plants (*toxa13*) of a DH population generated from Calingiri × Wyalkatchem (CW). Using *toxa13*, one QTL on 5DS was detected from all three data sets of adult disease severity of flag leaf-1 (F-1), flag leaf-2 (F-2) and flag leaf-3 (F-3) accounting for up to 30% of the variance in phenotype observed. The 5DS QTL was also found significantly contributing to disease incidence in seedling from the wild type and *tox1* isolates. A significant QTL was also found in the 5DS chromosome region from responses to culture filtrate of the *toxa13* isolate. In adult plant assay, an additional QTL was identified on 4AL which represented 81% of phenotypic variation in F-1 data set. Characterisation and functional studies of an effector candidate and its corresponding sensitivity in wheat are under investigation.



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Mapping and validation of *SnTox3-Snn3* as a major determinant of susceptibility to *Septoria nodorum* leaf blotch under field conditions in Norway

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Key message: The effect of the *SnTox3-Snn3* interaction was documented for the first time under natural infection at the adult plant stage in the field. Co-segregating SNP markers were identified.

Breeding for resistance to *Septoria nodorum* blotch (SNB) caused by *Parastagonospora nodorum* has been difficult due to large genotype × environment interactions and quantitative, moderate effects of each resistance locus. SNB is the major leaf blotch disease in Norwegian spring wheat. The discovery of host-specific interactions between necrotrophic effectors (NEs) and host sensitivity (*Snn*) genes in this pathosystem has raised new hopes for resistance breeding (Friesen & Faris 2010). However, the effects of these interactions under natural infection in the field have not been investigated. We saturated the genetic map of the recombinant inbred (RI) population SHA3/CBRD × Naxos using the Illumina 90K SNP chip. We infiltrated the population with purified *SnTox3* and used the sensitivity as a phenotypic marker to map *Snn3*. The population had previously been evaluated for segregation of SNB susceptibility in field trials (Lu & Lillemo 2014). We also conducted inoculation and infiltration experiments at the seedling stage with selected *P. nodorum* isolates. Re-mapping of quantitative trait loci (QTL) for field resistance showed that the *SnTox3-Snn3* interaction could explain more than 24% of the phenotypic variation in the field (Figure 1), and more than 51% of the variation in seedling inoculations. A collection of spring wheat for genome wide association mapping (GWAS), MASbasis, was also evaluated for field and seedling susceptibility to SNB and sensitivity to purified NEs. The markers co-segregating with *Snn3* in SHA3/CBRD × Naxos could be validated in the seedling trials. However, the markers were not significant in the field, although significant effect of *SnTox3* sensitivity on field susceptibility level could be detected when sensitive genotypes were compared to genotypes insensitive to both *SnTox3* and *SnToxA*. We also identified two distinct loci causing *SnTox3* sensitivity in MASbasis. One locus caused severe necrosis and corresponded to the reaction type and markers identified in SHA3/CBRD × Naxos. The other locus caused extensive chlorosis, but not necrosis, and we are currently screening mapping populations segregating for this chlorosis-causing sensitivity locus to map and validate its chromosomal position.

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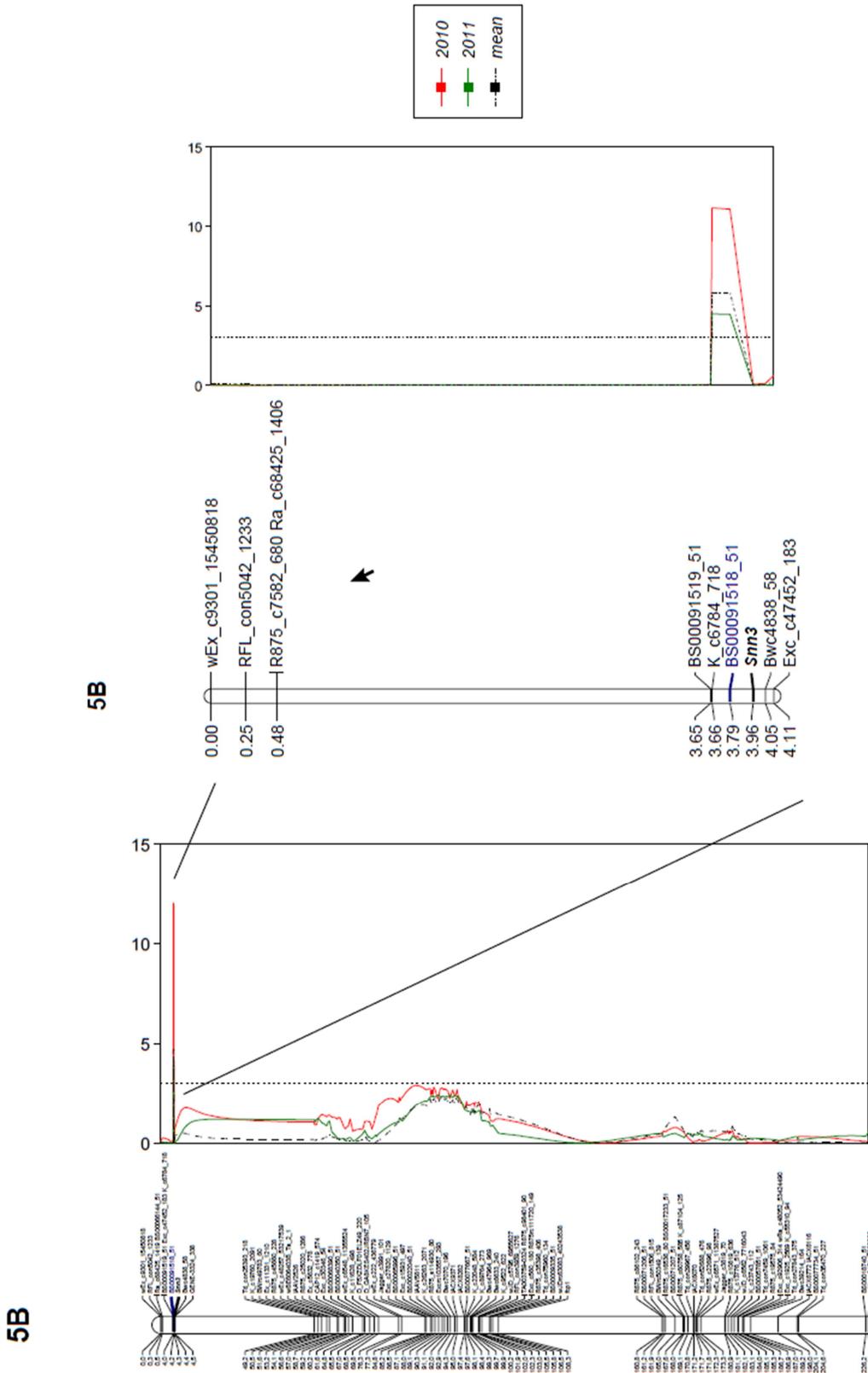


Figure 1: Linkage group 5B with LOD curves for the major QTL for field susceptibility to SNB at the *Snn3* locus detected in the field trials at Vollebekk, Ås, Norway in 2010, 2011 and across years (mean). Genetic distances are shown in centimorgans to the left of the chromosomes. A threshold of 3.0 is indicated by a dashed vertical line in the LOD graphs. The maps are drawn in Mapchart v.2.2.



P 212 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

The exploitation of wheat/*Amblyopyrum muticum* introgression lines carrying inter-genomic rearrangements, involving the D genome with the A and B genome of wheat, for durum wheat improvement to STB disease

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Key message: The effect of the D genome introgression in durum wheat on *Septoria tritici* blotch resistance and other traits for durum wheat improvement.

Septoria tritici blotch (STB), is an important foliar blight disease of wheat worldwide. Genetic resistance is the most effective and economical method of controlling STB. The aim of this study is to improve genetic resistance of two susceptible durum wheat varieties to STB disease through the exploitation of the D genome effect on the durum wheat specific strains. Forty plants coming from nine different introgression lines of wheat/*Amblyopyrum muticum* containing AD, BD and ABD chromosomal translocations were crossed in glasshouse conditions to the two durum wheat genotypes in an attempt to transfer the chromosomes carrying these intergenomic translocations involving the D genome into durum wheat. Five of those introgression lines were shown to also carry one or two segments of *Am. muticum* (King et al. 2017). Sixty crosses were made between these nine lines and two durum wheats to produce 488 F₁ seed. The presence of D genome translocations was assessed in 120 F₁ plants using multi-colour genomic *in situ* hybridisation technique. Only 40 plants were shown to carry the D genome translocations. Most of the D genome introgressions identified were located on the A genome especially in the telomeric regions, indicating that frequent associations of the A-D type occur in hybrid lines between wheat and the alien species *Am. muticum*. The F₁ plants selected will be back-crossed to the recurrent durum wheat parent and the BC₁ seed showing the presence of the D genome translocations will be selfed until they reach the tetraploid chromosome number with a potential homozygous introgression of the D genome. At this stage the selected plants will then be phenotyped in field conditions for STB disease resistance and other traits in two different environments. This will be achieved through a shuttle-breeding programme between Tunisia, a hot spot for STB disease, where the pathogen is durum wheat specific, and Mexico (CIMMYT). Phenotyping at these two locations will also enable selection for other traits in advanced generations. The aim is to map any potential STB resistance loci using genotyping with the 820K Axiom array SNP markers to identify D-genome specific contribution to such disease resistance.

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P 214 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

QTL mapping for spot blotch resistance in two bi-parental mapping populations of bread wheat

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Key message: In the current study, resistance to spot blotch is controlled by a QTL on chromosome 5AL at *Vrn-A1* and *Sb2* on 5BL and conditioned by multiple minor QTL.

Spot blotch (SB), caused by *Cochliobolus sativus* (anamorph *Bipolaris sorokiniana*), is a major fungal disease of wheat in South Asia and South America, causing significant yield loss in epidemic years. Inheritance of resistance to SB is generally taken as quantitative, although major resistance genes have been identified, including *Sb1* on chromosome 7DS, *Sb2* on 5BL, and *Sb3* on 3BS. In order to identify SB resistance QTLs and their flanking markers for the potential use in marker assisted selection, two bi-parental mapping populations with 232 F_{2:7} progenies each were generated for this study. Resistant parent of the first population was a CIMMYT breeding line SOKOLL//W15.92/WBLL1 and that of the second population was WHEAR/KRONSTAD F2004, whereas Ciano T79 was used as susceptible parent in both populations. The two populations were evaluated for field SB resistance in CIMMYT's Agua Fria station from 2012-2013 to 2014-2015 cropping seasons. Artificial inoculation was performed by scattering *B. sorokiniana* colonized sorghum grains in the field, and disease evaluation was done three to four times at weekly basis with the double-digit scale. Area under disease progress curve (AUDPC) was calculated based on SB evaluation data and was used for subsequent analysis. Two additional traits, plant height (PH) and days to heading (DH), were also scored due to their close relationships with SB. Genotyping was done with the DArTseq genotyping-by-sequencing (GBS) platform and approximately 1500 high quality and non-redundant GBSs were used for QTL mapping. Several additional SSRs and SNPs that had been reported for SB resistance and phenological traits were also scored. In both populations, a major QTL was found on chromosome 5AL at *Vrn-A1*, explaining phenotypic variations of 14-26%, which turned up to be less- or non-significant when DH and PH were used as covariates in the analysis, implying a disease escape mechanism. Another major QTL was located on 5BL in the SOKOLL//W15.92/WBLL1 population, most likely being *Sb2*, accounting for 9-22% of phenotypic variation. Minor QTL were found on 4A and 4B in SOKOLL//W15.92/WBLL1 and on 1B, 4B, 4D and 7A in WHEAR/KRONSTAD F2004. Additionally, minor QTL contributed by Ciano T79 were identified on 1B, 2B, 4B and 6D.



P 216 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Resistance to spot blotch in two CIMMYT wheat breeding lines is conditioned by multiple QTL of minor effects

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Key message: Multiple QTLs with minor effects were identified for field resistance to spot blotch in two bi-parental mapping populations involving CIMMYT germplasm as resistant parents

Spot blotch (SB), also known as Helminthosporium leaf blight or foliar blight, is a major fungal disease of wheat in South Asia and South America. Host resistance is regarded as an economical and environmentally friendly approach of controlling SB, and the inheritance of resistance is mostly quantitative. CIMMYT develops and distributes regularly Helminthosporium leaf blight screening nurseries (HLBSN, previously known as CSISA-SB) to help breeders/pathologists in South Asia and South America to cope with this disease. HLBSN entries are elite breeding lines with promising resistance to SB, good agronomy and high yield potential. In order to gain a better understanding on the SB resistance mechanism in CIMMYT germplasm, two bi-parental mapping populations were generated for this study, both comprising 232 F_{2:7} progenies. Elite CIMMYT breeding lines, BABAX/LR42//BABAX/3/ER2000 and WAXWING*2/CIRCUS, were used as resistant parents, respectively, whereas Ciano T79 was used as susceptible parent in both populations. The two populations were evaluated for field SB resistance in CIMMYT's Agua Fria station for three years, i.e. from 2012-2013 to 2014-2015 cropping seasons. Artificial inoculation was done by scattering *Bipolaris sorokiniana* colonized sorghum seeds in the field, and SB scoring was made three to four times at weekly basis with the double-digit scale. Phenological traits like plant height (PH) and days to heading (DH) were also scored. Genotyping was done with the DArTseq genotyping-by-sequencing (GBS) platform and approximately 1,500 high quality and non-redundant markers were used for QTL mapping. Selected SSRs and SNPs for previously reported SB resistance QTL and phenological traits were also used in genotyping the populations. The most prominent QTL in both populations was found on chromosome 5AL at *Vrn-A1*, explaining phenotypic variations of 6-27%. However, when DH and PH were used as covariates in the analysis, the effects of this QTL decreased significantly, implying a disease escape mechanism. Minor QTLs were found on 1A, 1B, 3A, 3B, 4B, 4D, 5B and 6D in BABAX/LR42//BABAX/3/ER2000 and on 1B, 2A, 2D and 4B in WAXWING*2/CIRCUS, all showing phenotypic effects less than 10 %. Additionally, minor QTL contributed by Ciano T79 were identified on 1B, 1D, 3A, 4B and 7A. In summary, resistance to SB in the two mapping populations was conditioned by multiple minor QTL, with strong influence of *Vrn-A1*.



P 218 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Identification of ergot resistance QTL in durum wheat

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Key message: QTL analysis of ergot resistance in two durum wheat populations identified QTL on chromosomes 1B, 2A, and 5B. SNPs linked to these QTL may facilitate breeding for ergot resistance.

Ergot, caused by the ascomycete *Claviceps purpurea* (Fr.) Tul., has emerged as an important disease of durum wheat in Canada. Ergot contamination has increased since 2000, reaching a peak in 2013 with 50%, 30%, and 25% of wheat delivered to elevators being downgraded in the provinces of Alberta, Saskatchewan, and Manitoba, respectively. Ergot sclerotia contain toxic alkaloids, which can cause severe health problems and even death if ingested. Seed cleaning equipment can remove sclerotia from grain, but this process is not completely effective and represents an extra cost to the farmer. The durum wheat line 9260B-173A, identified as a source of ergot resistance (Menzies 2004), was studied in an AC Avonlea/9260B-173A recombinant inbred line (RIL) population. Subsequently, a doubled haploid (DH) population was studied, AC Avonlea/RIL-3, where RIL-3 was an ergot resistant line from the AC Avonlea/9260B-173A population. These populations were phenotyped for honeydew and sclerotial production by inoculating florets with a 10⁴ conidia per ml suspension of six *C. purpurea* isolates in growth chamber experiments. Genotyping was performed with the wheat 90K Infinium SNP beadchip assay (Wang et al. 2014) and with the KASP SNP assay. Linkage maps were developed for each population and QTL analysis conducted. A major QTL on chromosome 2A reduced honeydew production and percent infected florets (i.e. sclerotia number). Another major QTL on chromosome 5B reduced sclerotia mass per spike, percent infected florets, and mean sclerotia mass. A third QTL on chromosome 1B reduced mean sclerotia mass. These results will enable marker-assisted selection of ergot resistance and guide future genetic research.

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P 220 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Evaluation of resistance of winter wheat and spelt wheat genotypes against common bunt and dwarf bunt

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Key message: The contribution presents data on cultivars tested for common bunt resistance and dwarf bunt resistance.

The contribution presents data on common bunt and dwarf bunt resistance from the Crop Research Institute in Prague-Ruzyně. Common bunt inoculation was done by shaking seed with teliospores in Erlenmayer flasks for 1-2 min. Inoculation and sowing (1 m long rows, 4 replications) was carried out in early October. For dwarf bunt tests rows 1 m long with 8 replications were sown in late October. Teliospores were evenly spread on the soil surface after sowing. In absence of a snow cover the plots covered with straw or white nonwoven fabric. The resistant checks 'Globus' and 'Bill' and the susceptible check 'Batis' were included in the tests. Out of the recently registered winter wheat cultivars in the Czech Republic 'Genius' proved common bunt resistance in two years of testing. 'Sailor' proved high resistance to common bunt only in one year, while in the other years it was more or less susceptible. 'Saturnus' and 'Potenzial' showed the lowest bunt incidence in the trials with dwarf bunt. The tested sources of resistance proved high resistance to dwarf bunt. As they were recorded resistant to common bunt as well, they offer a suitable genetic material for resistance breeding both to the common and dwarf bunt. In addition to winter wheat cultivars, 80 varieties of the winter form of spelt of a different origin were evaluated for common bunt. On the basis of the results from the Czech Republic, Austria and Switzerland it is possible to consider 'Albin' and Sofia 1 to be those with the highest resistance to common bunt.

Acknowledgement

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P 222 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Comparative mapping of common bunt and dwarf bunt resistance QTL in winter wheat

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Key message: We report the identification of novel major QTL conferring common bunt and dwarf bunt resistance in winter wheat

Common bunt (*Tilletia tritici*) and dwarf bunt (*T. controversa*) are destructive diseases of wheat (*Triticum aestivum* L.) that greatly reduce grain yield and quality. During the last two decades bunt diseases have re-emerged throughout Europe due to the steep increase in organic winter wheat production and a lack of adapted *and* resistant varieties. To large extents, the genetic basis of bunt diseases is still unknown: Few bunt resistance *Bt*-genes and QTL were mapped to specific wheat chromosomes, molecular markers that find use in marker assisted selection exist for *Bt-10* solely. Therefore, the identification of major bunt resistance QTL and closely linked molecular markers will help to speed up the development of bunt resistant varieties for organic farming. Three bi-parental recombinant inbred line (RIL) populations were derived from crosses of the bunt resistant North-American cultivars 'Blizzard' and 'Bonneville' - both carriers of unknown bunt resistance genes, and 'PI 119333' - a Turkish landrace and carrier of the highly effective bunt resistance gene *Bt-12*, with the susceptible cultivar 'Rainer'. Phenotypic reaction of all RIL populations to artificial inoculation with common bunt and dwarf bunt teliospores was evaluated in five trials over three years at two locations in Austria and the USA. In addition, all RIL populations were genotyped by single nucleotide polymorphism (SNP) markers using the Illumina 15K array. The combined statistical analysis of phenotypic and genotypic data allowed us to identify major QTL for common bunt and dwarf bunt resistance in winter wheat that explained large amounts of the total phenotypic variation (R^2): For Blizzard and Bonneville, two major QTL were found on wheat chromosome 1A ($R^2 = 20-28\%$) and 1B ($R^2 = 30-35\%$) that conferred common bunt *and* dwarf bunt resistance and common bunt resistance solely, respectively. For PI 119333, a carrier of the bunt resistance gene *Bt-12* which confers highly efficient protection against common bunt and dwarf bunt across countries, one major bunt resistance QTL (i.e. *Bt-12*) mapped to chromosome 7D and explained around 40% of the total phenotypic variation. The identified major bunt resistance QTL on wheat chromosomes 1A, 1B and 7D and associated SNP markers will find application in marker assisted selection and accelerate the development of bunt resistant varieties for organic agriculture.

Acknowledgement

The project „COBRA Austria“ was funded within the framework of the ERA-Net project Core Organic 2 by the Austrian Federal Ministry of Agriculture, Forestry, Environment and Water Management (project number 100898).



P 224 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Application of novel genomic tools for genome-wide analysis of resistance to eyespot disease in European winter wheat

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Key message: Genotypes with recombination events in the *Triticum ventricosum* introgression on chromosome 7D allowed to fine map resistance gene *Pch1*, the main source of eyespot resistance in European winter wheat cultivars.

Eyespot (also called strawbreaker) is a common and serious fungal disease of winter wheat caused by the necrotrophic fungi *Oculimacula yallundae* and *O. aciformis* (formerly *Pseudocercospora herpotrichoides*). A genome-wide association study (GWAS) for eyespot was performed with 732 microsatellite markers (SSR) and 7761 mapped SNP markers derived from the 90K iSELECT wheat array using a panel of 168 European winter wheat varieties as well as 3 spring wheat varieties and phenotypic evaluation of eyespot in field tests in three environments. Best linear unbiased estimations (BLUEs) were calculated across trials and ranged from 1.2 (most resistant) to 5.73 (most susceptible) with an average value of 4.24 and a heritability of $h^2 = 0.91$. A total of 108 SSR and 235 SNP marker-trait associations (MTAs) were identified by considering associations with a $-\log_{10}(p\text{-value}) \geq 3.0$. Significant MTAs for eyespot-score-BLUEs were found on chromosomes 1D, 2A, 2D, 3D, 5A, 5D, 6A, 7A and 7D for the SSR markers and chromosomes 1B, 2A, 2B, 2D, 3B and 7D for the SNP markers. For 18 varieties (10.5%), a highly resistant phenotype was detected that was linked to the presence of the resistance gene *Pch1* on chromosome 7D. The identification of genotypes with recombination events in the introgressed genomic segment from *Triticum ventricosum* harbouring the *Pch1* resistance gene on chromosome 7DL allowed the fine-mapping of this gene using additional SNP markers and a potential candidate gene Traes_7DL_973A33763 coding for a CC-NBS-LRR class protein was identified.

Acknowledgement

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P 226 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Identification of molecular markers for soil borne virus resistance in wheat

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Key message: High density SNP map of resistance to WSSMV and SBCMV in bread wheat was constructed using the iSelect 90K and GBS allowing efficient marker saturation

Mosaic disease, caused by the bymovirus *Wheat spindle streak mosaic virus* (WSSMV) as well as by the two closely related furoviruses *Soil-borne cereal mosaic virus* (SBCMV) and *Soil-borne wheat mosaic virus* (SBWMV), is a serious constraint to winter wheat production in many regions of Europe, North America and Asia. All viruses are transmitted by the endoparasitic protist *Polymyxa graminis*, a eukaryotic soil-borne microorganism classified in the family *Plasmodiophoridae*. Since chemical measures are neither effective nor ecologically sound, due to the long-term survival of *P. graminis* resting spores in the soil, the only possibility of control of this disease is growing of resistant cultivars. In previous reports, based on field phenotyping experiments, WSSMV resistance was mapped to chromosome 2D, while a single gene controlling resistance to SBCMV and a major QTL controlling resistance to SBWMV, *Sbm1*, was assigned to the long arm of chromosome 5D. The aim of this work was to map the WSSMV/SBCMV/SBWMV resistance in non related breeding material, saturating the respective genome regions with molecular markers and developing molecular markers for effective marker-based selection. For this purpose, two DH populations each containing 143 DH lines, i.e. Fanion (r) × Prevert (s) and Goncourt (s) × Cordiale (r), and a set of 52 diagnostic wheat lines have been phenotyped at seven field locations in France, Germany and USA. The DH population Fanion × Prevert (F×P) was genotyped using the 90K iSelect Illumina array and Genotyping by Sequencing (GBS) on a MiSeq. WSSMV/SBCMV phenotypic data revealed a 1r:1s segregation ratio, indicative of a single major gene. WSSMV resistance was mapped to chromosome 2D, while SBCMV resistance mapped to the long arm of chromosome 5D. The SBWMV data generated will be used for QTL mapping. GBS genotyping of the F×P population revealed twelve and 18 polymorphic reads at the target intervals, after anchoring genetic data to the wheat reference sequence. Polymorphic GBS reads will be converted into KASP SNP markers which can be efficiently employed in the transfer of resistance into elite wheat lines and the construction of a high resolution map towards the isolation of these resistance genes. The results of this study demonstrated that modern genotyping technologies can be efficiently employed for marker saturation of resistance loci, and the development of PCR based markers for accelerating the transfer of resistance genes.



P 228 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Fine-mapping of *Q_{Sbm.ubo-2BS}=*Sbm2*, a major QTL for resistance to Soil-Borne Cereal Mosaic Virus (SBCMV)*

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Key message: *Q_{Sbm.ubo-2B}* in durum wheat is located in a 2 Mb interval including 12 putative resistance genes among which the possible causative gene(s) for SBCMV resistance could be present.

Q_{Sbm.ubo-2BS} = Sbm2, a major QTL controlling the response to Soil-Borne Cereal Mosaic Virus (SBCMV) in durum wheat, was characterized in two recombinant inbred line populations, namely Meridiano (resistant, R) × Claudio (moderately susceptible, MS) and Simeto (susceptible, S) × Levante (R). By means of meta-QTL analysis *Q_{Sbm.ubo-2BS}* was mapped as a unique QTL within a 2 cM-wide interval (LOD-2) in the distal region of chromosome arm 2BS (Maccaferri et al. 2011). The addition of the Illumina 90K SNPs array to the durum linkage maps allowed us to identify 36 transcripts-associated SNPs tightly associated with the mendelized QTL (Maccaferri et al. 2014). Five SNPs from the Illumina 90K wheat array were converted to KASP[®] markers, which provided fluorescent high-throughput assays spanning the QTL region. Marker-assisted selection (MAS) was performed with KASP markers on ≈2000 RILs from the Svevo (R) × Ciccio (S) population. MAS was performed with two KASP markers flanking the QTL interval, *KUBO 9* and *KUBO 13*, and identified 330 recombinant RILs, which were characterized for SBCMV response in the 2016 field nursery under severe and uniform SBCMV infection. Informative RILs were scored for symptom severity (SS) on a 0 to 5 scale and screened with five KASP markers distributed along the QTL interval (*KUBO 1*, *KUBO 3*, *KUBO 27*, *KUBO 29*, *KUBO 38*). The results confirmed the presence of the QTL in the interval (mean SS score of RILs with Svevo and Ciccio haplotype of 1.4 and 3.0, respectively) and narrowed the most probable support interval to ≈2 Mb between *KUBO 27* and *KUBO 3*. The gene space of the *Q_{Sbm.ubo-2BS}* interval in the wheat genome assemblies includes 43 genes, 12 of which are possible candidate genes for SBCMV response.

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P 230 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Silencing of *Diuraphis noxia* virulence gene through RNA interference using a novel siRNA delivery method

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Key message: Resistance in wheat is often counteracted by *Diuraphis noxia* (Russian wheat aphid, RWA) through the development of a new RWA biotype. This study explore the use of iRNA to develop broad spectrum against aphids.

Resistance to *Diuraphis noxia* (Russian wheat aphid) in wheat is often overcome by the emergence of a new aphid biotype. dsRNA production in crop plants to induce RNA interference (RNAi) in insect pests is an alternative to conventional breeding practices. To investigate this possibility in wheat, a novel siRNA delivery method was developed to induce RNAi in *D. noxia* as a proof of concept for the above mentioned method. Synthetic siRNA targeting the *cpr1-8* and *c002* genes of *D. noxia* was injected into wheat leaves on which aphids were contained and allowed to feed. siRNA concentration was measured relative to leaves injected with buffer or leaves that did not receive an injection (Figure 1). Aphid survival was severely affected when feeding on *cpr1-8*- or *c002*-siRNA injected wheat leaves; 50% less survived than the control in the case of *c002*-siRNA (Figure 2A). Nymph production however was less affected by feeding on siRNA (Figure 2B). The permanent expression of dsRNA (in this case of sequences identical to *D. noxia cpr1-8* or *c002*) in wheat will result in a continuous supply of siRNA which is expected to result in an even larger death rate when consumed by aphids. To this end, dsRNA-producing constructs were made for the intention of wheat transformation. The constructs targeting *cpr1-8* were designed with the potential to target not only aphids, but other insect pests as well. The results of this experiment will be presented and discussed.

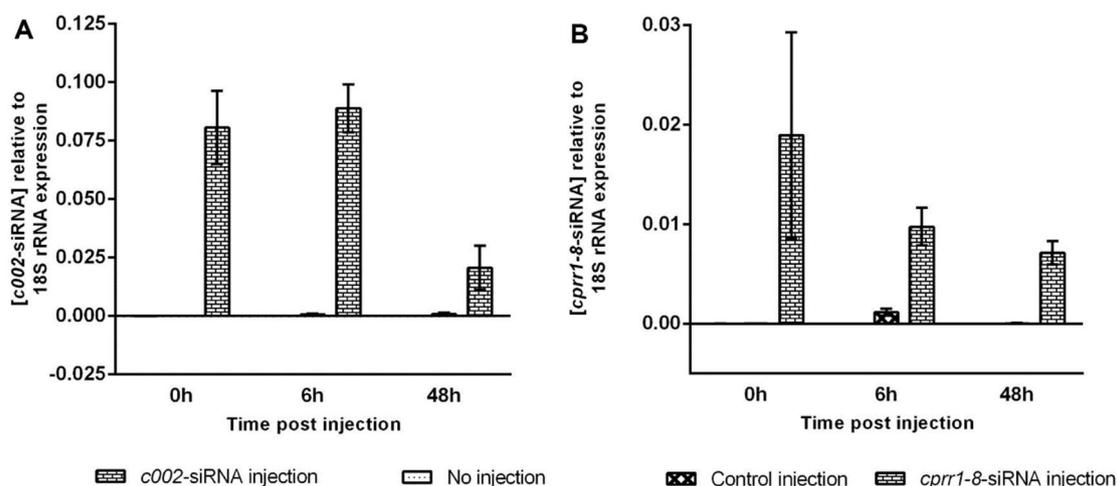


Figure 1: Relative concentration of *c002*-siRNA (A) and *cpr1-8*-siRNA (B) post injection in leaf area on which *Diuraphis noxia* biotype SAM was contained. Relative concentration was determined by comparing to wheat 18S rRNA expression. (no injection: wheat leaves without injection; buffer: 10 mM Tris (pH 7); *c002*-siRNA, *cpr1-8*-siRNA: siRNA targeting *c002* and *cpr1-8*, respectively, dissolved in 10 mM Tris).

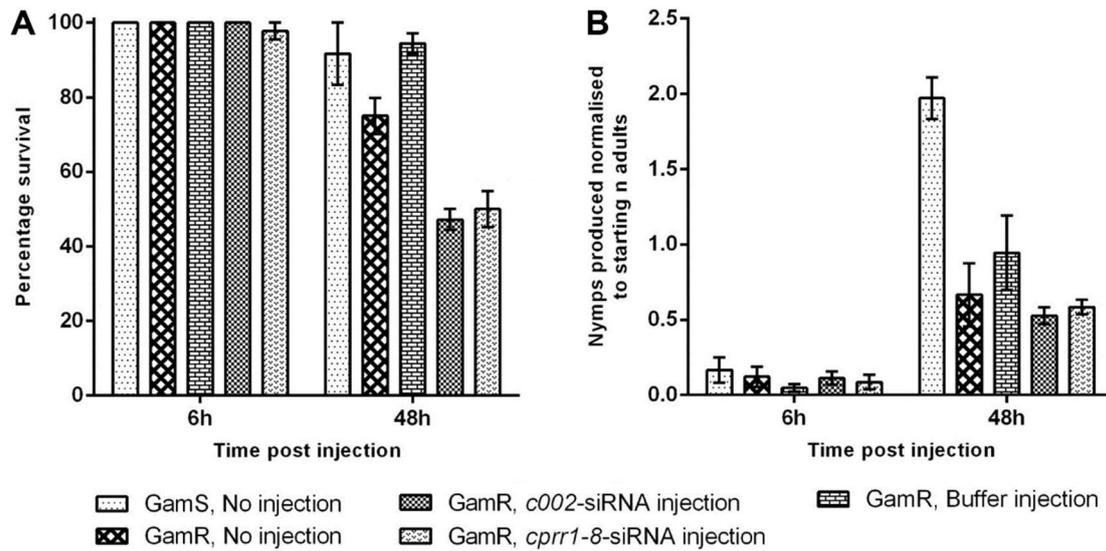


Figure 2: Percentage survival (A) and nymph production (B) of *Diuraphis noxia* biotype SAM after feeding on wheat leaves injected with siRNA (19 nt duplex region and a 2 nt 3'-overhang) that targets the genes *c002* or *cpr1-8*. Nymph production was normalized to the starting number of adult aphids. siRNA was dissolved in 10 mM Tris (pH 7) before injection. (GamS, GamR: *D. noxia* susceptible wheat cv 'Gamtoos-S' and resistant cv 'Gamtoos-R', respectively; no injection: wheat leaves without injection; buffer, 10mM Tris (pH 7); *c002*-siRNA, *cpr1-8*-siRNA: siRNA targeting *c002* and *cpr1-8*, respectively, dissolved in 10 mM Tris).



P 232 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Nutrient composition affects the crossover frequency in meiosis in wheat

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Key message: The stability of the *ph1b* mutant is affected by environmental factors, such as the nutrient composition of the soil which increases the crossover frequency in wheat and wheat-rye hybrid meiosis.

Bread wheat (*Triticum aestivum*) possesses three related (homoeologous) ancestral genomes, derived from three different diploid species. Each set consists of seven pairs of homologous chromosomes, with similar gene order and content. Despite similarity between homoeologues, wheat behaves as a diploid during meiosis, with every chromosome synapsing and recombining only with its true homologue. This phenotypic behaviour is predominantly controlled by *Ph1*, the most important locus on chromosome 5B. Wheat lacking *Ph1* is probably close to the initial situation where a new polyploid has arisen, but the meiotic process has not yet been modified to stabilise meiosis. Thus the *ph1b* mutant has been widely reported to accumulate extensive rearrangements, reducing fertility. In our laboratory however, the *ph1b* mutant has maintained a reasonable level of fertility for more than 30 years suggesting that environmental factors in different laboratories may affect the stability of the *ph1b* mutant. Genomic *in situ* hybridisation (GISH) in *ph1b* mutant revealed only three background translocations despite more than 10 generations of growth. The same original *ph1b* mutant line has also been grown independently in Córdoba, Spain, for over 30 generations. GISH analysis on this line showed the same three background translocations suggesting they were most likely present in the original *ph1b* line. RNA-seq comparison of the expression patterns of floral material in the presence and absence of *Ph1* were also performed suggesting only four additional smaller deletions. Thus, despite lacking the *Ph1* mechanism presumed to suppress synapsis and recombination between homoeologues, the Norwich and Córdoba *ph1b* lines have not accumulated extensive rearrangements or deletions. This implies that *Ph1* is not necessary for polyploid stability. Our *ph1b* mutant had not accumulated extensive rearrangements, suggesting suppression of homoeologous crossovers (COs) by another factor (or factors). One of the factors that varies markedly between research groups, and is known to affect CO formation during meiosis, is the nutrient composition of the soil in which wheat is grown. To assess the effect of nutrient concentration on homologous and homoeologous CO frequency in meiotic metaphase I, we added a modified Hoagland solution to the soil. The treatment significantly increased the number of ring bivalents between homologues in wheat and between homoeologues in the wheat-rye hybrids lacking *Ph1*, causing an increase in the CO frequency in meiosis. Nowadays, we are seeking the responsible nutrient (or nutrients) of this increase and effects of other environmental factors such as temperature, photoperiod and soils in the CO frequency.



P 234 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Comparative proteomics of stress-sensitive and stress-tolerant wheat and barley genotypes in response to abiotic and biotic stress factors using gel-based proteomic approaches

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Key message: Abiotic stresses induce profound alterations in plant cellular environments; however, plants actively respond to stress by adjusting their metabolism and regulatory processes to altered conditions.

Abiotic stresses induce an active plant response resulting in stress acclimation and enhanced tolerance. Two-dimensional differential gel electrophoresis (2D-DIGE) approach represents a complementary method to gel-free approaches and provides a visual representation of plant proteome based on pI and MW values. 2D-DIGE analysis also enables protein relative quantification leading to an identification of the protein spots revealing an enhanced abundance in stress-treated varieties which could be further tested as potential markers of stress tolerance. Summary of the major results of our team aimed at 2D-DIGE analyses of proteomes in stress-treated wheat and barley varieties is provided including studies aimed at wheat and barley proteome response to cold, drought, and salinity. Attention is paid to the proteins revealing differential abundance between stress treatments or genotypes revealing differential stress response such as spring and winter genotypes subjected to cold. The role of gel-based proteomic analysis in understanding plant stress response and acquisition of plant stress tolerance is discussed. The results of proteomic analyses are interpreted with respect to other physiological data such as parameters related to stress tolerance (LT50), phytohormone levels, water regime-related characteristics (water saturation deficit, osmotic potential), and others. Comparative proteomic analyses reveal that stress treatments induce relatively fewer alterations in proteomes of stress-tolerant genotypes when compared to stress-sensitive ones due to larger disturbances in cellular homeostasis. Therefore, tolerant genotypes can more efficiently remove reactive oxygen species (ROS) and other toxic metabolites and manage to adjust energy metabolism to altered conditions. They can thus acclimate to stress even in sensitive metabolic processes such as photosynthesis with minimum harm to cellular structures. Thus, differences in regulatory proteins were found between tolerant and sensitive plants under stress with tolerant plants revealing proteins associated with maintenance of stress tolerance and active cell growth and division while sensitive plants revealing stress damage as indicated by enhanced levels of programmed cell death (PCD)-related proteins.



P 236 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Allele distribution of *PPD-B1* and *PPD-B1* photoperiod sensitivity genes, and their effects on heading in wheat (*Triticum aestivum* L.)

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Key message: Detailed knowledge on the physiological and genetic factors influencing the start and length of the flowering period could contribute to breeding genotypes with better adaptation to present and future changes in the environment.

Flowering time is one of the most important adaptive characteristics of plants. The timing and duration of this plant developmental phase depends on the genetic background, on the environmental conditions (mainly temperature and photoperiod) and on their interactions. In wheat, the most important genes regulating photoperiod sensitivity are *PPD-A1* (2A), *PPD-B1* (2B) and *PPD-D1* (2D). In the case of *PPD-D1* a large deletion in the promoter region of the PRR gene results in insensitivity, while in the case of *PPD-B1* copy number variations stand behind the level of insensitivity. In this study our major aims were (i) to characterize a collection of 535 wheat germplasm, collected from Europe, for allelic variation at the *PPD-B1* and *PPD-D1* photoperiod genes using gene specific molecular markers and (ii) to evaluate the possible interactive effects between the alleles of this gene and two developmental phases (DEV49 and DEV59) in field experiments (2011, 2012). In the case of the *PPD-B1* gene, there are just three countries in the European region (France, Italy and Hungary), where all the copy number variations (1, 2 and 3 copies) of *PPD-B1* gene could be detected in the germplasms examined. Genotypes with 3 copies of the *PPD-B1* gene was the earliest, the ones with 2 copies the latest, while genotypes with 4 copies were more or less intermediate. In the eastern, southern and south-eastern regions of Europe, the photoperiod-insensitive allele of the *PPD-D1* gene were more frequent, while in Western Europe the photoperiod-sensitive allele of this gene was more common. In Central-Europe the photoperiod insensitive and sensitive alleles of the *PPD-D1* gene occurred to similar frequencies. Under the environmental conditions around Martonvásár, Hungary, *PPD-D1* had the largest genotypic effect on plant development under field grown conditions and the insensitive allele resulted in fastened plant development in both years. In spite of the largest contribution of *PPD-D1* allele type to the phenotypic variance, its overall effect was only 3-4 days difference in Central-Europe in both years, compared to the 7.8 day difference identified between these two alleles in the world wide wheat collection studied under field conditions in UK (Wilhelm et al. 2013).

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P 238 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Light quality- and quantity-dependent redox control of metabolism in wheat

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Key message: Changes in light intensity and spectrum affected growth, photosynthesis and the redox environment. Adjustment of several redox-responsive transcripts and metabolites ensured the adaptation to the new environmental conditions.

The redox-dependent adjustment of metabolism to changes of light conditions was studied in wheat. Seedlings were grown either at different light intensities (low, normal or high) or at different light spectrums (enrichment with blue, red or far-red components) in hydroponics for two weeks. The fresh weight of shoots was greater and their length was smaller at high light intensity compared to the lower one. Changes of the spectrum did not affect the growth. The photosynthetic electron transport rate (ETR) increased with increasing light intensity. ETR was also increased if the spectrum was enriched with blue component (higher energy) and decreased if the proportion of red and far-red light (lower energy) was high. Changes in photosynthesis led to the alteration of the glutathione-dependent redox environment. The size of glutathione pool (reduced + oxidised, GSH+GSSG) and the ratio of glutathione disulphide (GSSG) increased with increasing light intensity both in the roots and leaves. If the ratio of blue and red light was 1:1, the size of glutathione pool and the GSH/GSSG ratio was greater in the leaves and smaller in the roots compared to the values detected at a higher ratio of either of blue or red light. Changes in light quality and quantity modified the transcription of several redox-responsive genes identified by transcriptome analysis of H₂O₂-treated wheat. The expression of the genes encoding an auxin-responsive protein and UDP-glucuronate-4 epimerase was greater, while the transcript level of the genes of a nucleoredoxin and beta-carotene isomerase was lower at low light intensity compared to the values detected at high light. Higher red/blue light ratio resulted in greater expression of the genes of auxin-responsive protein and nucleoredoxin than the lower one. The transcription of UDP-glucuronate-4 epimerase was the greatest if the red/blue ratio was 1:1. The activity of nitrate reductase was greater at higher light intensities than at lower ones and at high red/blue ratio compared to the other spectrums. Thus, light condition-dependent changes can be also assumed during the planned examination of free amino acid and polyamine concentrations. Taken together, alterations in light intensity and spectrum modify growth because of the redox-dependent re-programming of metabolism. The planned investigation of light-dependent subcellular distribution of GSH will give deeper insight into this regulatory mechanism.

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P 240 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Can photoperiod sensitivity limit durum wheat adaptation to changing environments?

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Key message: Allelic combinations at *Ppd-1* loci reducing time to flowering were associated with increased HI and yield on a range of northern environments enlightening the world widespread of photoperiod insensitive germplasm.

Wheat adaptation to changing environments will be crucial for future food security under a climate-change scenario. Breeding strategies seeking to achieve large genetic gains will depend on the knowledge of both environmental and genetic yield-limiting factors in order to optimize the use of inputs by limiting the negative effects from stresses. This can only be attained by growing varieties with a flowering time suited to the environmental conditions. A population of spring durum wheat genotypes, involving different allelic combinations for *Ppd-A1/Ppd-B1* loci was jointly developed by IRTA and CIMMYT and grown on eight environments at latitudes from 19°N to 41°N. The number of days to flowering was strongly affected by the latitude. The attainment of potential yield was constrained due to the negative effect of environmental variables on yield components. A combination of minimum temperatures during growth and daylength after flowering limited the number of grains per unit area, while insufficient radiation during grain filling limited kernel weight (Villegas et al. 2016). Temperatures during grain filling strongly affected the balance between the contribution to yield of photosynthesis and the remobilization assimilates accumulated in vegetative organs prior to flowering. The phenotypic expression of alleles conferring photoperiod insensitivity at *Ppd-A1* increased at sites with average daylength from emergence to flowering lower than 12 h. The effect of the allele conferring photoperiod sensitivity at *Ppd-A1* was stronger than that at *Ppd-B1* (*Ppd-A1b*>*Ppd-B1b*). The effect of photoperiod insensitivity alleles was classified as GS-100>GS-105>*Ppd-B1a* (Royo et al. 2016). Cultivars carrying alleles *Ppd-A1b* and/or *Ppd-B1b* enlarging time to flowering produced more biomass at flowering but it did not result in superior yields. The contribution to grain filling of current photosynthesis after flowering was enhanced in early-flowering genotypes, which consistently had superior yields. Our results could provide genetic and physiological bases underlying the widespread success of photoperiod insensitive germplasm in many parts of the world.

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P 242 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Agro-morphological variability in Iranian durum wheat landraces under highland cold rainfed conditions

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Key message: In study of 269 Iranian durum landraces under cold and dryland conditions, it was found that genetically contrasting landraces would facilitate screening for high recombination and, ability to breed more adapted varieties for highland rainfed areas.

Among the Mediterranean ecosystems with exceptionally high number of endemic plant, Iran presents a great diversity of environments and is well known for its rich diversity of durum wheat (*Triticum turgidum* L. var. *durum*). The knowledge about the extent of variability, the distribution and the relationship between descriptors within local germplasm collection are a high value for the improvement and the efficient genetic diversity maintenance and utilization of plant species. The objective of this study was to evaluate the agro-morphological variability in a set of Iranian durum wheat germplasm collection maintained in the National Gene Bank of Iran. The 273 durum wheat accessions comprising 269 landraces and 4 improved varieties (as checks) were planted under cold and rainfed field condition. The study was carried out in DARI (Dryland Agricultural Research Institute) experimental research station at Maragheh, Iran (37°15' N, 46°15' E, 1730 m a.s.l.), during the cropping season 2011-2012. The experiment was laid out as an augmented block design. The recorded agro-morphological characters were cold tolerance, growth habit, days to heading, agronomic score, days to physiological maturity, grain filling period, plant height, thousands kernel weight and grain yield. Principle component analysis revealed that the first PCA explained 35% of the total variation where days to heading and maturity had more effects. The second PCA could explain 28% of variation where seed weight had the highest impacts. Grain yield had positive correlation with cold tolerance, growth habit, plant height and kernel weight, but there was negative correlation between grain yield and days to heading and maturity. Stepwise regression found that the combination of cold tolerance, days to maturity and kernel weight were the most important traits related to yield in the studied genotypes. It can be concluded that the existence of great variability among Iranian durum landraces is potentially useful in durum wheat breeding programs. Cold tolerance, early maturity and grain weight traits could be selection criteria in breeding programs to obtain varieties with higher yield in cold rainfed areas. The results of this study will support efforts of conservation and utilization of landraces in durum wheat breeding programs.



P 244 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Effects of ambient temperature in association with photoperiod on phenology in wheat (*Triticum aestivum* L.)

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Key message: Ambient temperature is an important environmental stimulus of the genetic regulation of flowering, determining the beginning and the rate of intensive stem elongation in wheat

In addition to its role in vernalization, temperature is an important environmental stimulus in determining later plant growth and development. The physiologically optimal temperature can be defined as the range in which the plant growth and development are proceeding at maximal rate but there are no strong negative effects on plant biomass production. Temperatures extending beyond (heat stress) or below (cold stress) of this range may severely affect plant fitness and survival. The majority of research to date has focused on understanding plant responses to these extreme temperatures, with limited attention directed to the dissection of the molecular mechanisms underlying plant responses to changes in the temperature range between the two extremes. We used factorial combinations of two photoperiods (12 and 16 h) and three temperature levels (11, 18 and 25°C) to study the temperature responses of 19 wheat cultivars with established genetic relationships. In this range, temperature practiced more significant effects on plant development than photoperiod, with strong genotypic components. Photoperiod insensitive wheat genotypes were relatively insensitive to temperature as well. When their vernalization requirement was saturated their development was not affected or was accelerated by higher temperatures at both long and short photoperiods. The photoperiod sensitive types however under non-inductive condition (12 h) were strongly delayed by higher temperature. This was already evident at the inductive photoperiod when their vernalization requirements were not completely saturated. The effect of temperature on plant development was not proportional; it influenced the process of stem elongation to the largest extent. Parallel to the higher ambient temperature levels the lag period between first node detectable and the beginning of intensive stem elongation lengthened significantly in the temperature sensitive genotypes. As the lag phase increased the disruption between the phyllocron and the stem elongation became more pronounced which was then the primary cause of the delayed heading.

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A novel wheat bZIP gene *TabZIP14* participates in salt and freezing tolerances in transgenic plants

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Key message: *TabZIP14* could function as a positive regulator in response to ABA and abiotic stresses.

The group C bZIP TFs involve in various biological processes, such as regulating seed storage protein production, responding to pathogen challenge and abiotic stress. However, our knowledge on the abiotic functions of group C bZIP genes is still poor in wheat. Here, we report the isolation and function analysis of a novel *TabZIP14* gene. The gene is a member of group C bZIP TFs, which encodes a nuclear localized protein. A transactivation assay showed that *TabZIP14* functions as a transcriptional activator and was capable of binding the ABRE *cis*-element in yeast. RT-qPCR analysis revealed that *TabZIP14* is expressed in different tissues. Expression of the *TabZIP14* gene is strongly induced by salt, cold, PEG and exogenous abscisic acid (ABA) treatments. Furthermore, *Arabidopsis* plants of overexpressing *TabZIP14* showed enhanced tolerance to salt, freezing stresses and ABA sensitivity. Overexpression of *TabZIP14* results in up-regulated expression of some abiotic stress-related genes and changes in several physiological indices. These results suggest that *TabZIP14* could function as a positive regulator in response to ABA and abiotic stresses.



P 248 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Modelling winter survival: an interactive tool

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Key message: Introduction of an interactive web application that simulates over-winter cold-stress responses of cereals. The tool allows users to model development and survival of different genotypes across diverse climatic conditions.

Successful adaptation of a crop species is dependent upon programming critical growth stages so the plant can optimize its response to environmental conditions during the growing season. The ability of winter cereal genotypes to survive over-winter low-temperature stress is an example of an adaptation that allows the plant to position itself to thrive when conditions are favorable for growth and development. Winter survival is dependent on environmental responses that affect just about every measurable morphological, physiological, and biochemical characteristic of the plant. These changes are determined by complex genotype×environment interactions that are not clearly understood. Simulation models offer a constructive method for advancing our understanding of these complicated plant responses in a so-called ‘systems approach’. In addition to providing a framework for the integration of the information accumulated from detailed physiological, genetic and genomics studies, a well-designed simulation model facilitates the investigation of production risks, cause-and-effect processes, and the evaluation of genetic theories. We have extended our winter survival model to allow researchers to independently upload soil temperature data and change model parameters to simulate cultivar differences and environmental conditions that may be of special interest. In addition to encouraging more widespread use, opening access to interested researchers will serve as a means of model testing and validation, which will in turn allow further development and refinements. This will hopefully lead to increased prediction accuracy over a wider range of conditions and a better understanding of the dynamic interactions involved in over-winter survival of cereal crops.



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Are the degrees of winter-hardiness of wheat varieties affected by their geographical and/or time period of origin?

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Key message: The average winter hardiness potential of wheat varieties of any respective country can serve as a breeding goal, or as an optimum of winter hardiness for that country.

Since the 1970s, the winter hardiness of different wheat varieties has been periodically evaluated using the wooden-box 'provocation' method at Crop Research Institute in Prague. This method is based on the wintering of plants in wooden-boxes placed at different heights above the ground during the entire winter (standard heights are 0 and 50 cm). The differences among the winter survival rates of the samples have been obtained every year; therefore, a database of the winter survival of several thousands of wheat varieties is available. Processing of these data by the 'classification of varieties' method enables a comparison of all samples on a 9-point scale which describes a variety's winter hardiness potential (WHP; 9 = very high, 1 = very low). We compared the WHP of 1550 winter wheat varieties here. We studied how the distribution of WHP and average (avgWHP) of varieties of wheat are affected by their geographical origin from 16 European countries. Moreover, within these groups, we divided the varieties according to their time of origin into the three subgroups: (i) obsolete landraces and varieties, (ii) advanced varieties from the second half of the 20th century, and (iii) most recent varieties registered in the 21st century. We have shown that the size of the avgWHP value of wheat varieties was primarily affected by their geographical origin. A significant decrease in the values of avgWHP was detected moving in Europe from the northeastern countries to the southwestern countries. This trend could be caused by the selection of varieties associated with different hardness of winters in particular countries (regions). A smaller decrease in avgWHP was observed when comparing varieties that originated in different periods; however, the avgWHP of varieties from different countries still showed some differences. For example, the decrease in avgWHP from 4.9 to 2.6 for obsolete to recent varieties from France, or the minimum changes in avgWHP for the varieties of Czech and Slovak origin over the 20th century (5.5-5.8). These differences could be explained by the differences in climate change during the last two centuries and/or by breeding for winter hardiness in some countries. In conclusion, we can summarize that the avgWHP of wheat varieties of any respective country can serve as a breeding goal, or as an optimum of winter hardiness for that country.



P 252 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Transcriptome profiling of crowns during seasonal cold acclimation revealed similarities and differences in transcriptional regulations in winter and spring wheat

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Key message: Global transcriptome profiling and network analysis revealed conserved and unique transcriptional regulations in winter and spring wheat during cold acclimation and vegetative to reproductive transition under field conditions.

Cold acclimation and timing of the vegetative to reproductive transition (VRT) are survival mechanisms that enable fall-planted wheat to acquire freezing tolerance and survive winter stresses. Both mechanisms are highly integrated and regulated by interrelated genetic systems, making it difficult to separate causes and effects to environmental cues that signal seasonal changes and the onset of cold stress. We employed the winter habit genotypes 'Norstar' (*vrn-A1/vrn-A1*), spring 'Manitou' (*Vrn-A1/Vrn-A1*), and their reciprocal near isogenic lines (NIL), spring Norstar (*Vrn-A1/Vrn-A1*) and winter Manitou (*vrn-A1/vrn-A1*), to investigate the transcriptional reprogramming in developmental and cold signals under autumn field conditions at Saskatoon, Saskatchewan, Canada. Crown transcriptome data and LT50 (temperature at which 50% of the plants are killed by LT stress) were determined for plants sampled at regular intervals from the recommended time of planting in the fall (2 cm soil temperature 17°C) to just before soil freeze-up at the start of winter (soil temperature 2°C). A total of 151 672 genes were identified, in which 16 674 were differentially expressed (likelihood-ratio test, FDR<0.01) across all treatments after eliminating environmental differences between years. Hierarchical clustering analysis was performed to separate genotype differences (11 097 genes) and cold responses (5577 genes). Specific effects of *VRN-A1* in Manitou and Norstar background were identified through comparisons between Manitou and winter Manitou as well as Norstar and spring Norstar. To further gain a system-level understanding of the relationship between gene expression changes and cold acclimation status, we applied weighted gene expression network analysis (WGCNA) to examine potential similarities and differences between four wheat lines. In total, we identified 14, 11, 11, and 13 modules in Manitou, winter Manitou, Norstar and spring Norstar respectively. Seven modules overlapped significantly between RIL lines which suggested a high degree of between-genotype module preservation. On the other hand, variety-specific modules were also identified. The similarities and differences between modules in each variety were further assessed and provided novel insights into the regulatory mechanisms during cold acclimation and VRT in wheat.



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Wheat breeding programs designed for extremes in winter weather

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Key message: A large number of interacting factors determine winter survival of wheat. Many new screening tools have been identified, but field performance still remains the definitive test for regional winter adaptation.

The ability of a winter wheat genotype to survive, grow, and develop during the winter is a primary factor determining its area of adaptation and distribution for commercial production. As a result, modification of the genetic systems that determine low-temperature (LT) responses is one of the first objectives for breeding programs. This can present difficult challenges because LT tolerance is determined by a complex, developmentally regulated, and environmentally induced genetic system that is expressed in anticipation of and during exposure of plants to low and freezing temperatures (Fowler et al. 2014). In this system, transition from the vegetative to reproductive growth stage is a critical switch that initiates down regulation of the LT stress response. This complicating factor means developmental genes (vernalization, photoperiod, etc.) determine the duration of expression of LT tolerance conferring genes while the rate of LT acclimation is determined by genotype dependent expression levels of these genes. These interactions among the early stages of phenological development and LT tolerance gene expression are of critical concern in variety development programs for production in high winter stress environments. While perhaps not as obvious, they must also be emphasized in breeding programs targeting adaptation in less severe winter environments, where they bring a whole new level of complexity. Recognition of these complicated relationships has allowed us to design strategies to optimize selection for regional differences in over-winter LT extremes. Molecular studies have identified QTL for a few major genes and a number of small effect candidates; however, a large amount of unexplained heritability remains (Fowler et al. 2016). Indirect selection methods and a wide array of new genetic tools (Miller et al. 2010) offer promising ways of expanding the strategies, but screening under field conditions is still required to identify agronomically superior lines adapted to the extremes in LTs expected for different wheat production regions of the world.

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Light-quality and temperature dependent regulation of the freezing tolerance in cereals

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Key message: Lowering red/far-red ratio in the incident white light affects freezing tolerance by temperature dependent manner.

Cold acclimation is a relatively slow, adaptive response during fall, when the temperature, day length and light intensity decrease gradually. The proper integration of light and temperature is vital for acclimatization to reach the appropriate frost hardiness level to survive the freezing in winter. During cold acclimation many molecular processes are conserved in the dicot and monocot lineages. One of the best examples of this phenomenon is the central role of the C-repeat Binding Factor or Dehydration-Responsive Element Binding Factor (CBF/DREB1) regulon in the cold acclimation process both in *Arabidopsis* and in cereals. Light-quality regulation of freezing tolerance has been described in *Arabidopsis*, and its interaction with *CBF* genes was also published (Franklin & Whitelam 2007). Repeating this experiment using winter cereals by lowering the red/far-red ratio in white light at 15°C, it was possible to induce *CBF14* expression and increase the freezing tolerance in winter wheat and barley genotype, but not in einkorn with relatively low freezing tolerance (Novak et al. 2016). It is well established that the high level of the *CBF14* transcription factor in wheat and barley is important for winter survival. Plants are sensing the changes in light quality with photoreceptors. From them we studied the expression profile of *PHYA*, *PHYB* and *PHYC*. Low red/far-red ratio enhances the expression level of *PHYA* in all three species, but induces *PHYB* expression only in einkorn. Based on our results, a model is proposed to illustrate the positive effect of *phyA* and the negative influence of *phyB* on the enhancement of freezing tolerance in cereals in response to spectral changes of incident light. Our model was confirmed in a very recent paper by Wang et al. (2016) on tomato.

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P 258 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Identification and characterization of the expressed wheat crown miRNAs during cold acclimation and vernalization under field conditions

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Key message: This work provides valuable resources of expressed wheat miRNAs that will help in elucidating the regulatory mechanisms involved in freezing tolerance and the vegetative to reproductive transition.

MicroRNAs (miRNAs) are small non-coding RNAs that play important regulatory roles in plant development and abiotic stress responses. Recent NGS studies have expanded the number of miRNAs identified in wheat, but the limited experimental design did not allow a clear understanding of their role in freezing tolerance adaptation and the vegetative to reproductive transition. To circumvent this limitation we designed a large multi-year (2010 and 2013) autumn field experiment with four genotypes, the winter habit 'Norstar' (*vrn-A1/vrn-A1*), spring 'Manitou' (*Vrn-A1/Vrn-A1*), and their reciprocal near isogenic lines (NIL), spring Norstar (*Vrn-A1/Vrn-A1*) and winter Manitou (*vrn-A1/vrn-A1*). Crown tissues were sampled from five time points between the beginnings of September and November in Saskatoon. miRNA-seq of 80 libraries were used to identify conserved, novel and differentially expressed miRNAs. Here, we report the accurate prediction of 706 miRNA families including 217 well described miRNA families from miRBase. Gene Ontology (GO) functional analysis of their predicted targets indicates that they function as diverse regulatory factors, including MADS-Box, GRF, SCARECROW-like, AP2-like, MYB, NAC, class III HD-Zip protein, DEMETER-like, and SPLs. They also may play a role in the regulation of lipid and sugar metabolism, auxin signaling and the epigenetic regulation. The results will help identify the miRNAs targeting key transcripts involved in both freezing tolerance and the vegetative to reproductive transition. This may lead to improving these two important traits in cereals.



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Overexpression of ERF1-V from *Haynaldia villosa* can enhance the resistance of wheat to powdery mildew and increase the tolerance to salt and drought stresses

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Key words: Abiotic stress, AP2/ERF, powdery mildew, transcription factor, transgenic wheat

AP2/ERF gene family has been found to be widely involved in the biotic and abiotic stress regulation. *Haynaldia villosa* (VV, 2n=14), a wild species of wheat, is an excellent gene pool for wheat improvement. It has been identified to confer high resistance to several wheat diseases and high tolerance to some abiotic stresses. In this study, *ERF1-V*, an ethylene-responsive element-binding factor gene of AP2/ERF gene family from wild *H. villosa*, was cloned and characterized. Sequence and phylogenetic analysis showed that *ERF1-V* was a deduced B2 type ERF gene. *ERF1-V* was first identified as a *Bgt* up-regulated gene, and later was found to be also induced by drought, salinity and cold stresses. As to the responses to hormones, it was up-regulated by ethylene and ABA, but was down-regulated by SA and JA. Overexpression of *ERF1-V* in wheat could improve the resistance to powdery mildew, salinity and drought stresses. The chlorophyll content, MDA content, SOD and POD activity showed significant differences between the recipient Yangmai158 and the transgenic plants after salt treatment, and the expression level of some stress responsive genes also showed differences after drought or salinity treatments. Although *ERF1-V* was activated by the constitutive promoter, the agronomic traits, including flowering time, plant height, effective tiller number, spikelet number per spike and grain size, were not changed significantly. *ERF1-V* is a valuable gene for wheat improvement by genetic engineering.

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P 262 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Drought tolerance studies of advanced wheat (*Triticum aestivum* L.) genotypes

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Drought tolerance studies of sixteen wheat genotypes during the year 2015-16 including four checks were conducted at Tando Jam, Pakistan. Three irrigation treatments were used, i.e. treatment-I (zero), treatment-II (two irrigations) and treatment three (four/full irrigations). Each treatment consisted of six rows of 4 m length in a randomized complete block design with three replicates. In treatment-I genotype V3-10-32 (1.35 kg/plot) had the highest grain yield, probably due to its early heading date and long spike length. In treatment-II, genotype V3-10-12 (1.87 kg/plot) had the highest grain yield which could be probably explained by a higher number of grains with increased main spike yield. In treatment-III genotype V3-10-29 (2.13 kg/plot) had the highest grain yield which can be explained by the early heading date and the higher number of grains per spike. Genotype V3-10-32 (1.68 kg/plot) had the highest average grain yield across all treatments. Parental genotype Bhattai (1.28 kg/plot) had the lowest average grain yield across treatments. With respect to treatments, treatment-III had significantly the highest average grain yield (1.86 kg/plot), followed by treatment-II (1.56 kg/plot) and treatment-I (0.94 kg/plot). Generally, treatment-I had the poorest performance for all investigated traits, whereas treatment-III had the highest performance for all traits.



P 264 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Proteomic analysis of proteins responsive to drought and low temperature stress in a hard red spring wheat cultivar

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Key message: Drought and low temperature stress caused a significant differential expression of proteins with peptide sequence similarity to HMW-GS DY12, PW212, PC256, HMW-GS DX5 and DY10, which will affect baking quality.

Drought stress is becoming more prevalent with global warming, and has been shown to have large effects on gluten proteins linked to wheat bread making quality. Likewise, low temperature stress can detrimentally affect proteins in wheat. This study was done to determine the differential expression of high molecular weight (HMW) gluten proteins in a drought and low temperature stressed high quality hard red spring wheat cultivar (PAN3478), against a control. The three treatments were applied in the greenhouse when the main tillers of each pot were at the soft dough stage, on 15 pots per replication, three replications and three plants per pot for each treatment. Seed of main tillers were bulked and randomly sampled. Removal of gliadins was done with 50% (v/v) 1-propanol. The HMW proteins were then extracted and separated by 2-dimensional gel electrophoresis. Gels were analyzed with SameSpots Progenesis (version 4.6.1.218). The protein spots that had *p* values lower than 0.05 and fold value equal to or greater than 1.2 were considered significantly differentially expressed. Spots were excised from the gel, digested with trypsin and analysed by mass spectrometry. The proteins were identified by matching the spectra to theoretical data from the Swissprot protein database. There was a 1.3 to 1.8 fold change in 19 protein spots due to the cold treatment. The drought treatment caused 1.3 to 3.8 fold change in 20 spots that were significantly differentially expressed. Two spots, one with matching peptide sequences to HMW glutenin proteins DX5, DY10 and PC256, and one to HMW glutenin protein PW212, were differentially expressed under both drought and cold stress. Other proteins differentially expressed under cold stress included proteins with similar peptides to alpha/beta-gliadin A-IV, fructan 1-exohydrolase w3, a number of HMW-GS P212 and HMW-GS DY12 proteins and HMW-GS DY10, chloroplastic Nad(P)H-quinone oxidoreductase subunit 2 A, alpha/beta-gliadin and low molecular weight-GS PTDUCD1. Under drought stress ATPase, HMW-GS PW212, HMW/GS (HMW subunit 12) and DY10, Ubiquitin and Eukaryotic translation initiation factor isoform 4G-2 were differentially expressed. The HMW glutenins are known to have a large effect on baking quality, and clearly protein spots with similar peptide sequences with especially HMW-GS DY12, PW212, PC256, HMW-GS DX5 and DY10 were responsive to cold and drought stress, and could directly influence baking quality.



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Identifying superior durum wheat genotypes (*Triticum durum* Desf.) under rain-fed conditions

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Key message: An important objective of wheat improvement programmes is to breed high-yielding varieties tolerant to heat- and drought, with high yield stability.

Climate scientists project (Urrutia & Vuille 2009) that global warming will raise temperatures and reduce rainfall in many parts of the world, not only decreasing agricultural productivity but also inducing changes in plant life. Nowadays drought is often associated with heat stress in late spring, which may occur increasingly earlier and more frequently, affecting the plants in earlier phenophases. An important objective of wheat improvement programmes is to breed high-yielding varieties tolerant to heat- and drought, with high yield stability. Analyses from multiple years allow us a comprehensive, comparative understanding of the evaluated germplasm and the environments where the trials were conducted. The present study was carried out to (i) evaluate the performance of elite varieties and landraces of durum wheat under different water regimes, aimed at enhancing productivity and determining the heritability of the examined traits, and (ii) to identify superior genotypes better adapted to drought conditions, therefore being useful for breeding germplasm tolerant to drought stress. A total of 100 accessions of spring durum wheat (*Triticum durum* Desf.) were evaluated under rain-fed and well-watered conditions in the nursery of the Agricultural Institute (MTA-ATK) at Martonvásár, Hungary (2011-2013). The experiments were laid out in an unbalanced, incomplete alpha lattice block design with three replications for the rain-fed experiment and two for the well-watered one. Considerable genotypic variability was detected by variance analysis, and the effect of irrigation was highly significant for all the traits in all the years. The trait with the lowest broad-sense heritability was test weight (0.44), while heading date (0.89), thousand-grain weight (0.85) and the protein content (0.85) exhibited high heritability values. For grain yield a moderate level of heritability (0.53) was identified across the three years due to the strong yearly genotype-by-environment interactions. PCA revealed that grain yield (t/ha) positively associated with the fertile tiller number, chlorophyll content values at early waxy ripeness stages (Z83) and plant height parameters. Based on biplot analysis BLK-2 and Cimmyt73 proves to be the best durum cultivars in terms of yield, genotypes Aghrass-1 and Ouaserl-1 preceded them with their good yield stability. The results suggested, that the balanced morphological, physiological parameters and yield components are more important than selecting just for high yield to develop cultivars more tolerant to environmental stresses.

Acknowledgements

Funding from the EU FP7-244374 DROPS and EU BONUS 12-1-2012-0017 projects is gratefully acknowledged.

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Effect of drought stress on lipid peroxidation level and ascorbate peroxidase activity in the inter-varietal single chromosome substitution lines of wheat (*Triticum aestivum* L.)

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Key message: Six hours of water deficit cause decrease of lipid peroxidation level in wheat tissue. Chromosome 3A is linked with strong increase of ascorbate peroxidase activity during quick response to drought.

Drought is one of the major factors limiting crop production worldwide. Because of climate change, nowadays water deficit has started to be the biggest challenge for plant researchers and breeders. Drought induces the accumulation of reactive oxygen species (ROS), what can cause oxidative damage, plant growth inhibition and grain yield decrease. One of the main cellular components susceptible to damage by ROS are lipids (by peroxidation of unsaturated fatty acids in membranes). In plant cells ROS are rapidly detoxified by various mechanisms based on different classes of enzymes (catalases, peroxidases, superoxide dismutases, etc.). The aim of presented study was to investigate the effects of drought stress on changes in the lipid peroxidation level, as well as ascorbate peroxidase (APX) enzymatic activity in leaves of the common wheat (*Triticum aestivum* L.) substitution lines. In examinations population of the 18 Janetzki Probat (JP) × Saratovskaya 29 (S29) (drought sensitive and tolerant, respectively) single chromosome substitution lines were used. Seedlings of analyzed wheat plants were growing 5 days in the MS medium in controlled condition of hydroponic culture. The drought stress was induced by 10% polyethylene glycol (PEG 6000) addition to the medium. After six hours of exposure to stress, the leaves were harvested for analysis. As a control, plants growing in the medium without PEG were used. The level of lipid peroxidation was estimated by thiobarbituric acid (TBA) test described by Uchiyama & Mihara (1978). Ascorbate peroxidase activity in tissue was assayed from the decrease in absorbance at 290 nm as described by Nakano & Asada (1981). Majority of tested genotypes showed lower level of lipid peroxidation (LPO) after 6 hours of drought condition in comparison to respective untreated forms. The lowest LPO level was observed for S29 and lines with substitution of 3A and 6A chromosomes. Results obtained for APX activity analysis showed that drought stress resulted in its upregulation in drought tolerant parent (S29) and in 9 substitution lines. The strongest, over twofold, activity increase was observed for line with 3A chromosome substitution. For drought sensitive parent (JP) and 5 substitution lines (4A, 5A, 5B, 3D and 5D) strong decrease of APX activity after stress factor application was noticed.

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Influence of the water deficit on proline accumulation in wheat (*Triticum aestivum* L.) inter-varietal single chromosome substitution lines

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Key message: Proline content is a factor linked to water deficit tolerance in plants. In wheat, major genetic sources of increased proline accumulation in response to drought are localized on B-genome chromosomes.

Drought is one of the major factors responsible for reduction of global crop production. A physiological level it causes plant growth reduction, reactive oxygen species (ROS) production as well as modification of antioxidant enzymes activity. Emerging ROS are responsible for cellular damage by oxidation of nucleic acids, proteins and lipids. One of the defensive physiological mechanism of ROS quenching in plant cells is based on proline. Proline is known as an excellent quencher for singlet oxygen. Moreover, it has a hydroxyl radical scavenging activity as well. In normal conditions proline provides less than 5% of the pool of free amino acids in plants. During oxidative stress, the proline concentration increases and can reach over 80% of the total free amino acids pool. Literature data indicate, that plants with deficiency of proline present significantly lower stress tolerance (e.g. Nanjo et al. 1999). The aim of presented study was determination of the proline content in the tissue of the wheat inter-varietal single chromosome substitution lines under drought condition. Population of Janetzki Probat (drought sensitive) × Saratovskaya 29 (drought tolerant) substitution lines were used. Seven-day-old wheat seedlings grown in MS (Murashige-Skoog) medium in controlled condition of hydroponic culture were exposed to stress. In presented study, chemically induced osmotic stress caused by the addition of 10% polyethylene glycol (PEG 6000) to the medium was applied. After six hours leaves were collected for experimental analysis. As a control plants growing in medium without PEG were used. Free proline content in tissue was estimated according to Bates et al. (1973) and reported as $\mu\text{mol g FW}^{-1}$. Obtained results revealed, that proline content increase in drought tolerant 'Saratovskaya 29' cultivar under stress conditions was almost twofold higher in comparison to drought sensitive cultivar 'Janetzki Probat' (1.15 vs. 0.65, respectively). For majority of analyzed substitution lines increase of proline content after stress application was noticed as well. The strongest response was noticed for lines with 2B, 3B and 6B substitution. However, for majority of lines D-genome chromosomes substitution resulted in downregulation of proline accumulation in response to water deficit. The strongest negative effect was noticed for 5D substitution line.

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Varietal difference and proteomic response to drought stress in wheat (*Triticum aestivum*)

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Wheat yield must be increased to meet the demand of the growing world population. Drought is a consistent stressful factor impacting yield and quality of wheat in Northern China. Breeding drought tolerant cultivars plays a vital role to minimize losses caused by water stress. Commercial wheat cultivars such as Liangxin 99, Yunhan 618, Luohan 6, Xi-nong 538, Bainong 1306, Jinmai 90, Pubin 151 and core germplasm resources including kangdongzao, Jinmai 47 were screened for seed germination and seedling growth response to osmotic stress after incubating seeds on 25% PEG6000 and 2% NaCl for 10 days. The results showed that Luohan 6, Liangxin 99, Jinmai 90, Pubin 151 and Jinmai 47 are highly drought tolerant, produced higher germination rates, better growth of seedling shoot and root and whist Xi-nong 538, Bainong 1306 are sensitive. High levels of salt tolerance have been obtained with Luohan 6, Kangdongzao, Jinmai 90, Pubin 151 whilst Yunhan 618, Liangxin 99 are sensitive. Wheat plants of Liangxin 99 and Bainong 1306 with contrasting drought response were subjected to drought stress (D) and re-watering (W) treatments and proteomic changes were quantified using label-free mass spectrometry. With Liangxin 99 leaves a total of 2420 proteins were identified with 443 were common and 1483 were specific to D and 494 specific to W. From these differentially expressed (DE) proteins were identified with 134 up-regulated (fold change ≥ 2.0) and 110 down-regulated proteins including heat shock cognate 70 kDa protein 1,2-isopropylmalate synthase A, Protein IN2-1-like protein B, IAA-amino acid hydrolase ILR1-like protein 1 and Delta-1-pyrroline-5-carboxylate synthase. Enrichment analysis indicated that DE proteins are among carbohydrate metabolism, catalytic activity and signal transduction. *DE* genes and SNP molecular markers have been obtained in combination with transcriptome analysis.



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Genetic dissection of QTL conferring drought resistance derived from wild emmer wheat (*Triticum dicoccoides*)

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Key message: QTL conferring drought resistance transferred from wild emmer wheat into wheat cultivars were subjected to fine mapping to reduce linkage drag by identification of the shortest fragments bearing the QTL.

Drought, one of the major factors limiting global wheat production, is expected to increase in severity and frequency in the future, as a result of climate change. The genetic diversity concerning genes conferring resistance to drought or other abiotic and biotic stresses has been depleted due to domestication and modern wheat breeding. Therefore, wild relatives offer a valuable source for improving drought resistance in domesticated wheat. In previous work, QTL regions conferring drought resistance in wild emmer wheat (*Triticum dicoccoides*) have been identified on chromosome 2BS and 7AS and were transferred into elite wheat cultivars. These near isogenic lines (NILs) were shown to have higher productivity under water limited conditions than their recurrent parents. The main target of this project is to reduce linkage drag by narrowing down the size of these QTL-regions and to introgress the shortest fragments bearing drought resistance into Israeli and German elite wheat cultivars. For that purpose, 151 F₇ plants of the original F₆ mapping population (derived from the cross between *Triticum durum* cv. Langdon and *T. dicoccoides* G18-16) were genotyped with the 15K iSelect chip and a new high resolution map with 4118 polymorphic markers was constructed. Intervals of 15.67 (chr. 2B) and 25.62 cM (chr.7B) QTL-regions that were transferred into wheat cultivars were validated in the introgression of the NILs using SNP markers and were selected for fine mapping. The iSelect-SNP markers in the regions of the QTL-intervals were converted into various types of PCR based molecular markers, such as kompetitive allele specific PCR markers (KASP), cleaved amplified polymorphic PCR-markers (CAPS) and simple sequence repeats (SSR). A total of 82 and 159 heterozygous segmental recombinant F₂ inbred lines of QTL-region 2BS and 7AS were subjected to genotyping respectively. Markers revealed perfect co-linearity to the physical map of *T. dicoccoides*. Currently specific F₂ heterozygote recombinants, showing recombination events in the targeted intervals, were selected to screen their F₃ progenies to identify homozygous recombinant plants. Next, F₄ progenies of these plants will be phenotyped under water-limited vs. control conditions and validated for their reduced QTL interval length.



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Phenotypic characterization of French elite germplasm in response to drought stress

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Key words: Agronomic and physiological traits, drought stress, genotype-by-environment interaction

Water deficit is one of the main abiotic stress limiting wheat growth and productivity around the world. European temperate climate seems to be less subject to drought. However, recent studies show that water deficit events have led to reduced yields for at least two decades. Furthermore, many simulation studies have predicted an increase in the frequency and intensity of this abiotic stress in the future. In this context, several French research and breeding programs have devised a trial network for understanding the physiological and genetic bases of response to drought stress. Trials were conducted under optimal and water deficient conditions, during five years for a total of 13 experiments including two trials in field high throughput phenotyping platforms. A panel of 220 European winter elite lines, mostly from French, German and English origins, was screened. Environmental characterization and ecophysiological modelling were performed to identify the timing, intensity and history of stress. This allowed understanding and dissecting the genotype \times environment interaction. Physiological traits as date of earing, plant height or senescence dynamics were followed together with the basic agronomic traits. Visual senescence and normalized difference vegetative index (NDVI) were scored for identifying stay-green traits. In controlled conditions, the panel was also scored for specific traits, such as early root development or heat stress during grain filling. Preliminary analyses of the results show a large diversity for tolerance to drought in this panel. Analyses will be conducted to quantify the genotype \times environment interaction and identify traits correlated to drought tolerance. Selection for these traits will be our main goal to enhance genetic progress of yield in response to optimal and water deficient conditions in wheat.

Acknowledgements

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Improved salinity tolerance and yield in an advanced backcross population of barley

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Key message: A *Hordeum spontaneum* allele confers improved salinity tolerance in barley and increased yield in the field.

Soil salinity leads to significant yield losses worldwide, with barley being more salt tolerant than bread wheat and durum wheat. Salinity reduces shoot growth and yield potential by decreasing tillering and leaf growth and, if high levels of sodium and chloride accumulate, increasing premature senescence of leaves. Identifying the genetic components contributing to salinity tolerance in barley would not only contribute to breeding more salt tolerant barley, but also offers the possibility of transferring similar tolerance traits to wheat. Increasing salinity tolerance could be achieved by improving sodium and chloride exclusion from the shoot and minimising growth reductions in response to soil salinity. To identify genetic components contributing to these tolerance traits, we undertook large scale screenings of wheat and barley mapping populations in a high-throughput phenotyping facility. The Plant Accelerator at the University of Adelaide was used to (i) establish controlled growth conditions under saline soil treatments and to (ii) measure shoot growth rates in response to salinity over time. In addition, leaf samples were taken to establish sodium and chloride levels at completion of the screening experiments. In an advanced backcross population of the South Australian barley cultivar Flagship with a cross of the *Hordeum spontaneum* lines Bargiyyora × Tel shoqet, we identified a strong QTL for sodium exclusion on Chromosome 7H. Advanced backcross lines with the *H. spontaneum* allele at this locus had approximately 30% less leaf sodium compared to lines with the Flagship allele. To test the allele effect in the field, lines differing at the 7H locus were selected for subsequent field trials. The results from two years and multiple locations confirmed the reduced sodium accumulation phenotype, under moderate and high salinity. More importantly, the advanced backcross lines with the *H. spontaneum* allele also yielded higher, with up to 25% yield increase compared to lines with the Flagship allele. Since the tolerance allele is already in advanced backcross material, it offers an ideal starting point for subsequent breeding with work underway to introduce this allele into other elite cultivars.



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Discovery of novel traits and loci for combined drought and heat tolerance

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Key message: We used genome-wide association mapping to identify novel quantitative trait loci for tolerance to combined drought and heat stresses in a worldwide collection of diverse wheat genotypes.

Drought and heat stress often occur together during anthesis and grain filling and cause massive reductions in wheat productivity. Events are unpredictable, largely unmanageable and increasingly frequent in many crop growing regions of the world. These stresses have rarely been studied in combination, although mechanisms underlying increased tolerance to either stress may be mutually exclusive or incompatible. A collection of *Triticeae* accessions from around the world, including landraces, synthetic hexaploids and modern cultivars was analysed following genotyping using the 90K Illumina iSelect markers. Significant associations with quantitative trait loci (QTL) were found when lines were grown first in Australian, rain-fed field conditions and yield, yield components, harvest index and plant phenology were assessed over two years. The responses to combined drought and heat of this association mapping panel have now been investigated in purpose-built, semi-controlled and controlled facilities with the aim of defining new genetic loci to support marker-assisted selection for new, tolerant varieties. Over a more than 90-day flowering period, each genotype was exposed to drought or drought and heat treatment three days post-anthesis in order to uncover novel loci and mechanisms unrelated to flowering time. New QTL and traits for drought and heat tolerance during grain filling, and research into the physiological mechanisms underlying these are reported here.

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Regulatory role of *TaIRE1* mediated *TabZIP60* signaling pathway in heat tolerance of wheat

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Heat stress extensively limits crop growth and causes dramatic yield loss worldwide, particularly for chimonophilous crops, e.g. wheat. It is estimated that global wheat production will fall by 6% for per centigrade temperature increase based on the model prediction. It has been reported that *IRE1* mediated *bZIP60* splicing pathway is conserved and plays a crucial role in the signal transduction and gene regulation in response to endoplasmic reticulum (ER) heat stress (HS) among *Arabidopsis*, rice and maize. However, little information is known about its counterparts in wheat, especially, the biological relevance contributing to HS tolerance. In the present study, we observed that the expression level of *TabZIP60* is significantly up-regulated by HS, and correspondingly, the unconventional splicing of *TabZIP60* also occurs under HS condition, which is likely to depend on *TaIRE1* function. Our investigation indicates attenuation of *TaIRE1* mediated *TabZIP60* splicing pathway by knocking down of either *TabZIP60* or *TaIRE1* results in heat sensitivity in wheat. Interestingly, enhanced heat tolerance was attributed to the overexpression of spliced form of *TabZIP60* (*TabZIP60s*) but not unspliced form (*TabZIP60u*) in *Arabidopsis*. RNA-Seq data revealed 35 differentially expressed genes between WT and *TabZIP60s* transgenic line before or/ and HS treatment, which mainly enriched in 'response to endoplasmic reticulum stress' term indicated by AgriGO software. Moreover, ChIP-qRT-PCR results validated that *TabZIP60s* directly binds to 17 targets including *TabZIP60u*, which forms a positive regulation feedback. It is worth noticing that the protein abundance of *TabZIP60s* was balanced during HS response at post-translational level *via* 26S proteasome pathway. Therefore, our study suggests that *TaIRE1* mediated splicing of *TabZIP60* signaling contributes to heat tolerance in wheat by manipulating expression pattern of ER-related genes.

Acknowledgements

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Histone acetyltransferase GCN5 is essential for heat stress-responsive gene activation and thermotolerance in *Arabidopsis* and wheat

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Key words: *Arabidopsis*, epigenetic modification, heat stress, histone acetyltransferase, *Triticum aestivum*

As a consequence of global warming, high temperatures occur frequently, presenting major environmental challenges with respect to crop growth and reproduction. Over the course of evolution, plants have developed adaptive strategies to survive under conditions of high temperature stress in their environments. To date, several central genes responsible for HS responses have been identified, but the underlying regulatory mechanism remains unknown. Histone modifications are major epigenetic mechanisms that regulate gene expression and plant growth and development. A growing body of research has demonstrated that stress affects histone modifications and that abiotic stress responses require access to certain epigenetic regulators in plants. In this study, we first found that the loss of function of *Arabidopsis* histone acetyltransferase *GCN5* presents serious defects in terms of thermotolerance and considerably impairs the transcriptional activation of HS-responsive genes (Figure 1). Chromatin immunoprecipitation (ChIP) assays indicated that *GCN5* protein is enriched in the promoter regions of *HsfA3* and *UVH6* genes, and that *GCN5* facilitate H3K9 and H3K14 acetylation, which is associated with *HsfA3* and *UVH6* activation under HS (Figure 2). Moreover, we also isolated wheat *TaGCN5*, an ortholog of *Arabidopsis GCN5*. Sequence analyses show that the deduced amino acid sequence of *TaGCN5* is 66% similar to *GCN5*. *TaGCN5* mRNA levels rapidly increased in wheat after heat treatments were applied (Figure 3a). Furthermore, we found that *TaUVH6* expression increased upon heat stress (Figure 3a). Constitutive expression of *TaGCN5* almost fully restored heat-sensitive and other phenotypes of the *gcn5* mutant relative to those of young wild type seedlings and mature plants (Figure 3b,c). These results further indicate that *TaGCN5* functions in similar ways as *GCN5*. We performed qRT-PCR analyses to examine expression levels of the *Arabidopsis UVH6* gene in *35S:TaGCN5 gcn5* plants. The expression of *UVH6* was constitutively increased in *35S:TaGCN5 gcn5* plants relative to the wild type and *gcn5* plants (Figure 3d). These results suggest that *GCN5*-mediated thermotolerance may be conserved in *Arabidopsis* and wheat.

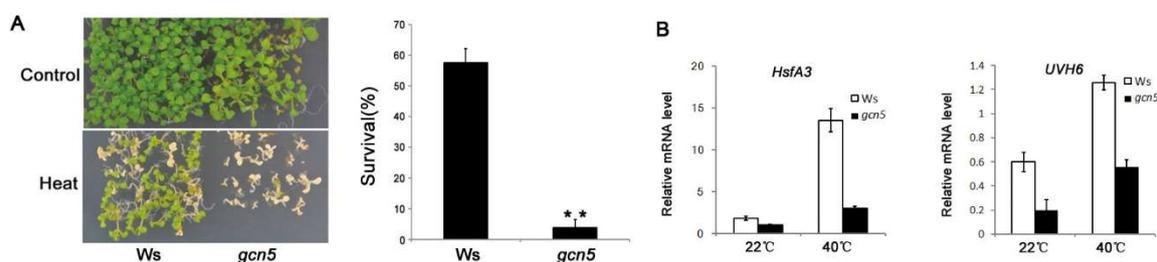


Figure 1: The *Arabidopsis gcn5* mutant displays defective phenotypes under heat stress treatment.

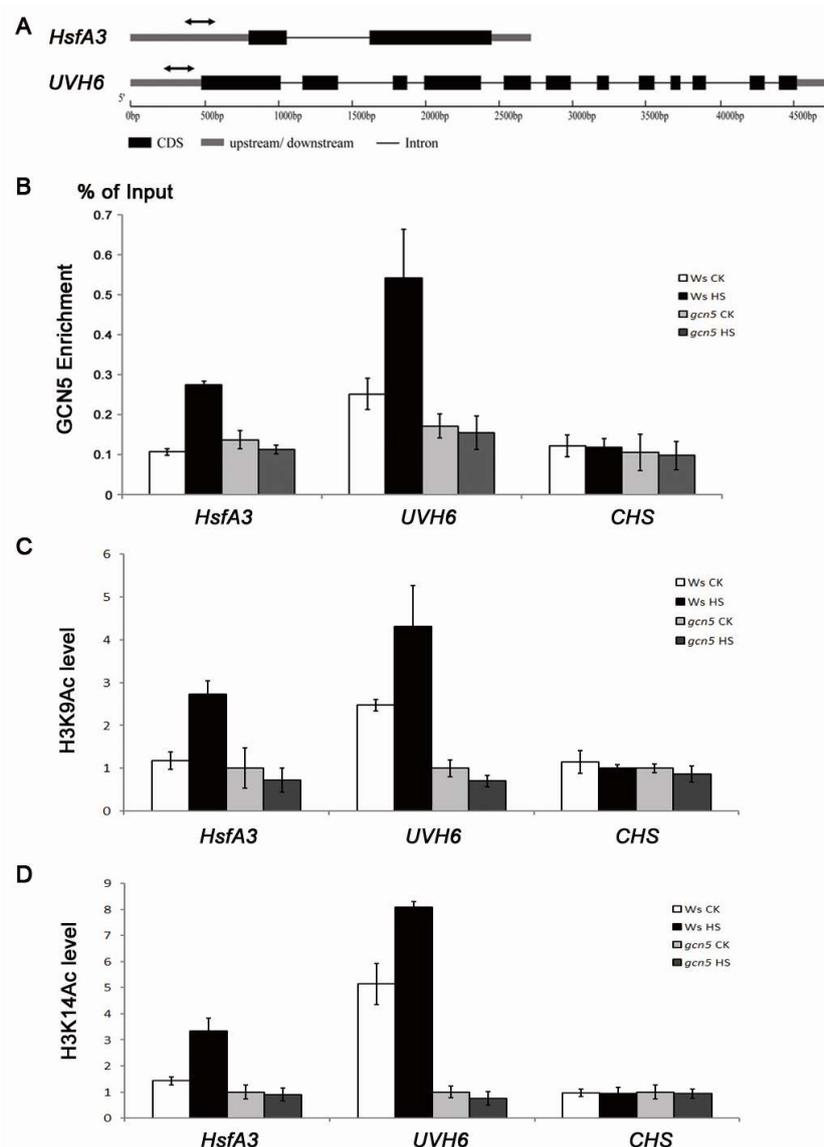


Figure 2: Analysis of GCN5 protein levels, H3K9 and H3K14 acetylation states of the promoter regions of *HsfA3* and *UVH6* genes in *Arabidopsis* wild type (Ws) and *gcn5* mutant plants and in response to HS.

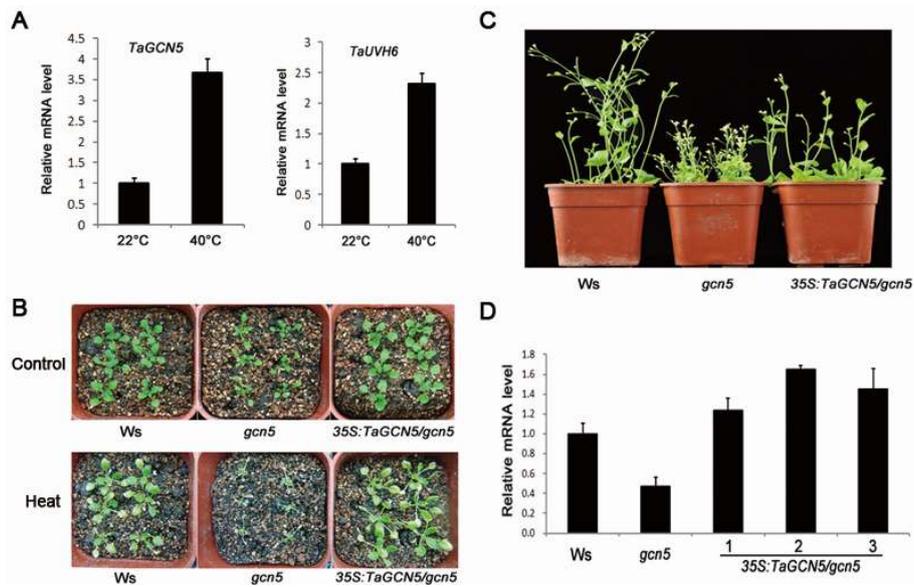


Figure 3: *TaGCN5* functions similarly to *GCN5*: (A) Expression of *TaGCN5* and *TaUVH6* after heat treatment; (B) Control and heat treated seedlings of wild type (Ws) and mutant lines; (C) Mature plants after heat treatment of wild type and mutant lines; (D) Expression of *UVH6* in wild type and *gcn5* mutant lines.



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Overexpression of wheat ferritin gene *TaFER-5B* enhances tolerance to heat stress and other abiotic stresses associated with the ROS scavenging system

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Key words: *TaFER-5B*, heat stress, abiotic stress, ferritin-lacking mutant, wheat

The yield of wheat (*Triticum aestivum* L.) is adversely affected by heat stress in many regions of the world. However, the molecular mechanisms underlying thermotolerance are largely unknown. A novel ferritin gene, *TaFER*, was identified from our previous heat stress-responsive transcriptome analysis of a heat-tolerant wheat cultivar (TAM107). *TaFER* was mapped to chromosome 5B and named *TaFER-5B*. Expression pattern analysis revealed that *TaFER-5B* was induced by heat, polyethylene glycol (PEG), H₂O₂ and Fe-ethylenediaminedi(o-hydroxyphenylacetic) acid (Fe-EDDHA). To confirm the function of *TaFER-5B* in wheat, *TaFER-5B* was transformed into the wheat cultivar Jimai 5265 (JM5265), and the transgenic plants exhibited enhanced thermotolerance (Figure 1). To examine whether the function of ferritin from mono- and dico-species is conserved, *TaFER-5B* was transformed into *Arabidopsis*, and overexpression of *TaFER-5B* functionally complemented the heat stress-sensitive phenotype of a ferritin-lacking mutant of *Arabidopsis* (Figure 2). Moreover, *TaFER-5B* is essential for protecting cells against heat stress associated with protecting cells against ROS. In addition, *TaFER-5B* overexpression also enhanced drought, oxidative and excess iron stress tolerance associated with the ROS scavenging system (Figure 3). Finally, *TaFER-5B* transgenic *Arabidopsis* and wheat plants exhibited improved leaf iron content. Our results suggest that *TaFER-5B* plays an important role in enhancing tolerance to heat stress and other abiotic stresses associated with the ROS scavenging system.

Figure 1: Thermotolerance assay of *TaFER-5B* transgenic wheat plants at the seedling stage: (A) 10 d old JM5265 and transgenic lines W-L1, W-L2 and W-L3 before heat treatment; (B) 5 d old JM5265 and transgenic lines W-L1, W-L2 and W-L3 treated with 45°C for 18 h after 5 d recovery at 22°C; (C) Ion leakage assay of seedlings (A) after heat treatment; (D) Maximum efficiency of PSII photo-chemistry (Fv/Fm ratio) in seedlings (A) after heat treatment at 38°C for 2 h; (data are mean values \pm SD of three experiments; * $p < 0.05$).

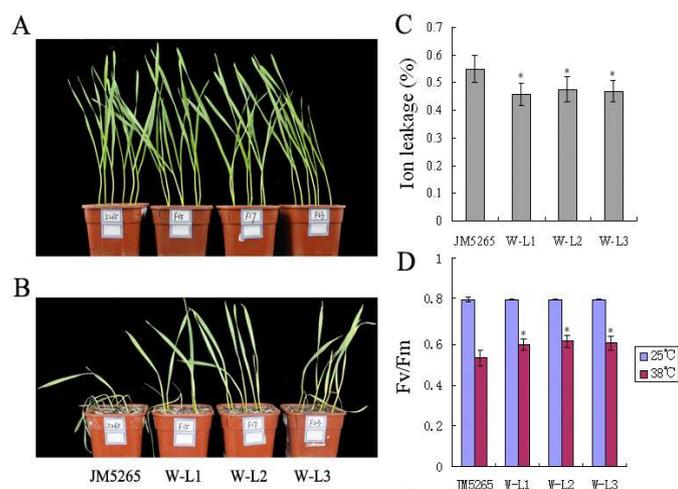


Figure 2: *Arabidopsis* ferritin-lacking mutants displaying a heat stress-sensitive phenotype by overexpression of TaFER-5B: (A) 6 d old seedlings of WT, *fer1-3-4* and *fer1-2-3-4* treated at 45°C for 2 h after 7 d recovery at 22°C; (B) 6 d old seedlings of WT, *fer1-2-3-4* and A-CL1 (complemented line) treated at 45°C for 2 h after 7 d recovery at 22°C; (C) Ion leakage assays of *fer1-2-3-4*, *fer1-3-4*, WT, overexpression lines A-L1 and A-L2 and complemented line A-CL1 seedlings after heat treatment; (D) Maximum efficiency of PSII photochemistry (Fv/Fm ratio) of *fer1-2-3-4*, *fer1-3-4*, WT, overexpression lines A-L1 and A-L2 and complemented line A-CL1 seedlings at 22°C and 38°C; (data are mean values \pm SD of three experiments; * $p < 0.05$; ** $p < 0.01$).

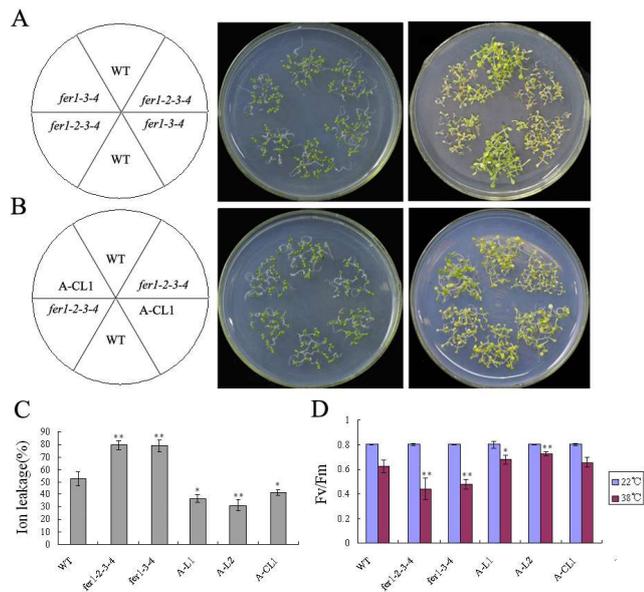
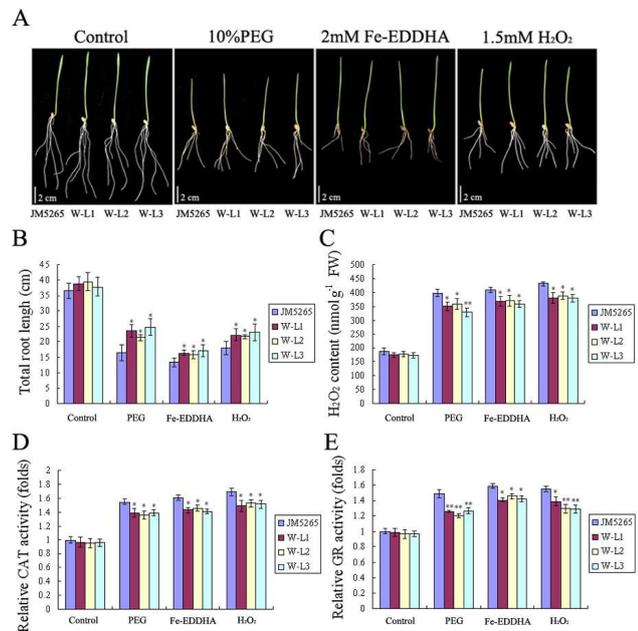


Figure 3: Drought, oxidative, excess iron stress tolerance assay and ROS accumulation analysis of TaFER-5B transgenic wheat plants: (A) 10 d old plants overexpressing TaFER-5B under control, mannitol, Fe-EDTA and H₂O₂ conditions; (B) Total root length of 10 d old seedlings in (A); (C) H₂O₂ content in 10 d old seedlings in (A); (D) Activity of antioxidant enzyme CAT in the seedlings in (A); (E) Activity of the antioxidant enzyme GR in the seedlings in (A); (data are mean values \pm SD of three experiments; * $p < 0.05$; ** $p < 0.01$).





P 288 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Multiple synthetic derivatives population: a new source to identify heat stress tolerance traits in bread wheat

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Key message: MSD population of bread wheat was tested across different locations in Sudan environments. Genome-wide association study (GWAS) was used for genotyping and identifying QTLs for heat tolerant related traits.

Heat stress is considered one of the most limiting factors to wheat production worldwide. Thus, development of heat tolerant cultivars is of paramount need in wheat breeding strategies. However, the genetic variability for heat tolerant in common wheat gene pool is scarce. Broadening the genetic variability of common wheat through introgression of the wild relative wheat gene(s) is proved to be an effective approach to coping with the heat stress. The objectives of this study were therefore to identify genetic variability for heat stress adaptive traits among multiple synthetic derivative population (MSD) and to detect QTL associated with heat tolerance-related traits using genome-wide association study (GWAS). Forty-three synthetic hexaploid wheat lines were produced by the crossing of *Aegilops tauschii* with *Triticum turgidum* var. *durum* cv. 'Langdon'. Subsequently, the synthetics wheat was crossed and backcrossed with the Japanese bread wheat cv. 'Norin 61' (N61) to produce the multiple synthetic derivative population (MSD). Of 400 BC₁F₄ lines were tested across four environments in Sudan. Heading, maturity, grain filling, chlorophyll content, canopy temperature, biomass, yield and yield attributes were measured across the different environments. DArT-seq markers were used for genotyping and associating the QTL for grain yield and heat tolerance related traits. Several QTL for heat tolerance related traits were identified. It could be concluded that the multiple synthetic derivative population (MSD) is a good candidate to improve the wheat breeding program for biotic and abiotic stresses.



P 290 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Linkage drag constrains the roots of modern wheat

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Key message: Selection for heading date has reduced variation for novel root QTL, highly syntenic to rice. Using high-throughput SNP markers, eroded diversity in elite germplasm could be recovered.

Roots, the hidden half of crop plants, are essential for resource acquisition. However, knowledge about the genetic control of below-ground plant development in wheat, one of the most important small-grain crops in the world, is very limited. The molecular interactions connecting root and shoot development and growth, and thus modulating the plant's demand for water and nutrients along with its ability to access them, are largely unexplored. Here we demonstrate that linkage drag in European bread wheat, driven by strong selection for a haplotype variant controlling heading date, has eliminated a specific combination of two flanking, highly conserved, haplotype variants whose interaction confers increased root biomass. Reversing this inadvertent consequence of selection could recover root diversity that may prove essential for future food production in fluctuating environments. Highly conserved syteny to rice across this chromosome segment suggests that adaptive selection has shaped the diversity landscape of this locus across different, globally-important cereal crops. By mining wheat gene expression data we identified root-expressed genes within the region of interest that could help breeders to select positive variants adapted to specific target soil environments.



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A candidate gene analysis of QTL for root growth angle in durum wheat

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Key message: A comparative analysis of *Triticum* spp. assemblies reveals putative candidate genes for root growth angle.

Root system architecture (RSA) plays a pivotal role in crop adaptation to adverse and/or specific growing conditions. Different RSAs are required to maximize the efficiency of the plant in water uptake/coping with drought stress, nutrient foraging and/or adaptation to different sowing density/managements. Therefore, optimization of RSA traits is an important goal for cereal and modern wheat breeding. In this study, genetic variation in elite tetraploid wheat germplasm has been investigated using two recombinant inbred line populations and one association mapping panel of 183 cultivars. These resources were searched for RSA QTL at seedling stage (seminal roots). QTL were mapped on a high-density tetraploid consensus map based on a transcript-associated Illumina 90K SNP assay, thus allowing for an accurate cross-referencing of RSA QTL. In total, 20 main QTL clusters for root length and/or number as well as 30 QTL for root growth angle (RGA) were identified. Based on their relative additive effects, allelic distribution in the AM panel and co-location with QTL for yield and kernel weight, the RSA QTL have been prioritized in terms of breeding value. Seven main RGA QTL were selected for investigating of gene content in the assembled wheat genomes: Chinese Spring *Triticum aestivum* (Release: 30/11/2015) assembled using the popseq map (Chapman et al. 2015) and the TriAnnot v4.3 gene prediction and annotation pipeline (Leroy et al. 2011) and the Zavitan *T. dicoccoides* genome assembly for integration and comparison (A. Distelfeld, unpublished). The chromosome regions ranged from 2.1 to 17.0 Mb and contained on average 146 predicted genes. The annotated genes are involved in auxin pathways (e.g. auxin-responsive factors, auxin biosynthetic process, auxin-induced proteins, etc.), PIN-formed protein family of auxin transporters and MYB transcription factors. The comparison between the *T. aestivum* and *T. dicoccoides* local gene content and the nature of the genes related to auxin biosynthesis and/or auxin signaling, and genes most likely involved in the RGA determinism, will be reported. A detailed study of functions, functional domains and tissue expression of the candidate genes is underway.

Acknowledgement

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P 294 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

***Triticum aestivum*/*Amblyopyrum muticum* introgressive lines polymorphism of AGL21 gene, which participates in regulation of root development**

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Key message: *Triticum aestivum*/*Amblyopyrum muticum* introgressive lines were investigated for polymorphism of *AGL21* gene, which participates in control of root development, and association with better persistency under winter stress was found.

Root system architecture is important for plant's adaptation to abiotic stresses including drought, nutrient deficiency in soil, and possibly low temperatures in winter (Kong et al. 2014). Development of wheat cultivars with optimal characteristics of root system for particular environmental conditions could provide better yields and possibility to use fewer fertilizers. Auxin is one of the main plant hormones regulating root development. MADS box transcription factors *AGL21* and *AGL14* were shown to influence auxin gradient in roots. *AGL21* positively regulates auxin accumulation in lateral root primordia through regulation of auxin biosynthesis (Yu et al. 2014). *AGL14* regulates auxin transport (Garay-Arroyo et al. 2013). *CBF-A14* is a wheat gene of one of the CBF transcription factors, which are the main regulators of low temperature tolerance in plants. Wheat introgressive lines with genetic material from wheat wild relative *Amblyopyrum muticum* (*Aegilops mutica*) were derived from the cross of common wheat cultivar Aurora (AABBDD) and genome substitution amphidiploid Aurotica (AABBTT), which combines in its genome AABB tetracomponent of Aurora genome and T genome of *A. muticum*. Parental genotypes Aurora and Aurotica were screened for polymorphism of *AGL21*, *AGL14* and *CBF-A14* genes using PCR with primers specific to the coding regions of wheat *AGL21*, *AGL14*, and *CBF-A14* genes, electrophoresis in PAAG in denaturing conditions, and visualized by silver staining. No polymorphism was found between Aurora and Aurotica for amplification products produced with primers to *AGL14* and *CBF-A14* genes. With primers to second part of *AGL21* gene Aurora and Aurotica produced polymorphic products, and these primers were used for analysis of introgressive lines; the results of this analysis were compared to lines' survival under winter stress in order to determine whether lines with Aurotica alleles differ from the lines with Aurora alleles for the rate of winter field survival. Introgressive lines with Aurotica allele of *AGL21* gene have shown better field survival under winter stress compared to introgressive lines with Aurora allele.

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P 296 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Response of wheat (*Triticum aestivum*) roots to nitrogen supply under drought

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Key message: Investigation of five Australian wheat genotypes under drought and well-watered conditions revealed treatment and genotype-specific responses of root traits and nitrogen uptake.

Nitrogen (N) fertilization is essential for wheat growth and productivity. However, unutilized nitrogen is lost from agricultural production systems by nitrate leaching, run off into waterways and emission of nitrous oxide or volatile ammonia. Moreover, nitrogen fertilizer production by the Haber-Bosch process is energy intensive and requires significant energy resources. Thus, in the agro-ecological context unutilized nitrogen is not only uneconomical but can have severe negative effects on ecosystems. For future food production, the question is how the increasing demand due to the growing population can be achieved without over-proportionally increasing N applications. The use of genetic variation by plant breeders for traits that increase nitrogen use efficiency is an important component towards a more sustainable agriculture. A relevant aspect that has to be considered is that nitrogen uptake efficiency is strongly influenced by abiotic constraints, such as drought, especially in Mediterranean environments. At the root level, there is still a lack in knowledge about the mechanisms and factors contributing to both, a better nitrogen acquisition and drought adaptability. Since drought events are predicted to occur more frequently and be more severe due to climate change, nutrient by drought interaction deserves more attention. The objective of this study therefore was to compare genotypes for nitrogen uptake in the presence of drought compared to well-watered conditions. For this, five wheat genotypes adapted to Australian growth conditions were grown in large plastic tubes filled with approximately 2 kg soil. At 14 days after sowing, a mild drought was applied for ten days. Subsequently, all tubes were re-watered to the same water-holding capacity and labeled nitrogen (¹⁵N) was applied for 30 min before root and shoots were harvested, weighed and processed for determination of nitrogen concentration. In addition, roots of the same plants were cross sectioned at 1.5 cm from the root base to investigate differences in root anatomy and diameter of the central cylinder and xylem vessels. The data showed that, as expected, the drought-treated plants had a significantly reduced nitrogen uptake, even after re-watering. Furthermore, the study revealed that individual genotypes had a significant longer shoot or root length and a different root/shoot ratio. Further analysis of root cross sections is ongoing to assess if genotype-specific differences in central cylinder diameter and other traits are associated with differences in ¹⁵N uptake.

P 298 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Genome-wide association analyses for the determination of floret fertility in wheatZifeng Guo¹, Dijun Chen², Ahmad M. Alqudah¹, Marion S. Roeder³, Martin W. Ganai⁴, Thorsten Schnurbusch¹

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Key words: Candidate genes, floret fertility, grain number, GWAS, maximum number of floret primordia, QTL

Increasing grain yield is still the main target of wheat breeding; yet today's wheat plants utilize less than half of their yield potential. Owing to the difficulty of determining grain yield potential in a large population, few genetic factors regulating floret fertility (i.e. the difference between grain yield potential and grain number) have been reported to date. In this study, we conducted a genome-wide association study (GWAS) by quantifying several floret fertility traits in 210 European winter wheat accessions. The results of this GWAS experiment suggested potential associations between floret fertility traits revealed by shared quantitative trait loci (QTL). Several candidate genes involved in carbohydrate metabolism, phytohormones or floral development colocalized with such QTL, thereby providing potential targets for selection. Based on our GWAS results, we proposed a genetic network underlying floret fertility traits, nominating determinants for improved yield performance.

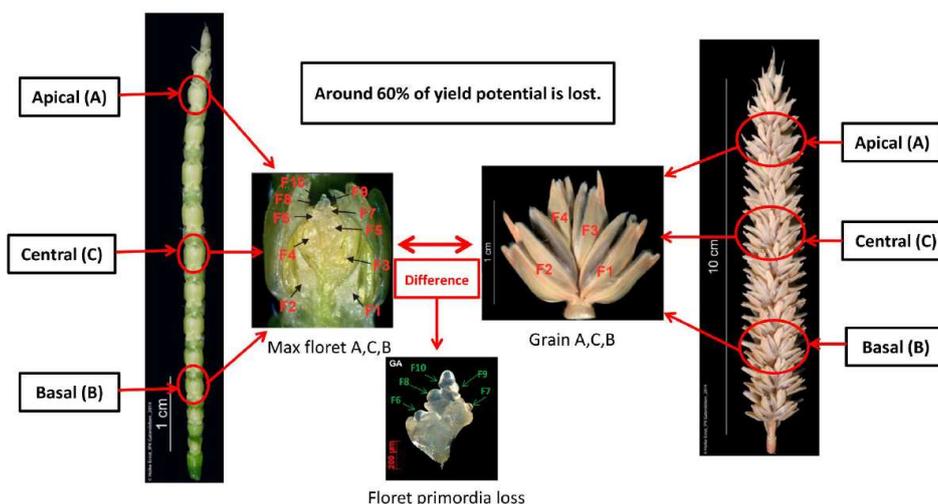


Figure 1: The explanation of floret abortion in wheat. The apical (A) and basal (B) spikelets are the third one from the top and bottom of spike, respectively. The central (C) spikelet is the one in the center of the spike.



P 300 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Is recruitment of SUMOylation the answer to climate resilience and increased crop yield?

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Key message: SUMO (Small Ubiquitin-like Modifier) class of molecules has emerged as an influential mechanism for target protein management. SUMO proteases are vital in regulating pathway flux and are therefore ideal targets for manipulating stress-response.

Post-translational modifications of proteins play a critical role in cellular signalling processes. In recent years, the SUMO (Small Ubiquitin-like Modifier) class of molecules has emerged as an influential mechanism for target protein management. SUMO proteases play a vital role in regulating pathway flux and are therefore ideal targets for manipulating stress-responsive SUMOylation. It was shown that SUMOylation could be recruited to dramatically improve plant growth during salinity stress, drought and high temperature stress by overexpressing the SUMO protease gene *OTS1* in *Arabidopsis*. Recently we identified and cloned wheat homologs of *OTS1*, *OTS2* and *ICE*, suggesting that SUMOylation may also be important in other crops. Thus, we transformed wheat lines with *OTS1* to enhance stress resilience. In a parallel approach, ethylmethanesulfonate (EMS) and sodium azide mutagenic lines were also developed, selected for water stress tolerance, and the mutagenic progeny tested for their stress responses (Figure 1). We also sampled wheatgrass, *Thinopyrum distichum* ($2n = 4x = 28$; J1dJ1dJ2dJ2d), a hardy, salt-tolerant maritime wheatgrass indigenous to southern Africa to compare its responses and genetic composition to that of our transgenic and mutagenic lines. To elucidate the possible contribution of SUMOylation to the increased drought tolerance observed in these lines, they are screened for SUMO targets (Figure 2). Data on observed yield increases, delayed water loss and senescence, changes in chlorophyll content, protein profiling, enzymatic activity, well as genotypic differences will be presented.



Figure 1: Phenotypic response of control and a chemically induced mutagenic line Ryno3936 after exposure to water stress. (A-C) Control plants after (A) day 7, (B) day 14 (C) day 14 without watering; (D-I) mutant line Ryno3936 after (D) day 7, (E, F) day 14; and after re-watering on (G) day 21, and after (H, I) 120 days.

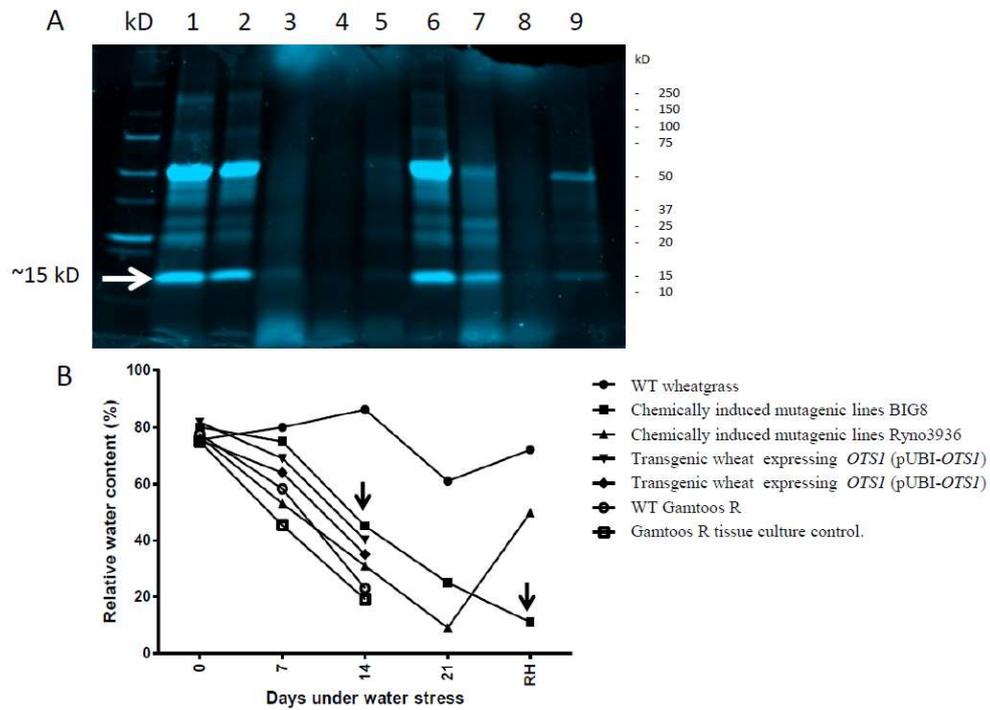


Figure 2: Responses of wheat lines exposed to different water stress regimes (0, 7, 14 d post watering, as well as 21 and 28 d post hydration). (A) Protein separated on a Mini-Protein TGX gradient gel (4-15%) where lanes 1 to 2 = chemically induced mutagenic line BIG8 and lanes 6 to 9 = chemically induced mutagenic line Ryno3936. All lanes were loaded with 150 μ g total protein. The arrow indicates the accumulation of free SUMO conjugates (\approx 15 kD); (B) Relative water content of the different wheat lines after exposure to water stress. Illustrated is the relative water content expressed as percentage of total water loss. Arrow indicates time interval where most of the plants were classified as completely dehydrated (senesced) and unable to recover/regrow after watering.



P 302 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

GWAS on yield components in Kazakh spring wheat accessions grown in three different environments

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Key words: GWAS, hexaploid wheat, QTL identification, spring type, SNP Illumina array, yield components

The recent introduction of Illumina single nucleotide polymorphism (SNP) arrays is an important step towards comprehensive genome-wide studies for wheat improvement worldwide. In this study the results of 90K SNP genotyping of 96 cultivars of hexaploid spring wheat growing in Kazakhstan based on Illumina array (Turuspekov et al. 2015) were used genome-wide association study of yield components. All accessions were grown in the field in three different regions of Kazakhstan ranging from Northern station in Karabalyk to Southern station in Kyzylorda region during 2012-2015. The study is allowed an identification of SNP markers for plant growth traits and yield parameters. Yield associated traits included plant height, peduncle length, number of productive stems per plant, number of kernels per spike, and thousand kernel weight. GWAS was performed using TASSEL 5 and outputs from STRUCTURE. The results provide important information on identification of key QTL associated with yield performances in three different regions of Kazakhstan.

Acknowledgement

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P 304 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Challenges and prospects of durum wheat breeding programs in Iran

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Key message: In spite of importance of durum for Iranian rural economies and pasta industry, the country has not all succeeded in its research and development efforts to substantially improve durum productivity under cold dryland conditions due to abiotic stress.

Durum wheat occupies about 300 000 ha mainly in warm and semi-warm areas of Iran. In spite of the importance of durum for Iranian rural economies and pasta industry, the country has not all succeeded in its research and development efforts to substantially improve durum productivity. The combinations of increasing demand for durum and durum products, and relatively low durum productivity made the country to import half of its demand (about 300 000 tons per year). The country is generally known of arid and semi-arid climate. Hence, about 4.0 million hectares (or 60%) of wheat cultivation area is totally reliant on rainfall. Durum is generally sown in marginal environments subject to great climatic fluctuations during the growing season. Abiotic (cold, drought, heat, salinity) and biotic stresses are major limiting factors for durum production. In addition to these stresses, lack of good agronomy is a major limiting factor to achievement of the genetic potential of improved cultivars. Under rainfed condition, average yields of durum is 600 kg/ha in 2011-12), where the country average was 2.5 t/ha. Over 80% of Iranian durum is produced in the four South-West provinces (Ilam, Khuzestan, Kohkiluyeh-Boyerahmad, Fars) with warm and semi-warm climates. There is also some potential for durum production in cold highlands depending on future varietal adaptation. But, frost damage along with drought and heat stresses are the most common adverse factors affecting autumn sown durum productivity in cold regions. The main factors causing winterkill can be summarized as: (i) inadequate hardening, due to late emergence in autumn or a sudden drop in temperature; (ii) long periods of cold-induced desiccation; (iii) prolonged periods of low sub-zero temperatures (below -15°C); (iv) alternate freezing and thawing. To overcome all of these constraints, durum breeding program was established in Dryland Agricultural Research Institute (DARI) in 1990s where it remains today as the lead agency of national program especially for cold and rainfed highlands. DARI in collaboration with ICARDA and CIMMYT could release some durum varieties (e.g. Seimareh, Dehdasht, Saji); however, accelerating of the process of releasing of new cultivars with high yield and good quality should be considered especially for cold regions. Hence, strong collaboration with the durum program at ICARDA needs to be reinforced based on exchange of germplasm with cold tolerance; and to have DARI more involved in the ongoing breeding programs in Turkey and the high plateaus in Atlas Mountains in Algeria and Morocco.



P 306 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Functional conservation and divergence among homoeologous genes at the *TaSPL20* and *TaSPL21* loci governing yield-related traits in hexaploid wheat in diverse water regimes

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Key message: *TaSPL20* and *TaSPL21* homoeologs underwent diversification in function. Favorable haplotypes of each set were selected and exploited due to their large effects on plant height and 1000-grain weight.

Maintaining high and stable yields has become an increasing challenge in wheat (*Triticum aestivum* L.) breeding due to the climate change over the past decades. Although *Squamosa promoter binding protein (SBP)-box* genes have important roles in plant development, very little is known about the actual biological functions of wheat SBP-box family members. Here, we dissect the functional conservation, divergence and exploitation of homoeologs of two paralogous *TaSPL* wheat loci during domestication and breeding. *TaSPL20* and *TaSPL21* were specifically expressed in the lemma and palea. Ectopic expressions of *TaSPL20* and *TaSPL21* in rice indicated similar functions in terms of promoting primary and secondary panicle branching without changing tiller number, and overexpression of *TaSPL20* led to larger seeds. We characterized all six *TaSPL20/21* genes located across the three homoeologous (A, B and D) genomes. They displayed diverse functions with each having distinctive characteristics revealed by analysis of natural variations in 20 environments, which were the combinations of year, location, water and heat treatments. Four favorable haplotypes were identified, namely *TaSPL20-7D-HapI*, *TaSPL21-6A-HapII*, and *TaSPL21-6D-HapII&III*. Together they reduced plant height by up to 27.5%, and *TaSPL21-6D-HapII* increased 1000-grain weight by 9.7%. Our study suggests that *TaSPL20* and *TaSPL21* homoeologs underwent diversification in function with each evolving its own distinctive characteristics. During domestication and breeding of wheat, favorable haplotypes of each set were selected and exploited to varying degrees due to their large effects on plant height and 1000-grain weight.



P 308 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Candidate genes for pre-harvest sprouting tolerance on wheat chromosome 4A

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Key message: More than one gene for dormancy is likely located within a QTL on 4A. Markers flanking the QTL as well as candidate genes have been identified.

A major QTL was identified on chromosome 4A for dormancy and pre-harvest sprouting (PHS) tolerance in the cross RL4452 × AC Domain (Cabral et al. 2014). The region contains several dormancy related candidate genes including plasma membrane protein PM19 identified in Australian wheat (Barrero et al. 2015) and a gibberellin-20-oxidase gene. Polymorphisms are found in both genes between the parental lines. The gene with the highest differential expression between dormant and non-dormant bulks in a microarray experiment revealed a gene which mapped to the QTL region distally to PM19. It was determined that AC Domain carries a large deletion in this gene. This gene and PM19 flank the QTL region. Genetic markers suitable for breeding purposes spanning the QTL region have been produced. Lines in the population that are recombinant between the markers were assessed for dormancy. Lines carrying the AC Domain allele at both markers were the most dormant. Lines carrying the AC Domain allele at one marker and the RL4452 allele at the other were intermediate but not significantly different from non-dormant and lines carrying the RL4452 allele at both markers were the least dormant. The evidence supports the theory that more than one gene in the 4A QTL contributes to the enhanced dormancy of AC Domain. The line RL4137 appears to be the source of the dormant haplotype at the first marker allele in Canadian germplasm while the second dormancy related allele has been present in Canadian wheat since Red Fife.

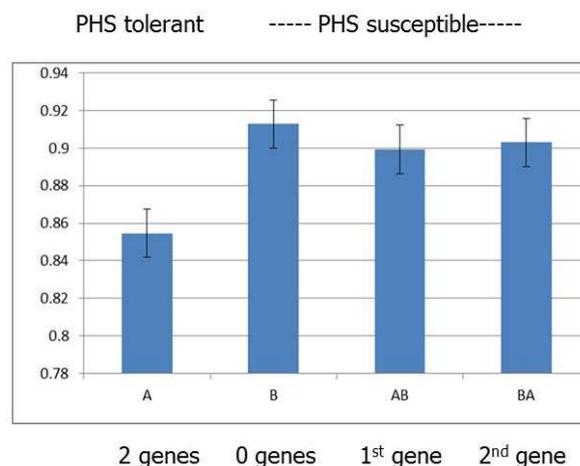


Figure 1: Germination index of lines carrying AC Domain alleles at none, one or both marker loci.

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P 310 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Morpho-anatomical and productive traits in wheat genotypes contrasting for peduncle water-soluble carbohydrates content

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Key message: High-WSSC genotypes had higher specific weight and biomass of the main stem, as well as larger parenchyma and phloem area compared to low-WSSC genotypes.

Water-soluble carbohydrates (WSC) assimilated during vegetative and early reproductive growth in wheat is temporarily stored in stem internodes and leaf sheaths, and can later be remobilized and transported to developing grain. In this study the WSC content and the WSC specific content (WSSC) were determined in the uppermost internode (peduncle) of the main stem at 10 days after anthesis (10 DAA) across 44 wheat genotypes in two-year field trials. The defoliation was done at 10 DAA by cutting off all leaf blades and these plants (DP) were grown along with the intact control plants (CP). The peduncle relative length was between 42 and 72% of the total stem length. The WSC content in peduncle varied from 3.03 to 7.08 mg/100 mg DW and from 1.72 to 6.86 mg/100 mg DW in the first and the second year, respectively. On the other hand, WSSC in peduncle varied from 4.41 to 14.10 mg/cm and from 2.05 to 12.97 mg/cm in the first and second year, respectively. The effects of genotype, environment and their interaction on both traits were significant, with the prevailing effect of a genotype (66% and 63% for WSC and WSSC, respectively). Genotypes contrasting for WSSC were compared for productive and other traits in DP and/or CP plants. Estimated contributions of peduncle (culm and flag leaf sheath) assimilate reserves to grain weight/spike in high-WSSC genotypes had significantly higher contribution ($p < 0.001$) in CP than low-WSSC genotypes (19.1 vs. 8.6%, respectively). The similar one-fold higher contribution ($p < 0.001$) to grain weight/spike from peduncle in high-WSSC genotypes than in low-WSC genotypes was estimated for DP plants (28.4 vs. 14.8%, respectively). Among 16 morphological, anatomical and developmental traits, the area of pith intercellular of peduncle, chlorophyll content in flag leaf and the flag leaf area appeared to be most important for WSC accumulation in peduncle, while biomass per main stem, area of parenchyma and again chlorophyll content in flag leaf had the greatest effect on WSSC. High WSSC genotypes tended to have higher grain weight per spike than low WSSC genotypes both in DP and CP plants.



P 312 - Topic: Genetics and Genomics of Biotic and Abiotic Stress Resistance

Ammonium nitrogen participated in the interactions of cadmium/zinc in dwarf Polish wheat (*Triticum polonicum* L.)

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Key message: NH_4^+ participated in the interactions of Cd/Zn.

The toxicities of cadmium (Cd) and zinc (Zn) have become serious issues in plant. Different intraspecific or interspecific wheat show different interactions of Cd/Zn. Previous studies indicated that N form (NO_3^- versus NH_4^+) affects the uptake of Cd and Zn, and also may participate in their interactions. Our previous results demonstrated that NO_3^- participated in the interactions of Cd/Zn in dwarf polish wheat (DPW). However, whether NH_4^+ participated in the interactions of Cd/Zn are unknown. In the present study, DPW (two leaves seedling) was cultivated in Hoagland nutrient solution with NH_4^+ starvation [CK (null), Cd (80 μM), Zn (500 μM) and Cd (80 μM) +Zn (500 μM)] and normal NH_4^+ (1 mM)[CK(), Cd (80 μM), Zn (500 μM), Wendi Shuai, Songyue Cai, Siyu Li, Xing Fan, Lina Sha, Houyang Kang, Haiqin Zhang, Yonghong Zhou, Yi WangM) and Cd (80 μM) + Zn (500 μM)]. Compared with NH_4^+ starvation, supplementary of NH_4^+ not only promoted the growth of DWP, but also significantly promoted the uptake of Cd and Zn in roots. Under Cd+Zn stress in roots, NH_4^+ significantly inhibited the uptake of Cd, but promoted the uptake of Zn, the uptake of Cd was significantly inhibited by Zn with NH_4^+ supplementary, the uptake of Zn was significantly inhibited by Cd without NH_4^+ . After treated with 1 mM $^{15}\text{NH}_4^+$ (a NH_4^+ tracer) for 24 hours, compared with control, Cd+Zn significantly promoted the uptake of NH_4^+ without NH_4^+ , Cd markedly inhibited the uptake of NH_4^+ with NH_4^+ supplementary, suggesting that Cd and Cd+Zn affected the uptake of NH_4^+ although the mode and efficiency were different. Therefore, these results indicated that NH_4^+ participated in the interactions of Cd/Zn.



P 73 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Functional genomics approaches reveal the gene network regulating senescence in wheat

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Key message: Combined forward genetic and gene network modelling approaches reveal that NAC transcription factors are key regulators of senescence.

Monocarpic senescence in crops is essential to enable nutrient remobilisation from photosynthetic tissues to the grain. This process must be tightly regulated to prevent premature senescence adversely affecting yields and to ensure nutrients are remobilised from vegetative tissues into the grain. However despite the importance of this process, few genes controlling senescence have been identified in wheat. We are using a combination of approaches to identify novel regulatory genes including forward genetics, protein-protein interactions, transcriptomics and gene regulatory network modelling.

Forward genetics

Through a forward genetic screen we have identified several mutants with delayed senescence. Two of these lines have mutations in *Nam-A1* the homoeolog of the *Nam-B1* NAC transcription factor known to regulate senescence and nutrient remobilisation. We are currently identifying the novel genes in other mutant lines using mapping-by-sequencing.

Protein-protein interactions

We have identified protein interacting partners of *Nam-B1* using a yeast 2 hybrid screen. These protein interacting partners consist mainly of NAC transcription factors including *Nam-A1*, a homoeologue of *Nam-B1*. Several previously uncharacterised NAC transcription factors were also identified and we are generating mutants to assess their effects on senescence and nutrient remobilisation.

Transcriptomics and gene networks

We have generated a high-resolution RNA-seq time-course of ten time-points from anthesis until the first visible signs of flag leaf senescence. We identified over 2000 genes differentially expressed during senescence, many of which are involved in known senescence-associated processes. To understand the key genes driving transcriptional changes we used a combination of gene regulatory network analyses to identify modules of co-expressed genes and hub genes regulating the transcriptional network. Several hub genes have been identified and knock-out mutants are being characterised to confirm the senescence-related roles of these genes.



P 75 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

RNA sequencing reveals genome-wide polymorphisms applicable to develop genetic markers in *Aegilops umbellulata*

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Key message: RNA-seq is quite useful to survey genome-wide polymorphisms and to develop genetic markers in a diploid wheat relative *Aegilops umbellulata*, whose genome information is unavailable.

RNA-seq is quite useful method to detect genome-wide polymorphisms for Triticeae species with the large genome size and repetitive sequence-rich genome. Using *de novo* assembly of RNA-seq data and conserved chromosomal synteny among Triticeae species, a large number of genome-wide polymorphisms including SNPs and indels have been detected even between genetically close accessions of *Aegilops tauschii* Coss., and the information was useful to design genetic markers (Nishijima et al. 2016). This RNA-seq-based method can apply to other wild wheat species with little genome information. *Ae. umbellulata* Zhuk., a diploid wild wheat relative, is distributed from Greece to Iraq, and provides a lot of unique and valuable traits such as a leaf rust resistance gene *Lr9* (Schachermayr et al. 1994). Owing to the valuable traits and high cross ability to tetraploid and hexaploid wheat, *Ae. umbellulata* is an important genetic resource for breeding of bread wheat. However, genetic variations of *Ae. umbellulata* have not been well studied and its genome information is quite limited. Here, we estimated the phylogenetic relationship of 58 *Ae. umbellulata* accessions based on the nucleotide sequences of some single-copy genes. Seedling leaf transcripts of the 12 selected accessions were deduced using *de novo* assembly of pair-end reads from RNA sequencing on the Illumina MiSeq platform. A large number of non-redundant SNPs and indels were found between the representative accessions, and many of them (66.4 to 90.8%) were anchored to the barley and *Ae. tauschii* chromosomes, and CAPS and indel markers were designed as molecular markers based on the identified SNPs and indels. These developed markers were successfully applied for validation of the intraspecific crossed F₁ plants and construction of a linkage map around the targeted loci. Therefore, the RNA-seq-based approach allows efficient development of genome-wide genetic markers in other Triticeae species which reference genome information is incomplete.

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P 77 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Genotyping-by-sequencing (GBS) revealed genetic structure and diversity of Iranian wheat landraces and cultivars

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Key words: Genetic diversity, population structure, single nucleotide polymorphism

Genetic diversity is essential for breeders to improve new cultivars with desirable characteristics. Recently, next generation sequencing (NGS) is a high throughput and cost effective molecular tool to speed up breeding processes and has caught a great attention of breeders worldwide. Genotyping-by-sequencing (GBS), a NGS technology that can simplify complex genomes, has now be used for routine breeding screening in many crop species including the species with a large genome. A diversity panel of 369 accessions of Iranian hexaploid wheat including 270 landraces collected from 1931 to 1968 in different climate zones and 99 cultivars released from 1942 to 2014 were genotyped using GBS. A total of 16 506 single nucleotide polymorphism (SNP) markers generated from GBS were analyzed across the diversity panel. After aligning all the SNPs to the CS and W7984 assemblies, the number of SNPs mapped to the three genomes are B genome > A genome > D genome (Table 1). Iranian wheat landraces revealed higher variation in D genome in compare with other resources in previous studies. Structure analysis divided the panel into three groups with landraces in two groups and most of cultivars in one group, suggesting high differentiation between landraces and cultivars (Figure 1). A similar grouping result was obtained using cluster analysis (Figure 2). When cultivars were clustered independently, cultivars developed in Iran or with Iranian pedigrees were separated from the cultivars originated from CIMMYT lines. For landraces, year of collection and their climate zones are the major factors for subgroup separation. Molecular analysis of variance showed larger intra-population variation than within population variation. Analysis of GBS-SNPs revealed obvious population structure and genetic diversity among Iranian wheat. High genetic differentiation between landraces and cultivars suggests that breeders can select divergent parents based on their genetic distance. The diverse landraces are rich genetic sources of tolerance to biotic and abiotic stresses to be used for improvement of wheat production in Iran and other countries.

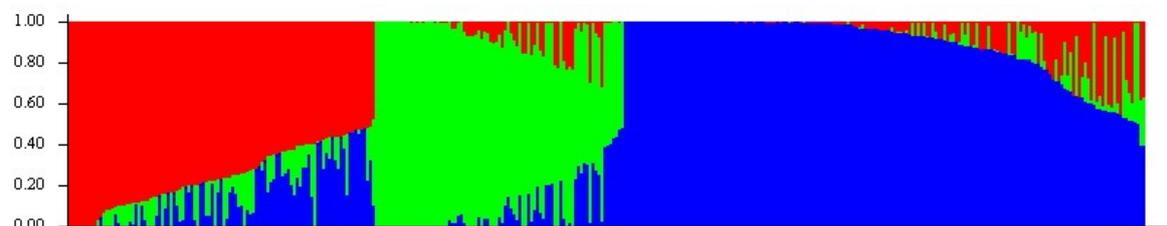


Figure 1: A structure plot of the 369 wheat Iranian landraces and cultivars determined by K=3 using 16 506 SNPs.



Table 1: A summary of single nucleotide substitutions identified in three homoeologous wheat genomes based on CSSS and W7984 assemblies.

Genome	CSSS				W7984				Total
	A	B	D	Unassigned	A	B	D	Unassigned	
No. of SNPs	4067	5425	2266	4748	5442	6449	2806	1809	16506
Chromosome size (Mbp)	5727	6274	4937	-	5727	6274	4937	-	16938
Density (SNP/Mbp)	0.710	0.865	0.459	-	0.950	1.028	0.568	-	0.974
A/G	1213	1564	651	1405	1648	1849	793	543	4833
C/T	1190	1527	582	1263	1576	1808	730	448	4562
G/A	131	203	80	173	175	238	108	66	587
T/C	125	171	84	141	165	212	90	54	521
Transition	2659	3465	1397	2982	3564	4107	1721	1111	10503
Ts %	65.38	63.87	61.65	62.81	65.49	63.68	61.33	61.42	63.63
A/T	195	253	136	223	273	309	150	75	807
A/C	310	411	192	367	410	481	234	155	1280
T/A	26	30	12	27	33	35	15	12	95
T/G	21	45	20	47	33	55	27	18	133
C/A	28	47	11	41	40	57	18	12	127
C/G	492	704	305	637	657	841	382	258	2138
G/T	280	383	162	332	363	457	201	136	1157
G/C	56	87	31	92	69	107	58	32	266
Transverion	1408	1960	869	1766	1878	2342	1085	698	6003
Tv %	34.62	36.13	38.35	37.20	34.51	36.32	38.67	38.59	36.37
Ts/Tv ratio	1.89	1.77	1.61	1.69	1.90	1.75	1.59	1.59	1.75

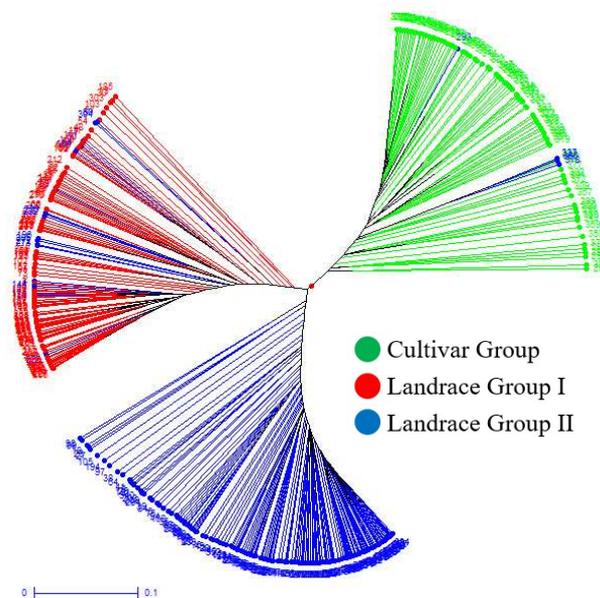


Figure 2: WPGMA clustering dendrogram generated using 16 506 SNPs and 369 Iranian hexaploid wheat accessions based on the result of structure analysis.



P 79 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Identification of candidate gene and linked marker for purple colored seeds in wheat (*Triticum aestivum* L.)

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Key message: We found based on GWAS analysis a marker linked to wheat purple colored seeds. Verification of the marker showed co-segregating. The candidate gene was identified based on the marker sequence.

Recently, purple wheat as well as other colored wheats have been a subject to public interest due to their content of cereals antioxidant, i.e. anthocyanin. The anthocyanins have attracted more and more interest from both researchers and food manufactures as health-promoting factors and disease-preventing effects. Thus, wheat is considered to be a useful source of dietary anthocyanin. Among thirteen major anthocyanins found in purple wheat, cyanidin 3-glucoside was the predominant anthocyanin with 42.6%, followed by peonidin-3-O-glucoside with 39.9% and malvidin-3-O-galactoside with 17.4% (Chen et al. 2013). *Pp3* mapped to chromosome 2A is known to have the main effect on the pericarp color in wheat, while *Pp-A1* (7A) and *Pp-D1* (7D) are newly uncovered genes of weak effect (Dobrovolskaya et al. 2005, Gordeeva et al. 2015). In this study, we aimed to find a SNP marker associated with the purple wheat and to use it in the marker assisted breeding. A collection of 149 wheat lines were employed in this study of which 11 were with purple colored seeds. Lines were genotyped with 15K wheat chip. Genome wide association analysis resulted in a very strong association with $-\log(P)$ value of over 22. T SNP marker mapped to 2A was associated with the trait. The marker is Tdurum_contig13653_47, which mapped to 2A at 58,092 cM. The marker was converted to kompetitive allele specific polymerase chain reaction (KASP) assays for further analysis. For validation, a collection of 18 more lines were ordered for The National Small Grains Collection (USDA-ARS). These 18 lines were registered as purple colored wheat. However, visual inspection of the seeds showed that some of them were not purple colored seeds. Genotyping the 18 lines with the linked marker validate the co-segregation of the SNP marker with the trait. Furthermore, this marker showed complete association with over 1200 wheat lines from Nordic Seed breeding materials. Blast search of the SNP sequence against the wheat genome revealed that the gene TRIAE_CS42_2AL_TGACv1_094284_AA0295290, could be the gene *Pp3* controlling the pericarp purple color in wheat. This gene composed of 9 exons with previously unknown function. However, the marker we found can be directly employed in wheat breeding program for developing wheat cultivars that have purple colored seeds with high anthocyanin content. Further investigation is on the way to identify the effect of the SNP on the protein function and to find other allelic variation in the gene.

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P 81 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Assessing diversity in *Triticum durum* cultivars and breeding lines for high versus low cadmium content in seeds using the CAPS marker *usw47*

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Key message: Cadmium (Cd) in food has toxic effects for humans. Durum wheat is used for pasta or bread. Therefore it is very important to minimize the concentration of Cd in grain.

Cadmium is a toxic heavy metal that occurs naturally in soils. Durum wheat is known to accumulate generally more Cd than other cereal crops. The uptake of Cd in durum wheat is governed by the gene *Cdu1*, which co-segregates with several DNA markers, such as the codominant marker *usw47* and the dominant marker *ScOPC20*. A panel of 314 durum wheat cultivars or lines originating from 16 countries or regions were assessed with *usw47*. The plant material was mainly comprised of cultivars and modern breeding lines. From this set, 165 durum wheat lines were classified as low-Cd accumulators, 144 high-Cd accumulators and five were heterogeneous. A smaller subset of 16 cultivars had previously been evaluated for Cd accumulation in replicated field trials in Germany. Lines with the high-Cd allele showed a 2.4-fold higher Cd content in the seeds than lines with the low-Cd allele. In most cases, low Cd-accumulating cultivars did not surpass the critical level of 0.2 mg/kg Cd in the grains, even when grown at critical sites with high-Cd availability in the soil. These results confirm that Cd accumulation in durum grains is controlled to a large proportion by the *Cdu1* gene. Marker-assisted selection appears as a robust and practicable tool for breeding durum cultivars with low-Cd content. For detailed results see Zimmerl et al. (2014).

Acknowledgements

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P 83 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Development and characterization of a whole genome radiation hybrid panel for tetraploid wheat

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Key message: A radiation hybrid panel for reference durum genotype Svevo (Sv-WGRH panel) was developed as resource for contributing to the positional cloning of genes and QTLs of important agronomic traits.

The recent release of high-quality sequence information from hexaploid wheat (IWGSC 2014) coupled with the availability of high-density consensus maps for tetraploid wheat (Maccaferri et al. 2014) has accelerated marker and gene discovery in durum wheat (*Triticum durum*), thus facilitating the genetic dissection of agronomic traits. This notwithstanding, the construction of genetic maps remains a bottleneck for the investigation of the durum wheat genome. Radiation hybrid (RH) mapping is a promising recombination-independent mapping approach, which involves the use of radiation-induced chromosomal breakage and marker segregation to reconstruct marker order (Tiwari et al. 2016). In this study a RH panel for tetraploid wheat was developed for reference durum genotype Svevo (Sv-WGRH panel). The Sv-WGRH panel was developed at Kansas State University (USA), according to the protocol reported by Tiwari et al. (2016). Freshly dehiscing pollen of Svevo was irradiated with γ -rays (10-Gy) and this was used to pollinate \approx 150 emasculated spikes of Senatore Cappelli (used as the female parent), which produced \approx 1000 RH₁ seeds, each representing an independent RH event. Greenhouse planting of these 1000 RH₁ seeds resulted in \approx 730 RH₁ plants, each representing a RH line of Sv-WGRH panel. Initial assessment of Sv-WGRH panel was performed based on 23 SSR markers. Results indicated that average marker retention of Sv-WGRH panel is \approx 87%. The Sv-WGRH panel will represent an important resource contributing towards the positional cloning, in particular in the centromeric regions, of important genes and QTL.

Acknowledgement

We acknowledge Dr. J.A. Geuther (Nuclear Reactor Facilities, Kansas State University) for contributing with radiation treatments.

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P 85 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Exploiting durum wheat germplasm to increase nitrogen use efficiency (NUE)

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Key message: Identification of wheat genotypes with higher NUE within a novel durum wheat germplasm collection.

Wheat is, among cereals, one of the most important worldwide in terms of production and utilization as staple food. An increase of 25% in the global production is demanded up to 2050 due to the exponential increases of the world population. The goal of wheat breeding is the development of new wheat varieties with increased production maintaining an high quality and high protein content. Low nitrogen content is one of the major constrain of wheat production, thus increased usage of N supply have been mandatory for increasing wheat grain yield and protein content. Several reports have shown that almost 50% of the nitrogen fertilizer applied remains unavailable to crops due to N losses. Many efforts have been done so far to obtained wheat varieties with an increased Nitrogen use efficiency (NUE). A possible strategy is to exploit the natural diversity available in landraces still cultivated *in situ* or stored *ex situ* in germplasm collections. To create and handy core collection with good representation of the genetic background we developed a durum wheat core collection of 452 genotypes between landraces and local varieties obtained by single seed descent, representative of more than 40 countries (Pignone et al. 2015) and currently characterized for several phenotypic traits. A number of genotypes of the SSD collection have been also selected to be part of the durum wheat reference collection (DWRC) recently constituted in frame of the Wheat Initiative. In this work a selection of 14 SSD genotypes plus two control cultivars have been tested in randomized blocks for two years in field in normal and low nitrogen supply (0-100%) and a difference in NUE and its components have been observed between the selected genotypes. Yield, development and photosynthesis related traits have been investigated to dissect the mechanisms underlying the observed differences between genotypes.

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P 87 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Successful transfer and validation of the Fusarium head blight resistance QTL *Fhb1* in durum wheat – a game changer for enhancing food safety

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Key message: For the first time the QTL *Fhb1* was successfully deployed and validated in modern durum wheat background. The knowledge and the germplasm generated is directly applicable for durum wheat improvement.

Fusarium head blight (FHB) is a devastating disease which affects small grain cereals worldwide, and can be particularly damaging on durum wheat. FHB also affects food safety due to mycotoxin contamination of infected grains. Development of resistant cultivars is pivotal in integrated disease management. Breeding for FHB resistance in durum wheat (*Triticum durum* Desf.) is challenging due to the lack of variation available for breeders with most lines being highly susceptible and only few genetic studies are available (Prat et al. 2014). We evaluated resistance derived from common wheat (*T. aestivum* L.) in the background of modern European durum cultivars. Three mapping populations of 100 F₇-RILs were developed from crosses between the resistant durum wheat experimental line DBC-480, which carries the major common wheat resistance QTL *Fhb1*, and the durum wheat cultivars Karur, Durobonus and SZD1029K. The populations were evaluated during three seasons in field experiments at IFA-Tulln (Austria) using artificial spray-inoculation by *Fusarium culmorum*. The lines were genotyped with SSR and genotyping-by-sequencing (GBS) DArTseq markers. QTL mapping identified six FHB-resistance QTL located on chromosome arms 2BL, 3BS, 4AL, 4BS, 5AL and 6AS. DBC-480 contributed the resistant allele at all QTL. *Fhb1* was detected in all three populations, demonstrating for the first time its successful deployment in durum wheat. The effect of *Fhb1* on FHB resistance in durum wheat was further verified by evaluating specific type 2 resistance in one of the three populations. Plant height had a strong influence in modulating FHB severity. Although the semi-dwarf allele *Rht-B1b* was associated with increased FHB susceptibility, its negative impact on FHB resistance was efficiently counterbalanced in lines carrying *Fhb1*. Semi-dwarf lines with enhanced levels of resistance were selected and are readily incorporated in durum wheat breeding programs. All details are available from Prat et al. (2017).

Acknowledgement

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P 89 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Does breeding for *Fusarium* resistance in wheat just mask the mycotoxins?

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Key message: Metabolisation of mycotoxins *in planta* can lead to masked mycotoxins, which present a health hazard. Despite that resistance QTL enhance the conversion, resistance breeding reduces all mycotoxins, including masked.

The most important threat associated with *Fusarium* head blight (FHB) is the possible mycotoxin contamination. *Fusarium* spp. can produce a range of different mycotoxins, among which deoxynivalenol (DON) is most common. Metabolisation of mycotoxins in planta yields so called 'masked' mycotoxins, which are not routinely analyzed, but remain hazardous since endogenous hydrolases may cleave the compound and reactivate the toxin. Several conjugated forms of DON such as DON-3-glucoside and DON sulfates, but also of zearalenone and fumonisins have been identified to date. The prominent resistance QTL *Fhb1* is associated with resistance to fungal spread and the ability to inactivate DON by conversion into the less toxic DON-3-glucoside. This dependency initiated discussions on possible hidden risks when introgressing this QTL into wheat cultivars because a considerable fraction of the mycotoxin content might be just masked as glucoside but not circumvented from production. Based on published and own data we investigated the effect of FHB resistance breeding in wheat on DON and DON-3-glucoside levels (Lemmens et al. 2016) and studied the relationship of disease measures evaluated on the plants or the seeds and toxin contents (Buerstmayr & Lemmens 2015). The results show that all wheat lines have the ability to convert DON to DON-3-glucoside, independent from their specific FHB resistance level confirming that detoxification of DON to DON-3-glucoside is not a new trait introduced by recent resistance breeding efforts. Our experiments revealed high correlations of FHB symptoms on wheat heads, DON and DON-3-glucoside contents showing that selecting improved lines based on FHB symptoms or DON reduces simultaneously the DON-3-glucoside contamination. The amount of DON-3-glucoside relative to DON contamination varied between 5% and 30% and was influenced by genotypes and environments. Notably the most FHB resistant lines showed the lowest contamination with DON and with DON-3 glucoside, but relatively more DON was glycosylated (up to 30%) compared to susceptible cultivars. Specific resistance QTL (e.g. *Fhb1*) possibly enhance the speed or rate of DON detoxification. Taken together, breeding of new cultivars with reduced *Fusarium* disease severity will lead to reduced toxin contamination, for the prevalent toxins such as DON, but also for less abundant mycotoxins and masked mycotoxins.

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P 91 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Molecular mapping of QTL for deoxynivalenol accumulation resistance in spring wheat

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Key message: Five QTL conferring reduced deoxynivalenol accumulation have been identified. Pyramiding these QTL has the potential to improve the control of the mycotoxin accumulation in adapted wheat cultivars

Deoxynivalenol (DON) produced by *Fusarium graminearum*, the causal agent of Fusarium head blight (FHB), poses a serious health risk when ingested as human food or livestock feed and is the basis of downgrading. Genetic resistance to DON accumulation (Type III resistance) is essential to protect wheat quality. This study aimed to identify DNA markers associated with DON resistance in adapted Western Canadian Red Spring wheat. From a large doubled haploid population derived from the cross between the FHB moderately resistant cultivar Carberry and the FHB moderately susceptible cultivar AC Cadillac, we selected a subset of the most field resistant and susceptible lines and evaluated them for DON accumulation. A genetic linkage map consisting of 2408 SNP markers (Infinium iSelect 90k SNP wheat array) was generated and Multiple QTL mapping (MQM) was performed. Despite the tails being selected based on severity, continuous distribution of DON content was observed indicating resistance to DON is not the same as resistance to severity and is quantitatively inherited. Five significant QTL for DON resistance on chromosomes 2A, 2B, 3A and 6A were contributed by AC Cadillac and a QTL on 3B was contributed by Carberry. The level of phenotypic variation explained by the identified QTL was 5.1% for 2A, 8.1% for 2B, 9.9% for 3A, 5.5% for 3B and 4.7% for 6A. The SNP markers associated with resistance QTL could be utilized to facilitate combining the QTL and accelerating the development of DON resistant adapted wheat cultivars.



P 93 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Comprehensive investigation on the composition, variation, and evolution of the low-molecular-weight glutenin subunit gene family in common wheat and its progenitors

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Key message: A comprehensive molecular genetic and evolutionary analysis of low-molecular-weight glutenin subunits genes was performed in common wheat and its progenitor populations, *Triticum urartu* and *Aegilops tauschii*.

Low-molecular-weight glutenin subunits (LMW-GS), which are encoded by a complex multi-gene family, play an important role in the processing quality of wheat flour, but the allelic variation, composition and evolution of LMW-GS genes in common wheat are not well understood. Using the LMW-GS gene molecular marker system and the full-length gene cloning method, we conducted a comprehensive molecular genetic analysis of LMW-GS genes in a representative population of common wheat and its A and D genome progenitor populations, *Triticum urartu* and *Aegilops tauschii* from the Fertile Crescent region. In the micro-core collection of Chinese wheat germplasm, more than 15 LMW-GS genes were identified from individual accessions, of which 4-6 were located at the *Glu-A3* locus, 3-5 at the *Glu-B3* locus, and eight at the *Glu-D3* locus. LMW-GS genes at the *Glu-A3* locus showed the highest allelic diversity, followed by *Glu-B3* genes, while *Glu-D3* genes were extremely conserved. Expression and sequence analysis showed that 9-13 active LMW-GS genes were present in each accession. In the *T. urartu* population, eight LMW-GS genes were characterized, including four m-, one s- and three i-type, and in each accession 6-7 genes were detected, presenting the highest number at the *Glu-A3* locus. The i-type genes each contain more than six allelic variants, while only 2-3 allelic variants were detected for each m- and s-type gene (Figure 1A). Most accessions had three active i-type genes, rather than one or two in common wheat. In *Ae. tauschii*, each accession contains 8-9 genes and in the whole population one s-type (*D3-578b*) and nine m-type were characterized (Figure 1B). Three variations were detected for each of *D3-393*, *D3-394* and *D3-586*, two and four variations were comprised in *D3-385a* and *D3-441*, respectively, and *D3-522* had six variations. In each accession, six genes (*D3-578b*, *D3-385a*, *D3-394*, *D3-441*, *D3-522* and *D3-575*) were expressed conservatively (Figure 1C), and the rare variation/genes were active, which increased the expressed number of genes. The evolutionary analysis revealed that Southeastern Turkey might be the center of origin and diversity for *T. urartu* due to its abundance of LMW-GS genes/genotypes. The larger number of highly diverse LMW-GS genes and active genes demonstrate that *T. urartu* and *Ae. tauschii* might provide valuable genetic resources for LMW-GS genes to improve the quality of common wheat. These results also contribute to the functional and evolutionary analysis of LMW-GS genes in wheats.

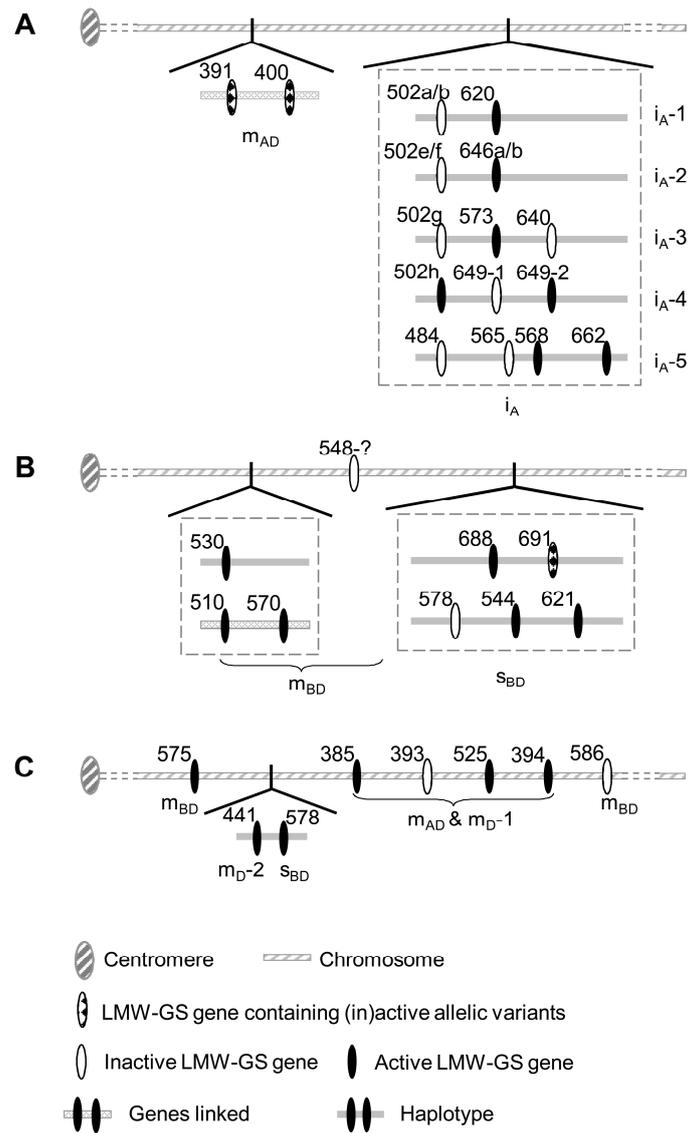


Figure 1: Organization of the LMW-GS genes in homologous group 1 chromosomes in common wheat.



P 95 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Molecular and protein analyses of *Glu-B1a1* allele in Korean wheat landrace

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Key words: Bread-making, *Glu-B1a1* allele, *Glu-B1x7* subunit, glutenin

The *Glu-B1a1* allele is related to strong gluten strength. Canadian and American cultivars with this allele show superior bread-making quality. The *Glu-B1a1* allele was also found in Korean wheat landraces using specific primer. Molecular and protein analyses were conducted to characterize this allele. Korean wheat landrace carrying the *Glu-B1a1* allele showed protein expression on SDS-PAGE similar to Canadian cv. Glenlea. The proportion of *Glu-B1x7* subunit in high-molecular weight glutenin subunit (HMW-GS) of Korean wheat landrace with *Glu-B1a1* allele (43.3%) was lower than in Glenlea (51.8%), but higher than in Chinese Spring (33.2%) using RP-UPLC (reverse-phase ultra-performance liquid chromatographic). The protein expression pattern in 2-DE (dimensional electrophoresis) was similar to that of Glenlea. LTQ-FT-MS (linear ion-trap and Fourier-transform mass spectrometry) is ongoing to determine the protein function of the Korean wheat landrace. The *Glu-B1a1* allele is caused by overexpression of *Glu-B1x7* subunit, known to be due to a gene duplication of the coding sequence and indels of the promoter region. Korean wheat landrace with *Glu-B1a1* allele had 43 bp indel of the promoter region, which is the same size as in Glenlea. The duplicated coding sequence in Korean wheat landrace was also same as in Glenlea. Korean wheat landrace carrying *Glu-B1a1* allele could be used to improve bread-making quality in Korean wheat breeding program because Korean wheats have a narrow genetic variation in glutenin compositions and most Korean wheat cultivars show inferior bread quality.



P 97 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Molecular characterization of novel y-type subunit on *Glu-D1* locus

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Key words: Glutenin, *Glu-D1* allele, HMW-GS, y-type, wheat

High molecular weight glutenin subunits (HMW-GSs) are encoded by *Glu-1* loci. At each locus, HMW-GSs are classified into x- and y-type subunits, which the x-type subunit is slower electrophoretic mobility and higher molecular weight than y-type on SDS-PAGE. Novel y-type subunit was found on *Glu-D1* locus, which wheat line with *Glu-D1y* allele was identified from F₉ lines crossed by two Korean wheat cultivars contained *Glu-D1d* and *Glu-D1f* alleles. This novel subunit showed faster electrophoretic mobility and lower molecular weight than *Glu-D1y12* subunit. Molecular and protein analyses were conducted to identify the characterization of this type subunit. Full length of sequences of this novel subunit was 1962 bp and 18 bp deletion and 3 SNPs, occurred at the 306, 1344 and 1685 bp, were found compared to the sequence of the *Glu-D1y12* (GI: 1036031968) subunit. Four indels, two insertions with 36 bp and two deletions with 24bp, and 21 SNPs were found compared to *Glu-D1y10* (GI: 164457872) subunit. Each protein spot of the novel subunit was very similar to the *Glu-D1y12* subunit using 2-DE and LTQ-FT-MS (linear ion-trap and Fourier-transform mass spectrometry). This novel subunit is probably due to the unequal crossover on the long arm of chromosome 1D. Therefore, more detailed mechanism during chromosome pairing, such as *Ph1* (pairing homoeologous) gene and other inhibitors, should be investigated to determine the cause of this novel subunit. The effect of this novel subunit on the dough rheology and bread-making quality will be also evaluated.



P 99 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Localization of the genes for high gluten content in grain in chromosomes of the second homoeologous group of bread wheat

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Key message: Chromosomes of the second homoeologous group in the lines of wheat Saratovskaya 29 with introgressions from *Triticum timopheevii* and old Siberian cultivars carry the genes responsible for high gluten content.

Gluten content in wheat grain which is closely correlated with protein content is an important trait for classifying the grain for end-use technological purposes. At present, only one gene, *Gpc-B1*, is reliably used in breeding for improvement of gluten content. Previously, we found that the tetraploid endemic species *Triticum timopheevii* can be a donor of high gluten content in grain for bread wheat (Pshenichnikova et al. 1995). Homoeologous introgression from that species in chromosome 2A of cv. Saratovskaya 29 (S29) significantly increased the gluten content in grain. By crossing this substitution line with the same recipient the recombinant lines were obtained. Fragments of introgression in the short arm of chromosome 2A have been marked with microsatellites. Two groups of the lines carrying contrasting parental alleles were tested for the target trait in greenhouse and field conditions. It was shown that the lines carrying the microsatellite alleles from *T. timopheevii*, exhibit a consistently high expression of the trait, 6-8% above the recipient. Another substitution line of S29 with 2G introgression from *T. timopheevii* into 2B chromosome was developed and studied for gluten content. It also showed high gluten content compared to the recipient. The third studied group of the lines was the lines of S29 with the replacement of 2D chromosome from the old Siberian cultivars. Introduction of these chromosomes increased gluten content by 8-10% compared with the recipient. The data obtained indicate the possibility of the existence of a set of homoeoallelic genes in the chromosomes of the second group homoeologous of cereals responsible for the biosynthesis of gluten in wheat grain. Development of isogenic lines for all three genes on the genetic background of cv. S29 is carried out.

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P 101 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Quantification of high molecular weight glutenin subunits in South African hard red wheat cultivars using reversed phase-high performance liquid chromatography

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Key message: Reverse phase-high performance liquid chromatography was used successfully to quantify high molecular weight glutenin subunits, and showed a poor relationship between HMW-GS 5+10 and baking quality in South African wheat.

High molecular weight-gluten subunits (HMW-GS) account for approximately 12% of the total seed storage proteins in wheat and play an important role in determining bread making quality. HMW-GS coded by the *Glu-D1* locus (2+12 and 5+10) have previously been used to predict baking quality in wheat. Subunits 2+12 were reported to correlate with poor quality and subunits 5+10 with good bread making quality. Fifty one commercial wheat cultivars from the South Africa cultivar adaptation trials were evaluated in three production areas of the country namely the dry land summer rainfall, dry land winter rainfall and the irrigation production areas. The aim of this investigation was to quantify the HMW-GS in the cultivars by reverse phase-high performance liquid chromatography (RP-HPLC) and to study the relationship between the quantities of HMW-GS and baking quality parameters, which can contribute to wheat genetic research and breeding practices in South Africa. Proteins were extracted using the method described by Marchylo et al. (1989) with modifications. RP-HPLC separated the HMW-GS in less than 30 min and the absorbance units for the different peaks were calculated according to Wieser et al. (1998). This technique fractionates the subunits by surface hydrophobicity and results were quantified under ultra violet absorbance at 210 nm. The relative eluting times were 1Ax > 1Bx > 1Dx > 1By > 1Dy and the specific eluting subunits for the different production areas were as follows: dry land summer rainfall cultivars 12, 10, 8, 18, 9, 5, 2, 7, 17, 1 2*; dry land winter rainfall cultivars 12, 10, 18, 9, 8, 16, 5, 2, 7, 13, 17, 1, 2*; and the cultivars in the irrigation region 12, 10, 8, 18, 16, 5, 2, 7, 13, 17, 1, 2*. Subunit 1Bx7 was overexpressed in most of the cultivars and 1Ax2* and 1Dx2 were easily separated and readily identified with RP-HPLC. The results revealed that RP-HPLC is an efficient method for the quantification of HMW-GS from different commercial wheat cultivars and is a valuable tool in wheat breeding and genetics since it opens up a gateway to explore the wealth of information encoded in the endosperm proteins.

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P 103 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Manipulation of high molecular weight glutenin subunits in bread wheat to reduce nitrogen fertility requirements for bread quality

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Key message: The objective of this study is to investigate the effect of the Bx7^{OE} allele on the bread-making quality in wheat with sub-optimal levels of grain protein.

High molecular weight glutenin subunits (HMW-GS) contribute to the unique bread-making qualities of wheat (*Triticum aestivum*) by determining gluten elasticity. Common wheat possesses three to five HMW-GS which are encoded by genes at the *Glu-1* loci on the long arms of chromosomes 1A, 1B, and 1D. Each locus consists of two linked paralogous genes that encode for x and y-type subunits (Shewry et al. 2003, Ravel et al. 2014). Wheat grown in the Pacific Northwest often produces high grain yield but low grain protein. Thus, the production of hard wheat used for bread-making usually requires high nitrogen input. The objective of this study is to evaluate whether a change in the gluten composition caused by an overexpression of the Bx7 allele (Bx7^{OE}) improves the bread-making quality in wheat with sub-optimal levels of grain protein. The Bx7^{OE} allele was introgressed into five different genetic backgrounds of breeding lines adapted to the growing conditions in the Pacific Northwest. Homozygous lines with and without the Bx7^{OE} allele were selected. A total of sixty lines were planted in replicated field trials over two years at two locations. A high and a low level of nitrogen was applied to investigate the allelic effect of Bx7^{OE} under different nitrogen fertility levels. Grain characteristics were measured using an NIR grain analyzer and a Perten SKCS 4100. Dough properties were evaluated using a mixograph and an extensograph. SDS-unextractable proteins were measured with HPLC. Protein sizing and quantification were performed using the Agilent 2100 Bioanalyzer. The end-product quality was assessed through baking tests. Preliminary results indicate differences for quality traits between environments, between fertilizer treatments, between lines with and without the Bx7^{OE} allele and among the genotypes tested.

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P 105 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Characterization of gliadins using aneuploids of Chinese Spring wheat reveals genome-specific gliadin regulation

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Key message: Gliadins in Chinese Spring wheat were characterized by 2D-PAGE. We found that specific gliadins harboring an epitope of celiac disease are suppressed by an unknown factor located on chromosome 2A.

The wheat seed storage proteins gliadin and glutenin comprise gluten, which influences the properties of flour, but causes allergic reactions including celiac disease (CD). Gliadins are classified into α/β , γ , and ω -gliadins and are encoded by multigenes. Genes encoding α/β -gliadins belong to a large multigene family at *Gli-2* loci on group 6 chromosomes. Other gliadins are encoded by *Gli-1* loci on group 1 chromosomes. To characterize gliadins, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) profiles were compared among wild-type and aneuploid Chinese Spring wheat lines. Gliadin proteins were selectively extracted from mature seeds and separated into 70 spots of 25-50 kDa by 2D-PAGE. Among them, 10, 10, and 16 spots were encoded on chromosomes 6A, 6B, and 6D, respectively, suggesting that they were α/β -gliadins (Table 1). Similarly, 3, 3, and 7 spots were encoded on chromosomes 1A, 1B, and 1D, respectively, indicating that they were γ -gliadins (Table 1). Spots that could not be assigned to chromosomes were sequenced at the N-terminal region and were all determined to be α/β -gliadins or γ -gliadins. The number of spots was slightly higher than estimated by an expressed sequence tag analysis (Kawaura et al. 2005). Interestingly, 2D-PAGE profiles showed that specific α/β -gliadin spots were lost in tetrasomy chromosome 2A lines, even though genes encoding gliadins are located on group 1 and 6 chromosomes. Furthermore, western blots against Gli- $\alpha 9$ peptide, an epitope of CD, suggested that a double dose of chromosome 2A suppressed α/β -gliadins harboring the CD epitope on chromosome 6D. To clarify whether the suppression occurs during transcription or post-transcriptionally, quantitative RT-PCR was conducted using chromosome-specific α/β -gliadin primers (Noma et al. 2016). Gene expression was measured in developing seeds in wild-type, tetrasomy 2A, and chromosome 6 aneuploid lines, revealing that the expression of α/β -gliadin genes on chromosome 6D was suppressed by an overdose of chromosome 2A. These findings suggest that trans-factor(s) that regulate the transcription of α/β -gliadin genes are located on chromosome 2A and contribute to repressing the cause of CD.

Acknowledgement

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Table 1: Number of gliadins encoded on each chromosome

Gliadin	Chromosome	No. protein spots	No. expressed genes
α/β -gliadin	6A	10	11 ^a
	6B	10	13 ^a
	6D	16	12 ^a
	unknown	12	
	Total		48
γ -gliadin	1A	6	2 ^b
	1B	3	
	1D	7	4 ^b
	unknown	6	5 ^b
	Total		22

Expressed genes were estimated by EST analyses (^aKawaura et al. 2005, ^bAnderson et al. 2013)

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P 107 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

The influence of high molecular weight-glutenin subunits on SDS sedimentation volume and Mixsmart characteristics in elite wheat lines grown at three locations

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Wheat (*Triticum aestivum* L.) is one of the staple cereal crops of humankind. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) is a useful method for estimating high molecular weight-glutenin subunits (HMW-GS) of hard red bread wheat genotypes. This study investigated the relationship between HMW-GS separated by SDS-PAGE and measured quality characteristics of 13 wheat elite lines across three locations. A total of nine alleles of HMW-GS were detected at three loci *Glu-A1*, *Glu-B1*, *Glu-D1*. Subunits 5+10, associated with good bread making quality, were expressed in three lines and subunits 2+12 were encoded in 10 lines. Genotype contributed the highest percentage to variation in SDS sedimentation volume. SDS sedimentation is an effective, fast and inexpensive method to perform, which has been widely used as a quality indicator in breeding programmes. When combining data for all locations, a highly and negatively significant correlation between subunits 13+16 and sodium dodecyl sulphate sedimentation volume was seen. Mixograph characteristics; envelope peak integral and envelope peak value were significantly and positively correlated with subunits 13+16. Envelope peak integral, envelope peak time, midline left integral, midline left time, midline peak integral, midline peak time and midline right time showed negative and positive significant correlation with subunits 2+12 and 5+10, respectively. There were significant correlations between subunits 7+8 and envelope peak integral, envelope peak time, midline left integral, midline left time, midline peak time and midline right time. In single locations, a negative significant correlation between SDS sedimentation volume and subunits 13+16 in Douglas and Marydale was observed. The genetic variation of HMW-GS can lead to different combinations of subunits which can influence the quality of the end-product.



P 109 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Mapping quantitative trait loci for gluten strength in Canadian durum wheat

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Key message: QTL located on chromosome 1A, 1B and 3A that were expressed consistently across environments can be exploited to enhance gluten strength to the targeted level in durum wheat.

Gluten strength determines the end product quality of durum wheat and is an important breeding target of durum cultivar. To characterize the quantitative trait loci (QTL) controlling gluten strength in Canadian durum wheat, a population of 162 doubled haploid (DH) lines segregating for gluten strength derived from Strongfield × Pelissier was used in this study. The DH lines, parents and checks were grown in two years and two seeding dates in each year and gluten strength of grain samples was measured by SDS sedimentation volume (SV). With the genetics map created using data from the Illumina Infinium wheat 90K SNP (single nucleotide polymorphism) chip, QTL contributing to gluten strength were detected on chromosome 1A, 1B, 2B, 3A and 6A. Two major and stable QTL mapped on chromosome 1A and 1B explaining 15-19% and 25-40% of the phenotypic variance respectively were consistently detected over two years and two seeding dates, with favourable alleles derived from Strongfield. One minor QTL located on chromosome 3A explaining up to 9% of the phenotypic variance, with beneficial allele derived from Pelissier, was detected across all environments as well. Another two minor QTL located on chromosome 2B and 6A were found in three out of four environments. The closely linked SNP markers can be combined with Kompetitive Allele Specific PCR (KASP) technique to develop more efficient marker assisted selection (MAS) for gluten strength of durum. Pyramiding QTL that were expressed consistently across environments could be used to enhance gluten strength to the targeted level in durum wheat.



P 111 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

GWAS and genomic prediction of baking quality in winter wheat breeding lines

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Key message: Correlations between observed and predicted phenotypes of wheat baking quality parameters show good potential for developing genomic predictions.

Wheat baking quality parameters are expensive and time-consuming to phenotype, so prediction of the phenotypes based on DNA markers is useful in breeding programs. In traditional marker-assisted selection, few DNA markers linked to QTL with large effects are used. To predict quantitative traits more accurately, genomic prediction can be used. Here, thousands of DNA markers across the genome are used to capture the effect of both large, intermediate, and small QTL. Training sets of lines that have been both phenotyped and genotyped are used to develop models for predicting phenotypes (or genomic values) of other lines based on their genotypes. Validation sets, where phenotypes of lines are predicted from the genotypes, are used to determine the predictive ability of the models based on the correlation between observed and predicted phenotypes. In this study 672 winter wheat lines from two breeding cycles of the plant breeding company Nordic Seed were phenotyped for the baking quality parameters grain protein content, flour yield, Zeleny sedimentation, falling number, and alveograph W, P, and L. All lines were genotyped using the Illumina 15K wheat SNP chip, and the informative SNP markers were used for GWAS and for genomic predictions. GWAS were done using single marker regression and using Bayesian Power Lasso models. In the Bayesian Power Lasso models, all SNPs were fitted simultaneously and their effects were assumed to follow an exponential power distribution. This might resemble the genetic architecture of the traits more realistically compared to effects from a normal distribution, since large SNP effects are shrunken less and small SNP effects are shrunken more. The GWAS indicated that the quality parameters are controlled by many QTL with small effects. Therefore, genomic prediction based on all SNPs seems to be a more effective strategy than selection based only on few SNPs. Accuracy of the genomic predictions were evaluated using several kinds of cross-validations. These showed that the genetic relationship between lines of the training and validation sets had a bigger impact on the predictive ability than the size of the training set. The genomic predictions for quality traits showed good potential with correlations ranging from 0.5 for grain protein content to 0.79 for Zeleny sedimentation based on Leave-One-Out cross-validations.

Acknowledgments

Phenotyping was done at Nordic Seed, Denmark, and at CIMMYT (International Maize and Wheat Improvement Center), Mexico. The study is funded by 'Innovation Fund Denmark' and 'Erstatningsfonden for Sædekorn'.



P 113 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Meta-QTL analysis for yellow pigment content in durum wheat

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Key message: This study reports several Meta-QTL for grain yellow pigment content in durum wheat and provides evidence for putative candidate genes based on the available sequence information.

Carotenoids are associated to lower risk of developing cancer and chronic diseases. The yellow colour of semolina is an important trait for durum wheat grain quality due to the carotenoid grain yellow pigment content (GYPC), a favourable feature for pasta and other products. As a result, GYPC is actively selected in durum wheat breeding programs. With the aim to provide an accurate inventory of QTLs for GYPC present in elite durum wheat, we analysed kernels of (i) F_{6:7} RIL populations Colosseo × Lloyd, Meridiano × Claudio, Kofa × Svevo and Simeto × Levante and (ii) a world-wide collection of 183 elite accessions (Durum Panel) evaluated in four different locations (in Italy, Mexico and Obregon). A genetic characterisation of RILs and Durum Panel was carried out using the SNP 90K iSelect Infinium wheat assay from the Illumina Platform and a QTL analysis based on single-marker analysis and multiple interval mapping (MIM) for RILs and a GWAS using mixed linear model (MLM) in Tassel v.5.2.7. The projection of the QTL onto a unique reference tetraploid consensus map identified 72 Meta-QTL, with major and stable QTL on chromosomes 1A, 1B, 3A, 3B, 6A, 6B, 7A and 7B. The use of a transcript-based SNP platform allowed us to cross-link SNPs, genes, homeologs, and anchor QTL-chromosome regions to well-defined syntenic intervals common to wheat, barley, *Brachypodium* and rice. Additionally, SNPs and corresponding gene sequences allowed us to anchor the same regions to the recently released Chinese Spring and W7984 assemblies which are being investigated for genes involved in the biosynthesis and degradation of carotenoids and non-mevalonate pathway. A remarkable number of QTL confidence intervals overlapped the location of the following candidates: All-trans-nonaprenyl diphosphate synthase (1A), farnesylcysteine lyase (2B), farnesyl diphosphate synthase (3A), farnesol dehydrogenase (4A), geranyl diphosphate synthase (1A), geranylgeranyl reductase (1B), lipoxigenase 3 (1B, 2B, 4A and 6B), prenylcysteine α-carboxyl methyltransferase (3A), phytoene synthase 1 (1A, 2B, 7A and 7B), 2 (2B) and 3 (7A and 7B), xanthoxin dehydrogenase (5B), 1-deoxy-D-xylulose 5-phosphate reductoisomerase (5A), 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (6B). Haploview software has allowed for the identification of conserved linkage blocks in the QTL/gene regions and their related effects.

Acknowledgement

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P 115 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

QTL mapping for some grain quality traits using DArTseq markers

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Key message: For efficient wheat quality breeding it is important to start with marker assisted selection as soon as possible.

Higher costs of wheat quality improvement are one of the main constraints in breeding programs, due to increased number of field trials to simulate all target environments and increased number of laboratory tests to assess quality. Despite some limitations, the effective use of molecular marker technology provides a viable and challenging way to alleviate the problem. A set of trials in 5 environments with 141 recombinant inbred lines from Bezostaya-1/Klara population was carried out in order to dissect phenotypic variation for some wheat quality traits such as grain protein content, wet gluten content, test weight and thousand kernel weight. The standard ANOVA and AMMI analysis, using R and MATMODEL software, revealed significant contribution of environmental (most important), genotypic and GEI components to total variation for grain protein content and test weight (94%), wet gluten content (94.7%), test weight and thousand weight kernels (90%). Quantitative trait loci associated with grain quality traits will be mapped using *DArTseq* markers, which will give us information about residing markers possibly exploitable in selection for wheat quality in early generations and thus minimizing trade-off between yield and quality.

Acknowledgements

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P 117 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Two tightly linked genes controlling grain length underlie a major grain weight QTL in polyploid wheat.

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Key message: Two tightly linked genes underlying a major grain weight QTL in wheat act early in grain development to increase final pericarp cell length, translating to an increase in grain length.

Wheat provides ≈20% of the calories and ≈25% of protein consumed by humans. However, a step-change in yield increase must be delivered to keep up with current and future demand. Final grain yield is a highly complex trait influenced by many interacting genetic and environmental factors and as such is not well understood. Grain weight, determined by grain length and width, is an important yield component: we hypothesise that identifying the genes controlling grain weight will provide a targeted route to manipulating yield in wheat. We have identified a stable quantitative trait loci (QTL) on chromosome 5A for thousand grain weight (TGW) and validated it using BC₄-near isogenic lines (NILs) across several years and environments (Figure 1A). The effect on TGW is specifically driven by an increase in grain length (Figure 1B) with no significant detrimental effects on other yield components. Fine mapping of the QTL suggests that there are two tightly linked genes, *GL-A1* and *GL-A2*, which are linked in *cis* and have an additive effect on grain length (Figure 1C,D). Characterisation of the *GL-A1* and *GL-A2* NILs suggests that these genes affect early grain development and grain filling rate, and act to increase the length, but not the number, of pericarp cells (Figure 1E,F). Transcriptomic analysis has identified genes from distinct biological pathways which are differentially expressed between NILs. These pathways include the ubiquitin-mediated protein degradation pathway, which has previously been shown to be important for the control of grain size in wheat (Simmonds et al. 2016). These genes, together with candidate genes from the physical interval, are being investigated using mutant lines identified from the wheat exome-captured TILLING platform. Haplotype analysis using exome capture data from a wide range of germplasm is being conducted to understand the allelic variation of candidates across the *GL-A1* and *GL-A2* regions. Pyramiding of *GL-A1* and *GL-A2* with a previously identified QTL for grain width on chromosome 6A (Simmonds et al. 2014) leads to additive effects on TGW.

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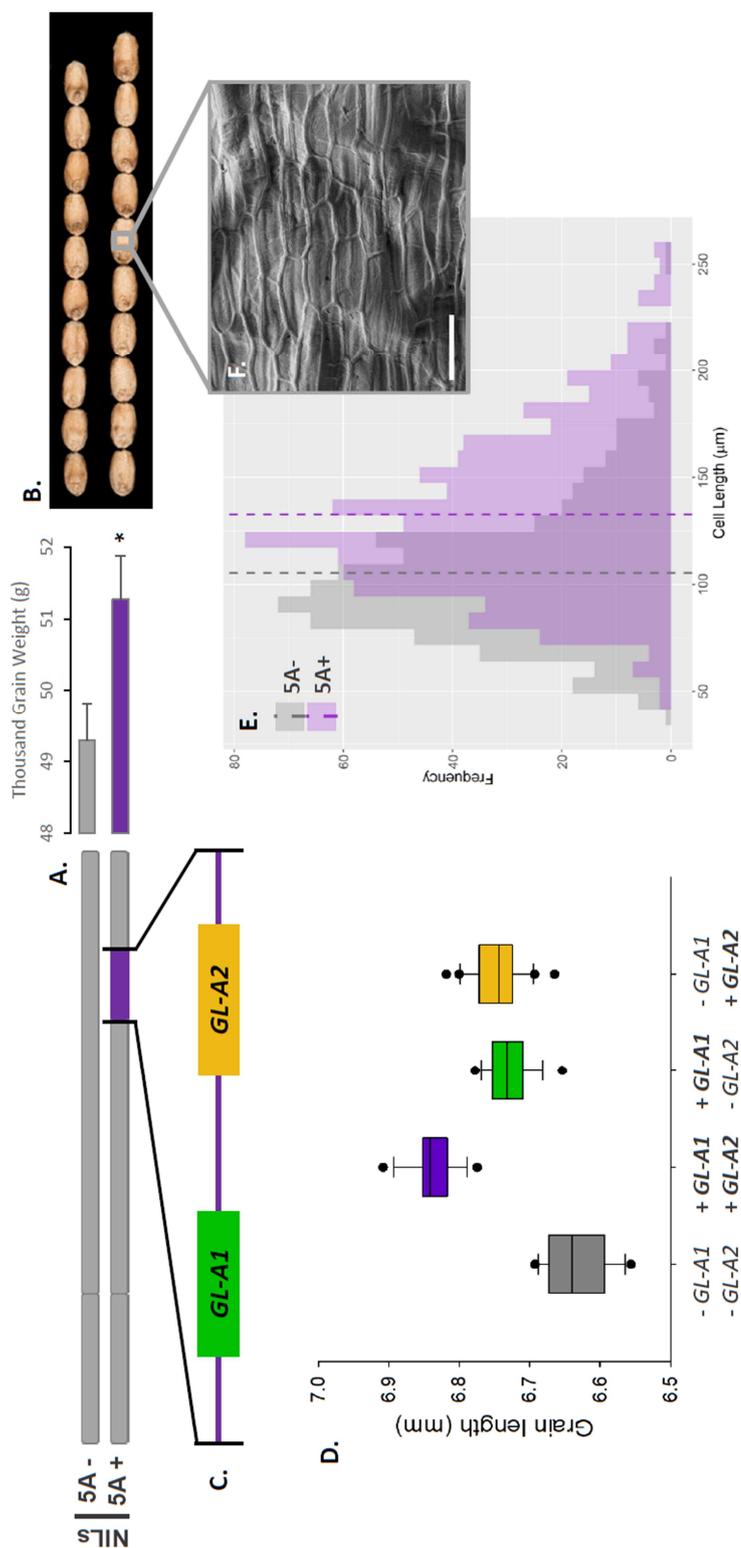


Figure 1: Two tightly linked genes controlling grain length underlie a major grain weight QTL. Near Isogenic Lines (NILs) developed for a major grain weight QTL show a ~5% increase in thousand grain weight (TGW) ($*=p<0.05$) (A) driven by an increase in grain length (B). Fine mapping of the QTL suggests that there are two tightly linked genes, *GL-A1* and *GL-A2*, (C) that have an additive effect on grain length (D). Imaging of the mature grains of NILs using scanning electron microscopy (F, scalebar=100 μm) suggests that these genes increase cell length in the pericarp (E, distribution of cell length in the pericarp of NILs with mean shown by dashed line).



P 119 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Wheat quality evaluation: from the seed to the final product

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Key message: Time and sample size are two important criteria for wheat quality evaluation. The development of small-scale rapid methods for predicting wheat performance is of great interest for breeders and millers.

The rheological properties of wheat are considered of great importance for determining technological performance and useful tools for predicting process efficiency (e.g. dough yield, extensibility and leavening conditions) and product quality (e.g specific volume) have been proposed along decades. However, most of the procedures available are time consuming and require a large amount of samples. Over the years, the needs along the value chain of wheat have changed. Breeders look for reliable methods to test the functional quality of wheat lines at early stages, with just a limited amount of sample. The milling industry needs fast and reliable methods for checking wheat quality right at the receiving station. Finally, the baking industry is looking for suitable methods that could predict end product quality for production and product development. In this frame, the GlutoPeak test has been recently proposed for the evaluation of wheat flour quality. It is a high shear based technique that measures the aggregation behavior of gluten upon addition of water and high-speed mixing. The test is rapid (<5 min) and it requires less than 10 g of sample, and it provides two main attributes of gluten quality: (i) torque that is an indication of strength of gluten; (ii) time to peak that is an indication of kinetics of gluten aggregation. This presentation will provide an overview of the points of strength and weaknesses of this new test and its potential application along the wheat value chain. Various case studies on the use of this test for the characterization of wheat flour and durum wheat semolina will be presented, to fill the knowledge gap between gluten composition, its aggregation kinetics, and flour/semolina performance. Results will show: (i) why flours with similar protein content and dough rheology have different bread-making performance; (ii) the correlation between the GlutoPeak indices and many of the conventional parameters which are currently used for flour characterization; (iii) the relation between gluten aggregation properties of wholegrain flour and the baking performance of refined flour; (iv) the relation between gluten aggregation properties and semolina pasta-attitude and the potential use of the test in breeding programs.



P 121 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Grain quality of synthetic wheats and their relatives

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Di-, tetra- and hexaploid wheat, *Aegilops* and synthetic forms of winter (24 lines) and spring wheat (34 lines) were studied for grain protein content. In the various species different protein fractions were prevalent: globulin for *Aegilops triaristata* grain (40.6% of total) and *Triticum militinae* (35.7%); gliadin for *T. dicoccoides* grain (38.9%), *T. dicoccum* (34.5%) and *T. timopheevii* (33.7%). Gluten content was in the range of 14.3-17.7% for *T. kiharae* up to 26% for *Ae. triaristata*. For technological processing the most preferred grain ratio N:S was in the range of 1 : 13-15. This criterion is in line with *T. compactum* (15.1); *T. timopheevii* (15.2); *T. turgidum* (15.3) and *T. aethiopicum* (15.8). A ratio >17 is regarded as deficient in sulfur in the formation of high protein. Classification of wild, cultivated and synthetic forms of winter wheat grain by cluster analysis based on the biochemical composition allowed to group the genotypes in three clusters: (1) synthetic and variety Komsomolskaya 1; (2) wild species and (3) varieties plus two synthetics. Wild relatives and synthetics were also analyzed for content and quality of gluten, Zeleny sedimentation, test weight, grain hardness, vitreousness and ash content of flour. According to the physical properties of flour and dough the synthetics varied from 80 to 170 alveograph units which corresponds to 'filler' and 'weak' wheat. Best values for the valorimetric evaluation were observed for Bezostaya 1 × *Ae. triaristata* and ErythrospERMUM 350 × *T. militinae*. Bakery assessment showed that synthetics baked bread with loaf volumes comparable to varieties including Almaly (720-760 ml) and Karahan (800 mL) (Figure 1). Using cluster analysis of gliadin electrophoresis spectrum we classified wild relatives. The most remote of all were *T. monococcum* and *T. ispahanicum*. Distribution of species into 3 clusters was determined by the absence of (full or majority) α-gliadin for *Aegilops*, and absence of ω9-gliadin or ω89-gliadin for tetraploid wheat and *T. compactum* (A^bGD). The synthetic forms revealed the presence of gliadin components specific to *T. kiharae* and *Ae. cylindrica* in ω-zone; *T. militinae* and *T. timopheevii* in α-gliadin zone. Identification of synthetic wheat by SSR-markers *Xgwm312*, *Xgwm135* and *Xgwm304*, respectively enabled to detect between 2-7 alleles per locus (Turuspekov et al. 2016) that allows to certify DNA genotypes for further use in the monitoring and selection.

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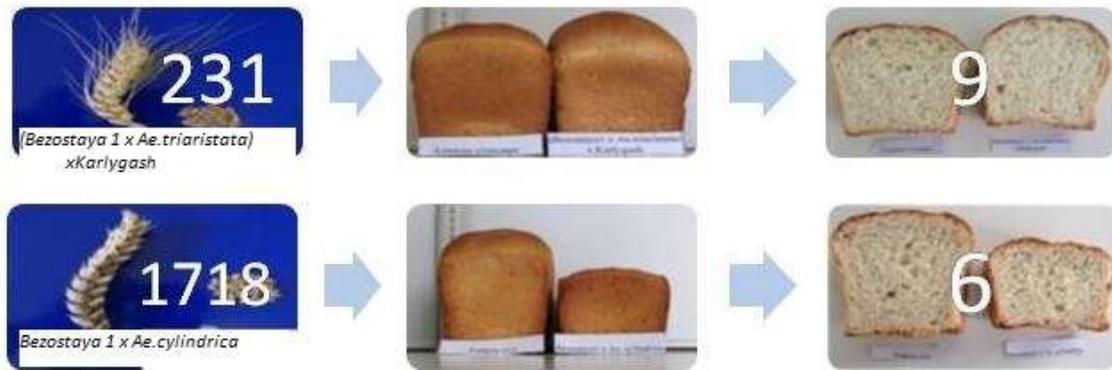


Figure 1: Bread volume and Payne score according to the HMW glutenin composition (Payne 1987) for wheat introgression lines 231 (*Bezostaya 1/Ae. triaristata//Karlygash*), and 1718 (*Bezostaya 1/Ae. cylindrica*).



P 123 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Effects of breeding in the 20th century on the morpho-physiology, yield and quality of Italian and Spanish durum wheat

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Key message: Breeding in the past century increased yield, adaptability and pasta-making quality by improving inputs use-efficiency, reverting assimilates to reproductive organs, fitting phenology and selecting for the most favorable quality alleles.

Understanding how past genetic gains were achieved gives clues to orientate future breeding strategies. This study summarizes the results of a comprehensive analysis including 12 Italian and 12 Spanish cultivars representative of the grown in those countries along the 20th century, over 13 field and 3 glasshouse experiments. Genetic gains for yield were 16.9 kg ha⁻¹ y⁻¹ in Italy and 23.6 kg ha⁻¹ y⁻¹ in Spain (0.51% y⁻¹ and 0.72% y⁻¹ in relative terms). Despite both countries followed different breeding strategies, yield gains were related to the same plant attributes. Up to 70% of yield gains were due to the introduction of the *Rht-B1b* dwarfing allele, which reduced plant height -0.81% y⁻¹ and increased harvest index (HI) 0.48% y⁻¹, with no effect on total biomass at maturity. Plant height and HI did not change beyond 1980. Plant biomass at anthesis was reduced above and below ground (-7.6% and -28.1%, respectively) by the introduction of *Rht-B1b*. When considering only semidwarf cultivars, no significant relationship existed between root and aerial biomass with yield, as improved cultivars were more efficient in remobilizing pre-anthesis assimilates to grains. Despite their reduced root system, modern cultivars were more responsive in terms of yield and number of grains per spike to environments with high water input after anthesis. Grain yield improvements were based on linear increases in the number of grains per m² (0.55% y⁻¹) with no significant changes on grain weight. Plants per m², spikes per plant and grains per spike contributed 20%, 29% and 51% respectively, to raise grains per m². Both, the number of fertile florets at anthesis (0.17% y⁻¹) and the percentage of florets setting grains (0.17 y⁻¹), contributed to improve the number of grains per spike. Grain setting could benefit from the reduction on 7 days, on average, in the length of the cycle to anthesis, as reduced the possibility of heat stress during grain filling. Breeding also improved overall pasta-making quality, the EU index increased by 0.13% y⁻¹ in Italy and 0.06% y⁻¹ in Spain. Gluten strength was the trait with a major contribution to this advance (IT: 0.64% y⁻¹, ES: 0.49% y⁻¹), followed by grain color (IT: 0.15% y⁻¹, ES: 0.10% y⁻¹), as protein content decreased (IT: -0.14% y⁻¹, ES: -0.19% y⁻¹) and no significant changes were observed in test weight. Progress in gluten strength was attained by the selection for the most favorable LMW-GS.

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P 125 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Mapping of agronomically important quantitative trait loci in diploid wheat (*Triticum monococcum* L.)

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Key message: 78 QTL affecting 13 agronomically important traits were identified in a diploid wheat (*Triticum monococcum*) mapping population.

Bread wheat (*Triticum aestivum* L.) is a major crop providing staple food for 40% of world's population. The main obstacles hampering the identification, mapping and cloning agronomically important genes are its genome size (1C~17 Gb), complexity of the allohexaploid genome (2n=6x=42, AABBDD), and high repetitive DNA content (80%). One option to simplify the problem is the use of diploid *T. monococcum* L. (2n=2x=14) as surrogate. Using a recombinant inbred line (RIL) population derived from a cross between *T. monococcum* ssp. *monococcum* DV92 and *T. monococcum* ssp. *aegilopoides* G3116, we have genetically mapped 13 agriculturally important quantitative traits (grain protein, grain weight, grain shape, spikelets per spike, kernels per spikelets, spike length, density and shattering, tiller number, ear emergence time, plant shape, plant height, and leaf hairiness). In total the map comprises 406 DArT, IRAP, SSR and STS markers. Phenotypic data were collected over four years at three geographical locations, and 78 quantitative trait loci (QTLs) were mapped to the seven chromosomes. While a majority of QTL were identified for 'grain shape' (27), the least number of QTL were mapped for 'tiller number' and 'kernels per spikelet' (two for each trait). The highest number of QTL (18) was detected on chromosome 2A^m and only three QTL were mapped to chromosome 6A^m. The identified QTL will be validated and used for characterization of genes responsible for variation in the traits.

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P 127 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Wheat SPA gene regulate quality character

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Key message: We examine SPA RNAi transgenic plants' SPA, high molecular weight glutenin subunit and gliadin genes expression. *Glu-1* (except *glu-B1-1*) and SPA genes expression peak at the same time, *glu-B1-1* and gliadin genes expression continue increase.

It has been reported that transcription factors, such as storage protein activator (SPA) in wheat could regulate the transcription of wheat grain storage protein (GSP) genes. SPA belongs to the bZIP TF of the Opaque2 subfamily, and can interact with the GCN4 motif upstream of the GSP genes. In order to investigate the effect of SPA on wheat quality, transgenic plants with SPA overexpression and RNAi vector were generated by *Agrobacterium*-mediated method, and 5 positive overexpressing plants and 26 RNAi transgenic plants were obtained. SDS-sedimentation test of transgenic plants showed that SDS sedimentation of RNAi strains (the average value is 24 ml) were lower than that in control (28 ml). The gene expression of SPA, high molecular weight glutenin subunit (HMW-GS) and gliadin by qPCR showed that *Glu-1* (except *glu-B1-1*) which encode HMW-GS gene and SPA got peak at 12 or 15 days post anthesis (DPA), while the gene expression of *glu-B1-1* and gliadin continued to rise from 5 to 20 DPA (Figure 1). The results provided indirect evidences that SPA gene played an important role in the regulation of wheat quality.

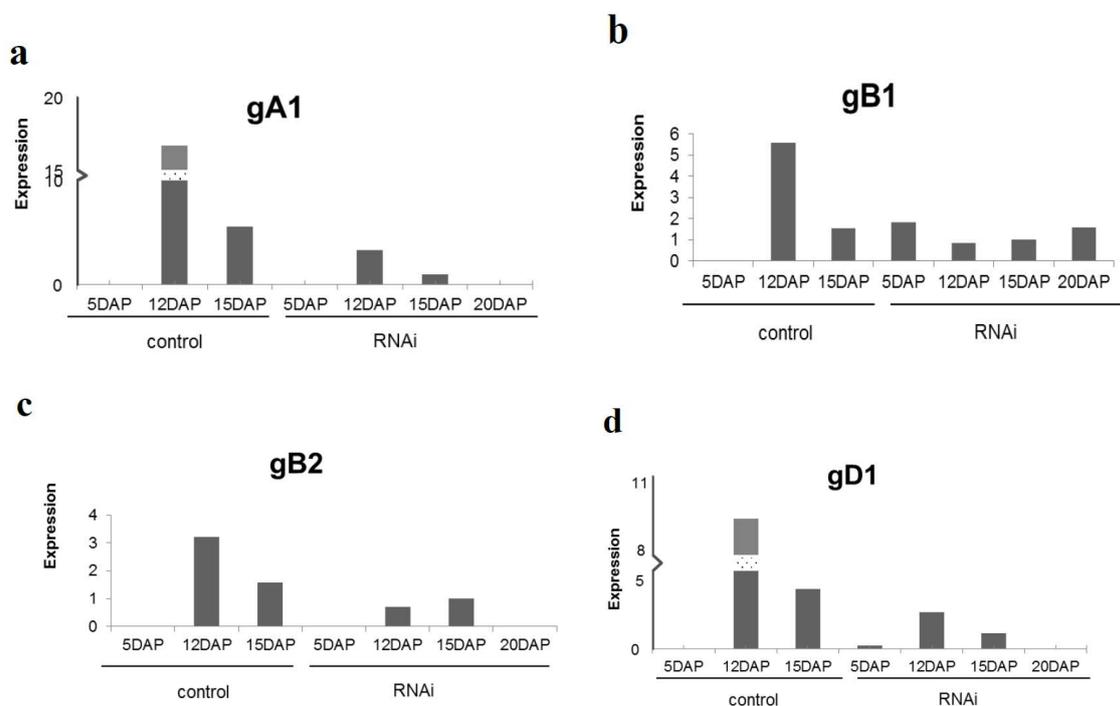


Figure 1: The relative expression of HMW-GS (a-e), α -gliadin (f) and three copies of SPA (g-i) genes during the development of wheat grain endosperm (continued next page)

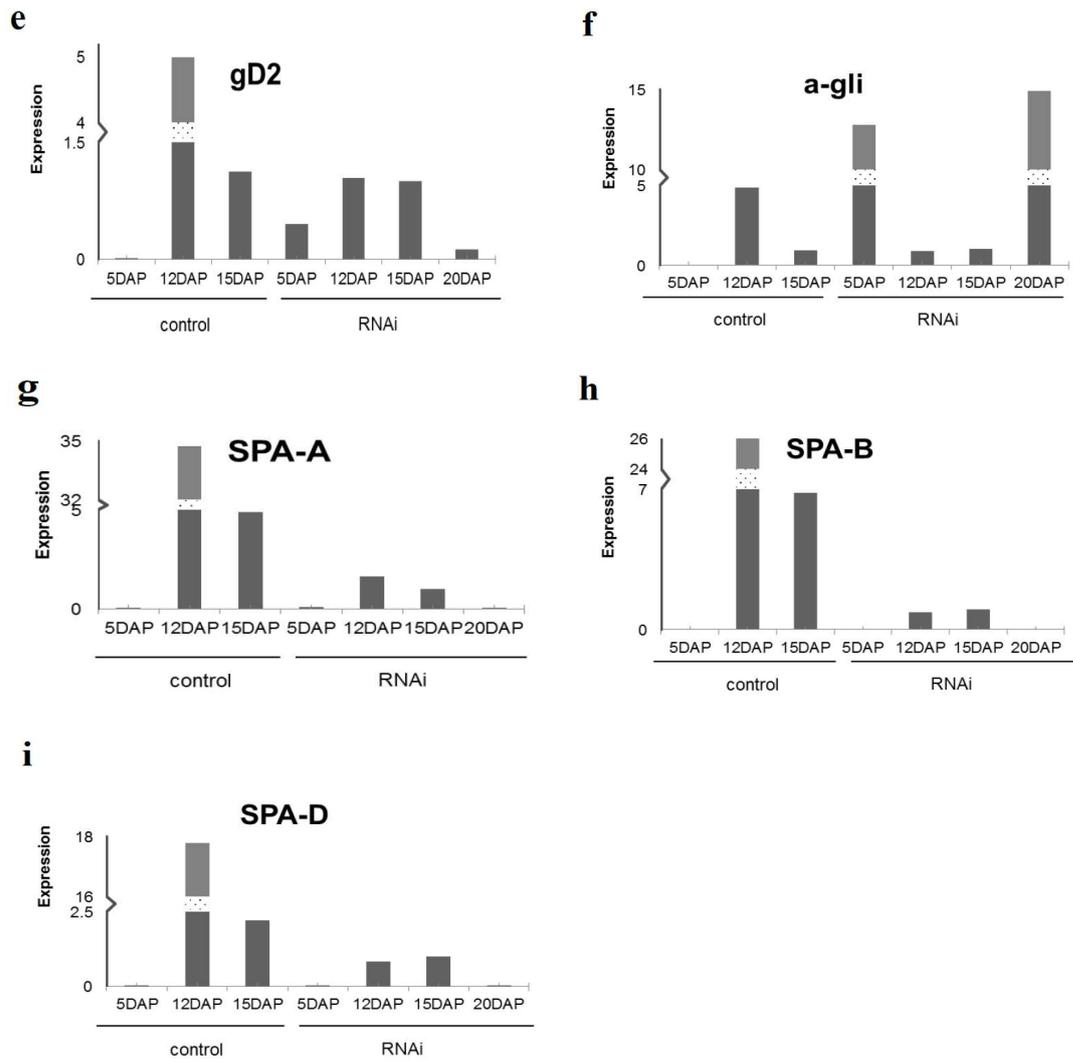


Figure 1 (continued): The relative expression of HMW-GS (a-e), α -gliadin (f) and three copies of SPA (g-i) genes during the development of wheat grain endosperm.



P 129 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Genomic prediction of baking quality in winter wheat

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Key message: Genomic prediction appears to be a promising strategy in breeding programs for improving baking quality traits in winter wheat.

In this study 672 winter wheat lines from two breeding cycles of the plant breeding company Nordic Seed were phenotyped for the baking quality parameters grain protein content, flour yield, Zeleny sedimentation, falling number, and alveograph W, P, and L. All lines were genotyped using the Illumina 15K wheat SNP chip, and the informative SNP markers were used for GWAS to identify SNPs with large effects on the quality parameters, and for genomic predictions using GBLUP models. SNPs significantly associated with Zeleny sedimentation, flour yield, alveograph W and P were identified. For grain protein content, falling number, and alveograph L, SNPs close to the significance threshold were found on several chromosomes. The GWAS indicate that the quality parameters are controlled by many QTLs with small effects. Therefore, genomic prediction based on all SNPs seems to be a more effective strategy than selection based only on few SNPs. Accuracy of the genomic predictions were evaluated using several kinds of cross-validations to determine the effect of genetic relationships between lines in the training and validation sets and of the size of the training set. Decreasing the number of lines in the training set had a small negative effect on the correlations between observed and predicted phenotypes corrected for fixed effects, while reducing the genetic relationship between the lines of the training and validation sets had a bigger negative effect. The genomic predictions for quality traits showed good potential with correlations ranging from 0.5 for protein to 0.79 for Zeleny sedimentation based on Leave-One-Out cross-validations. Hence, genomic prediction of baking quality in wheat using dense DNA markers can enable selection of lines at early stages of breeding programs and save resources compared to phenotyping.

Acknowledgements

Phenotyping was done at Nordic Seed, Denmark, and at CIMMYT (International Maize and Wheat Improvement Center), Mexico. The study is funded by 'Innovation Fund Denmark' and 'Erstatningsfonden for Sædekorn'.



P 131 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Mapping QTL controlling kernel morphology and weight in wheat

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Key message: Using the RIL mapping population derived from the cross of the key wheat breeding parent Nanda2419 with landrace Wangshuibai, QTL controlling kernel morphology and weight were identified and analysed.

Wheat (*Triticum aestivum* L.) kernel morphometry affects both yield potential and quality. Here, a recombinant inbred line (RIL) population, created by crossing the key wheat breeding parent Nanda 2419 with landrace Wangshuibai (Xue et al. 2008), was evaluated for kernel length, width, thickness and grain weight in five trials at two different geographical locations. As expected, the kernel thickness, kernel width and length were significantly related to the grain weight. The correlation coefficient of grain weight with the kernel thickness was the highest and with the kernel length the lowest. Through whole genome scan, eleven quantitative trait loci (QTL), distributed on five chromosomes, were identified in more than three year-location combinations, individually explaining 8.0% to 42.2% of the phenotypic variations. Nanda 2419 contributed all alleles associated with higher grain weight and wider kernels. The Nanda 2419 alleles of the two 4B and 5A QTLs had the strongest positive effects on kernel width and thickness, and conditioned wider, thicker, rounder and heavier kernels (Jia et al. 2013, Huang et al. 2015). These results could contribute to further improvement of wheat quality and yield.

Acknowledgments

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P 133 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

End-use quality of soft kernel durum wheat

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Key message: Soft kernel durum wheat possessing the *Hardness* locus exhibits milling and baking characteristics consistent with soft hexaploid wheat. Various durum parents contribute significant variation to soft durum wheat end-use quality.

Kernel texture is a major determinant of end-use quality of wheat. Durum wheat is known for its very hard texture, which influences how it is milled and for what products it is well suited. We developed soft kernel durum wheat lines via *Ph1b*-mediated homoeologous recombination with Dr. Leonard Joppa. The *Hardness* locus from Chinese Spring was successfully transferred to cv. Svevo durum wheat; Svevo was back-crossed 3 times to produce 'Soft Svevo' (Morris et al. 2011). Soft Svevo had SKCS kernel hardness, break flour yield, flour starch damage, and flour particle size similar to soft hexaploid wheat (Murray et al. 2016). Compared to Svevo, Soft Svevo had much reduced Solvent Retention Capacity (SRC) -water, -carbonate, and -sucrose; whereas SRC-lactic acid was similar to Svevo. Similarly, Mixograph, Farinograph and Alveograph results indicated much reduced water absorption, but similar gluten strength. Cookie diameter of Soft Svevo was markedly larger and similar to soft wheat (Murray et al. 2017). The energy required to produce flour was dramatically reduced: 624±200 kJ/kg flour for Svevo vs. 146±20 kJ/kg flour for Soft Svevo. When Soft Svevo was crossed to 10 CIMMYT durum parents, half-sib families and full-sib lines within families showed significant differences in SKCS hardness, break flour and total flour yields, starch damage, SRC-water, -carbonate, -sucrose, and -lactic acid, and flour SDS sedimentation volume. Cookie diameters ranged from 8.68 to 9.57 cm. Mean bread loaf volumes for families ranged from 680 to 838 cm³. Results illustrate the significant effect of the *Puroindoline* genes and the *Hardness* locus on kernel texture and end-use quality, and demonstrate that soft kernel durum wheat has properties similar to soft hexaploid wheat. Further, the hard durum parent has a significant effect on end-use quality traits by contributing superior alleles for soft wheat milling, flour properties, dough and bread quality.

Acknowledgements

Jeff Wilson, Gail Jacobson, Janet Luna contributed to this work. This research was supported by USDA NIFA AFRI Grant No. 2013-67013-21226.

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P 135 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Improving end-use quality, protein stability and immunogenic potential of wheat flour through biotechnology

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Key message: Elimination of omega gliadins in transgenic wheat plants by RNA interference yields flour with improved end-use quality, reduced immunogenic potential and increased protein stability.

The omega gliadins are a complex group of gluten proteins that comprise 5-10% of flour protein. These proteins contain unusually high proportions of glutamine and proline and consist almost entirely of repetitive sequences. In controlled environment studies, omega gliadins show some of the most notable responses to high temperatures² or fertilizer applied during grain development and thus may contribute to variability in flour end-use quality that occurs when wheat is grown in different environments. However, it has been difficult to determine the roles of omega gliadins in quality because their genes are tightly linked to genes encoding gamma gliadins and LMW-GS. There are two types of omega gliadins in wheat flour with distinct repetitive motifs, the omega-5 gliadins encoded on chromosome 1B and the omega-1,2 gliadins encoded on chromosomes 1A and 1D. Both are highly immunogenic. Omega-5 gliadins are major sensitizing allergens in the serious food allergy wheat-dependent exercise-induced anaphylaxis (WDEIA) while the omega-1,2 gliadins contain immunodominant epitopes involved in celiac disease. To assess the importance of omega gliadins in flour end-use quality and determine the feasibility of reducing the immunogenic potential of wheat flour, RNA interference was used to generate transgenic wheat plants in which either omega-5 or omega-1,2 gliadin genes were silenced. Lines were selected in which all target proteins were significantly reduced in flour with few changes in other proteins (Figure 1). End-use quality was assessed in omega-5 gliadin suppressed lines using mixing and baking studies and the allergenic potential of the flour was evaluated by 2-dimensional immunoblot analysis using sera from a collection of WDEIA patients. Both control and transgenic lines also were grown in greenhouses with and without post-anthesis fertilizer and changes in the accumulation of flour proteins were determined by quantitative two-dimensional gel electrophoresis. The data indicate that elimination of omega-5 gliadins results in flour with improved end-use quality, decreased allergenic potential and more stable protein composition. Similar analyses are underway with omega-1,2 gliadin suppressed lines. RNA interference proved to be an effective strategy to eliminate multiple closely related proteins and improve wheat flour quality. However, the wheat is transgenic and unlikely to reach the marketplace because of consumer acceptance issues. Future studies will employ gene editing to develop wheat with improved end-use quality and reduced immunogenic potential that can be rapidly deployed in breeding programs.

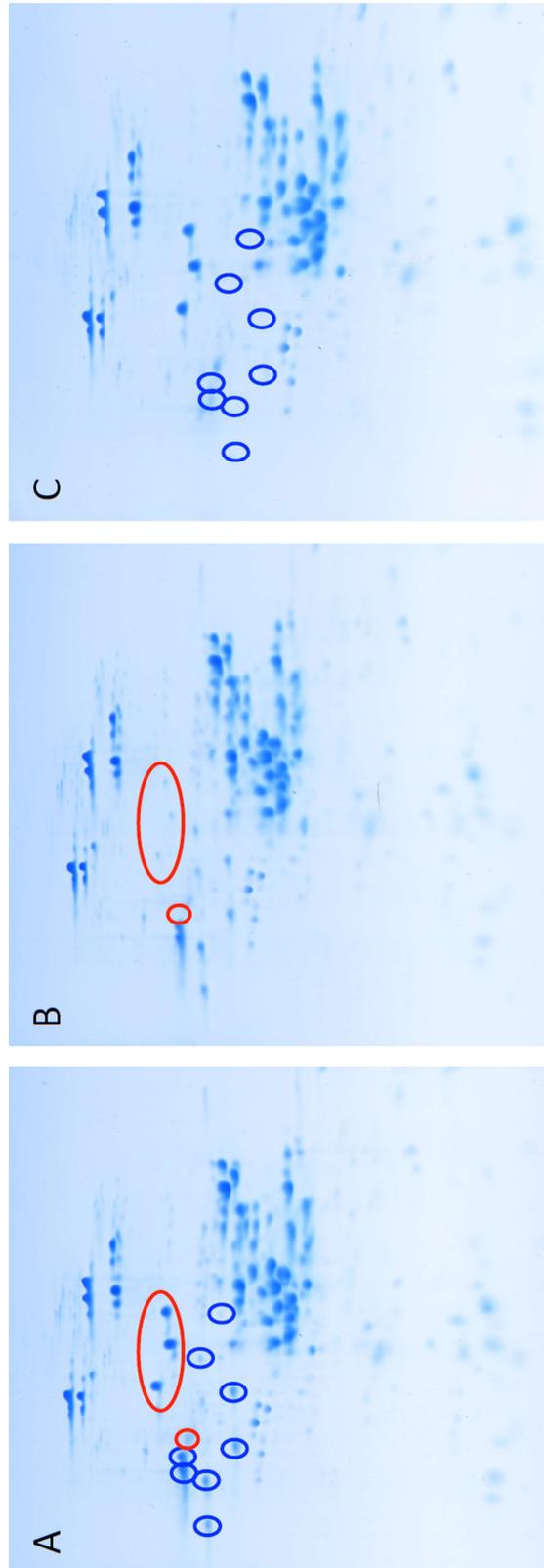


Figure 1: Non-transgenic (A) and transgenic (B, C) wheat lines in which omega-5 (red) or omega-1,2 (blue) gliadins were suppressed by RNA interference.



P 137 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Breeding progress in wheat: dissecting the principle components of grain yield

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Key message: Yield progress in European winter wheat has been strongly influenced by the numbers of kernels per spike. Detailed phenotyping of spikelet morphology helps explain the observed improvement in seed yield.

In spite of the documented breeding progress in winter wheat, grain yield has been reported to stagnate on the farm level in Germany and other countries. Several reasons are discussed for the stagnation of yield, e.g. (i) the recently observed lower precipitation and rising temperatures during the grain filling period in spring-early summer, (ii) the expansion of winter wheat growing into suboptimal sites, (iii) changes in crop rotation leading to a higher frequency of wheat cultivation. However, results from repeated multi-locational exact field trials conducted as part of the collaborative BRIWECS project with a set of 220 genetically diverse wheat cultivars, including winter and semi-winter types of different origin and released in different years, shows a significant genetic improvement in grain yield and give no hint that the yield stagnation observed in farmers' fields would be due to a stagnation of the genetic amelioration of wheat cultivars over the time. Moreover, the observed results demonstrate that the grain yield progress in European winter wheat is strongly influenced by a higher number of kernels per spike in modern cultivars. In this regard detailed phenotyping of different spike morphology traits, e.g. spikelets per spike, spikelet fertility, etc. are undertaken to obtain profound knowledge on the genetic control of this major yield component. Finally, these results will be used for quantitative-genetic studies to determine the genetic basis as well as to describe the breeding history of this fundamental grain yield parameter.

Acknowledgement

The BRIWECS (Breeding innovations in wheat for resilient cropping systems; www.briweecs.de) project is funded by the BMBF (IPAS-Project).



P 139 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Increasing wheat yield by focusing on light interception

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Key message: The great question behind breeding progress is which traits are most relevant for yield formation: how do we design the future wheat cultivars to achieve the needed grain yield increase?

One of the most crucial factors for yield formation is the amount of intercepted radiation which defines grain yield together with light use efficiency and harvest index. Canopy architecture as well as the post-anthesis available green leaf area influence radiation interception and we hypothesize that an extended kernel filling duration accompanied by a more translucent canopy architecture had an influence on the increased yields of modern in comparison to old cultivars. With our experiments we tested, whether these traits led to yield increase. The next step is to evaluate whether a further improvement of these traits could help to raise future grain yield if breeders intentionally consider them in the selection process. Within the framework of the collaborative research project BRIWECS ('Breeding innovations in wheat for resilient cropping systems') we investigate among others a selection of 100 varieties which originate from 50 years of cultivar registration spanning from 1966 to 2013, which makes it possible to investigate the breeding history in one field trial. In 2015 and 2016 we did measurements with two different sensor systems which provide leaf area index and light interception nondestructively. Furthermore, the percentage of green leaf area was assessed by scorings and SPAD meter. Measurements were done several times from stem elongation to ripening in order to investigate both pre- and post-anthesis growth. All cultivars were genotyped with the 15K iSelect SNP chip and genome wide association studies should give insight into the genetic background of these traits and the selection process. The poster will show the development of leaf area and light interception from the two years of field trails. Relating the yield parameters to details about the grain filling duration and canopy architecture will reveal whether it is worth focusing on these traits for the purpose of yield increase.



P 141 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Introgression of the high grain protein gene *Gpc-B1* in Turkish durum and bread wheat cultivars through marker assisted backcross breeding

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Key message: The results showed *Gpc-B1* locus differentially affected the grain protein, Fe and Zn concentration and genotypic structure may be effected on the gene action of this locus.

This research was conducted to transfer *Gpc-B1* locus previously reported to be associated with grain protein, Fe and Zn concentration to the spring bread and durum wheat varieties by using marker assisted backcross selection breeding method. In the present study Yecora Roja bread wheat variety which include *Gpc-B1* loci was crossed with Özkan, Balatilla and Genç-99 spring bread wheat varieties and UC1113 durum wheat variety including *Gpc-B1* loci was crossed with Balcalı-85, Balcalı-2000 and Zenit and F₁ generations improved. F₁ plants backcrossed during the BC₄F₁ generations and marker assisted selection applied to identify backcross lines carrying *Gpc-B1* locus. Bread and durum wheat backcross lines which carrying *Gpc-B1* locus and parental genotypes grown in the field conditions to consider agromorphological properties and grain protein, Fe and Zn concentration. The results show that *Gpc-B1* locus differentially affected the grain protein, Fe and Zn concentration and genotypic structure may be effected on the gene action of this locus. To conclude more validated results, bread and durum wheat backcross lines carrying *Gpc-B1* locus and parental genotypes should be grown in the different locations.



P 143 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Vernalisation and photoperiod sensitivity in wheat: impact on canopy development, floret fertility and yield components

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Key message: In near isogenic lines combining *VRN1* and *PPD-D1*, longer stem elongation led to more fertile florets, but vernalised plants or spring genotypes were more effective at producing more grains.

The impact of different allelic combinations of *VERNALIZATION1* (*VRN1*) and *PHOTOPERIOD1* (*PPD1*) on dynamics of canopy development, reproductive structures, spike dry weight (SDW) and its links to the duration of stem elongation (SE) and yield components was assessed using near isogenic lines of the cultivar Sunstate for both genes, including new alleles for *VRN1-A1*, in complementary field trials (low latitude) and controlled conditions. Allelic differences in *VRN1* had a stronger effect on the duration of the vegetative phase, while photoperiod sensitivity at *PPD-D1* lengthened the stem elongation phase (SE) by up to 23%. The level of response to daylength during SE by photoperiod sensitive alleles was dependent on *VRN1* composition and vernalisation status. A longer SE under short days was achieved by *PPD1* sensitive genotypes when one *VRN1* spring allele was present and plants were vernalised. The duration of SE was weakly related to SDW at anthesis in the field but did not translate into higher grain number. In the field, lines with two to three *VRN1* spring alleles had shortest development phases, close flowering dates, sampled similar temperature environments, and achieved high yields. Yield advantage was explained by higher biomass, harvest index, grain number m⁻² and thousand kernel weight. Allelic differences in both genes caused large variation in leaf and tiller number generation but also tiller mortality and individual leaf size, lessening the impact on leaf area. Investigation on differences in grain number showed that in vernalised plants, number of fertile florets in the main shoot spike was positively related to number of fertile spikelets, duration and sum of radiation during SE and anthesis SDW. No associations were found under non vernalised and slow vernalising field conditions. Vernalised plants produced more grains in the average tiller spike, particularly under short days. Both main shoot and tiller grain number per spike contributed to higher grain number per plant observed in vernalised plants, as spike number was similar. In the field, higher yield of lines with spring alleles of *VRN1* was underpinned by longer spikes, fewer spikelets but more main shoot spike fertile florets per spikelet, and more grains per tiller. When non vernalised, winter lines had a slow growing apex, shorter spikes and higher spikelet density. The utilisation of sensitivity to *VRN1* or *PPD1* to increase the duration of the SE and the number of fertile florets and grains is a more complex process than originally proposed.



P 145 - Topic: Applying Novel Tools to Practical Wheat Improvement

KODA mediated greater harvest in Japan wheat core collection harvest under low fertilizers field

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Key message: Overall α -ketol octadecadienoic acid (KODA) treatment pushed up approximately 12% greater grain yield under low fertilizer condition when grown at late autumn season.

KODA isolated from duck weed (*Lemna paucicostata*) was found to be involved in enhanced root growth and plant development in wheat under environmental stress such as alkalinity and drought (Haque et al. 2016). In this study, we investigated effects of KODA in wheat cultivars under low nitrogen field condition over different growing seasons. We used Japanese wheat core collection consisting of 94 cultivars. Seeds were imbibed with 5 μ M KODA for 2 days at room temperature in the dark. Seeds were sown in spaced planting at the experimental farm of Kihara Institute for Biological Research, Yokohama City University, Japan. First sowing of 69 cvs. was on late autumn (Dec. 4th, 2015) and second 25 cvs. on early spring (Feb. 4th, 2016). First nitrogen fertilizer was applied in the soil before sowing. Second application of nitrogen was withheld until the leaves become yellow as nitrogen deficiency confirmed with normalized difference vegetation index (NDVI) measurements. As shown in Figure 1A grain yield of KODA treated plants was increased by 12% in general in late autumn sowing. Interestingly, top yielding eight cvs. with KODA treatment were increased 38% of grain weight/plant, 50 cvs. In middle class by 12% and 8 genotypes showed slight effects of KODA by 7% increasing when the data were plotted in descending order of KODA treated order (+base). In the case of mock treated order (-base), genetically low yielding cvs. showed great KODA effect by 57% yield increasing. Among yield components studied, generally 6% of productive tillers (Figure 1B), 9% of seed numbers and 11% of biomass were increased, respectively. However, 1000 grain weight didn't changed suggesting that grain yield gained due to greater seed number and productive tillers. Regarding early spring sowing, yield alteration wasn't found in general, however, five genotypes increase by 12% grain weight as a top 12.5% plotting. Therefore, overall KODA responded better at late autumn sowing to bear up their yield under the stressed condition than in early spring sowing.

Acknowledgement

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Reference

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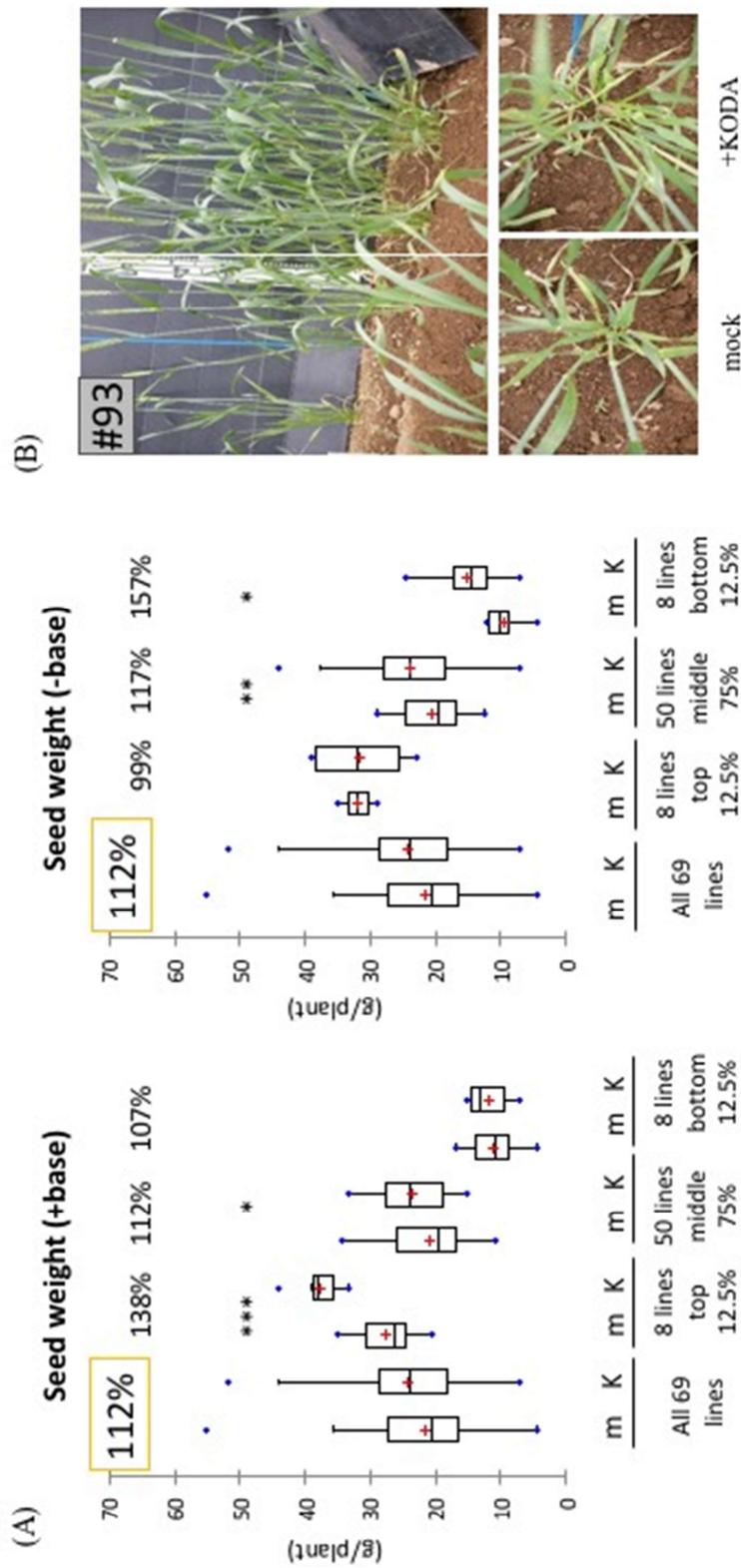


Figure 1: Box plots (mock/+KODA) for KODA effects in comparison of grain weight changes in late autumn sowing (A) and phenotypic difference of productive tiller (cv. #93) at flowering stage in late autumn sowing (B).



P 147 - Topic: Applying Novel Tools to Practical Wheat Improvement

Efficiency of agro-morphological and physiological criteria in screening drought tolerance in bread wheat

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Key message: The current study suggested that number of grains, thousands grain weight, canopy temperature depression and vigor can be suggested as reliable phenotypic secondary traits in selecting drought tolerant genotypes.

The development of varieties more suitable for water deficit conditions is one of the paramount strategies to overcome drought. This research was carried out to identify efficient indirect selection criteria for drought tolerance among ten agro-morphological (thousand kernel weight (TGW), biomass, number of grains (NG), number of spikes (NS), height) and physiological characters (vigor, cover, chlorophyll fluorescence (CF), chlorophyll content (CC), canopy temperature depression (CTD)). Forty contrasting bread wheat genotypes were evaluated under 4 drought intensities (DI) (0, 0.25, 0.35 and 0.57) using a randomized complete block design for each environment. The yield expressed large diversity over all the environments and among genotypes ($p < 0.001$). The heritability of grain yield was only 6%. Thus, the improvement of yield under stress must combine a reasonably high yield potential with specific factors which would buffer against a severe yield reduction under stress. The combined analysis of variance showed high genetic diversity among environments and genotypes for all the criteria, except CF. The heritability, correlation analysis and stepwise regression provided statistical evidence for relevant criteria. The NG (67%), TGW (80%), vigor (60%) and CC (65%) had the highest heritability values, followed by CTD (35%). Regarding correlation, the NG had the highest positive correlation with yield at all the stress intensities ($r > 0.8$). However, this correlation decreases (0.6) when the stress becomes very severe (0.57). TGW was significantly correlated with yield ($r = 0.77$) only when stress was very limiting (0.57). For physiological traits, none of the parameters were correlated to yield at non-stress and slight stress (0.25). At 0.35 DI, the cover and vigor were moderately positively correlated to yield (0.40 and 0.46 respectively); while CTD had negative correlation ($r = -0.46$). At 0.57, only vigor was still correlated to yield ($r = 0.36$). Finally, the stepwise regression identified especially NG followed by TGW for agro morphological characters at all stress intensities. For the physiological criteria, none of them has explained yield variation at non stress and very slight stress (0.25 stress intensity). However, at 0.35 DI, the CTD explained 21.1% of yield variation; while at severe stress (0.57), vigor and cover explained 24.1%. Thus, our results suggested that NG, TGW can be more reliable traits than yield for classification and separation of drought tolerant genotypes. Also, vigor and CTD can be used as supplementary criteria for accurate selection for drought tolerance. Molecular analysis is planned to discover the genes related to these traits.



P 149 - Topic: Applying Novel Tools to Practical Wheat Improvement

Effects of expression of rice alanine aminotransferase gene on nitrogen utilization efficiency of transgenic wheat

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Key words: Wheat, nitrogen efficient, transgene, Antiquitin, alanine aminotransferase

Nitrogen is the most abundant nutrient in wheat growth and development. The use of a large amount of nitrogen fertilizer has played an important role in raising wheat yield and ensuring food security in China. However, heavy use of nitrogen fertilizer also brings more and more serious environmental problems. Therefore, how to reduce the amount of nitrogen fertilizer under the premise of raising and maintaining the high yield of wheat is an urgent problem to be solved. Increasing nitrogen utilization efficiency of wheat is an important way to reduce the amount of nitrogen fertilizer. Some important progress has been made by using genetic engineering approaches to improve crop nitrogen use efficiency. Through expressing barley alanine aminotransferase gene which was driven by promoters of rape and rice antiquitin genes, the nitrogen utilization efficiency of transgenic rapeseed and transgenic rice was greatly improved. Whether this approach is feasible in other crops remains to be verified. In this study, we cloned the antiquitin gene promoter from wheat and the alanine aminotransferase gene from rice, and constructed two kinds of plant expression vectors with wheat antiquitin promoter driving the expression of rice alanine aminotransferase gene and Gus gene respectively. Through transformation of tobacco, it was found the Gus expression was mainly located in the microtubule tissue of root and shoot which was similar to that of transgenic rape and rice. Expressing the rice alanine aminotransferase gene stimulated the growth of transgenic tobacco under low nitrogen. On this basis, wheat was transformed. Plot yield comparison test was carried out with three stably inherited wheat transgenic lines and their control. Under the condition of high nitrogen, the yields of transgenic lines were at the same level as that of the control, but under the low nitrogen condition, the yields of transgenic lines were 5% to 14% higher than the control, indicating that using this approach to cultivate nitrogen efficient wheat varieties is feasible.

Acknowledgement

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P 151 - Topic: Applying Novel Tools to Practical Wheat Improvement

Fine mapping of the 'Chogokuwase (extra-early flowering)' gene in wheat

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Key message: Candidate region of early flowering gene found in 'Chogokuwase', an extra-early flowering wheat cultivar, was narrowed down to 1.4Mb region on the long arm of chromosome 3B, by fine mapping.

For stable production of wheat under changing global environment, early flowering is an important trait to avoid various kinds of damages caused by adverse environment such as rain, high temperature and drought during the later stage of grain filling. For the breeding of early flowering cultivars, marker assisted selection is practically employed for several major genes (*Vrn*, *Ppd*, and *Eps* genes). However, for fine tuning of flowering time, new genes and novel alleles of the known genes should be exploited. A Japanese breeding line 'Chogokuwase' developed from a cross between a Japanese spring type cultivar 'Minaminokomugi' and a Korean winter type cultivar 'Geurumil' is extremely early, and its heading time in the field is earlier by 18 to 27 days compared with the standard cultivars. Our previous studies indicated that extra-earliness of 'Chogokuwase' is caused by recessive alleles of at least three loci, of which two were located on the distal part of the long arm of chromosomes 3B and 3D. In this study, we focused on early flowering gene on chromosome 3B and developed mapping populations from a cross between 'Chogokuwase' and 'RIL-54' which derived from 'Chogokuwase' × 'Kinuiroha' (intermediate heading similar to 'Minaminokomugi'). These populations were grown in the field or growth chamber and heading date was recorded. Clear segregation of early and late types was observed in each population. Among a total of 385 plants of F₅ and F₆ populations, as an example, 84 and 301 plants were classified as early and late types, respectively, and the segregation ratio fitted to 1:3 (Figure 1). By fine mapping using SSR and SNP markers, early flowering gene was located in 2.7 Mb region in distal part of the long arm of chromosome 3B. Segregants having recombination in this candidate region were selected and their selfed progenies were grown to confirm the presence or absence of segregation in heading time. Based on the result, the candidate region was narrowed down to approximately 1.4 Mb flanked by two markers *Xpsm54* and *Xcfp1822*. An orthologue of Arabidopsis *LUX/PCL1* (*PHYTOCLOCK 1*), which is known as early flowering gene in diploid wheat *Triticum monococcum* (Mizuno et al. 2012), was also localized in this chromosome region, and is considered as the strong candidate.

Reference

Mizuno N, Nitta M, Sato K, Nasuda S (2012) A wheat homologue of *PHYTOCLOCK 1* is a candidate gene conferring the early heading phenotype to einkorn wheat. *Genes Genet Syst* 87: 357-367.

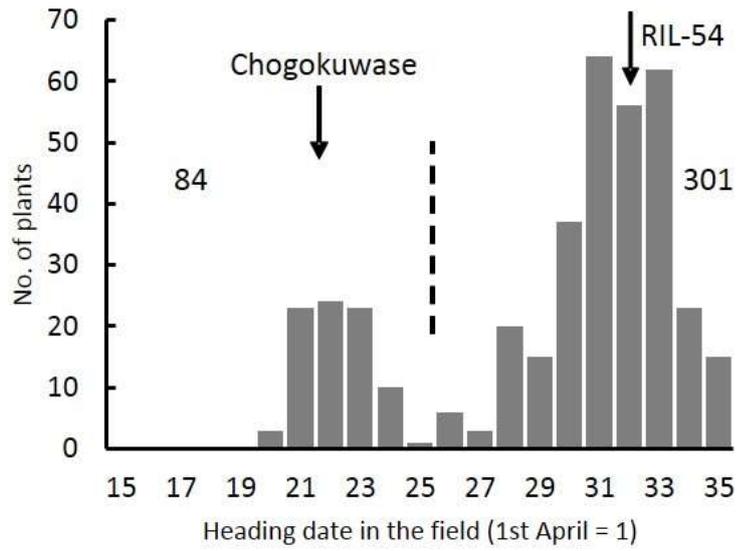


Figure 1: Frequency distribution of heading date in F₅ and F₆ populations derived from a cross between Chogokuwase and RIL-54 which carries late allele of 3B gene.



P 153 - Topic: Applying Novel Tools to Practical Wheat Improvement

Effect of interaction between *LUX/PCL1* genotypes on heading time of wheat, revealed by the analysis of a wheat DH population derived from Chogokuwase and Kinuiroha

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Key message: *PCL1* genotypes associated with heading time in wheat DH population derived from Chogokuwase and Kinuiroha, indicating the possibility of fine tuning of heading time by introducing non-functional allele of *PCL1*.

Flowering time of wheat is governed by three genetic traits, vernalization requirement, photoperiodic response, and earliness *per se*, and several major genes controlling these traits have been identified (*Vrn*, *Ppd*, and *Eps* genes). However, to improve the adaptability and to ensure the heading at the best timing in wheat breeding program, additional genes related with flowering time should be identified. A Japanese breeding line 'Chogokuwase' is extremely early, and its heading time in the field is earlier by 18-27 days compared with the standard cultivars. According to our previous studies, extreme earliness is a recessive trait and controlled by at least three genes of which three are located on the distal part of 3A, 3B, and 3D chromosomes. An orthologue of Arabidopsis *LUX/PCL1* was localized in these chromosome regions, and its deletion causes early heading in diploid wheat *Triticum monococcum* (Mizuno et al. 2012). Therefore, we analyzed *PCL1* genotype and their association with heading time by using 109 lines of doubled haploid (DH) derived from 'Chogokuwase' (extreme early) and 'Kinuiroha' (intermediate). For DNA marker analysis, three primer sets were used to determine *PCL1* genotype (Mizuno et al. 2016). 'Chogokuwase' has non-functional alleles of *PCL1-3A*, *PCL1-3B*, and *PCL1-3D* (designated as EEE type), while 'Kinuiroha' has non-functional allele of *PCL1-3A* and functional alleles of *PCL1-3B*, and *PCL1-3D* (ELL type). The segregation of *PCL1-3B* in 109 DH lines fitted to 1:1 ratio, while that of *PCL1-3D* was distorted (early:late=37:72) (Fig. 1). Therefore, the number of EEE type DH lines was 11 and less than expected (27.3). Heading date of 'Chogokuwase' and 'Kinuiroha' was 2.4 and 24.0 April, respectively, and transgressive segregation was observed in DH lines, suggesting the segregation of additional gene(s). Heading date of DH lines differed significantly among *PCL1* genotypes and EEE type (average = 0.5) headed earlier than other genotypes, strongly suggesting the association between extreme earliness and *PCL1* genotype. ELE type proved to head earlier (11.3) than EEL type (16.3) and ELL type (16.4), indicating interaction between *PCL1-3B* and *PCL1-3D*. Non-functional allele of *PCL1-3D* accelerated heading in the presence of functional allele of *PCL1-3B*. In contrast, earliness effect of non-functional allele of *PCL1-3B* was not observed in the presence of functional allele of *PCL1-3D*. These results suggested that heading date can be finely adjusted by introducing non-functional allele of *PCL1*, and further study is required to reveal genetic interaction including *PCL1-3A*.

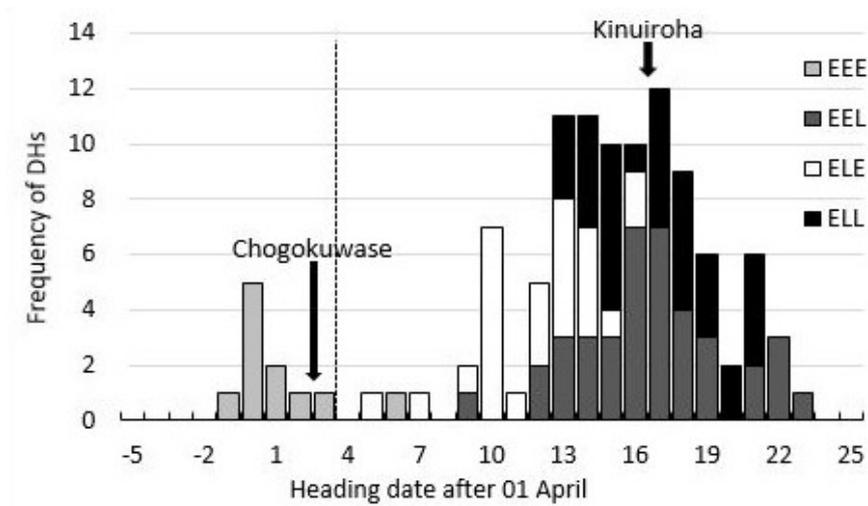


Figure 1: Heading date of 109 DH lines derived from Chogokuwase and Kinuiroha.

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- Mizuno N, Kinoshita M, Kinoshita S, Nishida H, Fujita M, Kato K, Murai K, Nasuda S (2016) Loss-of-function mutations in three homoeologous *PHYTOCLOCK 1* genes in common wheat are associated with the extra-early flowering phenotype. *PLoS ONE* 11. e0165618: doi: 10.1371/journal.pone.0165618



P 155 - Topic: Applying Novel Tools to Practical Wheat Improvement

New growth regulating genes in wheat

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Key message: Growth is controlled by a suite of both positive and negative gene regulators, by characterising mutants obtained from forward and reverse screens we will gain understanding of the molecular and cellular basis of growth

In the 'Green Revolution' wheat yields increased markedly due to the introduction of mutant DELLA genes that reduced plant growth, and allowed both an increased response to nitrogen fertiliser and more carbon to be partitioned to the grain. However, in dryland, water-limiting environments these mutant DELLA genes constrain genetic progress and longer term capacity to deliver growing global food needs because they reduce early seedling growth. In these environments a key objective is to improve water productivity by improving early growth and crop establishment. New dwarfing genes were previously generated by mutagenesis that do not compromise early growth and are independent of DELLA (Ellis et al. 2005). Agronomic assessments have shown that some of these genes have good potential to replace conventional DELLA dwarfing genes globally. We focused on the *Rht18* mutant as one of the most promising new dwarfing genes. The mutation was induced in tetraploid wheat, it is genetically independent of DELLA and reduces stem growth without compromising early growth. Dominant gene action suggested that a suppressor screen may generate new tall variants. We re-mutagenised the *Rht18* mutant resulting in several overgrowth mutants which are taller than *Rht18*. Genetic analysis confirmed that these mutants were allelic and were likely to carry second site mutations within the *Rht18* gene that are responsible for stem growth promotion. The target chromosome in the *Rht18* and overgrowth mutants was isolated using flow cytometry and sequenced using Illumina next-generation sequencing. The ability to sort specific chromosomes by flow cytometry dramatically reduces the genome complexity and allows deep sequencing at reasonable cost. We are currently testing the hypothesis that overexpression of a candidate gene involved in growth suppression resulted in the *Rht18* dwarf while second site mutations within the same gene generated the overgrowth phenotypes. There will be substantial scientific impact from identifying a new growth-regulating gene which is genetically distinct from DELLA and determining how mutations in the gene can be responsible for both growth suppression and promotion. We have extended the approach of generating overgrowth mutants, identify genes and study mechanism of growth regulation to early developmental stages.

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P 157 - Topic: Applying Novel Tools to Practical Wheat Improvement

A forward genetics approach to identifying novel-senescence related genes in *Triticum turgidum subsp. durum*.

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Key message: Phenotypic screening of TILLING mutant lines has identified two mutants which may contain novel genes involved in regulating senescence and confirmed the role of *NAM-A1* in senescence.

Understanding the growth and development of wheat is critical to breed new varieties with greater yield and other beneficial characteristics. A particularly important period of wheat development is senescence, characterised by a tightly regulated and coordinated death of green tissue and the drying of seeds. During this period, nutrients are remobilised from photosynthetic tissue to the developing grains. As such, understanding the processes driving senescence in wheat is central to our attempts to increase nutrient content in grain. Understanding complex traits, such as senescence, has been hampered to date by the lack of genetic resources in wheat. Previously, a NAC transcription factor (*NAM-B1*) and its homoeologs have been identified as positive regulators of senescence (Uauy et al. 2006), but little else is known about the molecular processes regulating senescence in wheat. Here we propose to take advantage of novel genetic resources in wheat, including sequenced TILLING populations, to identify new genes involved in senescence (Krasileva et al, unpublished). The Kronos TILLING mutants were screened for senescence phenotypes over two field seasons in the UK. Based on extreme phenotypes, four segregating populations were developed, three with late senescence phenotypes and one early (Figure 1a). Two of these populations were found to contain mutations in *NAM-A1*, the homoeolog of *NAM-B1*, which co-segregates with the late senescence phenotype (Figure 1b). This supports the role for *NAM-A1* in senescence and validates the use of TILLING populations to identify genes involved in senescence. The gene(s) responsible for the phenotypes in the remaining two populations are unknown. One population is also late senescing, while the other has an early senescence phenotype which co-segregates with a dominant chlorophyll-degradation phenotype (Figures 1c,d,e). DNA from these two populations was bulked based on phenotype (either senescence or leaf colour), and exome capture was carried out on the DNA bulks. Identification of the gene(s) responsible for the phenotypes is aided by the previously characterised coding sequence SNPs for each TILLING line in question (Figure 1d). This will allow the rapid identification of candidate genes in the genetic region of interest, which will be further characterised in the field.

Reference

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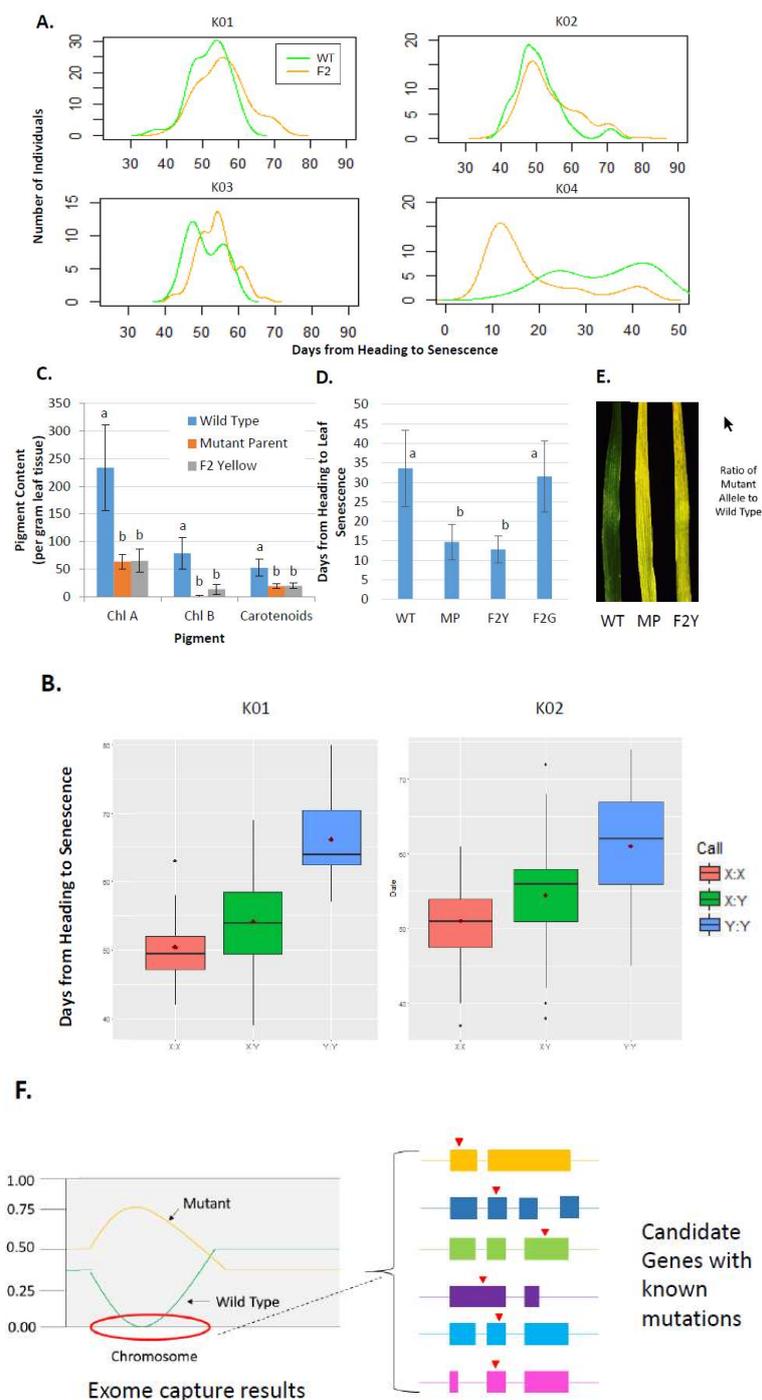


Figure 1: A forward genetics approach to identify novel-senescence related genes in *Triticum durum*. Four F₂ populations of Kronos TILLING mutants segregated for early or late senescence in the field (A). Lines K01 and K02 contain a mutation in *NAM-A1* which segregates with the senescence phenotype (B; X:X is homozygous for the wild type allele, Y:Y is homozygous for the mutant allele, and X:Y is heterozygous; differences between all genotypes are significant $p < 0.01$). Line K04 shows both early senescence (A) and a chlorophyll degradation phenotype (C, E) which co-segregates with the early senescence (D; MP: mutant-parent or M₄ seed from the TILLING population used for the initial backcross to wild type; F2Y: yellow plants in the F₂; F2G: green plants in the F₂; significance at $p < 0.05$ is shown by different letters). Exome capture of the bulks for K03 and K04 will identify a genetic region that contains the gene(s) involved which will be correlated with the known coding sequence mutations in the TILLING line to rapidly obtain a list of candidate genes (F).



P 159 - Topic: Applying Novel Tools to Practical Wheat Improvement

Expression analysis on flowering-related genes by RNAseq in a Japanese breeding line 'Chogokuwase' and its progenitor lines

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Key message: Triple mutation in *WPCL1*, a member of evening complex, was suggested to alter the expression patterns of circadian clock genes, especially *Ppd-1*, resulting in extremely early flowering.

A Japanese breeding line 'Chogokuwase' shows an extremely early-flowering phenotype. 'Chogokuwase' was developed by crossing two intermediate-flowering varieties: a Japanese variety 'Minaminokomugi' and a Korean variety 'Geurumil'. Previous study strongly suggested that the extremely early-flowering phenotype was conferred by the deployment of non-functional alleles at all of three *PHYTOCLOCK1* (*WPCL1*) homoeoloci (Mizuno et al. 2016). Since each non-functional allele has a moderate effect on flowering time, it would be useful for fine tuning of early-flowering phenotype in Japan, which is essential to avoid preharvest sprouting and *Fusarium* damages. In this study, we focused on the effect of *WPCL-B1* on expression of other flowering-related genes, as the first step to disclose the function of *WPCL1* homoeoalleles. We selected the lines that carry functional *WPCL-B1* and have the same genotype for the rest of flowering-related genes with 'Chogokuwase' (hereafter, *WPCL-B1* lines) from a RILs population of 'Chogokuwase' × 'Kinuiroha' (Table 1). These lines, together with the control lines, were grown for two weeks under short (8 h) photoperiods at a constant temperature 20°C. Total RNAs were extracted from second and third leaves of each line which were collected at dusk and three hours after dusk, and were subjected to RNAseq analysis. In *WPCL-B1* lines, expression levels of circadian clock genes including *TaTOC1*, *TaLHY*, *TaPRRs* were different between two time points. These differences were consistent with the expression patterns in wild type plants in the previous study, the circadian clock genes were suggested to oscillate normally. On the other hand, in 'Chogokuwase', the expression levels were extremely higher in *TaTOC1* and lower in *TaLHY* at dusk, indicating the alteration in the expression patterns due to the lack of functional *WPCL1*. Interestingly, expression levels of *Ppd-1*, one of the *TaPRRs*, were higher in accordance with the decrease in number of functional *WPCL1* (Figure 1). However, in 'Geurumil', the only winter type in this study, expression level of *Ppd-1* was markedly low and was considered to be repressed by the vernalization requirement. Since the expression levels of *Ppd-1* were correlated with flowering time, *Ppd-1* was considered to play a central role in controlling flowering time through *WPCL1* and *Vrn* genes.

Reference

Mizuno N, Kinoshita M, Kinoshita S, Nishida H, Fujita M, Kato K, Murai K, Nasuda S (2016) Loss-of-function mutations in three homoeologous *PHYTOCLOCK 1* genes in common wheat are associated with the extra-early flowering phenotype. *PLoS ONE* 11. e0165618: doi: 10.1371/journal.pone.0165618

Table 1: Genotype of *WPCL1* and other flowering-related genes

	<i>WPCL-A1</i>	<i>WPCL-B1</i>	<i>WPCL-D1</i>	<i>Vrn-D1</i>	HD
Chogokuwase	nf	nf	nf	spring	2.4
Geurumil	nf	nf	nf	winter	17.2
Miniminokomugi	nf	f	f	spring	-
Kinuiroha	nf	f	f	spring	16.3
CKRIL54	nf	f	nf	spring	10.4
CKRIL186	nf	f	nf	spring	11.6

f and nf indicate functional and non-functional alleles, respectively; All lines share *Ppd-A1b*, *Ppd-B1b*, *Ppd-D1a*, *vrn-A1* and *vrn-B1*; HD: heading date in April, 2011. Sowing date is 17th November 2011.

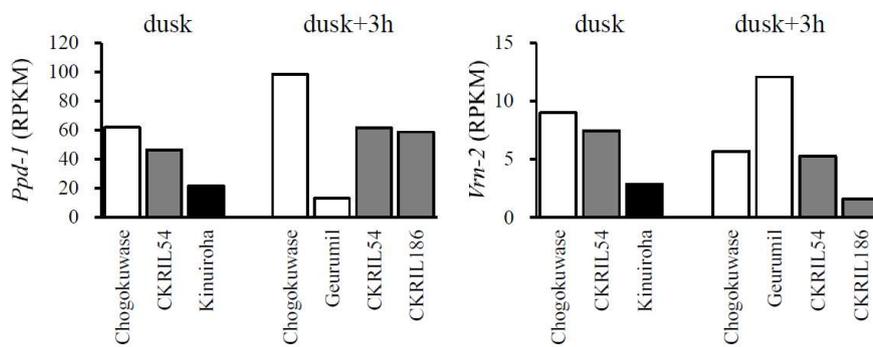


Figure 1: Expression of *Ppd-1* and *Vrn-2* at dusk and three hours after dusk. White, gray, and black bars indicate the functional *WPCL1* numbers of 0, 1, and 2, respectively.



P 161 - Topic: Applying Novel Tools to Practical Wheat Improvement

Vegetative growth and water use efficiency characterization of durum wheat near isogenic lines for the QTL *Qyld.idw-3B*

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Key message: The major yield and plant height QTL *Qyld.idw-3B* affect plants growth in late vegetative/reproductive stages but not at earlier stages.

The study of the genetic basis of grain yield is one of the major challenges of the scientific community because of both its complex genetic control and the strong interaction with environment and management practices. Furthermore, such interactions may affect yield during the entire life cycle of the plant. It is therefore crucial to consider yield as the result of multiple simpler traits and thus study their genetic control separately. In this study, we used the phenotyping platform PhenoArch in order to identify growth and water use related traits that may explain the segregation for yield and plant height observed at the QTL *Qyld.idw-3B* by Graziani et al. (2010). Four pairs of durum wheat near-isogenic lines (NILs) for the QTL *Qyld.idw-3B* were grown at three levels of drought stress: no stress (soil water potential > -1 bar), mild stress (soil water potential of -5/-8 bar) and severe stress (soil water potential \approx -13 bar). The stress was applied at the beginning of stem elongation until the end of the experiment (late milk stage, Zadok 77) on eight replicates per genotype per treatment. We recorded two main types of phenotypic data: (i) canopy images and (ii) weight measurements: every night, digital RGB images were collected. From these images we estimated several growth related phenotypes like biomass, leaf expansion and plant height; every plant was weighted to estimate the evapotranspiration at least once per day. Combining these data, it was possible to evaluate key physiological parameters like water use efficiency (WUE) and leaf transpiration. The QTL seemed to not affect vegetative behaviour and water use of plants during the early vegetative stages while majorly differentiating the NILs couples during the reproductive and ear ripening stages. This explains the segregation for final plant height previously observed by Graziani et al (2010). These results may provide useful information for further phenotypic as well as physiological and genetic characterization of the QTL *Qyld.idw-3B* with a main focus on mid to late stem elongation and reproductive/maturity stages.

Acknowledgements

We acknowledge the following projects: EU FP7 projects DROPS grant agreement# 244374, WATER4CROPS grant agreement# 311933.

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P 163 - Topic: Applying Novel Tools to Practical Wheat Improvement

KODA, an α -ketol derivative of linolenic acid isolated from duckweeds (*Lemna paucicostata*) provides wide recovery ability of wheat against various abiotic stresses

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Key message: KODA enhanced root growth and seedling survivability against soil alkalinity and drought, and tended to increase and stabilize wheat expansin genes in root.

Duckweeds was considered as a model for floating aquatic plants. It has been greatly used in fields of wide environmental adaptation, phytoremediation and biofuels production (Wang & Messing 2015). KODA (9-hydroxy-10-oxo-12(Z),15(Z)-octadecadienoic acid), a stress-inducing substance derived from duckweeds, was found to involve in flower formation and rate of rooting from stem cuttings (Yokoyama 2010). Here, we investigated KODA in terms of root growth, plant development and crop production in response to CaCO₃-imposed high pH (8.5) and drought, two major abiotic stresses for wheat production in Afghanistan (Figure 1). Two spring wheat cultivars, Japanese Yumeshiho and a cultivar from Kihara Afghan wheat landraces (KAWLR) with long root (LR-504; KU11202Bb), were used in CaCO₃ experiment. Another set of spring wheat cultivars, a KAWLR with short root (SR-823; KU7533), a KAWLR with long root (LR-744; KU7453), and a non-Japanese modern cultivar Lalmi-2, were used in drought experiment. KODA significantly enhanced root elongation in Yumeshiho under CaCO₃-imposed alkalinity condition. For the drought experiment, KODA significantly increased seed germination in all cultivars. KODA-treated plants had increased root and shoot growth in normal culture condition and greater recovery from drought, with the greatest root length in Lalmi-2 (43-51%). Interestingly, KODA-treated plants tends to increase grain yield and yield components. The recovery rates for plants that received KODA, in terms of grain weight per plant, were 2.5-3% in Lalmi-2, 13-15% in SR-823, and 11-13% in LR-744. In preliminary investigation, KODA-treated root showed moderate increase and stability in wheat root expansin genes under normal culture condition. These KODA-mediated physiological traits could be useful to efficiently uptake water and nutrients, and promote stable production in response to drought and high carbonate alkaline soils (Haque et al. 2016).

Acknowledgement

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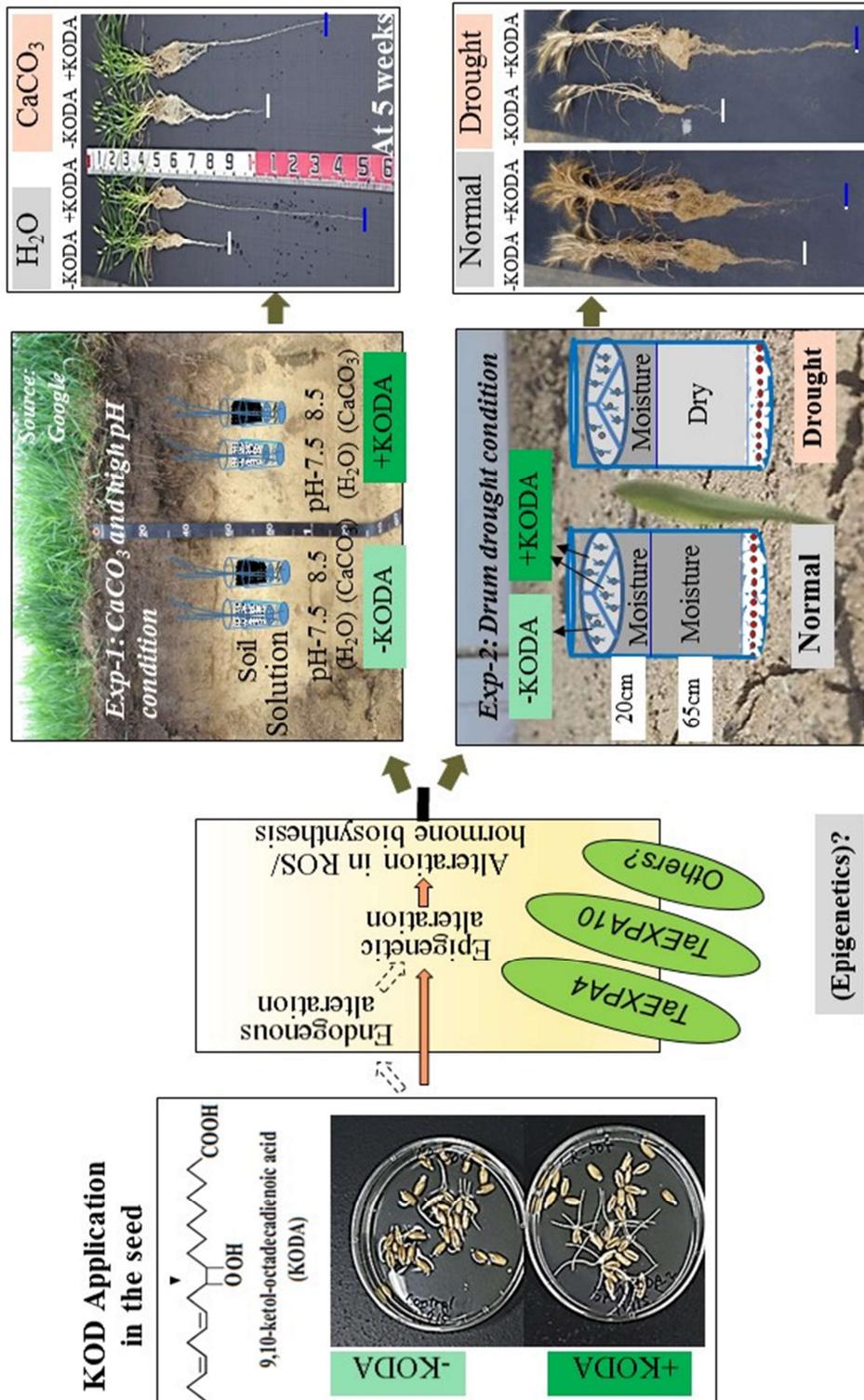


Figure 1: Model for the presumed mode of KODA action for greater root growth and plant development under CaCO₃ and drought conditions.



P 165 - Topic: Applying Novel Tools to Practical Wheat Improvement

Bioactive compounds from duckweed (*Lemna paucicostata*), can they provide food sustainability against adverse environments

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Key message: Overall α -ketol octadecadienoic acid (KODA) - and duck weed fertilizer (DWF) - treatment pushed up approximately 30 % greater grain yield under low fertilizer condition at late spring season.

While KODA was found to involve in enhanced root growth in Japanese wheat cultivar Yumeshiho against alkaline and dry soil (Haque et al. 2016), and its donor plant, duck weed played adaptive role against NH_4^+ toxicity (Wang et al. 2016). In late autumn and early spring sowing KODA enhanced yield in general under low fertilizer field (unpublished data). To see the season-specific recovery mode of KODA as well as DWF action against deficient fertilizer condition, we conducted a primary yield plot (2 m \times 1.3 m) experiment using Yumeshiho wheat on late spring (April). Seeds were imbibed with 5 μM KODA and DWF containing 5 μM KODA and sown in the experimental field of Kihara Institute for Biological Research, Yokohama, Japan. Whole plots were divided into two culture condition groups; half with optimum fertilizers and remaining half without fertilizers after sowing. Overall KODA and DWF treatments resulted in 27-34% higher grain yield improvement under low fertilizer condition than optimum condition (Figure 1). DWF showed slightly better performance. Seedling survival % as well as physiological traits (such as stomatal conductance and normalized difference vegetation index, NDVI) and seed number were higher in KODA and DWF treated plants. We suggested that KODA and DWF enhanced seedling survival and adjusted their underground part in the early stages of growth in response to hot-dry weather and low fertilizer condition. In autumn sowing study (unpublished data) KODA enhanced wheat branching but not found here. So KODA or DWF's respond to low fertilizer condition at late spring sowing different from winter as well as early spring wheat.

Acknowledgement

Financial support by Shiseido Co., Ltd, Japan is greatly acknowledged.

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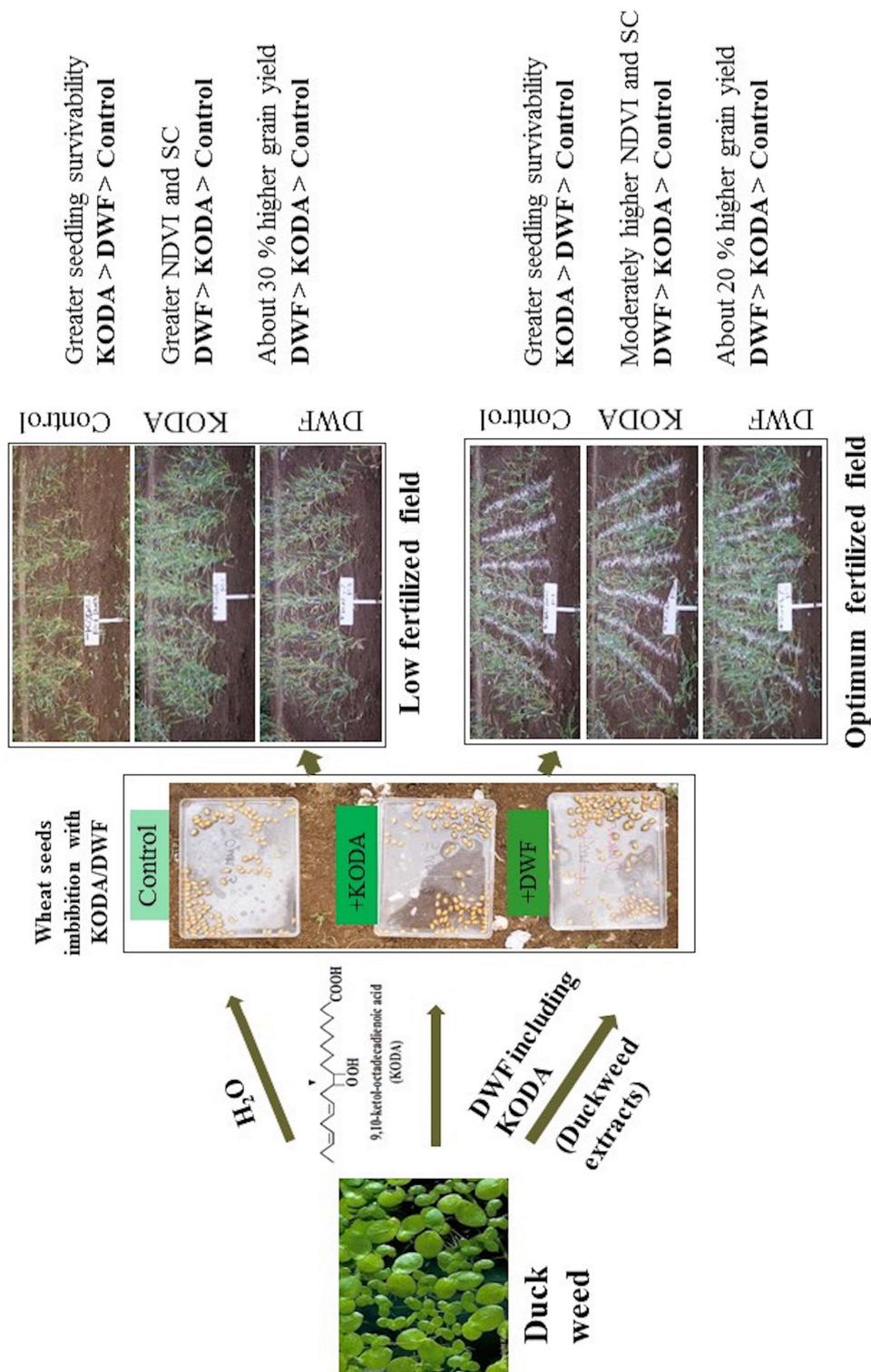


Figure 1: KODA (α -ketol octadecadienoic acid) and DWF (duck weed fertilizer) mediated over all higher growth and production in Japanese wheat cultivar Yumeshiho at late spring sowing under low fertilizer field condition.



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Phenotyping for rust resistance in wheat

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Key message: New methods for phenotyping for rust resistance in wheat.

The fungus *Puccinia striiformis* causes yellow (stripe) rust on wheat worldwide. New inoculation methods utilizing Novec™ 7100 as spore carrier facilitated a faster and more flexible application procedure for inoculation of both seedlings and adult plants. The new method allowed precise quantification of spore concentration and even distribution on both individual leaves and whole plants. Results were highly reproducible for both qualitative and quantitative assessment of disease parameters and resistance responses. Preliminary results suggests that the methods is applicable across different several host-pathosystems, including different species of rust and powdery mildew fungi on several species of cereals and grasses. New protocols for spray and point inoculation of *P. striiformis* on wheat is presented along with the prospect for applying these in rust research and resistance breeding activities.



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Development of wheat lines with complex resistance to rusts and Fusarium head blight

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Key message: Breeding for complex resistance to rusts and Fusarium head blight in South African spring wheat using phenotypic, molecular and biochemical (pathogen biomass accumulation) screening methods.

Wheat is an economically important food crop in South Africa and is affected by a number of fungal diseases, especially leaf rust, stem rust, stripe rust and Fusarium head blight (FHB). The aim of the study was to combine durable rust and FHB resistance into a single spring wheat line and to use different phenotypic and molecular screening methods to evaluate these lines. Wheat lines with resistance to the three rusts and FHB were developed from different breeding schemes using parental lines containing the following rust and FHB resistance genes/QTL: *Lr19*, *Lr34/Yr18/Sr57*, *Sr2*, *Sr26*, *Sr39*, *YrSp*, *QYr.sgi-2B.1*, *Fhb1* and *Fhb5*. Best lines were evaluated and selected throughout the breeding schemes using marker-assisted selection (MAS), phenotypic rust and FHB resistance evaluation in the greenhouse and under field conditions as well as by quantifying the biomass accumulation of the inoculated pathogen in each line using reverse transcriptase real-time polymerase chain reaction (RT-qPCR) analysis. Results indicated that RT-qPCR results correlated with infection type scores and the combination of genes/QTL present. Lines containing all nine rust and FHB genes/QTL were identified.



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Breeding lines of resistant to wheat yellow mosaic virus by marker assisted backcross

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Key message: Marker assist selection is useful in breeding program for resistant to wheat yellow mosaic virus.

Wheat yellow mosaic virus (WYMV) is one of critical diseases in Hokkaido, the northern area of Japan. WYMV is transmitted by a soil-borne micro-organism. In Hokkaido, the first occurrence was reported in 1991. Leading varieties of wheat in Hokkaido were susceptible to WYMV, and damage has occurred over a wide area in Hokkaido. Takeuchi et al. (2010) found that the North-American cultivar 'Madsen' was resistant to WYMV in field trials, and its resistance is governed by two complementary QTL, *Qym1* and *Qym2*, located on chromosome arms 2DL and 3BS (Suzuki et al. 2015). However, 'Madsen' is a late-maturing cultivar, susceptible to pre-harvest sprouting and snow mold. Therefore, we introduced the QTLs by marker assisted backcrossing using 'Madsen' as the donor parent and 'Kitahonami' as the recurrent parent. Kitahonami, the current leading variety in Hokkaido, is well adapted in Hokkaido because it has tolerance to pre-harvest sprouting and snow mold. Backcross lines were compared with Kitahonami about agronomic and quality traits for two years. Backcross lines and Kitahonami were similar for yield and almost agronomic traits, but 1000 seed weight were lower in backcross lines. Influence of resistant QTL introduction was not detected about flour milling characteristics and flour color. Negative effects of the resistant QTL were not serious, therefor marker assist selection is useful in breeding for resistant to WYMV.

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P 173 - Topic: Applying Novel Tools to Practical Wheat Improvement

Mutations in the branched head homoeo-allele *bh-B1* modify inflorescence architecture in tetraploid wheat

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Key message: QTL mapping, expression and haplotype analysis revealed that both homoeologous genes, *TtBH-A1* and *TtBH-B1* are involved in controlling inflorescence architecture in tetraploid wheat.

Although the gene underlying the *branched head* locus, *TtBH-A1*, has already been cloned (Poursarebani et al. 2015), the branched-spike phenotype is a quantitatively inherited trait showing considerable variation. Hence, modifiers are believed to be involved in controlling and modifying the phenotypic penetrance and expressivity of this phenotype. Besides the enormous variation in phenotypic plasticity of the branched-spike, QTL mapping identified 1A (*QSS.ipk-1AS*), 2A (*QSS.ipk-2AS*), and 2B (*QSS.ipk-2BS*) as carriers of QTL for supernumerary spikelet (SS) formation. From these three QTL, *QSS.ipk-2AS* and *QSS.ipk-2BS* were found to be major and medium effect loci, respectively, controlling the branched-spike phenotype. Mapping result revealed that besides controlling spike-branching and increased spikelet and grain number per spike, *QSS.ipk-2AS* and *QSS.ipk-2BS* negatively affected spikelet fertility showing lower grain number per individual spikelet. *QSS.ipk-2AS* and *QSS.ipk-2BS* were linked to CAPS markers derived from *TtBH-A1* and *TtBH-B1*, respectively, suggesting that *QSS.ipk-2AS* and *QSS.ipk-2BS* are homoeoloci. This has been further confirmed by expression analysis of *TtBH-A1* and *TtBH-B1* at the glume primordium, floret primordium, and terminal spikelet stages, where *TtBH-A1* was abundantly expressed in all stages followed by *TtBH-B1*. Furthermore, coding sequence analysis of *TtBH-B1* from 49 different wheat accessions revealed that all the mutant accessions carrying the *bh^t-A1* allele also carried a non-synonymous mutation in the homoeo-allele, *bh^t-B1*, which is frequently linked with the branching phenotype, indicating that both homoeologous genes are mutated. This could explain the strong phenotypic expressivity in the mutant accessions as well as in the RILs, which combined both alleles from TRI 19165. To further study the *bh* loci, Near Isogenic Lines (NILs) have been developed. Although the developed NILs (FL-*bh*-NILs) showed reduced phenotypic expressivity, on average FL-*bh*-NILs carry three to five additional spikelets per spike leading to 12 to 23.6% change in spike dry weight at harvest, FL-*bh*-NILs are valuable genetic materials to further study the source-sink relationship in wheat. Source-sink manipulation through de-tillering of FL-*bh*-NILs at BC₃F₃ generation suggested that spikelet fertility is partly connected with supply related problems which can be improved by partitioning more assimilates to the developing florets. One way of achieving this is by combining *bh* locus/loci with the *tiller inhibition* (*tin*) mutant, where more assimilate can be diverted to developing florets which otherwise are invested for side tillers.

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P 175 - Topic: Applying Novel Tools to Practical Wheat Improvement

An NIR-view into the underground: phenotyping architecture and functioning of durum wheat root systems

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Key message: The use of NIR imaging of the root system can highlight differences in root architecture and water uptake of wheat genetic resources.

Abiotic stresses explain around 30% of annual yield variability in major crops (Lobell & Field 2007) and are among the reasons underlying the lower yield advance since the 1990s in European wheat production (Brisson et al. 2010). A more targeted exploitation of plant traits contributing to stress resistance is thus expected to contribute to improved yield levels. This requires adequate methods for phenotyping stress resistance target traits. Effective resource uptake from soil is one path towards better stress resistance, depending critically on the root system. We present a novel approach for phenotyping root system architecture and root water uptake using hyperspectral imaging in the NIR wavelength range. NIR spectral imaging provides spatially resolved chemometric information to infer on both plant morphological properties and their functioning (Kim et al. 2015). Using a set of durum wheat genotypes (landraces) from different regional origin, we demonstrate the new phenotyping approach comprising (i) induced drought stress experiments in rhizoboxes, (ii) image acquisition with a hyperspectral root scanning system, and (iii) image processing to extract the targeted root and water uptake traits from spectral data. The approach is evaluated with direct root and transpiration measurement for its feasibility in detecting root system diversity of durum wheat genetic resources and its role for effective use of water in water limited environments.

Acknowledgements

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P 177 - Topic: Applying Novel Tools to Practical Wheat Improvement

Optimizing wheat root architecture by exploiting diverse germplasm

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Key message: Roots have a direct effect on wheat yield, but are currently non considered by breeding programs. Novel germplasm has been phenotyped for a variety of root traits.

Root architecture is key for efficient nutrient uptake, and thus has a direct effect on yield. Current wheat breeding and selection programs often do not directly consider root architecture due to the practical difficulties in its quantification. Preliminary analyses indicate that an unutilized reservoir of phenotypic variation in key root traits exists among current germplasm, landraces and wild relatives of modern wheat. As the availability of genetic resources in wheat increases, the bottleneck in improving yield is rapidly shifting towards phenotyping, especially with root traits which are in general, significantly more difficult to analyse than above-ground traits. Several high throughput, low cost root architecture and anatomy phenotyping pipelines have been developed at The University of Nottingham. These are currently being implemented on a variety of germplasm, with more detailed root analysis being conducted using micro X-ray computed tomography at the Hounsfield Facility for Rhizosphere Research. Another potential bottleneck facing wheat improvement is a lack of genetic variation stemming from its complex evolution and subsequent breeding. The introgression of genetic material from wild relatives into modern cultivars, has been proposed as a method for increasing the genetic variation in modern breeding programs for key agronomic traits. A population of doubled haploid lines containing introgressions from *Amblyopyrum muticum* into hexaploid wheat, produced by the Nottingham/BBSRC Wheat Research Centre, has been analysed using the phenotyping pipelines mentioned above with the aim of identifying introgressed segments conferring phenotypic improvements to root architecture and anatomy which, in turn, confer enhancements in above ground carbon acquisition. Phenotyping methodologies, together with preliminary data from the doubled haploid population will be presented.



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Dissecting wheat grain yield and yield stability using a genome wide association mapping approach in a large breeding germplasm

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Key message: Genome wide association mapping combined with epistatic scans in large breeding populations has the potential to exploit genomic regions for grain yield and yield stability

We discovered genomic regions associated with grain yield and yield stability using a large (720 lines) elite panel of CIMMYT wheat lines phenotyped in multiple environments (irrigated, drought and heat stress) performing a genome wide association mapping (GWAM) approach (Sehgal et al. 2017). Key genomic regions were located on chromosomes 2B, 3A, 4A, 5B, 7A and 7B. Furthermore, epistatic interactions among loci with and without main effects were explored followed by a stepwise regression of the most stable loci. This resulted in the identification of a combination of four markers that contributed to up to 17% gain in mean grain yield and up to 20% for yield stability (Figure 1). Twenty-one lines possessing the best allele combination of these four markers have been identified for future trials. Currently, KASP assays for these markers are being designed for their validation and identification of additional lines carrying the favorable alleles in CIMMYT international nurseries. Subsequent research will focus on the dissection of gain yield and yield stability using an even larger panel of 3000 wheat lines phenotyped across years. Results of this research will be presented.

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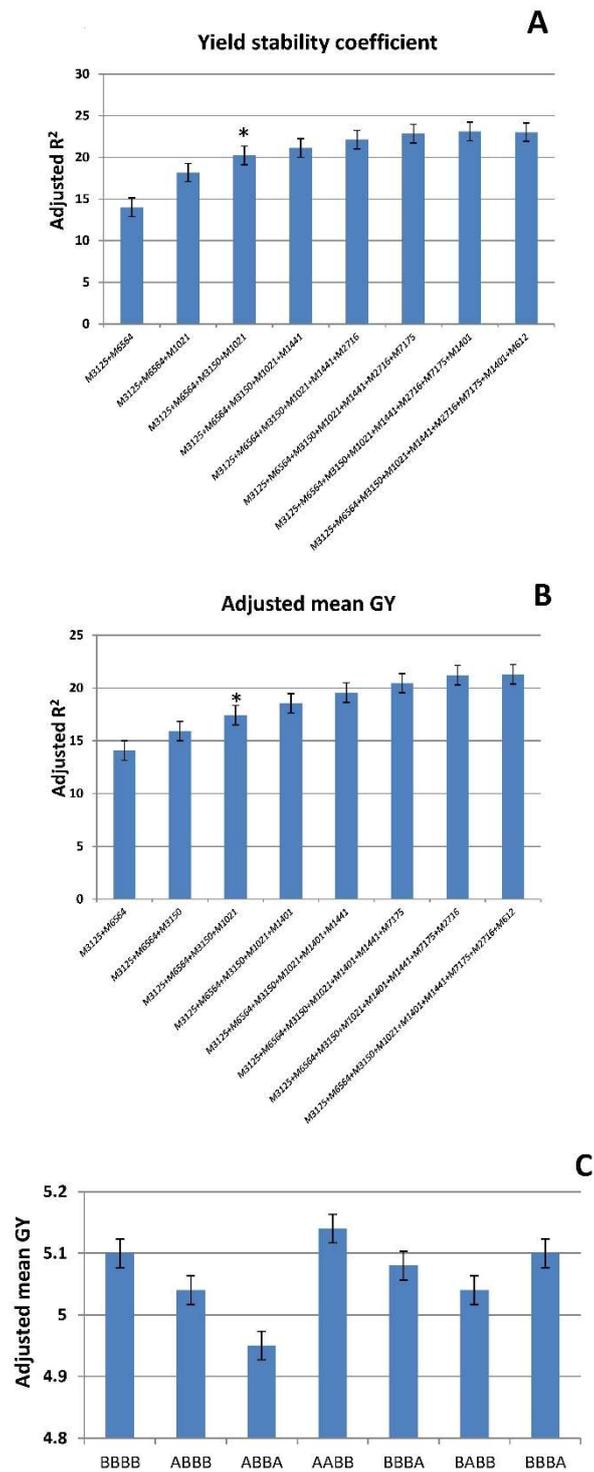


Figure 1: Stepwise regression of the nine markers for (A) yield stability coefficient and (B) adjusted mean GY across six environments. The starred column in (A) indicates the best marker combination that resulted in highest R^2 for yield stability; in (B) it indicates the R^2 of the mean GY for the four marker SNP combinations. The best allelic combination resulting in highest mean GY is shown in (C). Lines with 'A' calls for the SNPs associated with markers M3125 and M6564 and 'B' calls for markers M3150 and M1021 showed the highest mean GY.



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Association mapping in elite durum wheat reveals strong differential selection for a major root depth QTL according to water regime

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Key message: This study reports a QTL region with major effects on root depth and other root system architecture and shoot development traits, and shows how this QTL was differentially selected according to the prevailing water regime.

Root system architecture (RSA) is receiving increasing attention from the scientific and breeding communities because of its implications in water and nutrient uptake and thus on the capability of plants to cope with drought and nutrient starvation. This notwithstanding, phenotyping roots remains a major challenge because of its intrinsic difficulty. This study reports the characterization of 183 elite durum wheat (*Triticum turgidum* L. var. *durum* Desf.) for RSA and shoot developmental traits. Plants were grown in controlled conditions up to the 7th leaf appearance (late tillering) using the phenotyping platform GROWSCREEN-Rhizo at the Institut für Bio und Geowissenschaften Pflanzenwissenschaften. The depth (75 cm) of the rhizotrons of the platform and its automation allow for a quantitative, dynamic measurement of RSA parameters in 2D for most of the vegetative growth stage. The following RSA traits were measured: seminal root length, nodal root length, lateral root length, root system convex hull and root system width and depth distribution (twice per week). Measurements of leaf area, leaves number and tiller number were performed twice per week and SPAD measurements were collected twice along the experiment. Root dry biomass and shoot fresh and dry biomass were collected at the end of the experiment. A genome-wide association study (GWAS) based upon the Illumina Infinium 90K SNP assay identified 502 main loci associated with variation of RSA and/or shoot growth traits ($p < 0.0001$). GWAS confirmed a highly significant effect on adult plant root system width due to two QTLs on chromosome 6AL and 7A previously identified on seminal root at the seedling stage (Maccaferri et al. 2016). Furthermore, haplotype frequency at one of the main QTL cluster on chromosome 7Ac significantly associated with root depth, root system width, root specific weight and shoot/root ratio revealed a strong, contrasting selection pattern between the rainfed and the artificially watered breeding programs conducted at ICARDA and CIMMYT, respectively, suggesting an indirect but major role of RSA in durum wheat breeding.

Acknowledgements

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Canadian Wheat-NAM (Can-NAM): a next generation genetics platform for Canadian wheat improvement

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Key message: A nested association mapping (NAM) based next generation genetic and genomics platform for improving Canadian wheat performance will be presented and discussed

The linkage mapping approach to dissect complex traits has high statistical power but low resolution, and can only analyze two alleles. Genome wide association mapping (GWAS) is a complementary approach that has high resolution, gained by take advantage of historical recombination events, and can analyze many alleles, but with lower statistical power. Nested association mapping (NAM) is a powerful genetic platform that can dissect complex traits that combines the advantages of both linkage mapping and association mapping. NAM is designed as a structured multiple family approach by crossing a series of diverse founder lines to an adapted local elite line, and each resultant F_1 is selfed for several generations to provide homozygous recombinant inbred lines (RILs). NAM is an ideal genetically designed population to identify robust genotype and phenotype associations since such associations can be translated to breeding programs via the utilization of genetic markers to practise marker assisted selection (MAS). Recently, a 5 year Canadian wheat oriented NAM project started, titled 'Canadian Wheat-NAM (Can-NAM): capturing genetic variation for Canadian wheat improvement'. The goal of this largescale project is to develop a NAM based next generation genetics platform for Canadian wheat improvement. Here, we will present the NAM strategy, results about the establishment of this NAM resources, and phenotypic and genotypic analysis of NAM founder lines.



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Bavarian MAGIC winter wheat population (BMWpop): construction and genotypic data analysis

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Key message: First results from a novel eight-parent MAGIC winter wheat population indicate its usefulness as germplasm resource for analyzing the wide range of target traits in wheat breeding.

The Bavarian MAGIC (multiparent advanced generation intercross) winter wheat population (BMWpop) was constructed based on eight founder lines according to Cavanagh et al. (2008). The parental lines were selected on the basis of the following criteria: (i) variation in a range of phenotypic traits e.g. resistances to pathogens and quality parameters, (ii) derivation from diverse breeding programs and (iii) high performance within the respective quality group. During population development it was taken care to minimize selection. The final BMWpop consists of 400 recombinant inbred lines, which were selfed through single seed descent to the F₆ generation. The whole population was genotyped using the new 15k+5k Illumina® Wheat BeadChip (TraitGenetics GmbH), and a genetic linkage map was constructed according to Huang et al. (2012). Based on this linkage map analyses of recombination rates, genome-wide founder probabilities and genetic architecture, including haplotypes, will be conducted. First results of the genotypic data analyses indicate the BMWpop to be a good germplasm resource for genetic studies. The population will be used in further steps for different marker-trait association studies as well as for genomic prediction. It could already be shown that the population segregates well for a range of different traits like resistance to *Septoria tritici* blotch and powdery mildew, heading date, plant height, and leaf angle distribution. Survey of further traits of interest like grain yield and baking quality will follow.

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Lessons from MAGIC: a QTL cloning pipeline in wheat

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Key message: CSIRO two wheat MAGIC populations have been successfully deployed to improve our understanding of the underlying genetic basis of quantitative traits through QTL analysis and the development of an efficient QTL to gene pipeline.

Multi-parental populations are becoming increasingly popular in crops for their potential to dissect gene-trait relationships. This power derives from their fine-scale genomic structure resulting from many generations of genome mixing. CSIRO has developed two wheat Multi-parent Advanced Generation InterCross (MAGIC) populations that are being utilized to improve our understanding of the underlying genetic basis of a range of quantitative traits. The population with four founders has been extensively deployed for more than five years, while the population with eight founders has become more heavily utilized in recent years. This has resulted in extensive phenotype and QTL data for a large range of complex traits allowing for an understanding of the interactions between genotypes, environments and traits. As a result of the volume of QTL data produced, a novel QTL validation and cloning pipeline has been implemented. The pipeline exploits the increased allelic diversity, power and mapping resolution afforded by large multi-parent mapping populations, whilst reducing complexity by using multi-allelic contrasts at the targeted QTL region. The key aspects of the pipeline are presented using the successful identification of candidate genes for pre-harvest sprouting as an example.



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The NCCR multi-parental mapping population reveals a major QTL for number of grains per spikelet in durum wheat

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Key message: A multi-parental durum wheat mapping population representing a valuable asset to map QTL of agronomic value, including a major QTL for grains per spikelet (+0.54 grains/spikelet) is presented.

In the elite durum wheat germplasm, due to the overall low diversity, traditional bi-parental mapping and QTL discovery are inefficient. Therefore, a multi-parental recombinant inbred mapping population resource has been developed. Based on a survey of the available elite germplasm worldwide, four durum wheat parents (Neodur, Claudio, Colosseo and Rascon/2*Tarro) from the French/North American, Italian and CIMMYT genetic pools were chosen and crossed to produce 338 F_{7:8} lines (NCCR population). NCCR linkage map includes 7594 single nucleotide polymorphisms (SNPs) from the Illumina 90K SNP array. The four parents are diversified in phenology, grain yield, grain fertility components, partial resistance to pathogens and quality. Based on four field trials in Emilia Romagna (Po Valley), QTL analysis was carried out by both interval mapping on founder haplotype probabilities and SNP bi-allelic tests for heading and maturity, plant height, grain yield and components, biomass development, response to diseases. NCCR showed a highly transgressive distribution for all traits. Based on the known allelic variation at major *PPD/VRN* loci, we showed that QTL results based on estimated founder haplotypes closely matched the functional alleles. Despite the four founders, only 2.1 different functional haplotypes were estimated per QTL, on average. A major QTL for grain fertility ('grains per spikelet') was mapped on the short arm of chromosome 2A, with R² value of 39.2% and a sharp confidence interval of 3.0 cM, clearly distinct from *Ppd-A1* (35 cM proximal). The QTL allele from Rascon/2*Tarro parental line (CIMMYT origin) considerably increased the grain number per spike (+0.54 grains/spikelet on average) over the other three parents. Based on the local haplotype analysis, the positive allele at this major fertility QTL traces back to the CIMMYT hallmark founder Altar 84 and is present in some of the recent top yielding CIMMYT and Italian varieties. The combined transcript-based SNP map and the availability of the tetraploid and hexaploid wheat genome assemblies are being used toward the cloning of this QTL. NCCR has also been used to explore the marker × environment interaction model for genomic-enabled prediction and marker-trait associations for four representative traits (grain yield, grain volume weight, 1000-grain weight, heading date). The Bayesian B model proved suitable to accomplish both tasks. The NCCR durum wheat population provides a mapping resource for detailed genetic dissection of agronomic traits in an elite background typical of breeding programs.

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P 191 - Topic: Applying Novel Tools to Practical Wheat Improvement

Dissecting the genetic architecture of old Mediterranean durum wheats for yield formation by association mapping

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Key message: QTL identification for yield formation in Mediterranean durum wheat landraces as a breeding tool under a climate change scenario.

Durum wheat (*Triticum turgidum* L. var. *durum*) is a major crop in the Mediterranean Basin, which is the largest durum producing area worldwide. Natural and human selection occurring during its migration from the Fertile Crescent (10 000 BP), resulted in the establishment of local landraces specifically adapted to a diversity of agro-ecological zones (Nazco et al. 2012). The cultivation of local landraces was progressively abandoned from the early 1970s, however, evidence supports the hypothesis that landraces can provide new alleles for the improvement of commercially valuable traits (Lopes et al. 2015). A collection of 172 durum wheat landraces (LRs) from 21 Mediterranean countries and 20 modern cultivars were phenotyped in locations of northern and southern Spain for yield and yield components. The genetic structure of the collection was ascertained with 44 SSR markers that identified 448 alleles (Soriano et al. 2016). Total genetic diversity was $HT=0.7080$ and the genetic differentiation value was $GST=0.1730$. STRUCTURE software allocated 90.1% of the accessions in five subpopulations (SPs), one including all modern cultivars, and the four containing LRs related to their geographic origin: eastern Mediterranean, eastern Balkans and Turkey, western Balkans and Egypt, and western Mediterranean. The extent of linkage disequilibrium was estimated up to ca. 8 cM. Association mapping in the landrace collection was carried out to identify genome regions affecting yield formation under drought stress environments. The collection was genotyped with 1149 DArT markers. A total of 86 marker trait associations (MTAs) ($p<0.01$) were detected.

Acknowledgements

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P 193 - Topic: Applying Novel Tools to Practical Wheat Improvement

MAGIC WHEAT WM-800 targeted breeding of winter wheat elite cultivars

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Key message: This study reveals the proof of concept by detecting causal SNPs for reduced height genes *Rht-B1* and *Rht-D1* through QTL mapping within the MAGIC WHEAT population WM-800.

The MAGIC-WHEAT project pursues the goal to develop new winter wheat (*Triticum aestivum* L.) cultivars with improved agronomic traits concerning yield, quality, pathogen resistance and nutrient content. Therefore, most recent methods such as SNP genotyping, haplotype construction, mixed-model-association of quantitative trait loci (QTL), marker assisted selection (MAS) and genomic selection (GS) are used to identify and select improved genotypes. The multi-parent population WM-800 is based on an eight-way-cross (Cavanagh et al. 2008) of modern German winter wheat cultivars with major relevance to German wheat production. Phenotypic performance concerning yield, baking quality, nutrient content and pathogen resistance will be tested under field conditions with two nitrogen treatments at up to five locations in Germany. So far, plant height was used as a *proof of concept* for QTL mapping with WM-800. For this, phenotypic data from 2015 and 2016 and 7,849 polymorphic SNPs from the Illumina wheat 15k SNP chip (TraitGenetics) with genetic positions according to Wang et al. (2014) were used for QTL analysis with Proc GLMSELECT (SAS 9.4) and a fivefold cross-validation ($p \leq 0.001$ as model selection criteria). Altogether, 15 QTLs were detected for plant height. The causal SNPs for *Rht-B1* and *Rht-D1* showed the greatest effects on plant height with an average deviation of the Julius allele from the non-Julius allele of 12.8 cm and -14.9 cm, respectively. Besides *Rht-B1* and *Rht-D1*, a QTL near the photoperiod response gene *Ppd-D1* was detected as well as 12, so far, unknown QTL, located on different wheat chromosomes. Our results confirm that WM-800 is a powerful multi-parental mapping population suitable to unveil the genetic architecture of complex traits in wheat.

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P 195 - Topic: Applying Novel Tools to Practical Wheat Improvement

Comparing single trait with multi-trait genome-wide association in winter wheat (*Triticum aestivum*)

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Key message: Multi-trait genome-wide association compare to single trait GWA has more power to detect QTL. Multi-trait GWA has the potential to reveal QTL with pleiotropic effect.

Genome wide association studies (GWAS) reveals the markers that are causative or linked to the causative mutations related to different traits. The identification and subsequent utilization of such markers can facilitate accurate selection of superior breeding lines at an earlier stage of breeding schemes. In addition, phenotyping can be reduced with the help of useful markers. The aim of this study is to compare markers derived from single and multi-trait GWAS. In total, 1322 advanced winter wheat lines from Nordic Seed breeding program were genotyped with 15K SNP wheat array. These lines belong to four different breeding cycles, and from each cycle around 340 lines were selected to be genotyped. After marker editing based on minor allele frequency >0.05, missing <20% and excluding the un-mapped markers, 5493 SNP markers were used for GWAS analysis. Traits included lodging, plant height and plant heading date. These traits show moderate to high phenotypic correlation with each other that make them suitable traits for multi-trait GWAS. Recording of phenotypes was done over 4 years from 2013 to 2016. In the statistical model, factor year×location×trial was used as fix effect and factors genotype, year×location×field-row and year×location×genotypes were random effects. The genotype factor with genomic relationship matrix as variance-covariance matrix was to correct for population structure, year×location×genotypes factor was to count for genotype by environmental interaction and the year×location×field-row factor was for field special effect. Single marker regression was applied for GWAS. Single trait GWAS identified two markers for each trait with LOD score above 5. The markers for lodging were mapped to chromosome 3B and 3A, for plant height to chromosome 4B and 4D and for plant heading date to 5B and 6D. When the multi-trait model was applied, more markers with LOD score above 5 for all three traits were found to be associated with each trait. Overall, multi-trait model can be very useful strategy for finding QTL that have an effect on multiple traits.



P 197 - Topic: Applying Novel Tools to Practical Wheat Improvement

Crossing value of wheat parents used in Grain for Gain (G4G) platform

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Key message: The parents used in G4G platform could generate valuable genetic variability for further enhancements in wheat breeding.

G4G platform is an approach to produce winter wheat crosses by using the best performing genotypes from East and West, chosen upon data gathered during 20 years phenotyping over numerous environments. The main aim is to generate new and promising genetic variability to be successfully used in many breeding programs performed in similar agro-ecological conditions. To evaluate the concept viability, a research was conducted in 2016, to determine the crossing potential of 90 varieties used as parents for 423 crosses produced in 2014. Each of F₂ populations has been scored in the field for overall breeding potential, using a scale from 1 to 5, where '1' stands for the worst and '5' for the best potential. For each parent the total number of produced crosses was divided by the cumulative score obtained, thus producing the Parents Crossing Value (PCV). PCV scores distributed the parents into 4 groups. First group comprised of 68 varieties with very high PCV confirmed their great potential to be used as parents for crosses. The best scoring parents were Pannonia, Simonida, Nicol, Emina and NS 40S, for which we already knew to possess excellent combining abilities. From the total parents set, merely 4 genotypes had very low PCV. These results are very encouraging, confirming that the initial choice of parents was appropriate and the proposed concept is fully viable. The results of our research will be presented.



P 199 - Topic: Applying Novel Tools to Practical Wheat Improvement

Genomic prediction of Fusarium head blight resistance in adapted bread wheat germplasm

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Key words: DArTseq, Fusarium head blight, genomic selection

Fusarium head blight (FHB) disease has negative effect on both wheat yield and quality, because of that, breeding for FHB resistance is highly important in sustainable agricultural production. Genomic selection (GS) can improve FHB resistance breeding. In our study we evaluated the genomic prediction for FHB resistance. The study is based on 700 wheat lines F₅ and F₆ generations. The data were collected in year 2015 and 2016 from location Tulln an der Donau. For enhancing the genomic prediction accuracy, we also evaluated the effect on predictions of other phenotypic traits and environmental variables. We assessed the effect of heading date, plant height and anther retention, humidity and temperature. Wheat lines were genotyped with DArTseq, we obtained 7687 SNP markers. The effect of marker numbers on prediction accuracy were also assessed. In general the number of markers could be reduced as low as 5 times without significantly decreasing the prediction accuracy, what in return decreased the calculation time. For calculating the prediction accuracy we used cross validation and more importantly across year validation. In cross validation the other phenotypic traits had lower effect, then the environmental variables on prediction accuracy. The effect of the other phenotypic traits and environmental variables in cross validation changed. Environmental variables rather decreased the prediction accuracy, but the other phenotypic traits increased it. Remarkable was the effect of the plant height, alone its effect was close to zero, but by combining either with the heading date or anther retention it elevated the prediction accuracy by a significant margin.

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P 201 - Topic: Applying Novel Tools to Practical Wheat Improvement

Linkage mapping of fertility-restoring genes in common wheat and spelt

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Key message: Using independent mapping populations, we identified a molecular marker suitable for analysing the distribution of the restorer locus *Rf3* in common wheat and spelt germplasm.

Cytoplasmic male sterility (CMS) based on the sterility inducing cytoplasm of *Triticum timopheevii* Zhuk. is a promising approach for an efficient hybrid seed production in wheat. As fertility restoration is a crucial aspect of this CMS system, the localization of effective restorer genes is a prerequisite to implement the *T. timopheevii* cytoplasm in hybrid breeding programs. Although several restorer genes were genetically mapped, currently there is no molecular marker suitable for marker-assisted selection. Little is also known about the distribution of restorer genotypes in modern breeding germplasm. In this study, we genetically mapped the restorer locus *Rf3* located on chromosome 1BS (Tahir & Tsunewaki 1969) using five BC₁ populations and evaluated its distribution in a sample of German common wheat breeding lines and European spelt cultivars. Furthermore, we developed three BC₁ populations to map the restorer loci *Rf1* and *Rf4* on chromosome 1A and 6B, respectively (Maan 1985). In a mapping population involving CMS-Sperber and the restorer line Primepi (*N*=193), *Rf3* was mapped on chromosome 1BS with single nucleotide polymorphism (SNP) markers *IWB72107* (cosegregation) and *IWB14060* (2.0 cM distal). The linkage between *IWB72107* and *Rf3* was validated in four BC₁ populations derived from common wheat and spelt (*N*=628) revealing map distances from 0.4 to 2.3 cM. Segregation into fertile and sterile plants followed a 1:1 ratio in all five populations, suggesting that fertility restoration was solely controlled by *Rf3*. Further validation of *IWB72107* showed that it is suitable for marker-assisted selection and related applications. Genotyping the common wheat breeding lines and the European spelt cultivars with this marker revealed that 8.8% and 68% of the accessions carried the marker allele associated with fertility restoration. In both sample populations, the *IWB72107* genotypes did not depend on population structure. Our results suggest that *Rf3* explains the restoration capacity of a large proportion of European common wheat lines. The SNP *IWB72107* can be used to facilitate the establishment of heterotic pools and the transfer of *Rf3* into elite breeding lines.

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P 203 - Topic: Applying Novel Tools to Practical Wheat Improvement

Genetic architecture of anther extrusion in spring and winter wheat

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Key message: Hybrid wheat shows better and static yield than line varieties. For hybrid seed production, cross pollination has to be boosted in wheat. Anther extrusion ensures higher pollen shed outside the floret to enhance cross pollination in wheat.

Hybrid wheat breeding is gaining prominence worldwide because it ensures higher and more static yield than conventionally bred varieties. The cleistogamous floral architecture of wheat (*Triticum aestivum* L.) impedes anthers inside the floret, making it largely an inbreeder. For hybrid seed production, high anther extrusion is needed to promote cross pollination and to ensure a high level of pollen availability for the seed plant. This study, therefore, aimed at the genetic dissection of anther extrusion (AE) in panels of spring (SP) and winter wheat (WP) accessions by genome wide association studies (GWAS). We performed GWAS to identify the SNP markers potentially linked with AE in each panel separately. Phenotypic data were collected for three years for each panel. The average levels of Pearson's correlation (r) among all years and their best linear unbiased estimates (BLUES) within both panels were high ($r_{(SP)} = 0.75, p < 0.0001$; $r_{(WP)} = 0.72, p < 0.0001$). Genotypic data (with minimum of 0.05 minor allele frequency applied) included 12 066 and 12 191 SNP markers for SP and WP, respectively. Both genotypes and environment influenced the magnitude of AE. In total, 23 significant ($|\log_{10}(P)| > 3.0$) marker trait associations (MTAs) were detected in both panels (SP = 11; WP = 12). Anther extrusion behaved as a complex trait with significant markers having either favourable or unfavourable additive effects and imparting minor to moderate levels of phenotypic variance ($R^2_{(SP)} = 9.75\text{-}14.24\%$; $R^2_{(WP)} = 9.44\text{-}16.98\%$). All mapped significant markers as well as the markers within their significant linkage disequilibrium ($r^2 \geq 0.3$) regions were blasted against wheat genome assembly (IWGSC1+popseq) to find the corresponding genes and their high confidence descriptions were retrieved. These genes and their orthologues in *Hordeum vulgare*, *Brachypodium distachyon*, *Oryza sativa* and *Sorghum bicolor* revealed syntenic genomic regions potentially involved in flowering-related traits. Moreover, the expression data of these genes suggested potential candidates for AE. Our results suggest that the use of significant markers can help to introduce AE in high yielding varieties to increase cross fertilization rates and improve hybrid-seed production in wheat.



P 205 - Topic: Applying Novel Tools to Practical Wheat Improvement

What makes a good male? Prospects for improving the efficiency of hybrid seed production

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Key message: Within Europe, variation for floral and flowering traits is present within existing germplasm. Our study has provided insight into genetic factors which will be beneficial in selecting for these traits.

In bread wheat (*Triticum aestivum*) our understanding of the genetic factors that govern floral and flowering traits, and their role as determinants of grain yield, is limited. Genotype level variation has previously been reported for pollen and anthesis traits (Beri & Anand 1971, Boeven et al. 2016). Exploitation of existing diversity is anticipated to be beneficial in the development of cost effective hybrid breeding programmes. The present study set out firstly to ascertain the extent of genotypic variation for floral and flowering traits, with emphasis being placed on the male component of flowering. Methods for phenotyping traits were initially tested using a test panel of seven winter wheat genotypes and three hybrid male parental lines under controlled growth conditions. Subsequent to this, a field panel of 111 genotypes were assessed in field trials for anther extrusion capacity, anther length, anthesis duration and anthesis pattern. A population of 307 F_{3:4} lines, generated from a moderate and a high anther extruder, were also phenotyped for anther extrusion capacity. Phenotyping methods for anther extrusion capacity and anther length were found to be reliable and amendable to high throughput screening. Within the small test panel clear genotypic variation for anther length, anther extrusion and pollen production capacity was observed. Similarly, within the field panel considerable variation was observed for all traits assessed; even amongst locally adapted varieties. A genome wide association study carried out using the field panel revealed significant marker trait associations (MTAs) between anther extrusion capacity and SNPs located on chromosomes 2A and 2B. Despite the absence of significant MTAs for anthesis duration and anthesis pattern, correlations with traits for which strong MTAs have been previously reported, support the feasibility of indirectly selecting for these traits within breeding pools. Linkage mapping in the F_{3:4} population detected quantitative trait loci (QTL) on 5B and 6B but none on the group two chromosomes. Our results provide further evidence for the polygenic nature of anther extrusion capacity (Boeven et al. 2016) and emphasize the need for the simultaneous selection for multiple genes towards the development of male ideotypes. The phenotypic effects of potential candidate genes and QTL will be confirmed by phenotyping tilling lines.

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P 207 - Topic: Applying Novel Tools to Practical Wheat Improvement

Characterisation of *TaMs1*: a wheat fertility gene

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Key message: *TaMs1* encodes a glycosylphosphatidylinositol-anchored lipid transfer protein necessary for wheat pollen coat formation.

In flowering plants, lipid biosynthesis and transport within anthers is essential for male reproductive success. Non-specific lipid transfer proteins (nsLTPs) are small molecules capable of transferring lipid compounds between membranes. Here, we report the functional characterisation of *Ms1*, a wheat fertility gene present on chromosome 4BS. *TaMs1* encodes for a glycosylphosphatidylinositol (GPI)-anchored LTP, necessary for male fertility. Microscopic observations of *ms1* anthers indicate a disruption of orbicule and pollen exine development resulting in non-viable pollen. Lipidomic profiling shows an accumulation of long chain fatty acids in *ms1* anthers. *TaMs1* expression analysis using β -glucuronidase fusions and homeologue specific qRT-PCR show only B-genome *TaMs1* transcripts to be expressed in anthers with microspores at pre-meiosis to meiosis. Taken together this data demonstrates that *Ms1* is necessary for correct pollen exine formation.



P 209 - Topic: Applying Novel Tools to Practical Wheat Improvement

Molecular identification of *Ms1* and its application for hybrid wheat breeding

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Key message: *Ms1* can be used for Seed Production Technology (SPT process), an innovative, proprietary seed production process that is expected to dramatically increase the efficiency of hybrid seed production.

The current rate of yield gain in crops is insufficient to meet predicted demands. Capturing the yield boost from heterosis is one of the few technologies that offers rapid gain. Hybrids are widely used for the cereals maize and rice, but it has been a challenge to develop a viable hybrid system for bread wheat due to the wheat genome complexity, both large and hexaploid. Wheat is our most widely grown crop providing 20% of the calories for humans (FAOSTAT 2013-2015). Here we describe the identification of *Ms1* by positional cloning (Figure 1), a gene proposed for use in large-scale, low-cost production of male sterile (*ms*) female lines necessary for hybrid wheat seed production (Driscoll 1972). We show that *Ms1* completely restores fertility to *ms1d*, and encodes a glycosylphosphatidylinositol anchored lipid transfer protein, necessary for pollen exine development. This represents a key step towards developing a robust hybridization platform in wheat similar to the maize Seed Production Technology (SPT) process (Wu et al. 2016).

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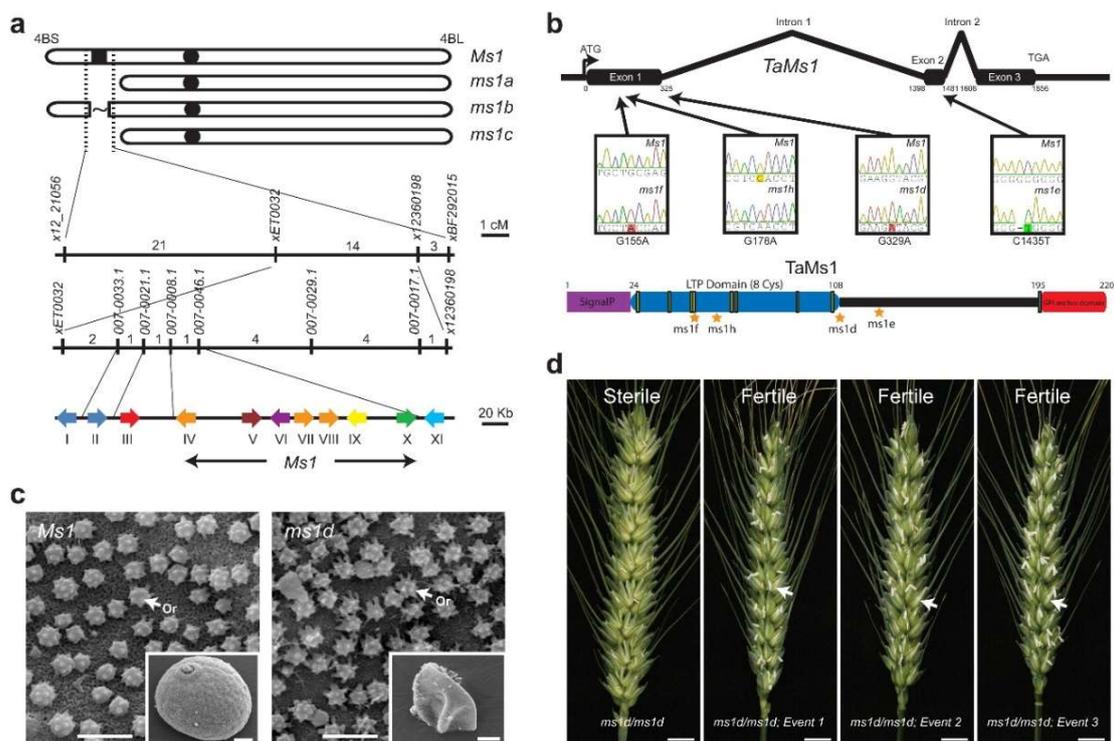


Figure 1: Map-based cloning of the *Male sterility 1* (*Ms1*) locus on chromosome 4BS (A). Schematic representation of the *TaMs1* (the three exons are shown as black boxes) gene depicting relative positions (indicated by solid lines with arrowheads) of EMS-derived lesions (*ms1d*, *ms1e*, *ms1f* and *ms1h*) and corresponding propeptide domain structure (B). Scanning electron micrographs showing defects in tapetal cell surface-localised orbicule (Or) structures and pollen coat (inset) within male sterile (*ms1d*) versus wild-type anthers (*Ms1*) (C). Stable complementation of the *ms1d* mutant by *TaMs1*. Mature inflorescences of male sterile *ms1d/ms1d*, and three independent transgenic lines (Events 1-3) each homozygous for *ms1d*, and showing self-seed set (arrow).



P 211 - Topic: Applying Novel Tools to Practical Wheat Improvement

Repeatability of genotyping by sequencing SNP markers in wheat

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Key message: We examined the proportion of markers representing common recombination bins from genotyping by sequencing of different wheat populations and found a large proportion of common markers compared to relatively few common SNPs mapped in the populations.

Genotyping by sequencing (GBS) provides a snapshot view of the genome and generates SNP markers that are free of ascertainment bias at a low cost. However, this approach suffers from data sparsity and high proportion of missing alleles upon multiplexing large number of samples leading to low repeatability of genetic markers. We genotyped two RIL populations and parent lines (replicated ≥ 6 times) using *PstI-MspI* enzyme combination in our GBS design. SNP calling was carried out using *bwa* and *Samtools* on the POPSEQ wheat reference genome sequence. Our analysis of GBS SNP markers in the two RIL populations showed that only 1185 markers (9.9% of 11 998 total) were common. However, a search for common markers within the same recombination bins in these two populations led to detection of over 5000 markers in common recombination bins that ranged in sizes of few kilobase pairs to several megabase pairs. We also found that using enzymes with higher cut frequency leads to a large amount of DNA fragments, which in turn increases the sampling of non-overlapping reads from different parts of the genome. This appears to be the main reason why not all genomic regions have uniform read coverage upon sequencing. We also investigated to what extent dense and sparse multiplexing levels exacerbate marker repeatability by simulating GBS library preparation and sequencing. As expected, multiplexing in higher order decreased the number of SNPs in 192-plex and 384-plex libraries relative to 48- and 96-plexed libraries. Read depth per SNP site reduced by a factor of $\frac{1}{2}$ each time to 2.5, 1.2, and 0.6 in 96-plexed, 192-plexed, and 384-plexed panels, respectively, from that of 5 in 48-plexed library. For best results, sequencing 92-plex libraries appears to give relatively good genome coverage while still keeping the cost low.



P 213 - Topic: Applying Novel Tools to Practical Wheat Improvement

Functional interactomics in crops through AP-MS

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Key message: Provide an AP-MS platform for functional analysis of wheat protein complexes in a developmental context with the ability to focus on the specific needs from the wheat research community.

Proteins and the interactions they acquire with other proteins, metabolites and nucleic acids form the rudiments for molecular functions and cell growth. Our research team runs a state of the art AP-MS platform for protein complex isolation both from *Arabidopsis* cell cultures and whole seedlings (Gadeyne et al. 2014). Through its high specificity and explanatory power, our platform steadily became a central -omics tool in our research. Several complexes involved in cell proliferation and phytohormonal pathways were isolated, leading to protein discovery, functional analysis of the protein complex and the mapping of protein networks. Crop plants are more suitable for the functional analysis of protein complexes in a developmental context due to their bigger organ size. Recently we developed applications in maize and rice, and obtained proof of concept for the study of protein complex dynamics during organ development in maize which demonstrate its use for organ growth engineering (Nelissen et al. 2015, Dedecker et al. 2016). Currently, we are translating our know-how to establish an AP-MS platform in wheat which will allow the scientific community to focus on in-depth analysis of wheat specific protein complexes during development. Furthermore, the platform will enable us to evaluate the dynamics of protein complexes when the crop plant is submitted to varying environmental conditions which will be a valuable asset for engineering.

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P 215 - Topic: Applying Novel Tools to Practical Wheat Improvement

Optimization of genotyping arrays for wheat genetics and breeding

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Key message: The development of optimized and cost-efficient genotyping arrays for wheat breeding and genetic analyses is a process that requires the continuous selection of optimal SNP marker sets from genotyping data

High throughput single nucleotide polymorphism (SNP) genotyping is now becoming a routine tool for wheat genetics including applications such as genetic relationship analysis, genetic mapping, marker/trait associations, genomic selection and others. Many currently available genotyping arrays contain a high proportion of markers that are not of high quality, are only useful in certain sets of germplasm (ascertainment bias), contain too many markers that create redundant information, lack markers in specific genes of relevance for breeding and are too expensive for routine use. In order to generate high quality genotyping arrays that contains mainly high quality and informative SNP markers, TraitGenetics used SNP genotyping and quality data from the Illumina 90K, an Affymetrix 35K breeders and other proprietary arrays generated for large sets of wheat varieties to generate an optimized marker set with respect to technical quality, extent of linkage disequilibrium, detected haplotype structure, linkage to specific genes of relevance for breeding, genetic and physical map position. Examples for the methods used in this continuous process of array content optimization are presented. Through the optimization process, such an array contains a relative minimum of markers which reduces the chip production costs. Combined interests from academic institutions and commercial entities reduce further chip production costs through the use of economics of scale. This makes such arrays ideal for breeding applications, the large scale characterization of germplasm and trait mapping. This is demonstrated through the annual analysis of several ten-thousands of wheat samples which such arrays.



P 217 - Topic: Applying Novel Tools to Practical Wheat Improvement

Development of DNA-based 2-D digital barcodes in wheats for reliable varietal identification and digital repository

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Key message: We developed unique digital barcodes for each of the 165 Pakistani wheat cultivars based on high-quality SNP markers and 100 functional genes

The objective of the current study was to develop unique 2-D digital barcodes for the identification of Pakistani wheat cultivars released since 1906. In total, 130 wheat cultivars were genotyped with high-density 660K SNP Affymetrix array and 77 functional genes using 124 Kompetitive Allele-Specific PCR (KASP) markers. A quality-control (QC) procedure was adopted to narrow down highly informative SNP markers from 660K array. In first step, SNP markers with minor allele frequency (MAF) between 0.4 to 0.5 were selected and used to calculate linkage disequilibrium (LD). On each chromosome 4-5 SNP markers with LD value <0.01 were finally selected with a total number of 100 SNP markers. Genetic distance based on neighbor-joining (NJ) method was calculated between SNP markers, and finally 50 SNP markers were selected. These 50 SNP markers were converted into KASP assays, out of which 41 SNPs were successfully converted and gave consistent results to the 660K array on 130 wheat cultivars. This core set of 41 KASP markers was used to genotype 165 historical wheat cultivars along with 124 KASP markers for functional genes. The information generated was used to develop 2-D digital barcode using online tool (<http://www.qr-code-generator.com>). This barcode will allow for the rapid and precise identification of wheat germplasm resources, developing a wheat varietal catalogue for breeders in Pakistan, digital and Genbank repository and will further assist in protecting plant breeder's rights (PBR) recently introduced in Pakistan.



P 219 - Topic: Applying Novel Tools to Practical Wheat Improvement

The Ethiopian durum wheat nested association mapping (NAM) population. High definition QTL mapping through breeding

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Key message: We developed and partially characterized a nested association mapping (NAM) population by intercrossing 50 Ethiopian durum wheat landraces with an international durum wheat improved line

Upon characterizing the molecular and phenotypic uniqueness of Ethiopian durum wheat, we established a nested association mapping (NAM) population (McMullen et al. 2009) by choosing 50 diverse landraces and crossing them with a recurrent parent with an international allele pool (Asassa). Each of the original crosses established a bi-parental family that underwent a single seed descent procedure, providing altogether 6280 recombinant inbred lines (RIL) in F₇. The NAM design jointly considers the interconnected families to provide high-definition quantitative trait loci (QTL) mapping through increased allelic diversity and recombination frequency. Our Ethiopian NAM aims at a dual objective. On the one hand, it aims to provide the durum wheat scientific community with a powerful QTL mapping tool, available to collaborators that will side the increasing availability of genomic tools for efficient candidate genes identification. On the other hand, the Ethiopian NAM represents the first systematic effort to incorporate Ethiopian farmer varieties in a pre-breeding panel, closing the gap between local and international material. The founders of the population were chosen not only to maximize genetic diversity, but also to include traits of agronomic relevance for local wheat cultivation. Here we present the preliminary characterization of twelve families (100 RIL each) belonging to the Ethiopian durum wheat NAM. We genotyped 1200 RIL and describe the genomic features of the population (Figure 1). The Ethiopian NAM shows a broad molecular and phenotypic variation. The selected families have also been sown in two replicas in two locations in Ethiopia, for a total of 4800 plots which have been characterized for phenologic and agronomic traits, as well as for farmers' appreciation (Figure 2).

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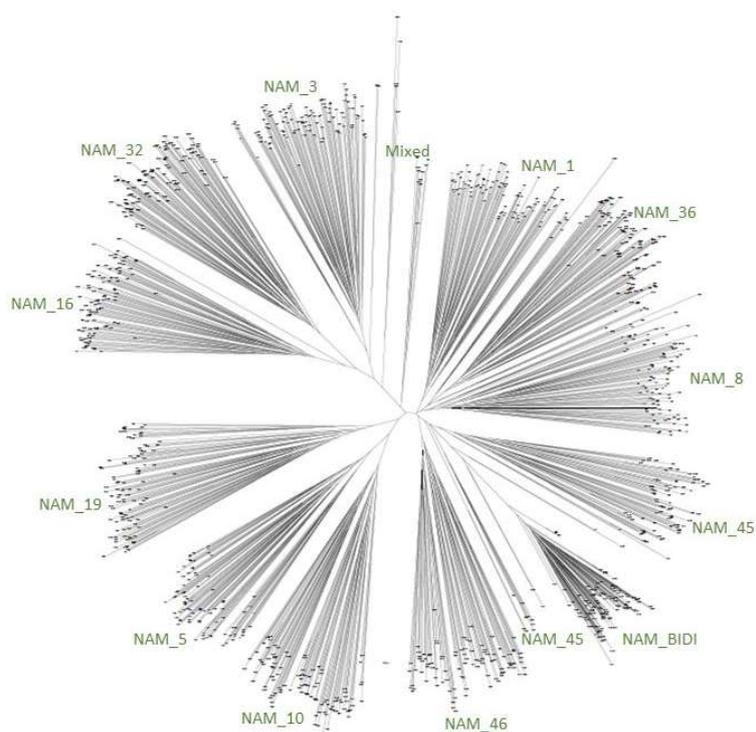


Figure 1: A neighbor joining phylogeny of the molecular diversity of 12 NAM families out of the 50 available.



Figure 2: The broad phenotypic variation of the population is visible in the experimental field run in Adet, Amhara, Ethiopia.



P 221 - Topic: Applying Novel Tools to Practical Wheat Improvement

The adoption of marker-assisted selection in South African wheat breeding programs: centralising resources to fast track wheat cultivar breeding

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Key message: In a resource-limited environment, the centralisation of molecular genetics infrastructure, knowledge and labour at a single facility is the most cost-effective way to apply marker-assisted selection in wheat breeding programmes.

Wheat breeding in South Africa has, for many years, relied on traditional phenotypic selection only. Until recently, there were no molecular marker-assisted selection (MAS) programmes or infrastructure available to wheat breeders. In 2010, CenGen (Pty) Ltd, an independent private laboratory, submitted a proposal to the South African wheat industry to establish a dedicated programme at their facilities to offer a MAS service to the local wheat breeding companies. The service, 'Marker Service Laboratory' (MSL), is tailor made to suit each breeding programme's individual needs and take into consideration its aims and status quo. All information regarding the breeding programmes is dealt with confidentially as not to compromise their competitive integrity. Since its inception in 2011, MSL has significantly expanded in terms of both infrastructure and the variety of traits that is screened for by mainly relying on information in the public domain. The MAS portfolio currently comprises of 48 genes/QTL. In the past year just over 120 000 datapoints have been generated for the different wheat breeding programmes of Pannar Seed and Sensako (Pty) Ltd. Advanced breeding lines already containing more than three genes/QTL after four generations also show great promise in the field, confirming the benefit of using MAS to incorporate desirable genes into agronomically acceptable backgrounds. In a country where the availability of resources is an omnipresent limitation, the centralization of MAS at a single facility gave all wheat breeders access to high-throughput instruments and sound scientific knowledge. This enabled their effective adoption of the technology. This strategy has proven to be the most cost-effective use of both human and laboratory resources. Furthermore, it laid a sound foundation to initiate the development of the breeding populations needed to test the feasibility of genomic selection in the South African wheat breeding programmes.

Acknowledgements

The South African Winter Cereal Trust (WCT/W/2009/02) are thanked for funding. The KASPTM SNP platform used in this project was supported by the UK Biotechnology and Biological Sciences Research Council (BBSRC) special initiative: Sustainable Crop Production Research for International Development (SCPRID) Project BB/J011525/1.



P 223 - Topic: Applying Novel Tools to Practical Wheat Improvement

Virtual selection for comparing wheat breeding schemes at constant total cost

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Key message: *In silico* simulations allow comparing various breeding schemes while genetic parameters are fixed to ensure approximately the same costs

We developed stochastic simulations in R language for comparing breeding schemes using either phenotypic or genomic selection. Simulations start from real genotyping value, therefore accounting for an actual genetic structure and linkage disequilibrium pattern. The model was calibrated using wheat genotyping data from the breedwheat French project (<http://www.breedwheat.fr>). The reference population of 760 breeding lines from the INRA-AGriObtentions programme was genotyped with a 420K Axiom Affymetrix Chip. It was evaluated from 2005 to 2014 in a highly unbalanced design. To generate virtual progenies according to recombination patterns, only 4260 markers with unique genetic map position were used. We simulated a two-step selection scheme, which aims to realistically mimic the actual scheme used by Agri-Obtention on doubled-haploid progenies (Figure 1). In one strategy, the first phenotyping step was replaced by genomic prediction using a reference population, from which the parents were initially sampled. Note that the phenotype data of the reference population was freely available (historical breeder's data), while its genotyping cost was explicitly taken into account. A subset of markers were randomly sampled and assigned as additive QTL effects, then a random noise was added to generate quantitative traits with the desired heritability. We evaluated genetic gain using the true breeding values defined as the sum of additive QTL effects. Crosses were made through pair mating, and homozygous lines were produced from the F₁, simulating doubled haploid progenies. Pair crosses were chosen either randomly, or according to an optimization process. The optimization criteria was the expected breeding value of the best possible line that could be produced from a given crosse. For the successive selection steps, QTL markers were removed from breeding values' estimations, but kept along the process to calculate the true breeding values. Budgets were calculated totaling the real costs of crosses, progeny development and multiplication, genotyping and phenotyping. With the genetic parameters observed in the real dataset, genomic selection gives more rapid genetic advance. This is due to the larger number of progenies that can be handled for the same cost, thus enabling an increase of section pressure.

Acknowledgement

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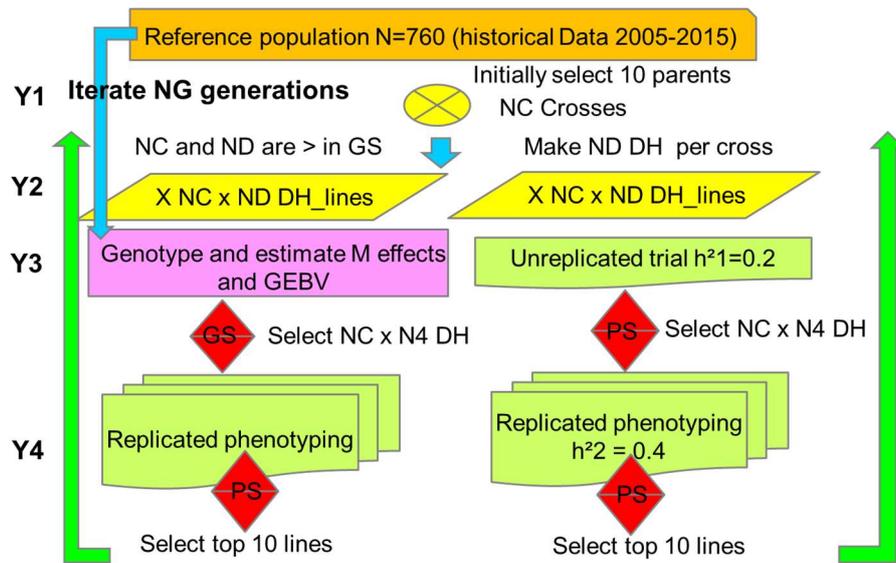


Figure 1: The alternative two steps breeding strategies used to compare genomic vs phenotypic selection at a given budget.



P 225 - Topic: Applying Novel Tools to Practical Wheat Improvement

A beginner's guide to wheat: www.wheat-training.com

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Key message: We developed a training website that aims to group practical information on wheat growth and genetics, along with an updated status of genomic resources, to facilitate access to this knowledge.

There has been a renewed interest from funders, students and researchers to work on crops. Wheat provides a great opportunity with the recent expansion of available resources to work with. However, training of new scientists in wheat is hindered by the dispersion of information across multiple sites and, ironically, by the step-changes in genomic resources that are difficult to keep up to date with. We have developed the 'wheat training' website (<http://www.wheat-training.com>) that aims to help both new wheat scientists as well as researchers looking to expand their work into wheat. The website is separated into three major sections and hopes to provide an initial entry point to understand basic principles of plant growth and husbandry, as well as genomic resources. The first section covers the basics of plant growth and crossing with ample detail and images. It also contains efficient protocols for simple wet lab tasks such as DNA extraction. The second part focuses on the available genomic resources in wheat and covers the available genome assemblies, gene models as well as expression and variation data. The last section is dedicated to the *in silico* TILLING resource for wheat which can be used to identify mutations in genes of interest. This section also covers creation of genome-specific markers and a guide to identifying the correct orthologue of an *Arabidopsis* gene in wheat. The entire website is indexed and can be searched for key words. Relevant passages are intended to be printed off to carry to the lab, glasshouse or field for reference. Additional information such as links to other important websites (e.g. CerealsDB) or online training guides is also available. We would be delighted to discuss ideas with IWGS attendees as we hope to expand the content further based on community feedback.



P 227 - Topic: Applying Novel Tools to Practical Wheat Improvement

The International Wheat Yield Partnership: an integrated science program focused on delivery

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Key message: IWYP is a new and uniquely coordinated funding and research approach with the goal of raising the genetic yield potential of wheat by up to 50% in the next 2 decades.

Wheat is the most important staple crop globally providing ≈20% of daily calories and protein. By 2050 wheat demand is expected to increase by 70% or more which will require annual potential wheat yield increases to double. The International Wheat Yield Partnership (IWYP) represents a new and uniquely coordinated funding and research approach with the goal of raising the genetic yield potential of wheat by up to 50% in the next two decades. IWYP builds on the Wheat Yield Consortium established by CIMMYT and contributes to the global Wheat Initiative as an Associated Programme. The IWYP goal is exceptionally challenging and requires a focused strategy and a collaborative approach to enable the best scientific teams across the globe to work together in an integrated program. Science projects selected by IWYP to address the challenge of significantly increasing wheat yields pursue new ideas and apply cutting edge technologies while discerning the genetics underpinning yield potential traits. IWYP science is largely based on optimizing photosynthesis in the wheat plant via a number of different pathways and traits that ultimately lead to substantial increases in grain yield. Approaches include exploiting variation in wild species, transgenes affecting photosynthesis and yield, variation for seed size and number, and variation for optimum phenology. IWYP's delivery strategy is structured around an integrated 'Science Program' consisting of research projects selected by IWYP and funded by IWYP Partners, ongoing projects that are funded outside of IWYP and formally align, public-private partnerships and the activities of the IWYP technical development platform (IWYP Hub) managed by CIMMYT. IWYP currently coordinates 15 IWYP sponsored research projects in 7 countries and has 5 other research projects that are formally aligned. The IWYP Hub began activities in 2014 and the first material from CIMMYT is in international trials this year. IWYP currently has 9 private industry partners. Lastly, a Competitive Call for Research Proposals is currently ongoing and we plan to select another set of projects by the end of the year. We expect exciting discoveries that lead to significant increases in wheat yields to come from these first projects. We further aim to generate substantial added value by integrating research at early stages and by combining the research outputs in various combinations at the IWYP Hub. In all, IWYP research will deliver new genetic resources to the global wheat breeding community to utilize to produce higher yielding locally adapted varieties.



P 229 - Topic: Applying Novel Tools to Practical Wheat Improvement

Wheat breeding using indirect selection in the genomics and phenomics era

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Key message: Indirect selection using high-throughput phenotyping and genomics can be a valuable tool for increasing trait values of interest within breeding programs for abiotic and biotic stress resistance.

Recent advances in genomics and phenomics have opened the path for many new strategies in plant breeding. Selection for many traits related to abiotic and biotic stress resistance was once only done using direct measures for selection. Genomics and high-throughput phenotyping now offers the advantage of making gains using indirect selection methods. The ability to select for these traits using indirect selection methods may advance the rate of gain for these correlated traits. Two large diversity panels of wheat were developed and grown over four years in the Pacific Northwest. Data was collected on both populations for agronomic traits, yield potential, canopy spectral reflectance, and disease resistance. Models built using one panel were used on the second panel to make selections. Analysis was completed to see if these models were able to indirectly select for resistance to abiotic and biotic stress and increased yield potential better than direct methods. Using a limited number of molecular markers associated with traits of interest for abiotic stress resistance, increased gains were not made. Utilization of whole genome approaches like genomic selection was able to select lines with improved performance. High-throughput phenotyping was also able to select the best performing lines, and was robust across years and locations. Selection using molecular markers was very useful in selecting for biotic stress resistance. Either direct or indirect selection methods can be useful in biotic stress resistance, mainly dependent on the difficulty of the direct disease selection method. The use of indirect selection can be as useful as direct selection when selecting for abiotic stress resistance. When doing abiotic stress resistance selection, whole genome or whole plant approaches appears to be more useful and can assist with selecting in earlier generations of plant breeding. Indirect selection using high-throughput phenotyping alone or in combination with genomic selection can be helpful in advancing breeding lines in wheat breeding programs.



P 231 - Topic: Applying Novel Tools to Practical Wheat Improvement

Exploiting trait correlations for next-generation grain yield and end-use quality improvement of U.S. hard winter wheat

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Key message: Prediction accuracy of production-related and end-use quality-related traits is significantly improved through exploitation of positive and negative trait correlations in multi-trait genomic selection prediction models.

Since the early 1980s, the land area planted to U.S. hard winter wheat and the share of U.S. wheat in global export markets have both declined dramatically. Improved profitability of other crops relative to wheat, declining or static domestic wheat flour consumption, and an increasingly competitive global wheat market are important factors underlying these trends. Although hard winter wheat breeding programs focus significant attention to end-use quality, the lack of direct market incentives for superior quality and the absence of a formal cultivar registration requirement often result in wheat cultivars with inferior functional quality being commercialized and, in some cases, gaining significant market share. Additionally, identity-preserved programs developed to segregate and maintain improved functional value have proven both difficult to establish and costly to maintain in a dynamic economic setting. In order to reverse the trends in declining plantings and market share, modern approaches that foster increased rates of genetic gain for production-related traits simultaneously with improved functional value from an end-use quality perspective must be implemented. Single-trait and multi-trait genomic selection (GS) approaches offer tremendous potential to accomplish these objectives. Phenotypic data collected within the context of a comprehensive public cultivar development program were utilized to develop GS-based prediction models for both production-related and quality-related traits. These data included spatially adjusted data from multi-year and multi-location yield trials and comprehensive experimental milling and bread-baking quality data. Genome-wide single nucleotide polymorphism (SNP) markers obtained using genotyping-by-sequencing (GBS, Figure 1) were used in ridge regression best-linear unbiased prediction (rrBLUP) approaches to develop GS-based predictions (Endelman 2011). Positive or negative trait associations between production-related traits (i.e., grain yield, plant height, grain protein deviation; Figure 2), between production- and quality-related traits (i.e., grain yield, grain protein concentration, water absorption), and between quality-related traits (i.e., water absorption, grain protein content, flour yield; Figure 3) were exploited to improve prediction accuracy of key traits through multi-trait genomic selection (Jia & Jannink 2012). Validation of predictions included both traditional cross-validation and forward prediction of not phenotyped individuals. Through focused integration of these approaches in a long-term cultivar development program we hope to be able to provide improved wheat cultivars that enhance both production- and quality-related traits (Oury & Godin 2007), optimize economic returns for both producers and product manufacturers, and reverse the decline in plantings and market share of U.S. hard winter wheat.

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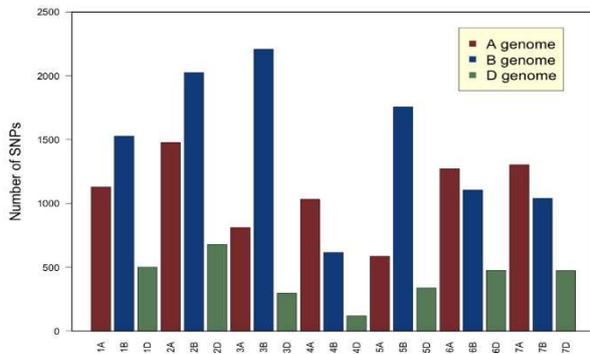


Figure 1: Distribution of 20 755 GBS-based SNPs across the 21 wheat chromosomes (1A-7D).

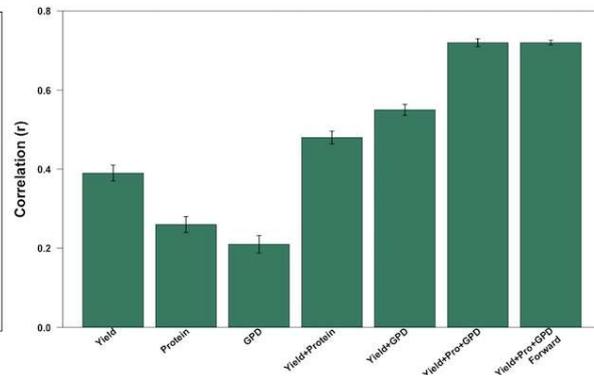


Figure 2: Univariate and multivariate (multi-trait) genomic selection prediction accuracy for production related traits.

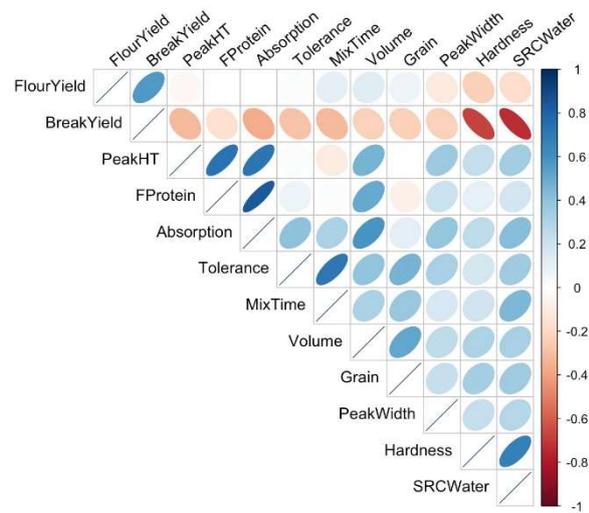


Figure 3: Correlations among end-use quality traits in wheat.



P 233 - Topic: Applying Novel Tools to Practical Wheat Improvement

A collaborative approach to pre-breeding in South Africa

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Key message: Pre-breeding at the SU-PBL (Stellenbosch University Plant Breeding Laboratory) contributing to the improvement of South African breeding programs

Pre-breeding is one of the most effective ways to achieve the introduction of existing and/or novel genes and/or traits into breeding programs. Typically material stemming from such a pre-breeding program (usually done in the public domain) is then introduced, usually, into (private/commercial) breeding programs as crossing parents and/or by direct selection. Thereby enabling breeding programs to achieve quicker, better results. The Department of Science and Technology (DST) of South Africa, Grain South Africa (GSA), the Technology and Human Resources for Industry Programme (THRIP), and the Winter Cereal Trust (WCT) have invested in a rust resistance and a yield related wheat pre-breeding program at Stellenbosch University (SU) since the late 1990s and 2014 respectively. The pre-breeding program is based on a male sterility (facilitated by a dominant sterility gene *Ms3*) mediated marker assisted recurrent selection scheme (MS-MARS) conceptualized, initiated, developed and implemented by the SU's Plant Breeding Laboratory (SU-PBL) (Marais & Botes 2003, 2009, Wessels & Botes 2014). Because of the funding received from industry and close interaction with all wheat breeding programs in South Africa (including Sensako, PANNAR and the ARC-SGI) the pre-breeding program of the SU-PBL has released ten annual rust resistance nurseries by 2015 consisting of several hundreds of lines that were used as crossing parents and/or as direct introductions by recipient breeding programs. All material was accompanied by marker information compiled during the selection process to facilitate the ease of use of material, and a routine (partially subsidised) service are also offered to (and used by) recipient programs to assist with routine marker assisted selection where crosses were made with SU-PBL nursery lines.

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P 235 - Topic: Applying Novel Tools to Practical Wheat Improvement

Long-range scaffolding enables rapid isolation of the leaf rust disease resistance gene *Lr22a*

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Key message: We used a novel gene isolation strategy based on complexity reduction and long-range scaffolding, which permitted the rapid and inexpensive cloning of the leaf rust resistance gene *Lr22a*.

To produce enough food for 9-10 billion people expected by 2050, the development of high-yielding crop cultivars is of paramount importance. One of the major challenges is to limit yield losses by improving abiotic and biotic stress tolerance. Map based cloning is a widely used method for the isolation of agriculturally important genes. Traditionally, repeated rounds of chromosome walking and BAC sequencing was necessary to get sequence information from a donor line containing the gene of interest. The availability of high quality reference sequences from particular cultivars has significant contributions to the understanding of genome structure and evolution but gene order and content, as well as gene sequences can differ dramatically between cultivars of same species. For gene isolation, it is thus important that high-quality sequence information is obtained from a genotype carrying the gene of interest. Here, we flow sorted the 2D chromosome from 'Lr22a Campala' and used Chicago long-range linkage to rapidly and inexpensively generate a high quality *de novo* assembly harboring the broad-spectrum *Lr22a* leaf rust resistance gene from wheat. *Lr22a* is a broad-spectrum resistance gene, introgressed into hexaploid wheat from *Aegilops tauschii* in the 1960s. Two closely linked SSR markers gwm455 and gwm296 enabled us to identify a single 6.39 Mb scaffold that contained both *Lr22a* flanking markers. The 1.79 Mb region between the flanking markers contained nine genes and two pseudogenes. A gene coding for an intracellular immune receptor with homology to the *Arabidopsis* RPM1 protein was validated as *Lr22a* using five EMS mutants that lost the *Lr22a*-activity. Our study demonstrates that it is now feasible to develop high-quality *de novo* assemblies for genomic loci of interest in particular genotypes even in species with complex genomes to facilitate cloning of agriculturally important genes.



P 237 - Topic: Applying Novel Tools to Practical Wheat Improvement

Lessons learned from the discovery of genetically modified wheat in Oregon, USA in 2013

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Key message: Proactive development of protocols to detect transgenic escapes can minimize the impact of an inadvertent release of a regulated trait/gene on the international grain trade.

Whenever field testing is done with a regulated plant such as a genetically modified (GM) crop there is the potential for an inadvertent release of that regulated gene into the environment. Such was the case in 2013 when wheat carrying a regulated gene for glyphosate resistance was found in a field in Oregon, USA. A second case occurred in 2016 when glyphosate resistant wheat was found in Washington State, USA. The preliminary identification that the herbicide resistant wheat was due to the presence of a transgene was done at Oregon State University using a strip test on leaf tissue that indicated the presence of the CP4 EPSPS protein present in Roundup Ready® crops. DNA was extracted from the leaf tissue of the plants and screened using PCR to detect the presence of the *CP4 EPSPS* gene, the *CaMV35S* promoter and *nos* terminator sequences, all components of the transgene in Roundup Ready wheat. The preliminary results were confirmed by USDA-APHIS. Using molecular markers, it was not possible to identify a specific cultivar as the source of the escape. Based on the molecular marker results it was determined that it was most likely a mixed genetic background since the samples varied for market class and growth habit. Results from USDA-APHIS were also inconclusive on the genetic background of the escape. The response to the 2013 event provides insights into how to respond to such events in the future. One challenge with the identification of the presence of the transgenic event in 2013 was the time it took to develop a protocol to provide to the grain market to screen for the presence of the transgene in commercial grain. This time lag caused disruption in the grain market. Having a more rapid method to identify potential transgenic escapes and provide screening protocols to determine presence or absence of the escape in wheat shipments would decrease concerns in the grain trade when a GM trait is discovered. One way to do this would be to have the gene (plasmid), identifying DNA sequences and seed of the transgenic plant on file in a country's regulatory agency for any transgenic cultivars that are submitted for deregulation. This would allow for more rapid identification of events when discovered and more rapid development of screening tests to provide to commodity customers to assure the public that a regulated GM crop has not inadvertently entered their food supply.



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High efficiency gene transfer to wheat mediated by *Agrobacterium tumefaciens* and particle bombardment

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Key message: Gene transfer methods for immature embryos were optimized further. Diverse cultivars are now transformed efficiently mediated by *Agrobacterium tumefaciens*, and gene transfer by particle bombardment is also an efficient process.

Cereal production needs to be significantly increased to support the growing population in the world. Biotechnology must play an important role, and transformation protocols had been well established in rice and maize by the end of the last century. However, wheat, the no.1 crop in many ways, such as in the global acreage for production and the amount of the grains internationally traded, had been left behind. Creation of transgenic wheat mediated by *Agrobacterium tumefaciens* was reported by Cheng et al. (1997), but not much progress followed. The frequency of transformation (independent transgenics/explant) observed was mostly less than 5% of the inoculated tissue pieces, which was much lower than that in other major cereals, unstable, and often not reproducible by other laboratories. The desperate need for wheat transformation technology urged us to identify and optimize key factors involved. Not surprisingly, the factors did not differ much from those in other plants, but the main difficulty was that the optimal window for each factor was very narrow. Wheat cv. Fielder, a spring cultivar, constantly showed high efficiency of transformation. Immature embryos of healthy plants of Fielder grown in a well-conditioned greenhouse were pretreated with centrifuging and co-cultivated with *A. tumefaciens* (Ishida et al. 2015). Transgenic wheat plants were obtained routinely from between 40 and 60% of the immature embryos and higher than 90% in the best cases. Most of the transformed plants were normal in morphology and fully fertile. More than 40% of the transformed plants carried a single copy of the transgenes, which were inherited in a Mendelian fashion. Then, nine cultivars, including spring and winter types, were examined and found all transformable in a preliminary study. Therefore, *Agrobacterium*-mediated wheat transformation technology is proven quite robust and good for a wide range of cultivars now. Furthermore, the same essence applied to a method of transformation by particle bombardment and the following regeneration. The frequency of transformation showed approximately 10% in Fielder. This method will be useful for developing gene editing technologies.

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Exome sequencing of EMS mutants provides a comprehensive resource for wheat functional genomics in tetraploid and hexaploid wheat

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Key message: We identified over 10 000 000 EMS-induced mutations in the protein-coding regions of the wheat genome and have organized them into public databases for research and breeding applications.

Comprehensive reverse genetic resources, which have been key to understanding gene function in model organisms, are missing in wheat. Given the relatively recent origin of polyploid wheat species, most genes in tetraploid and hexaploid wheat are present in multiple functional copies. These duplicated genes buffer the rapid natural changes occurring in wheat. Since loss-of-function mutations in any single wheat homoeolog are frequently masked by redundancy in other homoeologs, this variation remains hidden from natural and human selection. This drawback becomes an advantage for the development of mutant populations, since redundancy confers tolerance to high densities of induced mutations. We developed an 84-Mb exome capture platform comprising overlapping probes covering 82 511 non-redundant wheat genes. We used this platform to sequence the coding regions of 1535 EMS mutants from the tetraploid variety 'Kronos' and 1200 EMS mutants from the hexaploid variety 'Cadenza'. We identified over 10 000 000 EMS-induced mutations in the protein-coding regions of the wheat genome. We detected an average of 2705 and 5351 mutations per tetraploid and hexaploid line, respectively, which resulted in 35-40 mutations per kb in each population. With this mutation density, we found at least one truncation or deleterious missense mutation in more than 90% of the captured wheat genes with at least one mutation. Once mutations in individual homoeologs are identified, they can be combined to generate loss-of-function mutants and to overcome the masking effect of redundant homoeologs. To disseminate this resource, we organized mutations into public databases that can be queried using gene identification numbers or BLAST searches <http://www.wheat-tilling.com> and <http://dubcovskylab.ucdavis.edu/wheat-tilling>. Seeds can then be requested through public seed repositories and predesigned KASP primers are available to validate the mutations and to select them for downstream research and breeding applications. This public collection of mutant seed stocks and sequence data enables rapid identification of mutations in the different copies of the wheat genes providing a comprehensive functional genomic resource in polyploid wheat.



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The implementation of expVIP: a customizable RNA-Seq data analysis and visualization platform in wheat (www.wheat-expression.com)

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Key message: expVIP is a tool that enables simultaneous analysis of RNA-Seq experiments in an intuitive and interactive manner. We've analysed over 400 wheat RNA-Seq datasets and visualised them at www.wheat-expression.com.

Transcriptome expression sequencing (RNA-Seq) is a powerful tool that allows the analysis of active genes under a range of conditions. For this reason, RNA-Seq has become a common tool to characterize gene function in biological systems. The growing number of publically available wheat RNA-Seq datasets should allow exploratory analysis of candidate genes at different ages, growth conditions and tissues. However, the heterogeneous nature of the studies, analysis pipelines, and the disparate reference sequences hinders the comparison among them. To make accessible the information from previously published RNA-Seq studies we developed the expression Visualisation and Integration Platform (expVIP, Borrill et al. 2016). The gene expression is displayed in high-level factors to allow an initial assessment of candidate genes in the different set of conditions. To further explore the gene, it is possible to show fine-grained levels for each factor. Furthermore, if some studies or factors are not relevant to the comparison they can be filtered out and the plot can be sorted to produce high-quality figures for publication. All this can be done dynamically on a web browser. The expression can be shown as a single gene, with its homoeologs and paralogs (Figure 1), or in a heatmap (Figure 2) comprising several genes of interest. We have deployed expVIP with 16 gene expression studies, comprising 418 individual RNA-Seq samples of bread wheat, on www.wheat-expression.com. We mapped these data to both the IWGSC CSS gene models and the new TGACv1 gene annotation (Clavijo et al. 2016). We also prepared two virtual machine configured with expVIP: one ready to load private data and another with the expression values from the wheat studies to allow the inclusion of additional private RNA-Seq studies locally. ExpVIP is a novel approach to visualize expression experiments designed to compare homoeologous (from polyploids) and homologous (across species) genes. The design is flexible, being able to deal with different grouping factors and several transcriptome references.

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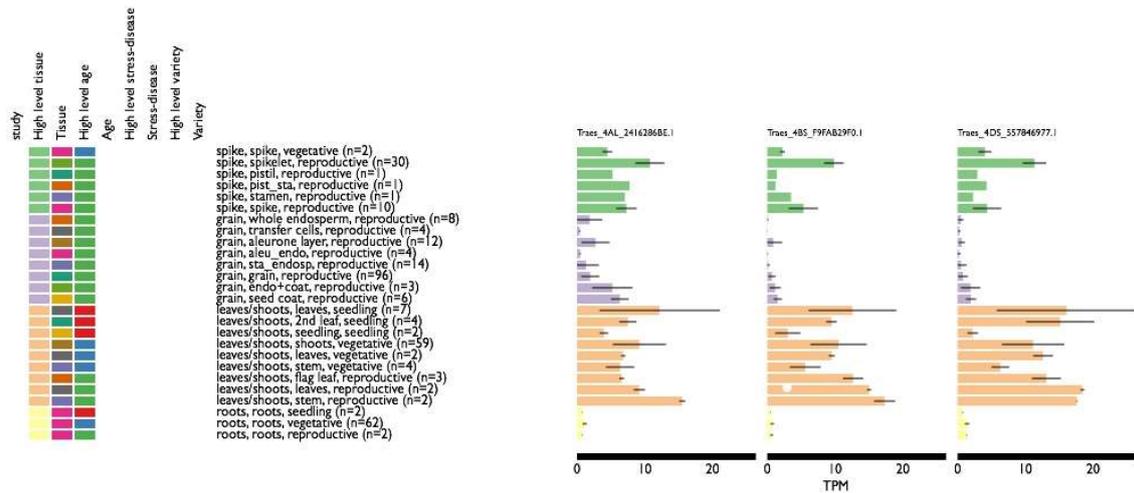


Figure 1: Gene expression visualisation using expVIP for the gene Traes_4AL_2416286BE.1 grouped by 'High level tissue', 'Tissue' and 'High level age'. The factors are sorted by 'High level tissue' and 'Age'. The label shows the number of samples used for each group. Genes Traes_4BS_F9FAB29F0.1 and Traes_4DS_557846977.1 are homoeologues to Traes_4AL_2416286BE.1, in this particular triplet none of the homoeologues has a dominant expression.

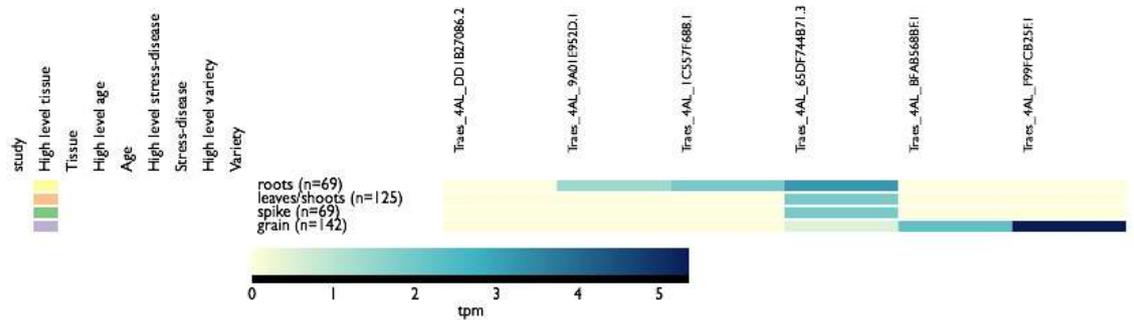


Figure 2: Heatmap produced by expVIP containing 6 candidate genes from a region in chromosome 4A (Traes_4AL_DD1B27086.2, Traes_4AL_9A01E952D.1, Traes_4AL_1C557F688.1, Traes_4AL_65DF744B71.3, Traes_4AL_BFAB568BF.1, Traes_4AL_F99FCB25F.1) with an unknown gene with an effect in grains. The plot suggests that either Traes_4AL_BFAB568BF.1, Traes_4AL_F99FCB25F.1 are better candidates to further research.



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Construction of complete DNA marker set for 32 Korean wheat cultivar identification

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Key message: The major subject of this study is development of DNA marker for Korean wheat cultivar identification.

DNA-based markers have been used in various fields like molecular biology and crop breeding program. DNA marker is able to be used for protection of genetic resource and quantification of specific cultivar. Eleven DNA markers have been developed for Korean wheat cultivar identification in 2013-2014 (Son et al. 2013, 2014). 27 of 32 wheat cultivars were distinguished by 11 DNA markers. In this study, we developed four DNA markers, KWSM0012, KWSM0013, KWSM0014 and KWSM0015, derived from SSR and SNP analysis. Consequently, 32 Korean wheat cultivars were identified by 15 DNA markers (Figure 1). We are convinced that these new DNA markers are very useful for wheat varieties DNA fingerprinting and are able to be applied to marker-assisted selection in wheat breeding program in Korea.

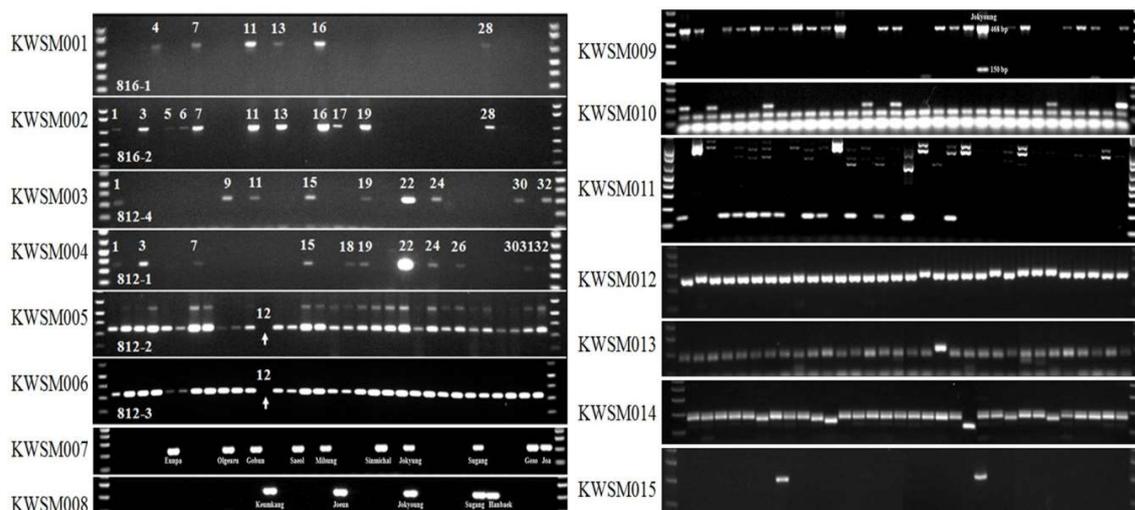


Figure 1: Polymorphisms of 32 Korean wheat cultivars amplified with KWSM001-KWSM015. KWSM001-006 was developed by ISSR analysis in 2013, KWSM007-0011 was developed by AFLP analysis in 2014 and KWSM012-015 was developed by SSR and SNP analysis in 2016.

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P 247 - Topic: Applying Novel Tools to Practical Wheat Improvement

Genotypic characterization of wheat wild relatives

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Key message: Genetic resources of wheat wild relatives (wheat CWR) were genetically characterized by wheat microsatellite markers and chloroplast markers. Interspecific relationships and intraspecific diversity was revealed.

Genetic resources of wheat wild relatives were analyzed for interspecies relationship and intraspecies relationship. The *Aegilops* species *Ae. bicornis*, *Ae. columnaris*, *Ae. comosa*, *Ae. crassa*, *Ae. cylindrica*, *Ae. geniculata*, *Ae. juvenalis*, *Ae. kotschyi*, *Ae. lorentii*, *Ae. markgrafii*, *Ae. neglecta*, *Ae. speltoides*, *Ae. tauschii*, *Ae. triuncialis*, *Ae. ventricosa* and the *Triticum* species *T. araraticum*, *T. boeoticum*, *T. dicoccoides*, *T. dicoccum*, *T. monococcum* were included. The accessions are a representative set of wheat CWR, selected by and obtained from Crop Research Institute, Prague, Division of Crop Genetics and Breeding. The origins of these accessions range from Europe, Middle East, Central Asia to China. The species were analyzed using 28 nuclear wheat microsatellite sequences (Korzun et al. 1997, Röder et al. 1998) as well as 10 chloroplast microsatellite sequences (Ishii et al. 2001). Genetic relationship between wheat CWR was analyzed. Results were mostly coherent with expectations from literature, with few exceptions most likely due to misclassifications in the gene bank. Further, accessions were divided in 8 species-specific groups and intraspecific genetic diversity and genetic structure was elucidated using the above-mentioned nuclear SSR markers.

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Physiological dissection and genotypic differences in growth stages in wheat under early sown conditions

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Key message: Physiological traits along with agronomic traits can play significant role in identification and selection of genotypes under early sown condition of wheat.

Grain yield along with yield attributing traits like productive tillers, days to heading, plant height, spike length and thousand grains weight and physiological traits Canopy temperature (CT) at heading, CT at 15 days after anthesis, leaf area index (LAI) and NDVI (Normalized Difference Vegetation Index) at eight different growth stages were recorded according to standard procedures (Reynolds et al. 2001) under early sown condition in twelve genotypes including three checks DBW88, HD2967 and WH1105 during 2015-16 crop season at IIWBR Karnal India. NDVI was measured through canopy reflectance with a Green Seeker. Statistical data was subjected to repeated measure analysis was done using GLM procedure of SAS 9.2. The study revealed significant genotypic variations in physiological and agro-morphological traits in wheat. The variation in NDVI values revealed that genotypes differed significantly during both chlorophyll accumulation and its decomposition. Canopy temperature at 15 DAA was positively associated with tiller number, days to heading, and NDVI values during grain filling duration. LAI was significantly and positively associated with the NDVI Values at 48 days after sowing. NDVI values during grain filling stages were positively associated with plant height. The repeated measure analysis showed significance of NDVI values recorded at different times on the given genotypes. The value of Wilks' lambda were small and value of Pillai's trace, Hotelling-Lawley trace and Roy's greatest root statistic was large, null hypothesis for the time effect was rejected at 1% level. This was also confirmed by probability of F value (<0.0001). The results showed that NDVI values significantly varied over time in the tested genotypes. High yielding genotypes showed more decline in NDVI values during grain filling duration thereby showing effective utilization of accumulated photosynthates. The less yielding genotypes had higher NDVI values during late grain filling stages revealing their cosmetic stay green habit.

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The cell state hypothesis like a 'Mendel's law' in plant tissue and cell culture

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For over 100 years, plant tissue and cell culture cannot be predicated. Inducing callus is not a problem, the main difficulty is to regenerate plant which depends on the type of callus determined by its major compositional cells. With wheat, rice, cotton and so on, it was found that different cell types in culture are the various displays of cell states. Seven typical types were founded: embryogenic cell (S_e), conservative cell (S_a), stimulating cell (S_b), multiplicative cell (S_t), conservatively degenerated cell (D_a), radically degenerated cell (D_b), hyperactively dividing cell (S_c). The other types are the transitional states of them. If callus is composed only by S_c or only by D_a or D_b , regeneration won't occur. When callus is mainly composed with S_a , it will regenerate plants via organogenesis. Callus with cells between S_a and S_b will regenerate plant via somatic embryogenesis. The key of culture is to regulate the cell state. The cell state hypothesis proposed here is abstracted the cell being controlled by a series of cell state factors. The roles of all the factors are understood as having physiological and biochemical effects. There are three categories: stimulating factors (E), conservative factors (I), and degenerate factors (D). E factors promote cell division, while I factors make cell differentiation. Both of them can be further classed into internal and external types. D factors accumulated in cell show some similar effects as I factors when at low level, and can be diluted by cell division. The changing of cell state follows the formula: $S = \{[\sum E_o] + [\sum E_s]\} / \{[\sum I_o] + [\sum I_s] + [\sum D]\}$ (S - cell state coefficient, $_o$ and $_s$ indicate internal or external factors respectively). Cell divide when $S \approx 1$, the higher both $[\sum E]$ and $[\sum I]$, the stronger the cell division. When S is away from 1, cell would be differentiated. Auxin and reduced nitrogen can be regarded as E factors; cytokine, oxidized nitrogen can be used as I factors; unfavorable matters to the cell can be listed as D factors. MS or AA medium can increase S value, and N6 or B5 medium will decrease S value. NH_4^+ plays E factor role for big callus, but show unfavorable effects in cell and protoplast culture. In this case, ammonia-N, amino acids should be recommended. When culture has strong nitrate reducing power, the effect of nitrate-N as I factor will be weaken.



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***In vivo* integrated biosensors to monitor in real-time physiological processes in wheat**

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Key message: A new *in vivo* integrated biosensor has been developed to monitor in real time the changing in wheat physiology during plant development and allow a direct and early detection of stress.

The ability to monitor the plant physiological activity using *in vivo* and integrated biosensors represents a key point for SMART farming. Different approaches have been proposed to analyse the plant physiological activity on a qualitative basis but few data are available in terms of quantitative data. Biosensors are tools that transform a recognition event such as the perception of a small molecule by a receptor into a signal that can be easily detected and quantified (Sadanandom & Napier 2010). So far, the developed biosensors limit their activity to cellular compartment and are based on indirect measurements through optical sensors exploiting fluorescent probes coupled with fluorescence resonance energy transfer (FRET) system or on electrochemical signals. Both lead to the disruption or damage the investigated tissue. Here we present for the first time, to best of our knowledge, a biomimetic integrated *in vivo* biosensor that monitors qualitative and quantitative changes in wheat sap during growth and development directly integrated within the plant tissues. The new biosensor is an active part of the device and resulted perfectly integrated within the plant tissues without altering wheat morphology. It has been inserted in different wheat developmental stages and monitors the biological activity of wheat plants by measuring the concentrations of electrolytes in the plant sap. The data analyses showed that the device detects the changes that occur in the sap composition and concentration and allowed to do a perfect match with the photoperiodic cycle. The performance of the biosensor following drought stress will be also presented. The developed user-friendly, low cost biosensor open new perspectives for wheat phenomics since can be directly integrated in automated phenotyping platforms. Understand the sap composition allows an early identification of nutrients or water stress and significantly contributes to precision farming.

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Fusarium damaged kernels notation on grains by digital picture analysis

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Key message: Easy and quick quantification of Fusarium-damaged-kernels (FDK) through a program which compares the surfaces of diseased and healthy kernels. It is a promising tool to enable larger scale FDK scoring.

Fusarium head blight (FHB) causes grain yield and quality reduction by the formation of shriveled and tan grains containing notably the mycotoxin deoxynivalenol (DON). Resistance to mycotoxin accumulation in grains is commonly approximated as a percentage of Fusarium-damaged-kernels (FDK) based on a visual notation. This process is useful to estimate DON content with the disadvantage of being time consuming and labor intensive. The objective of this study is to evaluate an alternative method for quantifying FDK by using digital image analysis. A program has been developed in Python which compares the surface of diseased kernels to the healthy grain surface (Figure 1). It has been developed specifically for triticale and is adaptable for other crops including common wheat. Designed to be easy and quick to use, it is a promising tool to enable larger scale FDK scoring. The quality of the program's notation was evaluated on a set of 50 F₄-RILs derived from a cross between the FHB resistant triticale G08.06 and the susceptible cultivar 'Tulus'. In 2014 and 2015 the lines were spray inoculated with *Fusarium culmorum* (isolate IFA 104, macroconidia concentration of 50 000 conidia/ml) in replicated field trials at the FHB disease nursery in Tulln, Austria. At several time points after inoculation the percentage of symptomatic spikelets per plot was estimated and AUDPC was calculated, for each genotype and replication, as a measure of FHB severity. For each year and replication genotypes were harvested and a set of 300 to 350 kernels by genotype was evaluated for both visual FDK notation and digital FDK notation. Correlation between visual FDK and digital FDK notation was high with $r=0.70$ ($p<0.001$). Correlation between AUDPC and visual FDK was of 0.74 and 0.85 ($p<0.001$) in 2014 and 2015, respectively, while correlation between AUDPC and digital FDK was of 0.62 and 0.68 ($p<0.001$) in 2014 and 2015, respectively. Measurement of DON content in the harvest samples will give additional information on the correlation between digital FDK notation and mycotoxin accumulation in the grains compared to visual FDK notation. Evaluation of the diseased surface versus counting of diseased kernels may allow a better approximation of the DON content. In other case, the program could be improved to allow a direct evaluation of the diseased grains number.

Acknowledgements

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Figure 1: Examples for two triticale seed samples analysed with digital image analysis for estimating Fusarium damaged kernes (FDK).



P 257 - Topic: Applying Novel Tools to Practical Wheat Improvement

SSR marker TSM106 a convenient tool for wheat- rye 1AL.1RS translocation selection

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Key message: SSR-TSM106 behaved like a dominant marker that clearly identified the distal wheat-rye 1AL.1RS translocation, coming from 'Insave' or similar rye chromatin, from other rye chromatin usually present in 1BL.1RS translocations.

Wheat continues to be one of the most cultivated cereals in the world. The research undertaken to find new genetic resources and new favorable alleles are a priority in the wheat breeding programs. Increasing genetic diversity is necessary to cope with the social, natural and economic challenges. Rye (*Secale cereale* L.) represents an important genetic resource for wheat breeding. The short arm of the rye chromosome 1 (1RS) contains several genes inducing resistance to biotic and abiotic stresses (Mirzaghaderi et al. 2011, Lu et al. 2014) and some authors (Howell et al. 2014) also, suggested presence of genes that increase yield, and/or are responsible for better adaptation. Therefore, identification of new tools for detection/selection of favorable rye alleles can help obtaining fast and reliable results. Molecular markers-SSRs are such convenient tools. Kofler et al. (2008) developed SSR (Simple Sequence Repeat) marker primer pairs, named TSM (Tulln Secale Microsatellites) specific for the short arm of rye chromosome 1. One of them is TSM106, the locus Xtsm106 being in distal position on 1RS. In our study, conducted on 17 genotypes (rye cultivar-Harkovskaya; five Romanian wheat cultivars; four 1AL.1RS translocation genotypes, including 'Amigo' derived from 'Insave' rye, and seven 1BL.1RS translocation genotypes), we used the TSM-106 marker to detect the presence of 1RS translocation. We found that the SSR-TSM 106(=170bp) marker clearly distinguished the wheat-rye 1AL.1RS translocation, coming from 'Insave' or related rye chromatin, from the wheat-rye 1BL.1RS translocation, coming from other rye sources. Therefore SSR-TSM marker could be used in MAS (Marker-Assisted Selection) for 1AL.1RS translocation.

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P 259 - Topic: Applying Novel Tools to Practical Wheat Improvement

A lipidomic, genetic and biophysical approach to improving breadmaking quality in wheat

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Key message: We are using genetic, lipidomic and biophysical analysis to identify lipids that play a role in determining breadmaking quality and to map the variation in lipid composition to quality QTL.

The breadmaking industry is worth £ 3.4 billion per annum in the UK alone and much research has been pursued on improving breadmaking quality. Lipids are believed to play a crucial role in breadmaking by adsorbing to the surface of gas bubbles, stabilizing them, allowing air retention within the dough. This stability mechanism provides the volume, crumb structure and fine texture that is associated with UK sliced bread. For this project six wheat lines grown at varying nitrogen levels between in 2012-13 were milled and analysed using a 'lipidomics platform' (Applied Biosystems 4000 QTRAP LC/MS/MS and associated equipment). This provided profiles of functionally active lipids in flour, allowing the use of multivariate statistics to identify the effects of genotype, environment or G×E on individual lipid species. A previous project identified a number of QTL (Quantitative Trait Loci) for milling and baking quality parameters using a doubled haploid (DH) population from a cross between the UK bread making cultivars Malacca and Hereward. Four robust QTL for gas cell number and loaf volume located on chromosomes 1B, 4D, 6A and 7A were selected, and near isogenic lines (NILs) were developed with good and poor quality alleles in the Malacca background. Lipidomic analyses were then carried out on material grown over two seasons to identify lipids associated with the allelic differences in quality. We will also determine the functional significance of any differences in lipid composition which are observed by determining the composition and surface rheological properties of dough liquor fractions which contain components present at the gas bubble interface. Finally, the availability of genetic maps for the Malacca/Hereward population will allow us to identify QTL for lipid composition and determine how these relate to quality QTL.



P 261 - Topic: Applying Novel Tools to Practical Wheat Improvement

Using the GlutoPeak to benchmark Ontario winter wheat

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Key message: The GlutoPeak has potential as a broad stroke tool in categorizing experimental lines and varieties in breeding programs.

The Brabender GlutoPeak has been used to establish insight to fundamental aspects of gluten quality and aggregation behaviour in previous studies and on-going research has been successful in relating agronomic management practices to flour quality. This information was used to provide a framework to benchmark the quality of 32 Ontario soft and hard winter wheat lines and varieties. Results revealed that there were statistically significant differences among the lines selected ($p < 0.05$). The differences between hard and soft wheat classes were not as large as would be expected, however, despite the range of quality parameters measured. The full data set displayed a significant correlation between bread volume and GlutoPeak torque ($r = 0.612$; $p < 0.01$). This relationship was still significant at the $p < 0.05$ level for the individual hard wheat ($r = 0.579$) and the $p < 0.01$ level for soft wheat ($r = 0.655$) subsets, respectively. Secondary structures identified in the GlutoPeak slurry including α -helices, β -sheets, and β -turns showed significant relationships with both GlutoPeak torque and bread volume, indicating that gluten protein structure may be an important driver of both GlutoPeak rheological parameters and bread making potential. Breaking the data set down into hard and soft winter wheat subsets revealed a correlation with GlutoPeak time ($r = -0.646$; $p < 0.01$) in the soft subset. PCA factors included most quality parameters measured, with the notable exceptions of SRC tests and total thiols. Interestingly, a clear separation of hard and soft winter wheat varieties was never achieved in various iterations of PCA plots, although the strong relationship between GlutoPeak parameters and gluten secondary structures was further defined. Closer evaluation revealed that a subset of soft winter wheat lines had comparable or better bread making performance compared to certain hard winter wheat lines. This is likely related to efforts in Ontario to breed for soft winter wheats with stronger gluten profiles over the past decade. The overall patterns from the study demonstrate that hard winter wheat quality improvements have lagged in Ontario compared to soft winter wheat; the GlutoPeak has potential as a broad stroke tool in categorizing experimental lines and varieties in breeding programs; and that further exploration of the role of gluten protein secondary structure in dough rheology and cereal products is necessary.

Acknowledgement

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P 263 - Topic: Applying Novel Tools to Practical Wheat Improvement

Genome-based prediction of falling number stability in wheat breeding material

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Key message: Genomic selection methods can enhance selection for preharvest sprouting tolerance in wheat.

Wheat cultivars for the milling industry have to meet several quality criteria. One of the quality descriptors for wheat is the falling number which can be used for determining the starch strength. Low falling numbers can be caused by preharvest sprouting (PHS) inducing high levels of α -amylase. The variation of falling number in field trials highly depends on the annual weather, i.e. hot and dry period during grain ripening and rain at harvest are required for differential response of wheat genotypes. Therefore, assessing falling number stability and, thus, PHS tolerance is time consuming and not possible in the early stage of wheat breeding programs. To increase the differentiation between lines for falling number, a test with wetting and drying of kernels was implemented to assess falling number stability under lab conditions independent of environmental effects. In recent projects, a diversity panel and biparental populations were analyzed and several molecular markers were identified to be associated with falling number, falling number stability, and PHS tolerance (Mohler et al. 2014, Albrecht et al. 2015). However, the application of these markers in breeding programs needs to be validated in unselected breeding material. Furthermore, genome-based selection strategies can be applied to predict PHS tolerance in wheat. In the ongoing project, 400 wheat lines from German and Austrian breeders were analyzed in two years for falling number stability and PHS tolerance. These lines were genotyped with the Illumina® 15k Infinium iSelect BeadChip and, in addition, with a self-customized Fluidigm® IFC array containing markers indicative for previously identified PHS QTL. The specificity of the selected markers was analyzed in the different breeding pools. Several prediction models including single markers, genome-wide markers or a combination of both were used to predict PHS tolerance in unselected material of the breeders. First results show that a genome-wide approach increased predictive abilities compared to a simple marker regression. However, including information of known marker-trait associations into the genome-based prediction models enhanced predictive abilities.

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P 265 - Topic: Applying Novel Tools to Practical Wheat Improvement

The contribution of non-prolamins (albumin and globulin) to dough properties in South African hard red wheat cultivars

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Key message: Non-prolamins (albumin and globulin) contribute to dough tenacity and dough extensibility. Contribution varied between genotypes and environments.

The aim of this study was to determine the contribution of albumin and globulin (AG) to alveograph tenacity (AlvP) and alveograph extensibility (AlvL). Seven South African dryland hard red bread wheat cultivars (BettaDN, Caledon, Elands, Gariiep, Komati, Matlabas and PAN3118) were evaluated for two years over two regions; North-Western Free State (NW-FS) and Eastern Free State (E-FS). The molecular weight distribution of unreduced wheat proteins was determined by means of size-exclusion high performance liquid chromatography (SE-HPLC). Protein fractions were measured as SDS-soluble and SDS-insoluble to determine proportional and quantitative variation. The stepwise multiple regression was performed, using PROC REG of SAS software (SAS Institute, Cary, NC), to determine the contribution of AG to variability. AG fractions contributed more to variation in NW-FS than in E-FS for AlvP and AlvL. Furthermore, these contributions were larger for BettaDN, Caledon, Elands, Gariiep and Komati than for Matlabas and PAN3118. The positive contribution of specific AG fractions on dough properties needs to be further investigated.



P 267 - Topic: Applying Novel Tools to Practical Wheat Improvement

Can some SSR markers associated with QTL for grain protein content be effective for common wheat improvement?

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Key message: Particular QTL possessed universal effect on GPC among varieties of different gene pool and their SSR markers are useful for application in MAS for GPC improvement in common wheat

Grain protein content (GPC) is considered one of the most important traits of common wheat. Selection for high GPC is expensive and time consuming due to its complicated genetic control and strong influence on its expression of biotic and abiotic factors. In recent years a number of QTL for GPC were identified on different wheat chromosomes by SSR markers. The aim of the present work was the validation of some SSR markers for GPC QTL among common wheat planted in Ukraine. For this purpose, 51 winter common wheat varieties were cultivated in different regions of Ukraine from 2007 to 2009. GPC varied among varieties from 11.9% to 16.8% and showed a normal distribution. All varieties were divided statistically into nine classes according to their GPC. 13 SSR markers associated with particular QTL for GPC (Prasad et al. 2003) were applied. The number of alleles for each marker varied from two (*Gwm614* on 2AS) up to 12 (*barc1005* on 7AS) among our population of varieties, but mainly 4-6 alleles per marker were observed. Most alleles showed a normal distribution, which means that they have no significant influence on GPC in this population. However, three exceptions were observed, i.e. alleles of *wmc415*, *wmc41* and *barc1005*. One *wmc415* allele was observed in the classes with higher GPC (average 14.3%) and another in classes with lower GPC (average 13.9%). The difference between the two groups of varieties was significant. Two alleles of *wmc41* were distributed among varieties with low GPC (<13%) and three other alleles were identified in the group of varieties with 14.1%-14.3% GPC. Two alleles of *barc1005* with higher quantity were distributed in the class with lower GPC 13.7%-14.1% and two other alleles were observed more frequently in varieties with higher GPC 14.6%-14.9%. Therefore, it was shown that some QTL are useful for the application in MAS for GPC improvement and possessed an universal effect on GPC among varieties of different gene pool, however, most SSR-QTL markers appeared to be useless.

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P 269 - Topic: Applying Novel Tools to Practical Wheat Improvement

Sequence differences associated with near isogenic spring wheat sister lines for grain yield, grain protein, and end-use quality

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Key message: We have identified sequence differences associated to grain yield, grain protein and end-use quality in near isogenic lines in an elite spring wheat cross.

Grain yield, grain protein concentration, and end-use quality traits (milling and gluten strength/extensibility) are economically important traits to improve in wheat (*Triticum aestivum* L.) through breeding. Given grain yield and grain protein concentration are negatively correlated, and protein quantity and quality contribute to rheological performance, understanding the genetic relationship among the traits is valuable. Five F_{6,8} sister lines were identified from an elite spring wheat breeding population named 'B1018' (BW928/BW431//Carberry) that differed in grain yield and grain protein level. Further investigation involved measuring grain yield, protein concentration, milling and gluten strength of grain from four field trials grown near Swift Current, SK (2015 and 2016), Indian Head, SK (2015) and Saskatoon, SK (2016). The sister lines and parents were genotyped with the 90K Infinium iSelect assay and the F_{6,8} lines with significant grain yield and grain protein differences were exome sequenced. Line B1018-LN04C03 had grain yield about 35% less than sister line B1018-LN04C02; however, the protein concentration of the lower yielding sister line was 2.2% units higher. Milling performance was low and gluten strength was weaker in the low yield/high protein sister. Preliminary analyses indicated the marker and sequence differences between these near-isogenic sister lines reside in intervals on chromosomes 5A, 5B, and 7B. The germplasm produced in this study will be useful in studying seed development and resource partitioning for grain yield, grain protein, milling and rheological properties.



P 271 - Topic: Applying Novel Tools to Practical Wheat Improvement

Improving the baking quality of bread wheat using rapid tests and genomic selection

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Key message: Genomic selection shows great promise for pre-selecting lines with superior bread baking quality in early generations, three years before labor-intensive and costly quality analysis could be conducting on the potential variety candidates.

Breeding winter wheat for baking quality is a challenge due to the large number of loci influencing the various properties of the dough as well as loaf volume, texture and structure. Wheat breeders usually select genotypes with superior bread-baking quality by traits like the protein content and sedimentation value or by parameters obtained from laborious and costly rheological tests. Selection for baking quality and favorable dough properties is therefore carried out very late in applied wheat breeding programs. Genomic selection with thousands of markers covering the genome with a high density makes a 2-3 years earlier selection for these traits possible, and has great potential of significantly accelerating the genetic improvement of baking quality in bread wheat breeding. We analyzed a population of more than 300 genotyped wheat inbred lines from an applied wheat breeding program, which were tested for their protein content and sedimentation value in multi-environment trials in 2009-2014. Multiple samples of all inbred lines were taken from these trials and analyzed in the lab for their rheological properties employing the Farinograph, Extensograph and Alveograph. The specific aims of this study were (1) to investigate the merit of genomic selection for baking quality related traits and (2) increase the prediction accuracy of these traits by integrating prior knowledge of their genetic architecture, and (3) exploiting the vast amount of protein content data that is routinely generated in wheat breeding programs by various multivariate genomic prediction strategies. Most of the rheological parameters could be predicted with an acceptable accuracy in three independent validation populations ($r = 0.20-0.54$). Depending on the strategy, the average prediction accuracy of the various traits was strongly increased ($r = 0.45$) in comparison to the baseline model ($r = 0.38$). Hence, our result show the great benefit of combining different sources of agronomic, genomic and rheological knowledge. The selection gain employing a genomic selection approach was furthermore 60% higher in comparison to indirect selection for quality traits using the protein content. Given these results, genomic selection proved to be a very promising approach to pre-select elite germplasm for superior baking quality in applied bread wheat breeding programs.

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P 273 - Topic: Applying Novel Tools to Practical Wheat Improvement

Complementary and reliable identification of HMW-GS using RP-HPLC and SDS-PAGE in common wheat cultivars

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Key message: This report describes improved RP-HPLC separation methods for the wheat HMW-GS that can be used in conjunction with conventional SDS-PAGE for efficient, accurate and cost-effective assessment of allelic compositions of wheat cultivars.

The accurate identification of alleles for high-molecular weight glutenins (HMW-GS) is critical for wheat breeding programs targeting end-use quality. RP-HPLC methods were optimized for separation of HMW-GS, resulting in enhanced resolution of 1By and 1Dx subunits. Statistically significant differences in retention times (RTs) for subunits corresponding to HMW-GS alleles were determined using 16 standard wheat cultivars with known HMW-GS compositions. Subunits that were not identified unambiguously by RP-HPLC were distinguished by SDS-PAGE or inferred from association with linked subunits. The method was used to verify the allelic compositions of 32 Korean wheat cultivars previously determined using SDS-PAGE and to assess the compositions of six new Korean cultivars. Three cultivars contained subunits that were identified incorrectly in the earlier analysis. The improved RP-HPLC method combined with conventional SDS-PAGE provides for efficient, accurate and cost-effective identification of HMW-GS and will contribute to efforts to improve wheat end-use quality.



P 275 - Topic: Applying Novel Tools to Practical Wheat Improvement

Study of the effects on processing and bread-making quality of the wheat bread making (*wbm*) gene in two bread wheat populations

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Key message: The effect of the recently described wheat bread making (*wbm*) gene was studied in two bread wheat populations

Bread-making quality is a core trait for wheat breeding programs. CIMMYT's wheat breeding program aims to develop new varieties that not only produce high yields for farmers but also satisfy subsequent actors in the value chain, including food manufacturers and consumers. Integrating bread-making quality in a breeding program is not simple, as quality analyses are expensive and time-consuming. Molecular markers can be useful for discriminating various wheat quality components and enhancing selection for bread-making quality. Recently Furtado et al. (2015) identified a new gene that is expressed in developing seeds, called the wheat bread making (*wbm*) gene. This gene codifies for a small sulphur-rich protein not previously associated with wheat quality. The *wbm* gene has shown highly differential expression in genotypes varying in bread-making quality: genotypes with high *wbm* expression all had good bread-making quality. The sequence variant in the promoter region of the gene associated with high expression of the gene presence (GWseqVar3) can be determined by a simple PCR marker. In another recent study, Guzman et al. (2016) showed that GWseqVar3 is present in several CIMMYT lines and it had a significant effect on different quality traits but smaller than other quality related genes. The objective of the current study was to know more about the specific effect of the *wbm* gene on quality traits. For this purpose, two populations composed each of 95 sibling bread wheat lines, derived each from a different cross (in each cross one of the parents carried the GWseqVar3), were grown during 2015-2016 in Ciudad Obregon, Mexico, under optimum conditions. Grain from one field replicate was analyzed for grain protein content, SDS-sedimentation volume, mixograph optimum dough development time and torque, alveograph gluten strength and tenacity/ extensibility ratio, and bread loaf volume. In addition, HMW glutenin composition and the presence of the 1BL.1RS translocation were determined by SDS-PAGE. To detect the presence of the *wbm* allele associated with high expression, PCR screening was carried out with primers NWPFor and NWPRev (Furtado et al. 2015). The results obtained revealed the contribution of the *wbm* to quality traits in the two populations.

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P 277 - Topic: Future of Wheat Improvement in Different Parts of the World

Wheat Initiative: achievements and challenges

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Key message: The Wheat Initiative, a global coordination platform to support and expand wheat research.

The Wheat Initiative (<http://www.wheatinitiative.org>) was established at the end of 2011 under the aegis of the G20 ministries of agriculture to coordinate international research efforts on wheat and provide opportunities for increased and efficient utilisation of resources through the alignment of national and regional activities and pooling of resources. Since its creation, the Wheat Initiative has built momentum by stimulating debate and discussion among its members, which has improved support for wheat research at the national level in several regions and facilitated understanding of on-going international wheat research. Members and non-members are represented in 11 Experts Working Groups and 4 Associated Programmes aiming at identifying and delivering research priorities of international relevance in the strategic areas outlined in the Wheat Initiative Strategic Research Agenda (SRA). The Wheat Initiative has contributed to sharing efforts and resources around initial priorities such as: the development of a Wheat Information System (<http://wheatis.org>), the establishment of the International Wheat Yield Partnership (<http://iwyp.org/>), the achievement of the wheat genome reference sequence by the IWGSC (<http://www.wheatgenome.org>), and the recent launch of a wheat 10-genome sequencing project. Other important activities include the circulation of information about wheat research through a regular Media Digest, and development of a information portal about wheat research and researchers. These activities are designed to raise the level of awareness in the wheat and related communities about opportunities and developments in wheat research. The momentum built so far by the Wheat Initiative now needs to extend to more countries, research organisations and industry. The objective is to support the delivery of research outcomes that will contribute to the sustainable increase in wheat production and contribute to global food security. The main challenges in the delivery of all the themes of the Wheat Initiative SRA will be to provide mechanisms to facilitate delivery and to avoid funding fragmentation by alignment or joint international funding, including public-private partnerships.





P 279 - Topic: Future of Wheat Improvement in Different Parts of the World

WheatME: Improve wheat production under climate changes in the Middle East region

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Key message: A Palestine-Israel-Jordan project expose local durum wheat landraces and cultivars with stable yield under drought. These will be used for new drought tolerance QTL introduction for better Mediterranean yield stability.

Water deficit is the major environmental factor limiting wheat (*Triticum* sp.) productivity and yield stability worldwide. Developing novel cultivars with greater drought tolerance is the most viable solution to ensure sustainable agricultural production and alleviating threats to food security. The overall aim of the current research is developing elite durum wheat (*T. turgidum* ssp. *durum*) cultivars with enhanced grain yield and improved water-use efficiency under climate change of the Middle East (WheatME: <http://wheatme.wixsite.com/wheatme>). We have established a core collection of landraces and modern durum wheat cultivars, from the Middle East region (Jordan, Palestine and Israel). Our research goals were to reveal the morpho-physiological and genetic adaptation to cope with water limited environments. In addition the shared material and knowledge could contribute for future breeding efforts as cultivars developed in neighbor countries. Field evaluation of the core collection was conducted during 2015-16 season in three contrasting locations: Till, (Palestine, 500mm), Irbid (Jordan, 315mm) and Bet-Dagan (Israel, 360mm). Analysis of variance showed a significant genotype × environment interaction ($p < 0.0001$) in plant phenology traits (plant height and heading date) and yield related traits (Number of spikes, 1000 kernel weight (TKW), and grain yield). In general, the Till environment sowed highest grain yield and productivity (dry mater, DM) while the Irbid environment showed the lowest values. Principal component analysis (PCA) uncovers three main groups (Figure 1): Cluster 1: High yielding lines (landraces and modern cultivars), Cluster 2: tall-late flowering landraces, across three environments and Cluster 3: landraces with high grain weight across three environments. Single landrace with high TKW and high stature was classified separately (Cluster 4). In parallel, a recombinant inbred lines (RIL) population derive from cross between elite durum wheat cultivar Svevo and highly drought tolerant wild emmer (*T. turgidum* ssp. *dicoccoides*) accession Zavitan was characterized across three environments. We have identified adaptive and constitutive quantitative trait loci (QTL) which confer morpho-physiological and yield related traits from wild emmer wheat. Selected lines from the core collection were crossed with RILs possessing promising QTL to establish pre-breeding genetic material with enhance yields and improved water use efficiency under the Mediterranean environments.

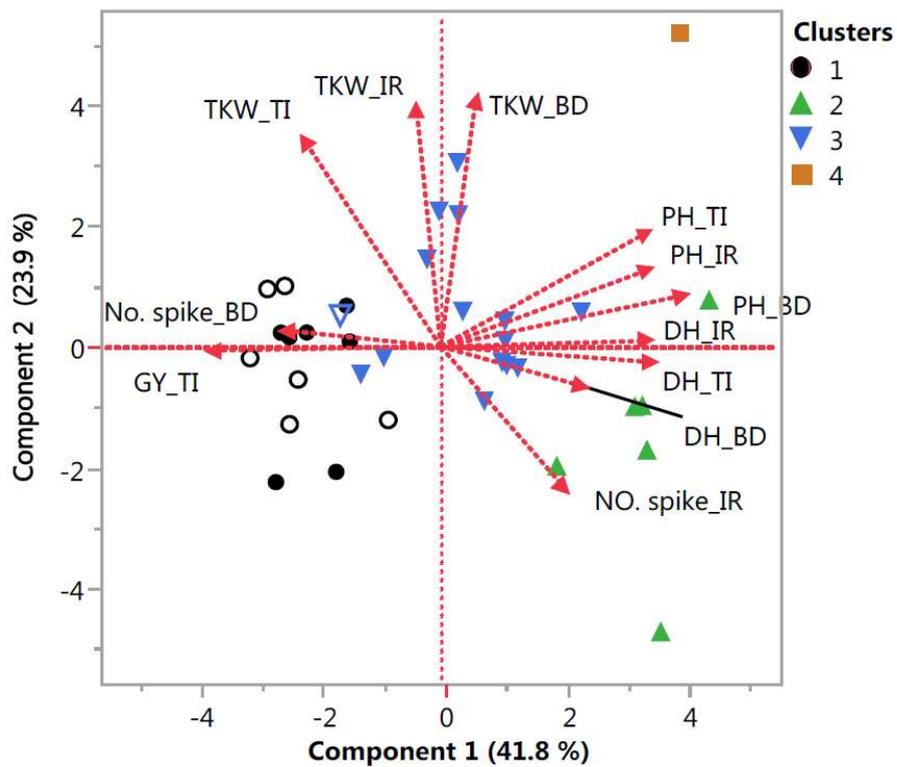


Figure 1: Evaluation of durum wheat core collection across three environments. Principal components analysis of phenotypic traits of a durum wheat core collection (36 landraces and cultivars) from the Mediterranean durum growing region. PC factor loadings are expressed as vectors (arrows) using the coordinates for PC1 and PC2 and represent mean data of traits from three environments: Till, Palestine (TI), Bet-Dagan, Israel (BD), Irbid, Jordan (IR). PC scores of individual lines are classified by markers and grouped into four clusters, which share common agronomic attributes. In addition the type of genetic material is also defined: modern cultivars (hollow symbols), landraces (filled symbols).



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International Winter Wheat Improvement Program: serving global breeding community

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Key message: IWWIP developed germplasm has been successfully utilized in Central and West Asia and contributes to expanding genetic diversity.

The International Winter Wheat Improvement Program (<http://www.iwwip.org>) was established in 1986 as a cooperative project between the Government of Turkey and CIMMYT. The main objective was development of winter and facultative wheat germplasm for the region of Central and West Asia and facilitation of global germplasm exchange. ICARDA joined the program in 1991. The breeding program in Turkey is incorporated into public breeding framework of the Ministry of Food, Agriculture and Livestock and utilizes 8-10 sites with variable environments, abiotic and biotic stresses. The germplasm developed by IWWIP is distributed globally to more than 100 cooperators in more than 50 countries through international nurseries: Facultative and Winter Wheat Observation Nursery (FAWWON) and International Winter Wheat Yield Trial (IWWYT). Important part of IWWIP activities is evaluation and distribution of the germplasm submitted by cooperators from other countries facilitating exchange of material. More than 65 varieties originating from IWWIP germplasm have been released and cultivated on area exceeding 2.5 mln ha. In addition to breeding activities IWWIP is involved in capacity building through training and biannual winter wheat travelling seminars conducted in different countries. Research is focused on abiotic and biotic stresses. Recent results can be found in Sharma et al. (2014), Morgounov et al. (2015, 2016) and Akin et al. (2016). In 2009-2014 IWWIP conducted national inventory of wheat landraces resulting in conservation and characterization of material collected from more than 1500 farmers from 50 provinces of the country.

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P 283 - Topic: Future of Wheat Improvement in Different Parts of the World

Kazakhstan-Siberia network on spring wheat improvement

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Key message: Kazakhstan-Siberia network on spring wheat improvement contributed greatly to diversity of the crop germplasm, adaptation and resistance to rusts through exchange of material and shuttle breeding with CIMMYT.

The region of Northern Kazakhstan, Southern Ural and Western Siberia collectively grows 17-18 mln ha of spring wheat (Morgounov et al. 2001). The crop is normally planted in May and harvested in September. The yield is relatively low (1.2-1.8 t/ha) due to extensive production system and dependence of rainfall. However, due to its scale, the region plays important role in regional and global food security. The Kazakhstan-Siberia Network on Spring Wheat Improvement (KASIB) was established in 2000 with the objective of germplasm and information exchange. KASIB comprises 12 breeding programs from Russia (mainly Western Siberia) and 10 programs from Kazakhstan (mainly Northern Kazakhstan). The network conducts KASIB yield trial for bread and durum wheat with each cooperator contributing 2 to 3 advanced lines or new varieties. Each trial is grown at all sites for two years. The data is collected, summarized and distributed to network participants. The data from these multilocational trials assist greatly in identification of superior genotypes with broad adaptation and resistance to diseases. Analysis of genotype × environment interaction using KASIB trial data from 2006 till 2016 allowed identification of sub-regions and 'ideal' sites for germplasm evaluation. Leaf and recently stem rust represent the major challenge among the biotic stresses. A set of stem rust (including Ug99) resistant germplasm has been identified and distributed to breeding programs in Russia and Kazakhstan (Shamanin et al. 2016). Shuttle breeding program was established between KASIB and CIMMYT to incorporate disease resistance. The crosses are made at CIMMYT utilizing KASIB germplasm, Mexican, US and Canadian germplasm. F₂ populations are subjected to selection under rust pressure and the best F₃ bulks are sent to the region for selection under local conditions. Shuttle breeding contributed substantially to diversifying the genetic basis of spring wheat in the region and resulted in competitive germplasm being tested as new varieties. KASIB conducts bi-annual meetings to summarize its activities and plan for the future.

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proWeizen - The German Wheat Research and Breeding Alliance

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Wheat is one of the most important crops and Germany is an important wheat producer. In Germany, 16 breeding companies are running independent wheat breeding programmes. The German Wheat Research and Breeding Alliance was founded in 2012 by the German wheat breeders to combine the scientific excellence in wheat research and breeding expertise in Germany. As a public-private partnership, the proWeizen alliance acts to foster wheat breeding and research on a national and international level as well as a platform for communication and coordination. The proWeizen platform is equally open to scientists and companies working in wheat breeding and research. Currently, 11 research projects, funded by the German Federal Ministry of Food and Agriculture (BMEL) as well as the German Federal Ministry of Education and Research (BMBF), are run within the proWeizen alliance and focus on breeding for yield increase and stability, better adaptation to environmental stresses and utilization of heterosis. In these projects, German universities and research institutes are working in close collaboration with wheat breeders who are vital partners and plan to implement project results in their future breeding programmes. In addition to support in project management and coordination, proWeizen liaises with wheat researchers and breeders and participates in wheat research and breeding on national and international levels, respectively. proWeizen also helps with mobilizing funding opportunities.



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Investigating traits of some wheat (*Triticum aestivum* L.) cultivars in temperate zone in Iran

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Key message: To investigate of yield formation in some wheat (*Triticum aestivum* L.) cultivars in temperate zone in Iran.

Wheat (*Triticum aestivum* L.) is one of the most important crops in Iran and plays a special role in peoples' nutrition. It is cultivated over a wide range of environments because of its wide adaptation to different conditions. It is a moderately salt-tolerant crop (Tester & Davenport 2003). Wheat yield in Iran is far lower than those recorded in other countries, therefore it is critical to increase wheat yield to meet food demand of its growing population. In Iran adequate amounts of rainfall occurs during fall and winter while rainfall is low during spring from mid-March to mid-May. Such uneven distribution of rainfall during wheat growing season affects wheat growth and yield. So, it is important to use a high performance cultivar to achieve stable and high yield in wheat production. To investigate the physiology of yield formation in some wheat (*Triticum aestivum* L.) cultivars in temperate zone in Iran, an experiment was conducted at the Agricultural Research School. Treatments included six cultivars of wheat. The present experiment was carried out at the Agricultural Research Institute in Iran in 2015. Treatments included 6 cultivars of wheat. The evaluation include morphological traits, yield and yield components. A randomized complete block design was used with three replications. Soil sample in different depths were collected to determine soil type in experiments were performed. The experimental field was a medium high land with sandy loam textured soil. During the grow season 5 time irrigation's the field (before sowing, after sowing, end of tillering, heading and end of flowering). The soil was prepared in September as usually done in commercial production. All other agronomic (cultural) practices were followed during the growing seasons as usually recommended in the surrounding commercial wheat production farms. Various traits were measured. Results (Table 1) showed that there were significant differences among different cultivars in the majority of studied traits. Also result shows that in most traits (leaf area index (LAI), crop growth ratio (CGR), chlorophyll, Pishtaz had minimum CGR between the wheat cultivars, because Pishtaz cultivar could not use the shoot organs to photosynthesis and it maintained the lowest remobilization proses. Also Sivand had minimum CGR but it had highest yield of other wheat cultivars. It could be concluded that there is significant genetic improvement occurred in Sivand cultivar (as a new cultivar) compare to other varieties.

Table 1: Mean comparison of yield and some traits of wheat cultivars

Treatment	LAI	CGR ((g/m ²)/d)	Grain yield (kg/ha)
Bahar	3.13 b	17.15 b	5644.1 a
Parsi	2.33 c	17.32 b	6576.8 ab
Pishtaz	2.09 c	13.82 c	7143.8 ab
Pishgam	1.32 d	16.00 bc	6572.3 ab
Sirvan	1.32 d	17.03 b	4940.2 c
Sivand	1.84 cd	16.69 b	7417.7 a

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P 289 - Topic: Future of Wheat Improvement in Different Parts of the World

Molecular characterization of some Romanian wheat cultivars using functional molecular markers for grain weight

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Key message: Functional markers for several genes associated with grain weight revealed high genetic variability among 26 Romanian wheat cultivars for most analyzed loci, and suggest presence of other important genes.

Food production and food security, influenced by climate changes, soil availability and accessibility, soil degradation, increase of the world population and other factors, lead to new challenges for farmers, breeders and scientist worldwide. Current estimations show that the food production must be raised by 70% from current level to sustain the estimated population of 9.1 billion by 2050. Wheat is one of the most important crops worldwide, directly providing about 50% of human food calories. In order to fulfill the estimated global demand, an annual increase of 1.6-2% in grain yield is required (Patil 2013, Faris 2014). Therefore, higher yield is one of the most important goals in wheat breeding. Higher yields directly relate to disease resistance, grain size and weight, protein content, drought tolerance and other key factors. Wheat yield is a trait controlled by numerous genes with additive and epistatic effects that are highly interactive with the environment. Larger grains are directly related with higher yield but also have favorable effects on seedling vigor and early growth, thereby promoting and stabilizing yielding ability. Grain size is negatively correlated with grain number, mainly due to competition for available assimilates. Large grain size has been an important trait and it is usually measured in plant breeding practice by one thousand grain weight (TGW). TGW, mainly determined by grain width, grain length and grain thickness, but also by grain shape and density, is a complex trait and a more detailed knowledge of its genetic control is useful for breeding programs and breeding efficiency worldwide. In this study we focused on the molecular characterization of 24 Romanian wheat cultivars and 2 breeding lines, released between 1933 and 2015, using functional markers for genes associated with TGW components such as: *TaSUS2-2B* (Jiang et al. 2011), *TaGW2-6A* (Su et al. 2011), *TaGS5-3A* (Ma et al. 2015), *TaTEF-7A* (Zheng et al. 2014), *TaCwi-A1* (Ma et al. 2012), *TaGS-D1* (Zhang et al. 2014), *6-SFT-A2* (Yue et al. 2015). Our results showed that some of the favorable haplotypes were present in Romanian cultivars since 1933. Analyzed genotypes showed genetic variability among all the loci except the *TaCWI-A1*, where all genotypes carried the favorable haplotype. Results suggest the presence of other important genetic factors with positive/negative influence on TGW. Also, we found, for *6-SFT-A2* gene, a new polymorphism in a breeding line F00628G34-1. Future research is needed to establish if this new polymorphism is associated with desirable agronomic traits.

Acknowledgment

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P 291 - Topic: Future of Wheat Improvement in Different Parts of the World

Heat adapted wheat cultivars in Pakistan are associated with high stem water soluble carbohydrates and chlorophyll content

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Phenotyping approaches are being used to develop new wheat plant types adaptive to heat or drought. The present study evaluated agronomic performance of seven different wheat cultivars including Sehar-2006, Faisalabad-2008, AARI-2011, Inqilab-91, Pasban-90, Lasani-2008 and Shafaq-2006 under heat stress in comparison to temperate sown conditions. As well as limited growth analysis, phenotyping was performed for canopy temperature, stem water soluble carbohydrates and stay green traits at anthesis or grain filling stages for their possible association with yield traits. Heat stress reduced tillers number per plant, thousand grain weight and maturity time while % stem water soluble carbohydrates at anthesis increased. However, no difference among biomass, number of grains per spike and chlorophyll contents during grain filling was found between environment types. Under heat stress, cultivars Sehar-2006, Inqilab-91 and Shafaq-2006 expressed high seed yield, harvest and spike indexes compared to temperate sown crop. These cultivars also expressed increase in stem water soluble carbohydrates at anthesis and flag leaf chlorophyll contents at grain filling stages up to 24% and 20% respectively with an earlier maturity of 15-17 days. Cooler canopy temperature during grain filling was also observed for these cultivars under heat stress. High seed yield observed in these cultivars was associated with high chlorophyll contents ($R^2= 0.41$) during grain filling and stem water soluble carbohydrates ($R^2= 0.54$) at anthesis. Association of stem water soluble carbohydrates was positive ($R^2= 0.30$) with chlorophyll contents. In crux, wheat cultivars (Sehar-2006, Inqilab-91 and Shafaq-2006) with earlier maturity, high seed yield, stem water soluble carbohydrates and stay green traits can be promising as heat adaptive sources in physiological breeding for future climate change.



P 293 - Topic: Future of Wheat Improvement in Different Parts of the World

Breeding innovations wheat for resilient cropping systems: genotype by crop management interactions in winter wheat

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Key message: Evaluating breeding innovations of the past 50 years in winter wheat with a gradient of intensities to predict their value and analyse the determinants for implementation under future conditions.

Wheat is not only one of the first domesticated food crops; it is also the basic staple food around the world. Therefore, it is of great importance to assure high yield and quality components of wheat under changing environment and future agronomic practices. The objective of the study is to determine the behavior of the different wheat genotypes under these conditions and determine correlations between allelic variation and environment and cropping systems. For that, 220 winter wheat German varieties from the last fifty years are tested under three different management systems varying in level of intensity of nitrogen supply and chemical plant protection ('low-input', 'semi intensive' and 'intensive') at Campus Klein-Altendorf, the experimental station of Bonn University. Several phenotypic shoot and root parameters are measured and the cultivars have been genotyped using SNP markers. Genome wide association mapping approach (GWAS), is used to identify genomic regions or candidate genes involved in breeding innovations and their interplay with the management system and the environment will be quantified. The concept of the project and the results from the two years of field trails will be presented.



P 295 - Topic: Future of Wheat Improvement in Different Parts of the World

A new opportunity for wheat improvement by exploiting desirable wheat variability derived from the crosses that combine the very best parents from East and West

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Key message: In August 2017, the F₂ seeds of more than 4000 winter wheat crosses obtained from combining gene pools of East and West will be available to be used in wheat breeding programs worldwide

Is wheat yield stagnation a reality or delusion? It seems wheat yield has reached its plateau, and presently used breeding approaches need immediate and innovative improvements. Today, it is estimated that 98.5% of potentially useful wheat genetic variability present in tens of thousands of genotypes are 'hidden' in genebanks, while numerous registered varieties with great breeding potential created worldwide remain quite unknown to broader wheat breeding audience. Also, the changes in breeding practices imposed by the Nagoya Protocol will have a huge impact on how we will proceed to do research and breeding in the future. To overcome such obstacles, we recently developed a concept to produce numerous crosses which will harbor new and desirable genetic variability to be used in breeding programs with intent to further enhance the yield potential. The parents for carefully planned crosses passed long lasting phenotypic evaluation over a range of different years and environments. For majority of the crosses one parent originates from public breeding programs in the Eastern Europe and Eurasia (Russia, Ukraine, Bulgaria, Romania, Hungary, Serbia, Croatia etc.), while the other parent is a newly developed variety released by private wheat breeding companies from Western Europe (Figure 1). We named this concept G4G (Grain for Gain), a platform that will be presented and discussed in detail.



Figure 1: Cross no. 691: Used parents and hybrid grains.



P 297 - Topic: Future of Wheat Improvement in Different Parts of the World

Assessing nitrogen deficiency tolerance of wheat varieties in registration process and breeding programs in France

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Key message: To better assess NUE, new indicators to phenotype nitrogen deficiency tolerance of wheat lines are necessary. We propose new combined indicators including a characterization of N deficiency applied on trials.

Optimizing bread wheat Nitrogen Use Efficiency (NUE) in most Western European countries pursues two objectives: reaching high yield and obtaining enough grain protein content to satisfy market requirements. Recent studies showed that NUE genetic progress has been significant since the 1950s in France. Nevertheless, this progress is not fast enough to reach the increasing challenges of French agriculture. To achieve them, we need new tools to characterize more precisely wheat lines during the breeding programs and registration process. Assessing new indicators to phenotype nitrogen deficiency tolerance is one of the main aims of two parallel research projects. Both projects are based on field trials network testing variety panels against at least two nitrogen fertilizer rates (estimated optimum rate [HN] and optimum minus 80 to 100 kg N.ha⁻¹ [LN]). The N-BW project evaluated varieties included in the French registration process while project BreedWheat evaluated large panels of already registered varieties. The measured variables are simple (grain yield, grain protein concentration). In order to assess the intensity of N deficiency applied on each trial, some additional measurements have been made on control varieties (grain weight, ears number, N uptake at flowering and harvest, soil N content). Three types of indicators have been studied. The first one relies on indicators including several ratios and the residues of the linear relation between [HN] and [LN] yields or grain protein concentrations. All indicators are strongly correlated. The indicator based on linear regression residues has been studied with a GGE-Biplot method allowing to consider the influence of the N deficiency really applied in each trial on the genotype classification. The second family of indicators considered a classical variance analysis of the trial network predicting the studied variable with [genotype × N rate] effects and some trial interactions random effects. The third type of indicators relies on a multi-environment model including [genotype × N uptake at flowering] interaction. The comparisons of indicators led us to think that a combination of them could be the best way to characterize genotypes in breeding program and that a precise characterization of N deficiency intensity is essential. Concerning their utilization in the registration process, the indicators chosen must be simple to calculate and communicate to stakeholders and farmers, the most stable across years and consider the probable antagonism between N deficiency tolerance regarding yield and grain protein concentration.

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Genome-wide association mapping of mineral contents in grains of bread wheat (*Triticum aestivum* L.)

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Key message: Wheat grains contain low amounts of minerals and billions of people affected by nutritional deficiencies. Our goal is developing mineral dense wheat varieties by genetic biofortification.

Wheat is among the most important three crops in the world and major food to most of the world's population. Wheat grains contain low amounts of minerals and there are around three billion people throughout the world affected by nutritional deficiencies especially of iron and zinc. Developing mineral dense wheat varieties by genetic biofortification is considered a long term remedy for mineral deficiencies in human nutrition. The work aims to explore genetic variation of mineral concentrations in the wheat grain using a collection of 353 wheat genotypes consisting of European winter wheat varieties. Moreover, our target is to identify quantitative trait loci (QTL) associated with these traits by using Genome Wide Association Study (GWAS) in order to identify the candidate genes of Fe and Zn content, and some other mineral elements, such as Ca, K, Mg, Mn, P and S. To this end, GWAS was performed using SNP (90k ILLUMINA and 35k Affymetrix chips) and SSR markers with the application of mixed linear models for two field experiments (2015 and 2016). Preliminary results have confirmed that there is genetic variation in mineral content between the genotypes which is controlled by a number of associated loci with positive and negative effects. The output showed some shared associations between 2015, 2016 and BLUEs. Further validation of these associations is required to reveal the candidate gene(s) of targeted traits.



P 301 - Topic: Future of Wheat Improvement in Different Parts of the World

Low genetic polymorphism and wide range of phenotypes in winter common wheat breeding for bread-making quality in Ukraine, understanding of mechanism and future challenge

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Key message: Three main genetic systems (Glu, Gli, Ha) which possess strong impact on bread making in wheat demonstrated low genetic variability among Ukrainian winter common wheat varieties with different end-use quality

It is well known that the wide range of genetic polymorphism by particular characteristics in breeding material is the key basis for successful selection for crop improvement by this trait. The main characteristics of common wheat is the bread-making quality. Its investigation since the 1960s and up to date allowed to assume that there are three main genetic systems in wheat: gliadins (*Gli*), glutenins (*Glu*) and grain hardness (*Ha*), which alleles predominantly can predict wheat end-use quality. Since the mid of the 20th century the main aim of winter common wheat breeding programs in Ukraine was to develop varieties with strong and extra strong quality characteristics without decreasing in yield. At most of breeding institutions MAS was applied for the selection of accessions with favorable gliadin and glutenin alleles. Such approaches increased the quality of registered varieties but in State Register there are still varieties with different bread-making characteristics i.e. fillers. The studies of 129 winter common wheat varieties planted in Ukraine in 2007-2009 by four wheat quality traits (grain protein content(GPC), gluten content(GC), bread loaf volume(BLV) and dough strength(DhS)) and allelic variations of *Gli*, *Glu* and *Ha* loci demonstrated the low level of polymorphism in three genetic systems: *Ha*(2 alleles), *Glu-A1*(a, b), *Glu-B1*(b, c, al), *Glu-D1*(d) and the same tendency observed in *Gli* loci, despite the wide range of variability of investigated varieties by GPC, GC, BLV and DhS. The allele variations of *Gli*, *Glu* and *Ha* loci did not explain high and low end-use characteristics of investigated varieties with exception of *Glu-B1al* allele (positive influence on DhS) and several *Gli-A1*(DhS) and *Gli-B1* (BLV) alleles. Application of induced mutagenesis in Ukrainian breeding programs since the end of 60th and up to date did not produce the new allele variation in those three main genetic systems which can be reviled in elite breeding lines and finally in realized varieties, which suggested about conservatism of those particular loci. A number of resent investigations demonstrated that a lot of QTL from different chromosomes of wheat which genetic nature still is unclear as well made their small input in final expression of end-use quality, therefore selected varieties are very promising sources for feather investigations of genetic and epigenetic mechanisms of end-use quality formation in wheat due to their high variability by investigated bread-making traits and low polymorphism in *Gli*, *Glu* and *Ha* loci.



P 303 - Topic: Future of Wheat Improvement in Different Parts of the World

Favorable alleles at genetic loci underlying the thousand-kernel weight of common wheat (*Triticum aestivum* L.) in the Huang-huai wheat-growing region of China

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Key words: Common wheat, grain yield, marker-trait analysis, superior alleles, thousand-kernel weight

Improving grain yield is a priority for common wheat breeding. Here, we report favorable alleles at four different thousand-kernel weight (TKW)-related simple sequence repeat (SSR) loci (*Xgwm259*, *Xcfe172*, *Xbarc186*, and *Xbarc322*) in a panel of 82 wheat genotypes from the Huang-huai main wheat-growing area (Table 1). Only two allelic variations were detected at each locus. Apart from the favored allele, *cfe172-122 bp*, with a frequency of 84.8% (Table 2), the frequency distribution of the allelic variations was nearly even at 50%, suggesting that the favorable alleles at these loci have not been strongly selected for and fixed. Moreover, the favored alleles have additive genetic effects. The highest mean TKW of 52 g corresponded to the presence of four favored alleles at 69 critical marker loci, whereas varieties with 0-3 favored alleles exhibited a lower mean TKW, ranging from 45.6 to 50 g (Table 3). More importantly, no modern wheat cultivar contained favored alleles at all marker loci. Therefore, there is still considerable genetic potential for yield improvement in wheat by identifying favorable alleles and pyramiding them into newly released cultivars. Our results allow for a better understanding of the complex wheat genome. The identified favored alleles will be useful in breeding high-yield wheat varieties in the future.



Table 1: Thousand-kernel weight (TKW, g) of 82 wheat varieties from the Huang-huai wheat-growing region of China.

#	Variety	TKW	#	Variety	TKW	#	Variety	TKW	#	Variety	TKW
1	Baiyingdong2	40.7	22	Jifeng703	51.2	43	Nongda135	49	64	Shixin828	46.2
2	Baofeng	53.7	23	Jing9428	54.7	44	Nongda179	35.5	65	Shiyou17	50.8
3	Baomai9	53	24	Jingdong10	54.5	45	Nongda211	49.7	66	Taima1	50.7
4	CA9722	45.5	25	Jingdong11	46	46	Nongda212	51.7	67	Tainong18	48.8
5	Cangmai028	45.5	26	Jingdong20	53.7	47	Nongda3488	50	68	Taishan9818	57
6	Cangmai6002	44.5	27	Jingdong24	50.3	48	Nongda3214	49	69	Tangmai8	50
7	Duofeng2000	49.2	28	Jingken49	45.8	49	Nongda3251	56.2	70	Weimai7	46.7
8	Gaoyou2018	49.7	29	Jingmai9158	49.3	50	Nongda3432	45.8	71	Wennong5	48.5
9	Hanyou3475	45.8	30	Jingshengmai1	59.5	51	Nongdaduoxil1	46.8	72	Xiaoyan81	57.5
10	Heimal	51.3	31	Jining12	46	52	Shannong12	49.5	73	Yan2415	49.3
11	Heng 6599	47.2	32	Jining13	48.8	53	Shannong14	51.5	74	Yannong19	49.3
12	Heng 7228	46.2	33	Jining16	55.8	54	Shannong15	52.8	75	Yannong24	47
13	Heng0628	52.2	34	Jinmai47	53.1	55	Shannong18	52.2	76	Zhongmai12	50.7
14	Heng4399	51.5	35	Jinmai54	47.7	56	Shannong8355	62.8	77	Zhongmai24	46.8
15	Heng9526	53.3	36	Jinqiang5	52.3	57	Shi4185	42.1	78	Zhongmai533	55.3
16	Henong4198	49.8	37	Jinyin159	47.2	58	Shijiazhuang10	49.2	79	Zhongyou206	53.3
17	Henong58-3	42	38	Laizhou950221	47.5	59	Shimai14	47.7	80	Zhongyou335	47.8
18	Henong822	44.3	39	Liamai16	54	60	Shimai16	46.7	81	Zhongyou9507	57.7
19	Henong827	44.7	40	Lunxuan061	51.5	61	Shimai18	40.8	82	Zhouyuan9369	50.5
20	Ji6358	48.7	41	Luyuan301	52.3	62	Shixin539	48.8			
21	Jidong3097	53.7	42	NC2	42.3	63	Shixin733	54.7			

Table 2: Genetic effects of four simple sequence repeat (SSR) loci on mean thousand-kernel weight (MTKW) in the Huang-huai wheat-growing area (PIC, polymorphism index content).

	Locus							
	<i>barc189</i>		<i>barc322</i>		<i>cfe172</i>		<i>gwm259</i>	
Allele (bp)	199	211	228	253	166	122	101	103
MTKW (g)	49.3	49.9	51.5	48.4	47.7	50.0	49.5	49.7
No. of alleles	35	46	18	43	12	67	32	42
Frequency (%)	43.2	56.8	29.5	70.5	15.2	84.8	43.2	56.8
PIC ^b	0.4908		0.4160		0.2577		0.4909	

Table 3: Effects of allele accumulation on mean thousand-kernel weight (MTKW).

No. of alleles	No. of genotypes	MTKW (g)
0	2	45.6
1	18	48.6
2	36	49.1
3	21	50.0
4	5	52.0



P 305 - Topic: Future of Wheat Improvement in Different Parts of the World

Introgression of three QTL for pre-harvest sprouting tolerance in a bread wheat line

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Key message: Each possible combination of three QTL for pre-harvest sprouting tolerance was introgressed in a wheat line. One of these QTL is sufficient to improve the tolerance clearly.

Pre-harvest sprouting (PHS) is a major problem in cereal production, especially in regions with relatively high rainfall before harvest. In Switzerland, a variable proportion of the harvest is downgraded from baking purpose to feed purpose almost every year. In the worst years, such as 2007 or 2014, more than 20% of Swiss bread wheat production was reclassified as feed wheat with a loss of around 13.50 CHF/dt. Breeding for pre-harvest tolerance is difficult. Screening for seed dormancy or low alpha-amylase activity, using the falling number method, is time-consuming. Nevertheless, some progress has recently been achieved in improving dormancy, especially in white wheat. The use of marker-assisted selection could improve breeding efficiency if the markers and genes or QTL are efficient. Some QTL involved in PHS tolerance or grain dormancy have been published. A QTL for dormancy (*QPhs.ocs-3A.1*) tracing back to the hard red wheat cultivar Zen (Mori et al. 2005) and two other QTL on chromosome 4AL and 5BL originating from the white-grained cultivar Aus1408 (Tan et al. 2006) were introgressed in a sprouting susceptible hard red wheat line (CH-111.14812) through five back-crosses (5BC). Twenty-two 5BC lines representing all possible combinations (zero, one, two or three of these QTL) were compared with the original line regarding their PHS tolerance. Scoring PHS tolerance was based on counts of sprouted grains on wet spikes, as described by Kumar et al. (2010). The lowest PHS was observed on BC lines possessing the QTL on 3AS, alone or in combination with other QTL. The second QTL on 4AL had a smaller impact than the 3AS QTL. No clear impact was observed with the QTL on 5BL. Combining the 3AS QTL with one or two other QTL did not improve sprouting tolerance. Further tests must be done to confirm these first results. Testing these QTL on other genetic backgrounds will give more confidence regarding their usefulness. The 5BC lines with the best QTL combinations will be used for future crosses in the Swiss breeding program.

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P 307 - Topic: Future of Wheat Improvement in Different Parts of the World

Enhancing and protecting wheat productivity in Pakistan through national and international collaboration

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Key message: Collaborative efforts to characterize wheat rusts pathogen and wheat germplasm for rust genes diversity and deploy those resistant genes through developing and disseminating rust resistant high yielding varieties for the benefits of farmers

Pakistan Wheat Productivity Enhancement Project (<http://wpepforpakistan.org>) which is known as “WPEP” in Pakistan is an outcome-driven science collaboration involving USDA, CIMMYT, ICARDA, and 11 Pakistani scientific organizations working in all provinces of Pakistan. WPEP’s overarching goals are to enhance and protect the productivity of wheat in Pakistan by increasing the capacity of Pakistani scientific institutions to minimize adverse effects of wheat rusts (including Ug99). The project strengthened Pakistan’s own wheat rust surveillance, conducted pre-breeding to enhance the diversity/utility of rust resistant wheat breeding parents, accelerated breeding to develop/test rust-resistant candidate varieties, conducted seed multiplication/distribution of improved varieties and enhance the capacity of national scientists in wheat research. The project enhanced Pakistan’s own capacity to collect, store, and characterize living rust culture collections in support of wheat rust resistance breeding, provided breeders with information on the nature of rust resistance genes in Pakistan wheat germplasm, modernized/mechanized breeding programs for efficient breeding, accelerated seed multiplication, and provided tools for enhanced farmer adoption of improved varieties. Results on the generation of more knowledge of host plant resistance genes in Pakistan germplasm which enabled breeders to make informed decisions leading to better varieties faster, pattern and distributions of rusts pathogens, release of improved varieties including resistant to Ug99, and the delivery of those varieties to farmers as a result of very effective collaboration which impacted farmers will be presented in the conference.



P 309 - Topic: Future of Wheat Improvement in Different Parts of the World

Field-based phenotyping for wheat diseases within a new multiple diseases platform in Uruguay: promoting germplasm sharing to increase resistance diversity

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Key message: Data from multiple traits obtained in this platform, complemented by molecular selection technologies, would increase the prediction value of phenotype/genotype data for new germplasm emerging from the partners breeding pipelines.

Breeding for durable disease resistance in wheat is a challenging task since it is usually quantitatively inherited, thus relying on the accumulation of QTL involved in resistance. This goal could be achieved by the use of a broad spectrum of resistance sources. Moreover, breeders usually need to test their materials in different abiotic and biotic stress conditions to know their adaptability to diverse environments. In order to improve the quality and speed of wheat breeding, CGIAR-WHEAT Initiative has promoted the establishment of field-based Precision Wheat Phenotyping Platforms (PWPP) accessible to public and private breeding partners. In 2015, a partnership between CGIAR and INIA launched the PWPP-Uruguay to test genotypes for multiple diseases: Fusarium head blight (FHB), Septoria tritici blotch (STB) and leaf rust (LR). These diseases are phenotyped each year in separate field trials. Trials are artificially inoculated with pathogen races identified as representatives of the pathogen regional population. Wheat material is sowed in plots; with susceptible checks every 50 entries. Disease severity and other variables characterizing the disease development are measured in internationally standard scales at dates when the expression of plant resistance is optimal. Disease variables are measured at more than one date, to determine the response of the material to the disease at different moments of the epidemic development. Plant height, heading date, growth stage at disease scoring dates and agronomic score are also measured. In 2016, 1544 genotypes were screened for the three diseases. These materials had diversified origins (ten different institutions, public and private, from six countries) and were of different types: from recent commercialized cultivars to ancient ones, advanced lines, International CIMMYT nurseries, mapping populations or association mapping panels. Disease variables were measured at three dates for all materials, except for FHB trial, with two measurements dates. Genotypes could be selected because of their high level of resistance for each set of material (from each institution) in the FHB, STB and LR trial. A 9% to 25% range of genotypes were found highly resistant when selected only from one disease. From these resistant genotypes, up to 5% were resistant against two diseases and near 2% were resistant to the three diseases screened. Data from multiple traits obtained in this platform, complemented by molecular selection technologies, would increase the precision and prediction value of phenotype/genotype data for new germplasm emerging from the partners breeding pipelines.



P 311 - Topic: Future of Wheat Improvement in Different Parts of the World

Yield capacity increasing in winter wheat by improvement of spike-stem-tillers (SST) complex

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Key message: The role of spike productivity, stem strength and tillering ability in combination with adaptation, stress tolerance and acceptable grain quality for wheat breeding success are discussed.

Wheat yield depends from many factors, but most important are tiller numbers bearing ear, spike grain productivity and stem strength. Standard Bulgarian wheat cultivars possessed high spike productivity and good grain quality, normal tiller numbers (1.5) and stem height 95-100 cm. These wheat varieties are well adapted to the semi-arid conditions in BG. They develop 550-600 spikes per square-meter. In case of drought the secondary additional tillers die without spike, this is a reaction of adaptation. Average yield of these cultivars is 5.5-7.5 t/ha with good grain shape and quality. But now the modern wheat cultivars should have low grain quality and yield potential 10 t/ha and more. This is the aim of our breeding strategy during last 12 years. This is wheat nonsense, but is the reality.

Spike capacity. Most useful genetic material for spike architecture is those from CIMMYT spring wheat type. The new winter wheat lines possessed their productivity (65-85 grains/spike) grain uniformity and weight, 38-42 g/1000 kernels and acceptable quality. The grain weight of the main spike is 2.5-3.0 g.

Tillers with ear. The CIMMYT lines are also useful to construct the new plant with ability to produce uniformed tillers bearing spike. Their grain weight is less than those of the main spike and they have the same height and time of development. The canopy is almost uniform.

Stem strength. The heavy spike needs strong stem with no lodging ability. The suitable genetic donors are some Italian and French varieties. Italian cultivars possessed such a stem, combined with normal earliness and grain filling rate and capacity.

The results. The new genetic combinations of spike productivity, stem strength and tillers ability (SST) with adaptation, stress tolerance and acceptable grain quality of the new cultivars is a basis to produce wheat varieties with high yield potential, stability, stress tolerance and good quality, typical for previous Bulgarian wheat breeding.



P 313 - Topic: Genetics and Genomics of Resource Efficiency; Quality and Composition

Genetic impact on protein polymerization in various applications

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Key message: Protein polymerization in wheat based applications is genetically determined by specific protein composition and plant development rhythm, making these genetic characters an asset in breeding to fine-tune end-use properties.

The ability of the wheat grain storage proteins to polymerize during processing contributes with the unique properties of wheat. Polymerization of the wheat grain proteins determines the end-use quality of the wheat grain. Differences in polymerization behavior affects the end-use properties in bread, pasta, and in materials such as films, sheets, foams etc produced from the wheat (Johansson et al. 2013). Polymerization behavior of the wheat grain proteins is determined both by genetic and environmental conditions, both being of equal importance. The genetic factors playing a significant role for the polymerization behavior of the wheat grain proteins are the specific protein composition i.e. the high molecular weight-glutenin subunits (HMW-GS) and the plant development rhythm. The HMW-GS composition shows a relatively simple inheritance, the proteins are encoded on genes present on the Glu-1 loci on the long arm of the chromosome group 1. The genetic back-ground of the plant development rhythm is more complex with a range of genes involved, indicating the importance of QTL for this trait. Among the HMW-GS, 5+10 is since long known to contribute more strength to the products as compared to 2+12 due to its higher number of cysteine residues. Of relevance for such a cross-linking is, however, a pre-treatment and treatment during the handling preventing unfavorable structures to be formed early during processing (Rasheed et al. 2016).

Research is currently in progress to increase the understanding of how different types of “green modifiers” (genetic material in combination with environmental treatments, pre-treatment and processing) can be applied to fine-tune the polymerization behavior of the gluten proteins at processing for various applications. Furthermore, we are modeling structures of various types of gluten protein in order to understand their individual polymerization behavior. The modeled protein structures are thereafter compared with structures of the proteins produced in bacterial systems and thereafter purified. An increased understanding of polymerization behavior of specific gluten proteins as well as how various proteins interact with each other while forming the polymers will lead to opportunities to fine-tune wheat quality for various end-uses through breeding of genotypes with suitable combinations of proteins.

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Short history of International Wheat Genetics Symposia (IWGS)

- 1st IWGS August 11-15, 1958 in Winnipeg, Canada
- 2nd IWGS August 19-24, 1963, Lund, Sweden
- 3rd IWGS August 5-9, 1968, Canberra, Australia
- 4th IWGS August 6-11, 1973, Columbia, Missouri, USA
- 5th IWGS February 23-28, 1978, New-Delhi, India
- 6th IWGS November 28- December 3, 1983, Kyoto, Japan
- 7th IWGS July 13-19, 1988, Cambridge, England
- 8th IWGS July 20-25, 1993, Beijing, China
- 9th IWGS August 2-7, 1998, Saskatoon, Canada
- 10th IWGS September 1-6, 2003, Paestum, Italy
- 11th IWGS August 24-29, 2008, Brisbane, Australia
- 12th IWGS September 8-14, 2013, Yokohama, Japan

FIRST INTERNATIONAL WHEAT GENETICS SYMPOSIUM

UNIVERSITY OF MANITOBA
Winnipeg - Manitoba - Canada
AUGUST 11th - 15th, 1958



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International Organizing Committee, Reviewers, and Supporters

Yasunari Ogihara

Kihara Institute for Biological Research, Yokohama City University, Japan
Role: Head of IOC

Yasunari Ogihara teaches Plant Genomics at the Department of Life and Environmental Sciences, Yokohama City University, Japan. He is former head of the Kihara Institute for Biological Research, Yokohama City University. He would like to contribute production of useful crops for sustainable cultivation with the novel biotechnology. His interest is focused on the functional genomics of polyploid wheat. He contributed to perform the nucleotide sequencing of chloroplast, mitochondria and nuclear genomes of Chinese Spring wheat. He carried out the research work on comprehensive gene expression patterns of common wheat in response to developmental and/or environmental conditions. He applies genomics to improve grain quality and omit allergens of wheat flour. He also aims to improve stresses-resistance of common wheat using genetic and genomic resources. His activities are available at: <http://pgenome.sci.yokohama-cu.ac.jp/>



Ahmed Amri

International Center for Agricultural Research in the Dry Areas (ICARDA,
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Role: IOC and Reviewer

Holds a PhD Genetics and Plant Breeding from Kansas State University (1989); worked at INRA-Morocco for 20 years as cereal breeder (release of 17 barley varieties, 5 triticale and 7 bread wheat and durum varieties resistant to Hessian fly). Ahmed Amri works at ICARDA since 1999 as regional coordinator for a GEF West Asia Dryland Agrobiodiversity project (1999-2005), ICARDA Regional Coordinator for West Asia (2001-2008), Coordinator Iran-ICARDA office (2005-2009) and since 2008, appointed as the Head of Genetic Resources Unit and Deputy Director of the Biodiversity and Integrated Gene Management Program. He has a total of 132 publications including 72 in refereed journals and advised 27 PhD and MSc. students. His expertise is in pre-breeding, breeding of cereals, *ex situ* conservation of plant genetic resources and on approaches for promoting the *in situ*/on-farm conservation of dryland agrobiodiversity.



Alexey Morgounov

International Maize and Wheat Improvement Center (CIMMYT), Winter Wheat Program in Turkey, Head of IWWIP (www.iwwip.org)
Role: Reviewer

Several achievements were accomplished in the last 10 years: 1) More than 70 IWWIP originated varieties have been released in the region and occupy more than 2.5 mln ha. 2) National wheat landraces inventory completed in Turkey with collections covering 60 provinces and thousands of lines characterized and evaluated. 3) Winter wheat germplasm resistant to Ug99, stripe rust, common bunt and soil-borne pathogens was identified and distributed through IWWIP international nurseries. 4) Winter hexaploid synthetics were developed, characterized and included into breeding.





Andreas J. Obrecht

Austrian Agency for International Cooperation in Education and Research (OeAD GmbH), Austria

Role: Facilitator for Public Evening Discussion

Andreas Obrecht is a social and cultural anthropologist, writer and sociologist; habilitation in sociology with an emphasis on developmental sociology (1997); head of the Interdisciplinary Research Institute for Development Cooperation (IEZ), Johannes Kepler University Linz (1998-2009); visiting professor for the thematic focus Sub-Saharan Africa and South Pacific at the Department for Contemporary History, Karl Franzens University Graz (1998-2013); since 2004 host for science and culture in the ORF-radio broadcast „Von Tag zu Tag“; since 2009 head of the Commission for Development Research (www.kef-research.at) at the Austrian Agency for International Cooperation in Education and Research (OeAD GmbH) and head of the Austrian Partnership Programme in Higher Education and Research for Development (www.appear.at)

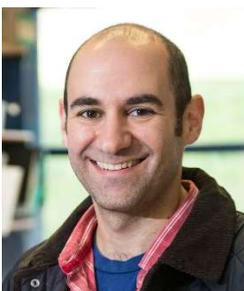


Bernd Friebe

Wheat Genetics Resources Center at Kansas State University, USA

Role: Reviewer

I received my Ph.D. in 1977 from the Free University of Berlin and after postdocs at the Technical University in Munich-Weihenstephan and the University of Manitoba, Winnipeg, I joined the Wheat Genetics Resources Center at Kansas State University in 1991. My research focusses on the molecular cytogenetics and evolution of wheat and its wild relatives with special emphasis on the transfer and characterization of agronomically useful alien genes from distantly related wild species into bread wheat using directed chromosome engineering. I am also involved in the management of wheat genetic resources, germplasms, and genetic stocks.



Cristobal Uauy

John Innes Centre, Norwich, UK.

Role: IOC and Reviewer

Cristobal Uauy is a Project Leader in wheat genetics and genomics at the John Innes Centre. He studied Agronomy in Chile and holds a PhD in Genetics from the University of California, Davis. His work was recognized as the most outstanding PhD dissertation in Biological and Life Sciences in the US and Canada (2007). His programme focuses on the identification of genes involved in wheat productivity traits, including grain size/yield, and the development of tools and resources to enhance scientific discovery. Uauy is using molecular genetic approaches to identify these genes and enhance the pipeline to translate new knowledge at the molecular level into improved wheat varieties for growers, industry and consumers. Cristobal's work has been recognized through the Bayer Foundation Early Excellence in Science Award (2012) and the Society of Experimental Biology President's Medal (2014).



Elena Salina

Laboratory of Plant Molecular Genetics and Cytogenetics at the Institute of Cytology and Genetics (IC&G) in Novosibirsk, Russia.

Role: IOC and Reviewer

Head of the laboratory, joined the Institute in August 1981 after graduation from Moscow University. Her main scientific interest was connected with reorganization wheat genome during remote hybridization, amphi-ploidization and evolution. The last years one of the research directions of her lab was focused on identification genes and alien translocations responsible for wheat agricultural traits such as resistance to disease, heading time, spike morphology. This information is then used for improving methods and strategies for harnessing allelic diversity of wheat relatives and hybrids and using its in wheat breeding. Elena Salina is a team leader in IWGSC since 2007 and responsible for physical mapping and sequencing of 5BS chromosome.



Eva Stoeger

University of Natural Resources and Life Sciences Vienna, Austria

Role: Speaker at the Public Evening Discussion

Eva Stoeger is Professor of Molecular Plant Physiology at the University of Natural Resources and Life Sciences in Vienna, Austria. Eva Stoeger holds a PhD from from the University of Vienna. Further stages in her career brought her to the University of Florida (USA), the John Innes Centre, Norwich (UK), and at the Aachen Technical University (Germany). Her main research interests are in the areas of cereal biotechnology, endomembrane dynamics and the production of high-value recombinant proteins in seed crops. <http://www.boku.ac.at/en/personen/person/EDCFD1D522BED587/>



Franziska Löschenberger

Saatzucht-Donau, Probstdorf, Austria

Role: Speaker at the Public Evening Discussion

Franziska graduated at the University of Natural Resources and Life Sciences Vienna, she did her PhD studies in the field of doubled haploids in wheat. She is working for Saatzucht-Donau, a medium sized Austrian plant breeding company as wheat breeder. She has attained 175 cultivar registrations of 100 bread wheat and durum wheat cultivars in 18 countries on three continents. She has till now developed 50 winter wheat cultivars from crossing to cultivar registration. Her breeding program covers wheat cultivars for conventional farming and for organic farming, with a particular emphasis on stable performance, resilience and superior end use quality. <http://www.saatzucht-donau.at/>





George Fedak

Ottawa Research and Development Centre, Agriculture and Agri-food Canada, Ottawa, Ontario, Canada

Role: Reviewer

George Fedak is a principal research scientist at the Ottawa Research and Development Centre of Agriculture and Agri-food Canada in Ottawa, Canada. His main research interests focus on cytogenetic studies of interspecific and intergenetic hybrids in wheat for progress of resolving genomic relationships and transfer of disease resistance from the alien species to wheat. Thus far QTL for resistance to Fusarium head blight from five alien species have been introgressed into wheat and mapped. These genes are being pyramided and used to augment FHB resistance in spring wheat breeding programs. For resistance to Ug99, pyramids of up to four known resistance genes have been built up by mean of DH technology and markers. The pyramids have been crossed and backcrossed into spring and winter varieties and widely distributed to wheat improvement programs. The screening of cytogenetic stocks and alien species for Ug99 resistance has revealed numerous sources of resistance. Procedures are underway to introgress these into wheat.



Hans-Joachim Braun

CIMMYT – Centro Internacional de Mejoramiento de Maíz y Trigo, Mexico

Role: speaker at the public evening discussion

He serves as the Director of CIMMYT's Global Wheat Program (GWP) since 2006 and Director of the CGIAR Research Program on Wheat (WHEAT) since 2015. Based in Mexico, Braun leads and manages 40 internationally recruited scientists as part of the GWP. His achievements include contributing to the development and release of 44 winter wheat varieties grown on 2 million hectares in Central and West Asia. Prior to his current position, Dr. Braun led the Turkey International Winter Wheat Improvement Program. During his 20 years in Turkey he was also involved in identifying Zn deficiency and soil borne diseases as production constraints for wheat production in rainfed areas of Central Anatolia and other regions in West Asia. In 2003 he received the Chinese Friendship Award for his contributions to wheat improvement in Gansu Province. Braun currently holds positions as a board member for the Wheat Initiative and the International Wheat Yield Partnership.



H el ene Lucas

Institut National de la Recherche Agronomique, France

Role: IOC and Reviewer

After a scientific career dedicated to the analysis of plant genomes organisation and evolution, with a special focus on retrotransposons, H el ene Lucas took the role of Head the Genetics and Plant Breeding Division of INRA (2005-2011). As its International Scientific Coordinator, she established successfully the G20-endorsed Wheat Initiative from 2011 to 2016, while chairing the Managing Board of the French "Plant Biotech" Public-Private Partnership Scientific Group. She is now Scientific Advisor to the President and CEO of INRA.



Helmut Haberl

Institute of Social Ecology, Alpen Adria Universitaet Klagenfurt/Graz/Wien,
Vienna Austria
Role: Keynote Opening Speaker



Helmut Haberl studied biology, ecology and mathematics at the Universities of Vienna and Salzburg. PhD 1995, Habilitation 2001, both University of Vienna. He currently serves as director of the Institute of Social Ecology at the Alpen-Adria Universitaet Klagenfurt, Wien, Graz in Vienna. His mission is to contribute to sustainability through inter- and transdisciplinary research on society-nature interaction, with a focus on society's use of biophysical resources such as raw materials, energy, and land. He has pioneered socioecological sustainability indicators such as the human appropriation of net primary production (HANPP) as well as indicators for the energetic metabolism of societies and contributed to the emergence of the research field of Long-Term Socio-Ecological Research (LTSER). He served on the SSC of the Global Land Project, the Scientific Committee of the European Environment Agency and in contributed to the Global Energy Assessment, IPCC's Fifth Assessment Report (AR5, WGIII) and the Austrian Panel on Climate Change's (APCC) first Austrian Climate Assessment Report 2014. Further information is available at: <http://www.uni-klu.ac.at/socec/eng/inhalt/885.htm>

Hermann Buerstmayr

University of Natural Resources and Life Sciences Vienna, Austria
Role: Head of LOC and Reviewer



He teaches Plant Breeding at the University of Natural Resources and Life Sciences Vienna. His mission is to contribute to sustainable improvement of crop production through genetics and genomics research and capacity building. His main research interests focus on disease resistance in crop plants, particularly on wheat. He is recognized as a leading expert in Fusarium head blight resistance research. He aims to combine germplasm improvement, with classical and molecular genetics and genomics in order gain novel knowledge. At the same time improved germplasm, sometimes from exotic sources and wild relatives, is generated and made available for practical breeding. Further information is available at: <http://www.ifa-tulln.boku.ac.at/en/institut-fuer-biotechnologie-in-der-pflanzenproduktion/>

Mark E. Sorrells

Cornell University, Ithaca, USA
Role: IOC and Reviewer



Mark E. Sorrells joined the faculty at Cornell University in the Department of Plant Breeding & Biometry in 1978. Currently, he is a Professor in the Department of Plant Breeding & Genetics. The primary focus of Dr. Sorrells' research program is on breeding methodologies incorporating new technologies such as high throughput phenotyping and genomic selection. His breeding program has released 18 small grains varieties. He has published more than 260 papers in peer-reviewed journals and served as major advisor to 37 PhD students and 15 M.S. graduate students. Further information is available at: <http://smallgrains.cals.cornell.edu>



Parveen Chhuneja

School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, India

Role: IOC and Reviewer

Dr. Parveen Chhuneja has been working on wheat hybridization for more than 20 years and presently is the Director, School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana. She has identified and transferred a number of novel alleles and genes from progenitor and non-progenitor Aegilops and Triticum species to cultivated wheat. Her group has transferred and mapped a number of alien genes for disease resistance, productivity and quality traits which are being used in the wheat varietal development programme for diversifying the breeders' germplasm base. Dr Chhuneja is responsible for maintaining and utilising the largest collection of wild species of wheat among all the National Institutes and SAUs in India. She has supervised 17 post-graduate students. Dr Parveen Chhuneja has worked as visiting scientist at Institute of Plant Molecular Biology, University of Zurich, Switzerland and John Innes Centre, UK. Dr Chhuneja has also been awarded Merit Certificate and Plaque by her University in recognition of her outstanding research contributions. She has been awarded Dr Gurdev Singh Khush Distinguished Professor award by her institution for 2016-19.



Peter Langridge

Wheat initiative and University of Adelaide, Australia

Role: IOC and Reviewer

Peter is Emeritus Professor at the University of Adelaide, Australia. Peter established the Australian Centre for Plant Functional Genomics (ACPGF) and was appointed Chief Executive Officer in 2003. In 2014 Peter resigned as CEO of ACPGF to focus on his role on the boards of several research organisations in Europe, North America and in developing countries. Peter's interests have focused on the role of modern technologies in crop improvement with a particular focus on the importance of science and education in helping to improve food security. Further information is available at: <http://www.adelaide.edu.au/directory/peter.langridge>



Peter Sharp

University of Sydney, Australia

Role: Reviewer

Peter Sharp teaches in the areas of genetics, plant breeding and biotechnology in the School of Life and Environmental Sciences at the University of Sydney. He is Head of the Plant Science Cluster in the School, and is Director of the university's Plant Breeding Institute, which is at two locations; Cobbitty near Sydney, and Narrabri, in the NW cereal growing area of NSW. His research in wheat is on molecular markers, mapping of grain quality and agronomic traits, and diversity generation –TILLING and use of wild relatives. Outputs from his research (linkages and germplasm) are being used by commercial breeders. Further information is available at: http://sydney.edu.au/agriculture/academic_staff/peter.sharp.php



Ravi Prakash Singh

Global Wheat Program, CIMMYT – Centro Internacional de Mejoramiento de Maíz y Trigo
Role: IOC and Reviewer



Dr. Ravi P. Singh has made highly significant contributions in the generation and application of science that has enhanced food production and security in numerous developing countries during his 33 years' scientific career at CIMMYT where he is Distinguished Scientist and leads the Wheat Improvement and Rust Research. He is also Adjunct Professor in Cornell and Kansas State Universities. Dr. Singh's research on rust epidemiology and durable resistance are widely recognized and he has contributed and led to the development of over 400 more productive, disease resistant, stress tolerant and nutritious wheat varieties released and widely grown by National program partners in many countries of Asia, Africa and Latin America. He has authored or coauthored 234 research and review articles in peer reviewed journals. Dr. Singh is also recipient of various awards and recognitions including Outstanding CGIAR Scientist Award, Crop Science Research Award by CSSA, E.C. Stakman Award by Univ. of Minnesota, and Friendship Award by the China State Council.

Ruth Wanyera

Kenya Agricultural and Livestock Research Organization Njoro, Kenya
Role: IOC and Reviewer



Ruth a Principal Research Scientist at Kenya Agricultural and Livestock Research Organization Njoro. She is head of Plant Pathology and National Wheat Coordinator. Ruth has extensive research experience in wheat rust diseases, including phenotyping, surveys and surveillance. She has contributed to the release of wheat varieties with adult plant resistance to the wheat stem rust race Ug99 and its variants. She also has good background and research knowledge on sunflower, soybean and canola diseases and seed health. She has coordinated research projects funded by International, regional and national bodies. Has won a number of awards including the 2015 Borlaug Global Rust Initiative (BGRI) Gene Stewardship Award, Sydney, Australia. A mentor of a number of university students (Msc and PhD). She aspires to mentor young women scientists, contribute to improving food security in her country by sharing knowledge, experience and learning from other scientists what they have in terms of current innovations and technologies.

Silvia Germán

Instituto Nacional de Investigación Agropecuaria, Uruguay (www.inia.uy).
Role: IOC and Reviewer



Principal Researcher at La Estanzuela Experimental Station, with main focus on wheat breeding for disease resistance, genetics of resistance and rust pathology. Works on the development of bread wheat germplasm resistant to multiple diseases, study of the basis of resistance to rusts and Fusarium Head Blight, and variation and evolution of wheat rusts.



Simon Krattinger

University of Zurich, Switzerland

Role: Reviewer

Simon Krattinger's main research interests focus on the molecular understanding of fungal disease resistance in cereals. One aim of the group consists in the development of novel approaches to rapidly isolate agriculturally important genes. In particular, the group works towards a better understanding of broad-spectrum and durable disease resistance.



Susanne Weber

University of Natural Resources and Life Sciences Vienna, Austria

Role: Symposium Secretary

Susanne holds a master degree from the University of Natural Resources and Life Sciences Vienna. She joined the Department of Agrobiotechnology Tulln only one year ago. She is the master mind behind the organization and practical implementation of IWGS 2017.



Thomas S. Payne

CIMMYT – Centro Internacional de Mejoramiento de Maíz y Trigo

Role: Reviewer

Tom Payne (Ph.D.) is currently in charge of the world's largest, publically available, collection of wheat and its related species, held by CIMMYT. The collection consists of over 125,000 accessions collected or donated by nearly 80 countries. He is also responsible for CIMMYT's international maize and wheat germplasm testing unit, which since the 1960's has dispatched annually hundreds of experimental varieties, free-of-charge, to public and private sector researchers globally for experimental testing and release. During the 1990's, Tom spent six years with CIMMYT based in Zimbabwe and Ethiopia, coordinating European Commission funded regional maize and wheat improvement networks, with other long-term postings in Mexico, Turkey, Syria and Yugoslavia.



Wolfgang Spielmeier

CSIRO Agriculture & Food, Canberra, Australia

Role: Reviewer

Research interests: Molecular genetics of important traits in wheat including rust resistance, crop establishment and carbon partitioning. Major focus is on using mutagenesis to generate novel variants and next-generation sequencing technologies to identify functional mutations and genes that generate basic knowledge of mechanisms and can be used to develop accurate selection tools for the industry.



Xu Liu

Chinese Academy of Engineering, Academician
Role: IOC and Reviewer



Xu Liu has been engaging in crop germplasm resources and genetic breeding for over 30 years. He leads and participates in many research projects that are mainly on the collection, conservation, evaluation and utilization of crop germplasm resources in China, which have provided solid and profound foundation for germplasm dissemination and utilization as well as relevant technology and largely improved China's research system of germplasm.

Besides, Xu Liu led the research on the basic and principal diversity and technical indexes of China's crop germplasm resources. In this research work, he conducted comprehensive investigations to verify the origins of China's crop resources, established technical criteria for crop germplasm resources and improved related information system, which significantly increase the efficiency and effectiveness of germplasm utilization.



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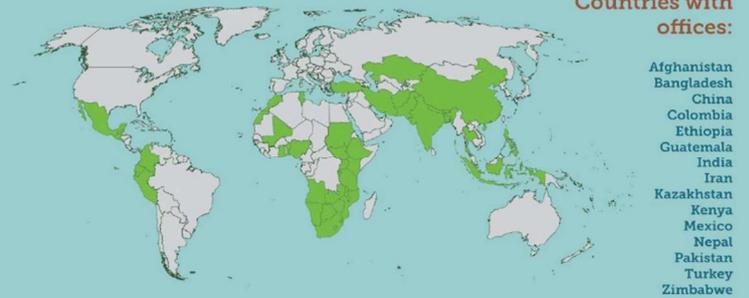
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CIMMYT at a glance

CIMMYT AROUND THE WORLD

Mission
Maize and wheat science for improved livelihoods

Vision
CIMMYT contributes to the development of a world with less poverty, healthier and more prosperous people, more resilient farming systems and fewer global crises.



Projects in over 40 countries

HOW DO WE CONDUCT OUR WORK?

CIMMYT transforms research into large-scale farm-level impacts through strong, long-established partnerships.



CIMMYT links scientific excellence, impact through partnerships and capacity building. "One CIMMYT" integrates these domains.



THE BIG IMPACT

CIMMYT GENERATES BENEFITS of **\$3.5-4.0 BILLION** annually

50% OF MAIZE AND WHEAT grown in the developing world IS BASED ON CIMMYT VARIETIES

More than **10,000** AGRICULTURAL EXPERTS AND SCIENTISTS have trained at CIMMYT



CIMMYT: Turning Research Into Impact

CIMMYT – the International Maize and Wheat Improvement Center (www.cimmyt.org) is the global leader in publicly-funded maize and wheat research and related farming systems. Headquartered near Mexico City, CIMMYT works with hundreds of partners throughout the developing world to sustainably increase the productivity of maize and wheat cropping systems, thus improving global food security and reducing poverty. The center operates regionally from offices based in 15 countries throughout Africa, Asia, and Latin America. CIMMYT is a member of the CGIAR Consortium and leads the CGIAR Research Programs on Maize and Wheat. The center receives support from national governments, foundations, development banks and other public and private agencies.

Notable facts about CIMMYT include the following:

- More than 70 percent of the wheat grown in developing countries and more than 50 percent of improved maize varieties derive from CIMMYT breeding materials. By conservative estimates, the value of the added grain from CIMMYT breeding contributions to wheat varieties worldwide reaches \$3.1 billion each year.
- CIMMYT alumni include a Nobel Peace Prize laureate and three World Food Prize winners.
- More than 10,000 scientists have trained at CIMMYT and gone on to become leaders in their own countries. The center empowers thousands of students, extension workers and farmers through courses, workshops and field days.
- The CIMMYT Germplasm Bank conserves, studies and shares samples from 28,000 unique seed collections of maize and over 140,000 of wheat.
- From its breeding programs, each year CIMMYT sends half a million seed packages to 600 partners in 100 countries.



Created in 2011 following endorsement from the G20 Agriculture Ministries, the Wheat Initiative provides **a framework to establish strategic priorities for wheat research** at the international level by **fostering communication between the research community, funders and global policy makers**.

The Wheat Initiative currently brings together **16 countries, 9 private companies and 2 CGIAR centres**, and welcomes continuously new public and private members. Individual membership is awarded through registration to the Wheat Initiative website.

The Wheat Initiative aims to encourage support and coordinate the development of **a vibrant global public-private research community sharing resources, capabilities, data and ideas to improve wheat productivity, resilience, quality and sustainable production around the world**.

To answer the challenges of wheat research internationally, the Wheat Initiative:

- Has developed a global **Strategic Research Agenda for wheat research** through the identification of priorities and challenges beyond the capacity of single research groups and countries, which can best be addressed by international coordination and collaboration between researchers, research institutions and funding arrangements
- Encourages **efficient investment in wheat research** based on the capabilities of, and synergies among, national and international programmes
- Initiates the development of new **collaborative programmes and coordinated actions** across developing and developed countries
- Develops and coordinates **knowledge sharing** amongst the international wheat community²
- Improves **access of all to resources, services and facilities**
- Supports **education of students and life-long learning of wheat researchers and farmers**
- **Stimulates public-private collaborations**

The **successful implementation** of the Strategic Research Agenda (SRA) depends on the **engagement of the global wheat community** and the support of funders and policy makers.

Coordinated public and private investments and development of global partnerships in the key priority areas identified in the SRA will ensure the stable and sustainable delivery of research outcomes for the benefit of farmers, industry and consumers.

WEB : www.wheatinitiative.org

EMAIL : wheat.initiative@inra.fr



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Wheat has a strategic position in the KWS portfolio of crops, with successful breeding programs on both winter and spring wheat in the key wheat markets in Europe and programs on Soft Red and Soft White Winter Wheat in the USA.

In addition, KWS invests significantly in associated research activities both internally and with external partners across the world. As an active member of both the Wheat Initiative and the International Wheat Yield Partnership, KWS plays a major role in assisting in the international coordination of wheat research into maximising yield potential, increasing biotic and abiotic resistance and developing hybrid wheat. We actively explore other research collaboration opportunities and are open for business for approaches from others in the area of wheat research.

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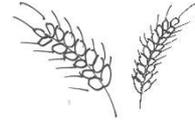


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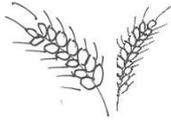


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