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# Book of Abstracts

## 6th Conference on Cereal Biotechnology and Breeding

**jointly organized by EUCARPIA Cereals Section**

3–5 November 2021 • Budapest, Hungary

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EUCARPIA

**CBB6**
**November 3–5, 2021**
**Virtually from Budapest, Hungary**

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**Please be aware that certain changes introduced in the Conference programme after editing has been closed may not be included in this Book of Abstracts due to the publishing deadline.**

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# Contents

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**Date and time of our industry talk:** Thursday, 4 November 12:50–13:50

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# Final Program of CBB6 / Virtual conference / 3–5 November 2021

| CET (GMT+1) | Wednesday, 3 November 2021 | | |
|---|---|---|---|
| 09:00–09:20 | **Opening remarks**<br>*Andreas Börner* (Germany) (EUCARPIA)<br>*János Pauk* (Hungary) (Editor-in-Chief, Cereal Research Communications)<br>*Gábor Galiba* (Hungary) (Agricultural Institute, Centre for Agricultural Research, ELKH) | | |
| 09:20–10:00 | **Congress Opening Lecture**<br>*Cristobal Uauy* (UK)<br>Unlocking the polyploid potential of wheat through genomics | | |
| | **Session I** | **Chair: Andreas Börner** | **Natural Diversity for Genetic Improvement** |
| 10:00–10:30 | Plenary talk | *Elena Khlestkina* (Russia) | The lessons from Vavilov's law for next generation breeding: emphasis on cereals |
| 10:30–11:00 | Plenary talk | *Luigi Cattivalli* (Italy) | Gene duplications and copy number variants: a new level of genetic diversity in barley |
| 11:00–11:30 | **Coffee Break** | | |
| 11:30–11:50 | | *Uğur Sesiz* (Turkey) | A novel genetic map provides insight into the genetic basis of grain size and shape-related traits in einkorn wheat |
| 11:50–12:10 | | *Eszter Gaál* (Hungary) | Widening the gene pool of bread wheat by dissection of added Agropyron cristatum chromosomes 5P and 6P using gametocidal system |
| 12:10–12:30 | | *Yeorgia Argirou* (UK) | Novel genetic diversity from synthetic-hexaploids and tetraploid-hexaploids in hybrid wheat |
| 12:30–12:50 | | *Svetlana Misheva* (Bulgaria) | Phenotypic variation for grain protein content and marker–trait associations in a worldwide collection of bread wheat |
| 12:50–14:00 | **Lunch Break** | | |
| 14:00–14:20 | | *Ana López Malvar* (Spain) | Study of the interrelation of cell wall components and its role in the maize exploitation |
| 14:20–14:40 | | *Salvatore Esposito* (Italy) | Multi-locus GWAS as a powerful approach to dissect complex traits in durum wheat |
| 14:40–15:00 | | *Michał Kwiatek* (Poland) | Development of tetraploid triticale (einkorn wheat × rye) pre-breeding germplasm with loci for steam rust resistance |
| | **Session II** | **Chair: János Pauk** | **Environmental Adaptation** |
| 15:00–15:30 | Plenary talk | *Ildikó Karsai* (Hungary) | Environmental driven genetic regulation of plant development in cereals |
| 15:30–15:50 | | *Mohamed Ahres* (Hungary) | The temperature dependent impact of modulated white light on hormonal responses, lipid composition and frost tolerance of barley plantlets at different age |

| CET (GMT+1) | Wednesday, 3 November 2021 | | |
|---|---|---|---|
| 15:50–16:10 | | *Yuzhou Lan* (Sweden) | Drought stress and genotypic variability - effects on early vigour, final yield and protein composition |
| 16:10–16:30 | | *Alexander Simonov* (Russia) | Influence of an introgression from Triticum timopheevii into chromosome 5A of bread wheat cultivars on diverse biological parameters under contrasting irrigation conditions |
| 16:30–16:50 | Coffee Break | | |
| | **Session III** | **Chair: Ildikó Karsai** | **Biotic Stress Response** |
| 16:50–17:20 | Plenary talk | *Brande Wulff* (Saudi Arabia) | Sustainable control of disease resistance – the case for GM wheat |
| 17:20–17:40 | | *Ahmed Fawzy Elkot* (Egypt/UK) | Exploring the genetic diversity in the Watkins wheat land race collection for wheat disease resistance in Egypt |
| 17:40–18:00 | | *Agnieszka Tomkowiak* (Poland) | Analysis of miRNA expression associated with gene Lr34 responsible for resistance mechanisms to leaf rust of wheat |
| 18:00–19:00 | **Poster Session – Elevator Pitch** | | **Sessions I–III** |

| CET (GMT+1) | Wednesday, 3 November 2021 | | |
|---|---|---|---|
| | **Chair: Andreas Börner** | | **Poster Session – Elevator Pitch** |
| 18:00–18:55 | **Session I** | | **Natural Diversity for Genetic Improvement** |
| | 18:00–18:05 | *Matias Schierenbeck* (Argentina) | Genetic dissection of grain architecture related traits in a winter wheat population |
| | 18:05–18:10 | *Matilde López Fernández* (Spain) | GWAS Analysis in a Collection of Spanish Bread Wheat Landraces for Agromorphological and Quality Traits |
| | 18:10–18:15 | *Helga Ochagavía* (Spain) | Phenotypic and genetic diversity in a Spanish barley landrace |
| | 18:15–18:20 | *María D. Requena Ramírez* (Spain) | Durum wheat (Triticum durum L.) landraces reveal potential for the biofortification of carotenoid esters in grain. |
| | 18:20–18:25 | *Petra Kis* (Hungary) | Molecular marker analysis of gene bank tetraploid wheat accessions |
| | 18:25–18:30 | *Noemi Gesteiro* (Spain) | Genomics of maize resistance to kernel contamination with fumonisins using a Multiparental Advanced Generation InterCross population (MAGIC) |
| | **Session II** | | **Environmental Adaptation** |
| | 18:30–18:35 | *Emmanuel A. Jampoh* (Hungary) | Evaluating the impact of heat and drought co-stress on the chlorophyll content and gas exchange of two barley (Hordeum vulgare L.) genotypes |

| CET (GMT+1) | Wednesday, 3 November 2021 | | |
|---|---|---|---|
| 18:00–18:55 | 18:35–18:40 | *Francesc Montardit-Tarda* (Spain) | Meta-analysis of agronomic-genotypic associations in a European field trial network of spring barley |
| | 18:40–18:45 | *Rita Armoniene* (Lithuania) | Transcriptome profiling of short-term low temperature-treated winter wheat |
| | **Session III** | | **Biotic Stress Response** |
| | 18:45–18:50 | *Katalin Jäger* (Hunagry) | Effect of heat and drought co-stress on functionality of generative organs and cells in wheat |
| | 18:50–18:55 | *Valérie Cadot* (France) | RustWatch : European rust surveillance supported by rust trap nurseries in wheat VCU trials |

| CET (GMT+1) | Thursday, 4 November 2021 | | |
|---|---|---|---|
| | **Session IV** | **Chair: Kerstin Neumann** | **Quality for Food and Industrial Use** |
| 9:00-9:30 | Plenary talk | *Katharina Scherf* (Germany) | WHEATSCAN – Fingerprinting of wheat varieties from 1891 to 2010 to study changes in protein composition |
| 9:30-9:50 | | *Sabrina Geisslitz* (Germany) | Influence of wheat species, breeding and environmental conditions on amylase/trypsin-inhibitors as triggers of wheat sensitivity |
| 9:50–10:10 | | *Sbatie Lama* (Sweden) | Striving for stable mixing qualities of dough from Swedish wheat in a varying climate: To breed or not to breed? |
| 10:10–10:30 | | *Uzzal Ahmed Liton* (Canada) | Genetic analysis reveals a major 4A QTL and a novel 1D QTL for pre-harvest sprouting resistance in red spring wheat |
| 10:30–10:50 | | *Tatyana Pshenichnikova* (Russia) | Genetic resources of bread wheat and relative species for the different end-use of grain and flour |
| 10:50–11:20 | **Coffee Break** | | |
| | **Session V** | **Chair: Gábor Galiba** | **Bioinformatics and Genomic Selection** |
| 11:20–11:50 | Plenary talk | *Gilles Charmet* (France) | Genomic prediction of agronomic and malting quality traits in six-rowed winter barley |
| 11:50–12:10 | | *Abdulqader Jighly* (Australia) | Doubling the prediction accuracy for non-reference unrelated genotypes and deviated environments |
| 12:10–12:30 | | *Pauline Robert* (France) | Identification of factors influencing predictive ability of phenomic selection and comparison to genomic selection in wheat breeding programs |
| 12:30–12:50 | | *Artem Pronozin* (Russia) | Prediction, classification and annotation of long noncoding RNAs |
| 12:50–13:10 | Industry talk | *Nick Jordan* (FLUIDIGM) | Extensive QTL analysis of rice grain size using a convenient and flexible microfluidics platform |
| 13:10–14:00 | **Lunch Break** | | |

| CET (GMT+1) | Thursday, 4 November 2021 | | |
|---|---|---|---|
| | **Session VI** | **Chair: Viktor Korzun** | **Genomic and Genetic Manipulation** |
| 14:00–14:30 | Plenary talk | *Jochen Kumlehn* (Germany) | Site-directed genetic engineering in cereals - principles and applications |
| 14:30–15:00 | Plenary talk | *István Molnár* (Hungary) | Chromosome genomics of Aegilops supports marker development and introgression breeding of wheat |
| 15:00–15:20 | | *Pooja Satpathy* (Germany) | Generation of haploidy inducers for Cas endonuclease-mediated mutagenesis in barley |
| 15:20–15:40 | | *Christian Hertig* (Germany) | Engineering of cereal spike architecture by site-directed mutagenesis |
| 15:40–16:00 | | *Diaaeldin Daghma* (Germany) | Towards rye genome editing - establishment of adventitious shoot formation in vitro and a genetic engineering platform |
| 16:00–16:30 | **Coffee Break** | | |
| 16:30–16:50 | | *Joanna Melonek* (Australia) | Developing strong restorer-of-fertility genes for hybrid breeding in wheat |
| 16:50–17:10 | | *Eleanor Brant* (USA) | CRISPR/Cas9 mediated targeted mutagenesis of *Liguleless1* in sorghum provides a rapidly scorable phenotype |
| 17:10–18:05 | **Poster Session – Elevator Pitch** | | **Sessions IV–VIII** |

| CET (GMT+1) | Thursday, 4 November 2021 | | |
|---|---|---|---|
| | **Chair: Andreas Börner** | | **Poster Session – Elevator Pitch** |
| 17:10–17:55 | **Session IV** | | **Quality for Food and Industrial Use** |
| | 17:10-17:15 | *Hermann G. Dallinger* (Austria) | Phs-A1 confers pre-harvest sprouting resistance independent of phenology in European winter wheat and multiple genomes reveal structural variation |
| | 17:15-17:20 | *Ramanpreet Ramanpreet* (Canada) | Genome-wide association mapping of preharvest sprouting resistance in spring wheat (Triticum aestivum L.) |
| | 17:20-17:25 | *Maria Chiara Piro* (Belgium) | A wheat-rye-triticale crossing platform to increase wheat white flour arabinoxylan content |
| | **Session V** | | **Bioinformatics and Genomic Selection** |
| | 17:25–17:30 | *Ivana Plavšin* (Croatia) | The prediction accuracy of genetic values is affected by imputation method within a wheat biparental population |
| | **Session VI** | | **Genomic and Genetic Manipulation** |
| | 17:30–17:35 | *Sara Miller* (Denmark) | Establishing new tools for genetic studies in cereals |

| CET (GMT+1) | Thursday, 4 November 2021 | | |
|---|---|---|---|
| 17:10–17:55 | **Session VII** | | **Phenotyping Technologies** |
| | 17:35–17:40 | *Svetlana P. Misheva* (Bulgaria) | Automated screening of wheat in environments, varying in nitrogen and water supply |
| | 17:40–17:45 | *Alejandra Cabeza* (Spain) | Diversity of root traits in seedlings of a barley RIL population |
| | **Session VIII** | | **Future Challenges and Innovations** |
| | 17:45–17:50 | *Michal Nowak* (Poland) | Identification of the genome regions associated with heterosis in hexaploid winter triticale |
| | 17:50–17:55 | *Stanislav Ježek* (Czech Republic) | Improving the phytosanitary efficiency of Gliding Arc plasma seed treatment by adding nitrogenous solutions to the process |

| CET (GMT+1) | Friday, 5 November 2021 | | |
|---|---|---|---|
| | **Session VII** | **Chair: Katharina Scherf** | **Phenotyping Technologies** |
| 9:00–9:30 | Plenary talk | *Kerstin Neumann* (Germany) | Phenomic infrastructure at IPK and applications |
| 9:30–9:50 | | *Madita Lauterberg* (Germany) | Precision phenotyping for shoot development under contrasting water regimes to further characterize wild emmer (Triticum turgidum ssp. dicoccoides) QTL that improve grain yield under drought in durum (T.turgidum ssp. durum) and bread wheat (T.aestivum) |
| 9:50–10:10 | | *Dmitry Afonnikov* (Russia) | Wheat spike morphometric characteristics analysis using 2D image processing |
| 10:10-10:30 | | *Danuta Kurasiak-Popowska* (Poland) | Estimation of stem-solidness in spring wheat genotypes: 50 varieties and hybrids of F1 and F2 generations |
| 10:30-10:50 | | *Wannes De Man* (Belgium) | Advances in the structural characterization of wheat flour arabinoxylan by high-resolution NMR technologies |
| 10:50-11:10 | | *Sara Petit-Jean* (Belgium) | A simple and cost-effective method to estimate arabinoxylan contents in wheat, rye and triticale flours |
| 11:10–11:40 | **Coffee Break** | | |
| | **Session VIII** | **Chair: Andreas Börner** | **Future Challenges and Innovations** |
| 11:40–12:10 | Plenary talk | *Victor Korzun* (Germany) | Future challenges and innovations |
| 12:10–12:40 | Plenary talk | *Renáta Sándor* (Hungary) | Combined effect of ploughing, minimal tillage and green manure managements in a temperate agro-ecosyste |

| CET (GMT+1) | Friday, 5 November 2021 | | |
|---|---|---|---|
| 12:40–13:00 | | *Irene S. Breider* (UK) | A multipart breeding strategy for introgression of exotic germplasm in elite breeding programs using genomic selection |
| 13:00–13:20 | | *Liina Jakobson* (Estonia) | Comparative study of powdery mildew resistance breeding strategies in barley |
| 13:20–13:40 | | *Ioannis Tokatlidis* (Greece) | Resilience to environmental uncertainty relies on crop spacing via improved plant yield efficiency |
| 13:40–14:00 | **Closing Remarks**<br>*Andreas Börner* (Germany) (EUCARPIA)<br>*János Pauk* (Hungary) (Editor-in-Chief, Cereal Research Communications)<br>*Gábor Galiba* (Hungary) (Agricultural Institute, Centre for Agricultural Research, ELKH) | | |

# Congress Opening Lecture

## Unlocking the polyploid potential of wheat through genomics

*Cristobal Uauy*

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**Keywords**: genomics, *VRT-A2*, *Triticum polonicum*, haplotypes

Developments over the past few years have radically changed the way we work with polyploid wheat. These developments have dramatically lowered the barriers to undertake biological research in polyploid wheat. For many purposes, wheat can now be treated (almost) like a model crop species. The next phase will be to start understanding the biological mechanisms underlying the most important traits in polyploid wheat and to design strategies to ensure this knowledge is quickly transferred to the field. I will argue that given polyploidy, breeders have exploited only a fraction of the potential genetic variation in the wheat genome. The recent breakthroughs in wheat genomics now allow us to make a decisive effort towards exploiting this under-utilised variation, thereby unleashing the full potential of the polyploid wheat genome.

# Oral Sessions

*Wednesday, 3 November*

## Session I – Natural Diversity for Genetic Improvement

### The lessons from Vavilov's law for next generation breeding: emphasis on cereals

*Elena Khlestkina*

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Nikolai Vavilov formulated The Law of Homologous Series in Hereditary Variability in 1920. The law is gaining more and more relevance today. The first logical explanation for the observed phenomenon was the genetic similarity of organisms, their common origin. This was evidenced by numerous results of cytogenetic, molecular genetic and later genomic studies. However, it became clear after decades of molecular genetic studies, that phenotype similarity may often be a result of convergence. Good example in cereals is reduced-height genes. The contributions of Vavilov's law to understanding of genomes synteny in related taxa is widely used in breeding, including next-generation breeding programs based on genome editing techniques. Good examples are the Mlo, DEP1 or ALS genes edited in various crops. Besides this simple and obvious way, which has its limitations, there is a prospective genome-editing based approach, implying the mechanism of convergence. Natural selection often uses different genes and different mutations for the same result in different species. Such "fast decision" is especially valuable for adaptation to dramatically changing environmental conditions. Convergence is a result of adaptation to adverse conditions, when natural selection uses silent mutations that become important in changed environment. Today, meeting the challenges of a changing climate and unstable weather conditions, it is important to learn these lessons from natural selection and adapt this mechanism for breeding. The model of natural process of adaptation via using silent genetic diversity is presented in the current report. It is the strategy we can follow in artificial selection. It is possible to create the silent genetic diversity using gene duplications and then test the mutants under different stress conditions. Another way is more precise. It is targeted reprogramming of duplicated copies. The possibility to edit genes with pinpoint accuracy having no effect on another gene from the duplications pair is demonstrated in the report on examples in cereals. The approach of targeted reprogramming of duplicated copies needs preliminary analysis of big data accumulated on gene and metabolic networks in different environment conditions. It may help breeders to go ahead with creation of new varieties for future climate conditions. The joint efforts on geneticists, bioinformaticians, genetic engineers and plant genetic resources scientists as well as breeders will provide this new approach for improvement cultivated plants through the simulation of natural evolutionary processes and accelerating them.

**Acknowledgments**

The work is supported by the Russian Science Foundation (Project No 21-66-00012).

# Gene duplications and copy number variants: a new level of genetic diversity in barley

*Luigi Cattivelli[1*], Davide. Guerra[1], Caterina Morcia[1], Laura Rossini[2], Gianluca Bretani[2], Alessandro Tondelli[1]*

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Genomic structural variants, such as gene and segmental duplications, copy number variants (CNVs), and presence absence variants (PAVs) are widespread in several animal and plant genomes and contribute to shape the genetic diversity of the species. We have exploited the large dataset generated in the frame of the FP7-Whealbi project (Wheat and barley legacy for breeding improvement) to study the extent of structural variation in barley, both at the genome-wide level and at single locus level. Using exome sequencing and read count data, we detected >16,000 deletions and duplications that affect gene content in a panel of 397 diverse barley accessions. Specific loci characterized by a phenotype potentially associated to CNVs were analysed in detail. The presence of CNVs at the locus coding for the C-repeat Binding Factors (*CBF*), the major determinant of frost tolerance in Triticeae, was screened across the whole panel. The CNVs observed were validated by digital PCR and associated to differences in spring barley resistance to low temperatures. Heterozygous mapping was then employed to detect gene duplications and/or paralogs at the *CBF* and at the black lemma and pericarp (*blp1*) loci.

# A novel genetic map provides insight into the genetic basis of grain size and shape-related traits in einkorn wheat

*Mihriban Taş[1], Uğur Sesiz[1,2], Hakan Özkan[1*]*

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[2]Department of Field Crops, Faculty of Agriculture, University of Çukurova, Adana, Turkey
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**Keywords**: einkorn wheat, genetic map, QTLs, grain size, grain shape

Einkorn wheat is the first cultivated crop that led to initiating agriculture. Cultivated einkorn is a valuable reservoir of genes for wheat improvement. Grain size and shape have an important impact on wheat yield and are two main breeding targets due to their direct relation to yield and milling quality. In the present study, 134 $F_8$ state recombinant inbred lines (RILs) derived from a cross between two advanced einkorn lines, ID432 and ID347, were used for constructing a genetic map and identifying grain size and shape related QTLs in einkorn wheat using DArTseq markers. The genetic map included 1,888 markers distributed across 563 loci on seven chromosomes, with a total length of 1089,49 cM and an average locus density of 1.93 cM across the map. The largest chromosome was 7A (206.79), whereas 4A (109.50 cM) was the smallest. Composite Interval Mapping (CIM) method was used to detect the quantitative trait loci controlling grain size and shape-related traits. A total of 31 QTLs were detected in all the einkorn chromosomes. Of these, 13 QTLs were environmentally stable and distributed on chromosomes 3A, 5A, 6A, and 7A, and explained a wide range of phenotypic variation ranging from 10.0 to 65.0%. As a result, this new genetic map and identified QTL regions will provide important genetic information to understand and find out the genetic basis of grain size and shape-related traits in einkorn wheat and other relative wheat species.

# Widening the gene pool of bread wheat by dissection of added *Agropyron cristatum* chromosomes 5P and 6P using gametocidal system

*Eszter Gaál[1*], Mahmoud Said[2], Alejandro Copete Parada[3], Adoración Cabrera[3], Petr Cápal[2], Éva Szakács[1], András Farkas[1], László Ivanizs[1], Edina Türkösi[1], Takashi Endo[2,4], Jaroslav Doležel[2], István Molnár[1,2]*

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[2]Institute of Experimental Botany, Centre of the Region Haná for Biotechnological and Agricultural Science, Olomouc, Czech Republic
[3]Genetics Department, ETSIAM, Agrifood Campus of International Excellence (ceiA3), University of Córdoba, 14071 Córdoba, Spain
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**Keywords**: *Agropyron cristatum*, COS marker, genome analysis, translocations

Wild relatives of bread wheat (*Triticum aestivum*) are promising source of new alleles and genes which can be used for wheat improvement. *Agropyron cristatum* (PPPP) represents a rich source of agronomically important genes providing resistance to rust diseases, tolerance to drought and salinity and they positively affect the yield quantity. Gametocidal (Gc) system is an efficient approach to transfer the useful agronomic traits to wheat. *Gc* genes induce chromosomal rearrangements in gametes lacking them in the monosomic addition of the Gc chromosome. The utilization of wild genetic diversity has been hampered by low throughput of selection methods and the lack of knowledge on the genomes of wild relatives. The COS (Conserved Orthologous Set) markers specific for genes conserved throughout evolution define orthologous regions, thus enabling the comparison of regions on the chromosomes of related species.

In this study, the wheat-Chinese Spring (CS)-*A. cristatum* 5P and 6P disomic addition lines were crossed with CS-*Ae. cylindrica* 2C addition line with gametocidal effect to induce structural rearrangements between the chromosomes of wheat and 5P or 6P. GISH and FISH analysis of the $BC_1$ $F_2$ lines detected seven and two wheat-*Agropyron* translocations for chromosomes 5P and 6P, respectively. Three 5P-wheat and one translocation involving the chromosome 6P were successfully transferred to the next generation. The morphological investigation of the selfed progenies indicated the 5P chromosome introgressions affected positively to the grain number per plant and the fertility relative to the wheat parental line.

We also used wheat–*A. cristatum* 1P-6P disomic and ditelosomic addition lines to map COS markers on *Agropyron* chromosomes and characterize syntenic relationships between *A. cristatum* and bread wheat. Out of 328 tested markers, 139 were polymorphic between *A. cristatum* and wheat. Sixty-nine markers were located on the chromosomes 1P–6P in wheat. Generally, COS markers of the same homeologous group were detected on similar arms in both *Agropyron* and wheat. However, some intragenomic duplications and chromosome rearrangements were detected in tetraploid *A. cristatum*.

These results provide new insights into the structure and evolution of the tetraploid *A. cristatum* genome and will facilitate the exploitation of this Triticeae species for introgression breeding of wheat.

**Acknowledgments**
This research was funded by Hungarian National Research, Development and Innovation Office (http://nkfih.gov.hu/english) (OTKA K135057) and 2019-2.1.11-TÉT-2019-00074. Grant award RTI2018-093367-B-I00 from the Spanish State Research Agency (Ministry of Science, Innovation and Universities), co-financed by the European Regional Development Fund (FEDER) from the European Union.

# Novel genetic diversity from synthetic-hexaploids and tetraploid-hexaploids in hybrid wheat

*Yeorgia Argirou*[1*], *Jacob Lage*[2], *Phil Howell*[1]

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**Keywords**: wheat, hybrid, synthetic-hexaploid, tetraploid-hexaploid, Yield components

Hybrid breeding has the potential to increase yield and yield stability in wheat (*Triticum aestivum* L.), but is currently limited by high production costs. Maximising yield by increasing the genetic distance between parents, and therefore increasing heterosis, could offset these costs. However, this is difficult due to the lack of genetic diversity within hexaploid wheat. In this project we compared yield and yield component traits among hybrids made from genetically diverse pre-breeding material versus commercial elite wheats.

Genetically diverse synthetic hexaploid (SHW) and tetraploid-hexaploid (THW) wheats, and less diverse commercial elite varieties were crossed to three female (male-sterile) testers creating 99 hybrids. All male parents were of a Robigus (a common parent in UK elite wheats) background, and all female parents were of a non-Robigus background to increase the genetic distance between parents. Yield and yield component traits were measured in five field trials in Cambridge, Norfolk, France, and Germany, conducted over two trial years to test the performance of the hybrids. The preliminary results show that many of the hybrids made from SHW and THW lines have higher yield component trait values than hybrids made from commercial elite varieties for traits such as thousand grain weight (TGW), seed number per ear, and tillering. This suggests that the increased genetic diversity in the SHW and THW lines has the potential to increase hybrid wheat yields. Further analysis will investigate the best parental combinations and look at the effects of yield components on yield.

**Acknowledgments**
We thank the University of Cambridge BBSRC DTP and KWS UK Ltd for funding this research, the KWS pre-breeding team and KWS trials team for their help with field trials, as well as Keith Gardner and Tally Wright for their help with statistical analysis.

# Phenotypic variation for grain protein content and marker–trait associations in a worldwide collection of bread wheat

*Tania Kartseva[1], Vladimir Aleksandrov[1], Ahmad M. Alqudah[2], Andreas Börner[3], Svetlana Misheva[1*]*

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**Keywords:** association mapping, diversity, grain protein content, SNPs, wheat

Grain protein content (GPC) is a key aspect of grain quality, a major determinant of the flour functional properties and grain nutritional value of wheat. It is a very important quantitative trait controlled by genetic factors but also influenced by the environmental cues. Large number of genomic regions affecting GPC is expected on many chromosomes because synthesis of grain protein involves plenty of structural genes and transcription factors, and depends on the nitrogen metabolism enzymes. For quantitative traits, finding associations with molecular markers is a potential tool to reduce length of selection cycles and opens new possibilities for advancing crop improvement. In this study, we used a collection of 255 diverse bread wheat accessions, consisting of advanced cultivars, breeding lines and double-haploid lines, originated from 28 countries from five continents. Our aims were: 1) to characterize the natural phenotypic variation in GPC, and 2) to identify molecular marker loci associated with GPC using the genome-wide association scan (GWAS) approach. Phenotypic evaluations were performed using field-grown seed samples from three consecutive crop seasons. Association analysis was performed with 17,093 SNP markers (Schierenbeck et al. 2021) using trait values for each year and the best linear unbiased estimations (BLUEs) over years. As expected for a quantitative trait, GPC means of the 255 accessions followed a normal distribution across the three years with a large number of accessions covering protein contents within the range 10-12% each year. The distribution of the calculated BLUEs also showed a wide genotypic variation for GPC in the range 10.4-12.6%. Significant positive Pearson's correlations *r* were observed for GPC across the years and with the BLUEs ($p<0.05$). Analysis of variance revealed significant genotypic and environmental (year) variation ($p<0.001$) with high broad-sense heritability estimation ($h_2 = 0.79$). Using Farm CPU model, 13 significant marker-trait associations (MTAs; $-\log_{10}$ (*p-value*) > 4.2 (above than FDR)) for GPC were detected on 10 chromosomes across the years. Five of the GPC-associated SNP loci were on homoeologous group 3. Most MTAs showed negative additive effect reducing the GPC (additive effect ranged from -0.86 to -0.32%), and only three MTAs were found to increase the protein content (additive effect ranged from 0.32 to 0.70%). The "year × association" interaction was apparent, and no stable associations (significant for at least two years) were found. Based on BLUEs, seven significant MTAs were identified, four of which on homoeologous group 3, and one MTA on chromosomes 1D, 4B and 5A each. All except one of these MTAs had negative effect on GPC (additive effect ranged from -0.39 to -0.30%). The obtained GWAS results will be complemented with a candidate search approach to identify underlying genes.

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# Study of the interrelation of cell wall components and its role in the maize exploitation

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**Keywords**: maize, saccharification, digestibility, pest resistance, cell wall, stem, biomass

Besides the use of maize grain as food and feed, maize stover can be a profitable by-product for cellulosic ethanol production, whereas the whole plant can be used for silage production. However, yield is reduced by pest damages, stem corn borers being one of the most important yield constraints. Overall, cell wall composition is key in determining the quality of maize biomass, as well as pest resistance. Besides, the framework that constitutes the cell wall is closely linked to plant performance fitness and development, and it is expected to determine the functional characteristics of the stalk, which are closely related to yield. This study aims to evaluate the composition of the four cell wall fractions (cellulose, hemicellulose, lignin and hydroxycinnamates) in diverse maize genotypes and to understand how this composition influences the resistance to pests, ethanol capacity and digestibility. In parallel, from the same subset of maize genotypes, we evaluated the influence of cell wall composition in the anatomical characteristics of the stem and its biomass yield. The following results can be highlighted: pests' resistant materials may show cell walls with low p-coumaric acid and low hemicellulose content; inbred lines showing cell walls with high cellulose content and high diferulate cross-linking may present higher performance for ethanol production and inbreds with enhanced digestibility may have cell walls poor in neutral detergent fibre and diferulates, combined with a lignin polymer composition richer in G subunits. Also, greater concentration of ferulic acid and greater proportion of subunits G and, on the opposite, lower concentrations of p-coumaric acid and subunits S are associated with greater rind puncture resistance and greater biomass yield and plant height. The inbreds that showed the greater resistance of puncture showed greater content of cellulose and lower of hemicellulose; in addition, crosslinking mediated by diferulates influenced negatively rind puncture resistance.

Lastly, the longest the internode the greater proportion of subunits H and the greater the diameter of the internode the lower the cellulose content. Results evidence that there is no maize cell wall ideotype among the tested for optimal performance for various uses, and maize plants should be specifically bred for each particular application. Along with this, the influence of cell wall on biomass yield and stem descriptions traits is confirmed.

# Multi-locus GWAS as a powerful approach to dissect complex traits in durum wheat

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**Keywords**: multi-locus GWAS, wheat, quantitative trait nucleotides, complex traits, straw composition

Since the establishment of the mixed linear model (MLM) method for genome-wide association studies (GWAS), a series of new MLM-based methods have been proposed (Yu et al. 2006; Feng et al. 2016). Most of the methods require Bonferroni correction, which often is too conservative. As a result, most of the small effects associated with complex traits, especially those with low heritability, are still not captured by these GWAS methods. To address this concern, here, we tested six different multi-locus models (mrMLM, FASTmrMLM, FASTmrEMMA, pLARmEB, pKWmEB, and ISIS EM-BLASSO) using the SNP-effect as a random factor and adopting a modified Bonferroni correction to calculate the threshold value for significance. To show the effectiveness of these models, we analyzed more than 40 phenotypic traits with high and low heritability in two different wheat panels, including wild and domesticated *Triticum turgidum* spp. accessions, and modern durum wheat cultivars. For the traits with high heritability (*i.e.*, plant height, PH), two reliable QTNs (detected by more than two different methods) were localized on chromosome 4B, ~ 9kb far from the main *Rht-1* gene controlling PH. Similarly, three reliable QTNs on chromosomes 2A, 3A and 7B coincided with the physical regions of known QTLs for the same trait, demonstrating the effectiveness of multi-locus GWAS methods. Interestingly, a reliable QTN, previously not reported in the literature on chromosome 1A (Excalibur_c6255_1119), had the highest LOD value (LOD = 17; $\log_{10}(P) = 19.77$). This marker was found nearby the *Receptor-like kinase (RLK)* gene, which could represent a good candidate gene, as it plays an important role during wheat growth and development (Ou et al. 2021).

For low heritability traits, the multi-locus approach identified genomic regions that the classic single-locus models failed to detect. For example, one reliable QTN was found associated with the grain protein content (GPC) on chromosome 6B, in the genomic regions previously identified in several studies (Blanco et al. 2002; Suprayogi et al. 2009). This leads us to argue that, the mrMLM approach provides a robust alternative for GWAS applications, especially for complex traits regulated by many small-effect loci.

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# Development of tetraploid triticale (eikorn wheat × rye) pre-breeding germplasm with loci for steam rust resistance

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**Keywords**: einkorn, fluorescence *in situ* hybridization, genetic diversity, molecular markers, resistance genes, steam rust, triticale, wheat

Non-GMO approaches in crop improvement programs are still widely used, because it is still not resolved how New Plant Breeding Techniques (NPBTs) should be regulated in UE. Therefore, an exploration of genepools of species related to cultivated plants still plays a crucial role in crop improvement. Distant hybridization can break species limits, increase genetic variation, and combine the biological characteristics of existing species.

In this study, we have focused transfer of stem rust resistance genes into triticale (×*Tritit-coseale* Wittmack ex A. Camus). Triticale as an artificial crop is characterized by low genetic variation. Moreover, the increasing harvesting area of this crop is associated with the rapid development of fungal pathogens which are continuously adapting to triticale. Among fungal pathogens, stem rust of wheat caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) seems to be one of the most devastating fungal diseases. Since aggressive new strains have emerged – such as Ug99, first detected in Uganda in 1999, this pathogen reveal the rapid expanse with the potential to result in a 100% yield loss on susceptible varieties.

Einkorn wheat (*Triticum monococcum* L.; 2n=2x=14 chromosomes, $A^mA^m$) is considered as a primary gene pool of cultivated wheats and can refer either to the wild species of wheat, *Triticum boeoticum*, or to the domesticated form, *Triticum monococcum*. It is widely used to improve their genetic variability, i.a. transfer of resistance genes to rusts, including stem rust resistance gene *Sr21*, *Sr22* and *Sr35* (two latter genes are effective against the *Ug99* race group).

In this lecture I am going to present the subsequent steps of our study, including: (a) the identification of molecular markers linked to *Sr21*, *Sr22* and *Sr35* loci in the collection of *T. monococcum* accessions; (b) the development of einkorn × rye amphidiploid forms through distant cross-hybridization followed by sell-pollinations, (c) karyotyping of parental forms, hybrids and amphidiplioids; (d) the molecular identification *Sr* resistance genes in einkorn × rye amphiploid form ($A^mA^mRR$); (d) the analysis of chromosome dynamics during meiosis of pollen mother cells of amphiploid forms; and (e) the evaluation of stem rust resistance level of amphiploid forms.

Considering the growing harvest area of triticale and the rapid expansion of *Ptg* races worldwide, there is a need to widen the genetic diversity of triticale considering the stem rust resistance genes. It is also possible that einkorn × rye amphiploid form can be utilized as a valuable germplasm for wheat resistance breeding.

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## Session II – Environmental Adaptation

### Environmental driven genetic regulation of plant development in cereals

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**Keywords**: vegetative-generative transition, stem elongation, ambient temperature, light spectrum

The complex genetic regulation of plant development, in constant perception of and response to the various environmental factors enables the plants to best adapt to their given environment. In addition to the primary developmental responses (vernalization requirement and photoperiod sensitivity), there are series of interconnected regulations continuously responding to various supplemental environmental factors, ensuring thus the fine tuning of plant development. The primary environmental factors (low temperature, photoperiod) have important roles in determining the vegetative – generative turning points leading to the basic developmental categories. The later phenological phases, however, are significantly influenced by various supplemental environmental factors (ambient temperature, light intensity and spectral composition). Our results have underlined that both ambient temperature and light spectral compositions practice very strong modulating effects on cereal plant development even under the fully inductive conditions (saturated vernalization requirement, long photoperiod) optimal for initiating flowering (Karsai et al. 2013, Kiss et al. 2017, Dixon et al. 2019, Monteguado et al. 2020). From these experiments the following important findings could be deducted: (1) the effects of the supplemental environmental factors were the most pronounced in influencing the process of stem elongation, (2) while the genotypic response to ambient temperature depended on the allele compositions of the major plant developmental genes, this association could not be proven in the case of the responses to light spectral compositions, and that (3) greater attention should be given to the functions of circadian rhythm in cereals.

In the light of climate change, the importance of this research area intensifies as the various weather anomalies can negatively affect plant developmental patterns, leading even to yield decrease.

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# The temperature dependent impact of modulated white light on hormonal responses, lipid composition and frost tolerance of barley plantlets at different age

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**Keywords**: phytohormones, frost-tolerance, low R/FR ratio, LED lighting, barley

Cold acclimation, the necessary prerequisite for promotion of freezing tolerance, is affected by both low temperature and enhanced far-red/red light (FR/R) ratio. In our experiment the impact of low R/FR ratio in the incident white light was studied on the lipidome, hormone levels, and on the key hormone metabolism-related genes in winter barley leaves at moderate (15°C) and low (5°C) temperature. FR-enhanced freezing tolerance at 15°C was associated with promotion of abscisic acid (ABA) levels, accompanied by moderate increase of indole-3-acetic acid (IAA) and cis-zeatin (cZ) levels. The most prominent impact on plants' freezing tolerance was found after FR pre-treatment at 15°C followed by cold treatment together with FR supplementation. Jasmonic acid (JA) and salicylic acid (SA) were transiently reduced. When the plants were exposed directly to a combination of cold (5°C) and FR supplementation, ABA increase was higher than in white light, and was associated with enhanced elevation of JA. After seven days of the combined treatment IAA and cis-zeatin also increased, which indicate stronger stress response and better acclimation. After serious alterations in phytohormone contents and in their related transcriptome by the decreased R/FR ratio and/or the low temperature, an elevated frost tolerance was also measured in all instances. The double-bond index of phosphatidylglycerol, phosphatidylserine, phosphatidylcholine ceramide together with total double-bond index changed when the plant was grown at 15°C as a function of white light supplemented with far-red light. Changes were observed in the levels of bilayer forming phosphatidylethanolamine, and non-bilayer forming phosphatidylserine under white light supplemented with far-red light illumination. Concerning the main building blocks of chloroplast membranes the balance between the bilayer forming galactolipid digalactosyldiacyloglicerol and non-bilayer forming monogalactosyldiacyloglycerol also altered as a results of FR supplementation. Cold hardening was more efficient when FR light was applied earlier (18 days old) than later (28 days old) in the vegetative phase.

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# Drought stress and genotypic variability – effects on early vigour, final yield and protein composition

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**Keywords**: wheat, early vigour, final yield, drought, stress tolerance index, protein composition

The global climate change is predicted to cause a rise in temperature and increasingly frequent spells of drought, which will severely impact wheat productivity. Therefore, it is urgent to ensure global food security, through improved yield performance and baking quality of wheat under drought conditions. This PhD project aims to evaluate the effects of drought stresses at different growth stages on wheat morphologic traits, yield, and protein composition. To do so, we have performed experiments on a wide array of spring wheat genotypes including 14 old, 9 modern and 50 wheat-rye introgression lines that were subjected to drought stress at various times during the plant development in a biotrol pot trial. The same lines were used in an early vigour experiment, in which phenotyping at seedling stage, and the early root and shoot growth were studied. All lines with different drought treatments are currently also under evaluation for their baking quality and nutritional quality through the use of high performance liquid chromatography (HPLC) and Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-OES), respectively. The lay-out of the experiment allowed comparisons to be made on effects of drought at separate stages during the plant growth and of genetic variation between different lines and between groups of genotypes including those being old or modern or with introgression of different rye chromosomes.

The preliminary results show that both flag leaf area and root biomass correlated with the final yield. A stress tolerance index could be a valuable tool to select valuable genotypes with good performance during drought stress. In general, early vigour was better in old Swedish lines than in modern genotypes. The combination of final yield traits and the novel phenotyping technique was able to identify drought-tolerant genotypes and also emphasized the potential of old lines as a valuable resource of superior adaptation. The outcome of protein composition analysis will help better understand the end-use quality of wheat affected by drought stresses at different growth stages.

# Influence of an introgression from *Triticum timopheevii* into chromosome 5A of bread wheat cultivars on diverse biological parameters under contrasting irrigation conditions

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The relative species enlarge a variability of bread wheat. From the species *Triticum timopheevii*, a line 821 (Budashkina, 1988) inherited an introgression in the subtelomeric region of the long arm of 5A chromosome flanked by the markers *Xgwm179* and *Xgwm291* (Leonova et al., 2000). It includes the gene for leaf pubescence $Hl_{tt}$ determining the formation of long, rare trichomes. As noted earlier, *T. timopheevii* has an outstanding leaf pubescence (Pshenichnikova et al., 2016). Leaf pubescence plays an adaptive role in the response to abiotic stresses by changing the processes of gas exchange. The drought-tolerant cultivar Saratovskaya 29 (S29) carries two pubescence genes that form a dense layer of short trichomes. The non-drought-resistant cultivar Diamant 2 (Dm2) is practically glabrous. The aim of this work was to assess the effect of introgression carrying the gene $Hl_{tt}$ on productivity and physiological and biochemical parameters under contrasting irrigation conditions. For this, substitution lines S29(821-5A) and Dm2(821-5A) were created (Figure 1). Under drought and irrigation conditions, the line S29(821-5A) showed a reduced productivity in comparison with the recipient: the number of grains per plant by 17-19% and the weight of 1000 grains by 15-28%. The line Dm2(821-5A) retained the yield under irrigation and the weight of 1000 grains increased by 9%. Under drought, this line surpassed the recipient for weight of 1000 grains by 1.5 times. These differences can be explained by the differences in physiological properties. In the line Dm2(821-5A), under both conditions, a decrease in transpiration rate and stomatal conductance was noted, and the efficiency of water use doubled. In S29(821-5A), under both conditions the photosynthetic parameters did not change. Under both conditions, S29(821-5A) showed a decrease of the total activity of the main antioxidant enzymes. Thus, the described introgression is favorable for Dm2, but negatively affects the drought tolerance of S29.

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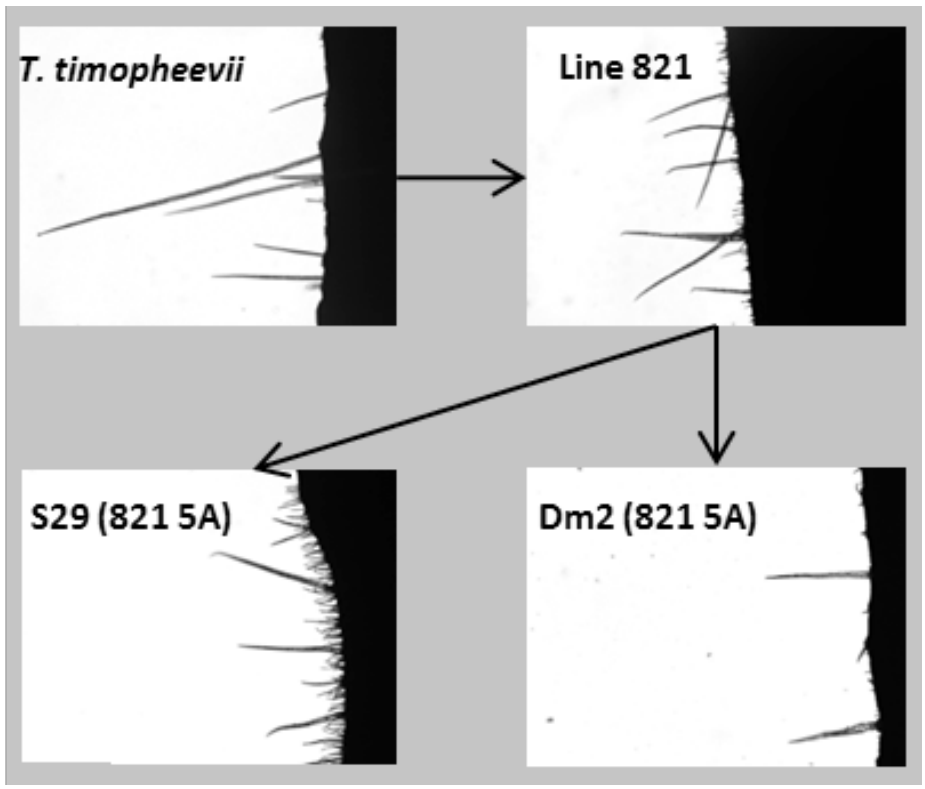


**Figure 1.** Creating of substitution lines with the pubescence from *T. timopheevii* determining by the gene $Hl_n$ (5A chromosome)

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## Session III – Biotic Stress Response

### Sustainable control of disease resistance – the case for GM wheat

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**Keywords**: resistance genes, wheat rusts, genomics, gene cloning

Worldwide, wheat yields are reduced by >20% every year due to pest and disease. Protecting the crop with pesticides is expensive, environmentally unfriendly and unsustainable. Wheat wild relatives represent a treasure trove of genetic resistance, but, introducing this resistance into our elite cultivars through traditional breeding is, at best, cumbersome. However, cloned resistance genes can be delivered as transgenes thus overcoming sexual hybridisation barriers and linkage drag. A stack of multiple resistance genes holds great promise for long-lasting, i.e. durable disease resistance. We have developed efficient methods for gene discovery and cloning which use mutant and natural population structures[1-5]. Our long-term aim is to engineer pyramids of resistance genes against major diseases of wheat [6-8]. I will present our enabling technologies and resources, and discuss a roadmap for sustainable, disease resistant GM wheat.

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## Exploring the genetic diversity in the Watkins wheat land race collection for wheat disease resistance in Egypt

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The use of genetic diversity from wheat wild progenitor species and landraces has made a significant contribution to improving wheat productivity and provide new sources of resistance to different wheat diseases. A diverse set of 300 Watkins bread wheat landraces were evaluated in Egypt in four different locations including Sids, Sakha, Nubaria and Gemmeiza Agricultural research stations, Agricultural Research Center during three consecutive seasons 2018-2019, 2019-2020 and 2020-2021 for disease resistance under natural field conditions. The obtained phenotypes were analysed by coupling association genetics to resistance gene enrichment sequencing (AgRenSeq) to define functional resistance genes in wheat. Candidate genes were identified for resistance to stripe rust on chromosomes 7A, 7D and 3B, leaf rust on chromosomes 1A and 6B and stem rust chromosomes 2B and 6B. In addition, one powdery mildew candidate resistance gene was identified on chromosome 5D which confers resistance at seedling and adult plant stages. Future work will focus on genetic confirmation of the identified genes and their incorporation into Egyptian wheat breeding programmes.

# Analysis of miRNA expression associated with gene *Lr34* responsible for resistance mechanisms to leaf rust of wheat

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**Keywords**: miR9653b, *Triticum aestivum*, *P. triticina*, APR, plant disease

Diseases caused by pathogenic fungi significantly limit the yield and quality of common wheat. The most dangerous fungal diseases of wheat include leaf and stripe rust and powdery mildew. Cultivating resistant wheat varieties is the most effective way to minimize the development of fungal diseases. Genetically determined resistance of the horizontal type (racially non-specific, adult plant resistance genes) is a preferred feature due to its more durable expression than that of the major (R) genes that determine race-specific resistance, which is often overcome by pathogens. So far, the hexaploid wheat resistance gene *Lr34/Yr18/Sr57/Pm38* (hereinafter referred to as *Lr34*) is the best-characterized gene determining horizontal-type resistance. The aim of this study was to analyze miRNA expression in selected common wheat cultivars, showing the presence of the resistance gene *Lr34*, in response to infection by the *Puccinia triticina* fungus responsible for leaf rust. The reference varieties contain slow rust resistance genes, including *Lr34* (Pavon'S', Myna'S', Frontana'S' and Sparrow'S' varieties), whereas the HN ROD control variant does not have these genes. The presence of *Lr34* in four reference varieties was confirmed using the polymerase chain reaction (PCR). In adult plants, biotic stress was induced by inoculation with fungal spores under monitored conditions in a phytotron. The differences in the expression of various microRNAs (miR9653b, miR9657b, miR9773, miR9677b) associated with gene *Lr34* were tested using the emulsion PCR (ddPCR) method. Plant material for analysis was collected before inoculation and after 6, 12, 24 and 48 hours after inoculation. Studies showed that an increase in miR9653b expression was observed in varieties carrying gene *Lr34* as a result of plant infection by *P. triticina*. For miR9657b, miR9773 and miR9677b, the ddPCR analysis resulted in too few copies to be able to properly infer. In a control variety (HN ROD) lacking this resistance gene, the expression level of miR9653b remained stable. This demonstrates that miR9653b may be involved in plant resistance mechanisms in response to leaf rust. According to the information contained in the psRNATarget database, miR9653b is associated with leaf rust resistance Lr34 gene, and differences in its expression caused by infection with the fungus seem to confirm this information. These small miRNAs are a crucial component of the cellular response to stress, in this case, biotic stress. They enable the cell to adapt to the external environment or survive its adverse effects.

*Thursday, 4 November*

## Session IV – Quality for Food and Industrial Use

### WHEATSCAN – Fingerprinting of wheat varieties from 1891 to 2010 to study changes in protein composition

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**Keywords**: celiac disease, gliadin, glutenin, non-celiac wheat sensitivity (NCWS), wheat allergy

Wheat products are one of the major pillars for nutrition security worldwide providing essential nutrients, vitamins and minerals. However, wheat proteins may trigger wheat-related disorders (WRD) such as wheat allergy, non-celiac wheat sensitivity (NCWS) or celiac disease in predisposed individuals. Recent evidence points to a rising prevalence of WRD, but the reason for this increase remains elusive. One hypothesis was that wheat breeding over the last century may have inadvertently changed the protein composition of wheat in a way that it became more immunoreactive. Therefore, our aim was to study plant characteristics, protein content and protein composition of German winter wheat cultivars from 1891 to 2010.

Five hexaploid wheat (*Triticum aestivum* L.) varieties most widely grown in Germany in each decade from 1891 to 2010 were selected from the German Federal *ex situ* Genebank of crops at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK, Gatersleben, Germany). The 60 varieties were grown at Gatersleben in three consecutive years from 2015 to 2017. The grains from three biological replicates were pooled and milled to wholemeal flours, respectively. The protein composition was characterized by reversed-phase (RP)-HPLC-UV after extraction of albumins/globulins, gliadins (ω5-, ω1,2-, α-, and γ-gliadins), and glutenins (high- and low-molecular-weight glutenin subunits) [1].

Next to this classification of gluten protein types, a more detailed integration method that captured the individual protein fingerprints more accurately was developed. This new approach was better in differentiating old and modern wheat varieties and helped identify four varieties that stood out because of their specific fingerprints [2]. Overall, the proportions of albumins/globulins and gluten did not change over time. The proportions of gliadins decreased significantly from 1891 to 2010 from 62% to 46%, whereas those of glutenins increased significantly from 17% to 33%, resulting in a decreasing gliadin/glutenin ratio from

3.6 to 1.4. Further, the content of four selected celiac disease-active peptides did not show any consistent changes from old to modern varieties [3]. All in all, our findings showed that the immunoreactive potential of old and modern wheat varieties appears to be comparable.

**Acknowledgments**
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# Influence of wheat species, breeding and environmental conditions on amylase/trypsin-inhibitors as triggers of wheat sensitivity

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**Keywords**: ancient and modern wheat, spelt, non-coeliac wheat sensitivity, mass spectrometry

Amylase/trypsin-inhibitors (ATI) activate innate immunity resulting in intra-intestinal (e.g., diarrhea) and extra-intestinal (e.g., headache, tiredness) symptoms typical of non-coeliac wheat sensitivity (NCWS) in susceptible individuals. Liquid chromatography tandem mass spectrometry (LC-MS/MS) is an appropriate method to quantitate the proteins belonging to the group of ATI. The aim of the current study was to answer the question if breeding of modern, high-yielding common wheat resulted in increased ATI levels compared to old common wheat cultivars and to ancient wheat species. Consequently, the aim was to find indications why the prevalence of hypersensitivities towards wheat increased during the last decades.

For this purpose, two comprehensive sample sets were analyzed for their ATI content by LC-MS/MS. The first sample set comprised eight cultivars each of the modern wheat species common and durum wheat and of the ancient wheat species spelt, emmer and einkorn cultivated at three different locations in Germany. The second one included 30 old wheat cultivars (licensed between 1890 and 1950) and 30 modern wheat cultivars (licensed between 1950 and 2010) cultivated at the same location over four years.

Ancient spelt had the highest ATI content of all samples investigated and no difference was observed between ancient emmer and modern common wheat (Figure 1A). Apparently, the evolution of common wheat from ancient wheat species was not accompanied by an increase of the ATI content. However, the analyzed ATI were either absent or only present in very low amounts in ancient einkorn. The old wheat cultivars were characterized by a slightly higher ATI content compared to the modern cultivars (Figure 1B), mostly due to the higher overall protein content. This shows that modern breeding during the last 100 years did not lead to increased ATI contents. The influence of environmental factors (year to year) was prominent for the old and modern wheat cultivars. In contrast, the different wheat species had a much higher impact on the ATI content than the location.

Taken together, the results indicate that the evolution of wheat and modern breeding did not lead to an increase of ATI. Thus, modern wheats are not any more likely to contribute to the increased prevalence of hypersensitivities than ancient wheats.

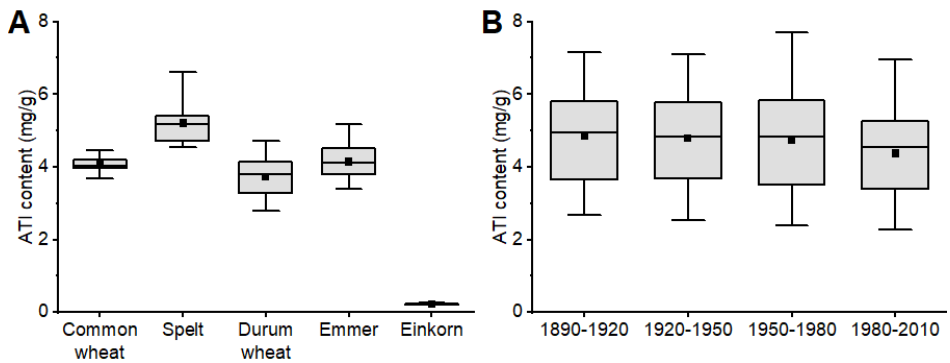


**Figure 1**: ATI content of A, modern (common and durum wheat) and ancient (spelt, emmer and einkorn) wheat species (n = 24 per box) and B, old (1890–1950) and modern (1950–2010) common wheat cultivars (n = 45 per box)

# Striving for stable mixing qualities of dough from Swedish wheat in a varying climate: To breed or not to breed?

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**Keywords**: bread wheat, dough mixing, rheology, prolonged drought and heat, breeding for stability

Climate is greatly changing and often causing large variations in the gluten protein quality of bread wheat. Heat and drought stresses causing huge problems to the breeding and baking industries that strive to develop wheat varieties with consistent qualities of gluten[1]. Therefore, a better understanding on the gluten quality stability of bread wheat in varying climates is needed.

The excessive heat combined with the prolonged drought occurred in Sweden during 2018 growing season of spring wheat. This decreased the grain protein content and increased the amounts of polymeric proteins (%UPP; gluten strength) in the flour for most of the studied wheat lines when compared to 2017 (cool and rainy) season in our recent study (Lama et al. unpublished results). Still several questions remain to be answered: can a genotype with attractive (and stable) gluten strength in flour deliver desired properties of dough for bakers? Is it enough to breed for stable gluten qualities in flour?

In regard to this, the aim of this study was to evaluate the gluten quality and mixing characteristics, and stability in dough of spring wheat lines grown in the varying climates in 2017 and 2018 in Sweden. The gluten protein parameters of flour of 294 genotypes were studied by SE-HPLC and stability was estimated by performing a multivariate principal component analysis (PCA). A selection of stable, intermediate stable and unstable wheat genotypes was based on differences in the polymeric protein parameters and mon/pol ratio in the flour between the years evaluated from PCA plot of 294 samples. Fifty-five wheat breeding lines from the three groups differing in stability in flour were selected for dough mixing study. The rheological characteristics of doughs mixed at optimum mixing time were studied using a mixograph (Rheomix,10 g)[2, 3] and parameters such as, peak time, height and width, as well as build-up and water absorption were compared between the genotypes from the different environments. The results indicated that the genotypes showed similar variation in peaktime, buildup, time1-2 and water absorption within the groups for both the environments, while a clear variation was found among the groups and the environments. The stable group between 2017 and 2018 showed a similar variation and the median of the peak development time, build-up and time 1-2, except the water absorption. This indicated similar dough development that could be designated as stability. The intermediate stable group showed similar median of build-up between 2017 and 2018, and variation in the other mixing parameters and water absorption. The unstable group showed a broad variation in all the parameters studied, and the variation for all the studied parameters was larger for the material from 2017, except for the build-up when compared to 2018 material. With this study we preliminary con-

clude that the gluten quality stability evaluation in flour by SE-HPLC and multivariate (PCA) analysis taking into account gluten polymeric proteins, mon/pol ratio and %UPP might be a suitable tool to evaluate genotypes for stability in dough parameters *e.g.* peaktime, buildup and time1-2 in fluctuating environments.

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# Genetic analysis reveals a major 4A QTL and a novel 1D QTL for pre-harvest sprouting resistance in red spring wheat

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**Keywords**: pre-harvest sprouting, seed dormancy, QTL, SNP marker

Pre-harvest sprouting (PHS) is one of the most important problems of wheat production globally. It reduces grain yield and quality significantly, resulting in huge financial losses to the producers. Given that PHS is a complex trait that is inherited quantitatively, this study was aimed at identifying quantitative trait loci (QTL) and the underlying genes for PHS resistance, which can accelerate marker assisted breeding, using a doubled haploid (DH) population of 330 lines derived from a cross between 'Roblin' (PHS susceptible) and RL4137 (PHS resistance) parental lines. To this end, the population was genotyped using a 90 K Infinium iSelect Custom Wheat Beadchip, and speed of germination index (SGI) of the DH lines was evaluated as a PHS trait in a greenhouse experiment and four field trials in three different environments over three years. Genetic analysis showed considerable variation in SGI, frequency distribution and heritability across the locations and years. The frequency distribution of DH lines was skewed towards PHS susceptibility for all trial environments while the distribution based on intermediate SGI value was normal, indicating transgressive segregation. A high density linkage map of 2343.9 cM consisting 8751 SNPs was developed where the marker density was 3.7 per cM. A total of five QTLs were detected on linkage group 1D, 4A.2, 6B.1, 6D and 7A across the five trials. Among them, the most consistent *QPhs.umb-4A* explained up to 50% of phenotypic variation and it is required for strong dormancy. The *QPhs.umb-1D* is novel and it explained up to 2.3% of phenotypic variation. The *QPhs.umb-6B* and *QPhs.umb-6D* explained up to 4.3% and 2.5% of phenotypic variation, respectively. Resistance allele for QTLs on 4A.2, 6B.1 and 6D was contributed by resistance parent RL4137 while susceptible parent 'Roblin' contributed resistance allele for the QTL on 1D. Combination of these four QTLs incrementally enhanced dormancy in our studied population. Thus, QTLs identified by this study could potentially be used in PHS resistance breeding program of red spring wheat.

# Genetic resources of bread wheat and relative species for the different end-use of grain and flour

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**Keywords**: bread wheat, introgressions, end-use of grain, gluten and protein content, grain texture, physical properties of dough

The world's population is expected to exceed 9 billion by 2050, which could cause global food shortages. Therefore, humanity is faced with the task of significantly increasing a food production, including the increasing up to 858 million tons of the production of wheat grain. This projected grain yield is intended for a wide variety of food and non-food uses. For the rational use of the grain harvest and the reasonable distribution of costs for its production, it is necessary to create cultivars, which give the grain corresponding to a certain technological purpose. The technological properties of grain are determined by protein and gluten content in grain, physical properties of gluten, and the form of starch aggregation in the endosperm of caryopsis. The results will be presented of using introgressions from the related species of bread wheat to create the lines with such grain properties that can be used for various end-use. Two species, *Ae. speltoides* and *Triticum timopheevii*, were used to obtain the new genotypes, as well as old spring Siberian cultivars. Molecularly marked limited introgressions were introduced into various chromosomes of bread wheat, which resulted in increasing of protein and gluten content in grain. The obtained genotypes retained the yield indicators and high physical properties of dough of the recipient cultivar. The lines with such grain quality indicators can be used for yeast baking. From the species *Ae. speltoides*, an analogue of the *Ha* gene of bread wheat was introduced into various spring cultivars, which determines the soft endosperm texture. Its presence in various lines significantly increased the sugar content in plant stems and leaves. It was also used to obtain the genotypes with a super-soft endosperm of the grain with a high starch availability. The grain of these lines may be used in the confectionery industry and for the distillation of alcohols and biofuel. The combination of introgressions from the species *Ae. speltoides* and *Triticum timopheevii* made it possible to obtain a high-yielding feed line that combines a consistently high protein content in grain with a high content of flavonoids in the grain hulls.

## Session V – Bioinformatics and Genomic Selection

### Genomic prediction of agronomic and malting quality traits in six-rowed winter barley

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**Keywords**: Hordeum vulgare L., genomic selection, malt quality, genetic improvement

While two-rowed barley is usually preferred for malting and beer-making, six-rowed malting barley varieties appear in Europe around 30 years ago, and several breeders have specific improvement programs on this specific germplasm.

In this study, we evaluated the feasibility of genomic prediction for yield and malting related traits using 679 breeding lines from two French barley breeders, as well as a set of recently registered varieties. These lines were evaluated in five locations and two harvest years in an unbalanced design. Although the germplasm from the two breeders does show some trend towards differentiation, globally the whole panel did not show any genetic structure. Predictive ability of GBLUP was evaluated through random cross-validation within and across breeder sets, and using cross-prediction between breeder sets. Results show moderate to high predictive ability (PA), particularly for malt friability and β-glucan content, for which predictive ability of 0.8 was obtained with training populations as small as 96 (registered varieties) and across breeding sets. The long range of useful linkage disequilibrium in this particular germplasm allows using as few as 2,000 to 5,000 markers to obtain high PA. Other prediction methods such as RKHS, Bayes Cpi or random forest do not improve predictive ability. These results are very encouraging for implementing GS prediction of malting quality traits in applied breeding programs

**Table**

| TRAIT | randomCV | BRE1+FO.CV | BRE2+FO.CV | FounderCV |
|---|---|---|---|---|
| Yield | 0.556 / 0.012 | 0.530 / 0.026 | 0.431 / 0.023 | 0.490 / 0.056 |
| Prot | 0.515 / 0.014 | 0.645 / 0.016 | 0.215 / 0.032 | 0.379 / 0.064 |
| TGW | 0.692 /0.010 | 0.763 / 0.014 | 0.540 / 0.022 | 0.585 / 0.048 |
| TestW | 0.661 / 0.014 | 0.722 / 0.016 | 0.578 / 0.016 | 0.658 / 0.050 |
| Cal | 0.714 / 0.012 | 0.697 / 0.016 | 0.598 / 0.020 | 0.350 / 0.072 |
| Head | 0.655 / 0.019 | 0.632 / 0.032 | 0.676 / 0.034 | 0.313 / 0.104 |
| Friability | 0.814 / 0.006 | 0.823 / 0.009 | 0.782 / 0.014 | 0.745 / 0.032 |
| Extract | 0.696 / 0.008 | 0.766 / 0.027 | 0.654 / 0.014 | 0.785 / 0.028 |
| Viscosity | 0.698 / 0.011 | 0.743 / 0.011 | 0.651 / 0.020 | 0.706 / 0.036 |
| BGlucan | 0.762 / 0.010 | 0.796 / 0.011 | 0.725 / 0.017 | 0.740 / 0.022 |

Predictive abilities, i.e. Pearson's correlation between observed phenotypes (main genotype effect from ANOVA) and genomic estimates of breeding values obtained by GBLUP for the 10 traits, using several validation methods Mean / standard deviation:
The first three columns are cross validation, with 50 replications of 10-fold random sampling:

- randomCV: random 10-fold sampling (9 for training, 1 for validation in turn) using the whole dataset (N=678), replicated 50 times
- BRE1+FO .CV (N=349): random cross validation using BRE1 + founder lines, thus evaluated in the same environments
- BRE2+FO .CV (N=410): random cross validation using BRE2 + founder lines, thus evaluated in the same environments
- FOUNDER.CV: random cross validation using founder lines only (N=95), thus evaluated in the same environments

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# Doubling the prediction accuracy for non-reference unrelated genotypes and deviated environments

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**Keywords**: genomic selection, genotype-by-environment interaction, GGE biplot

Accurate modelling of genotype-by-environment interaction (GEI) has major effect on improving the accuracy of genomic selection (GS). A recently developed model, named 3GS, that combines GS with genotype plus genotype × environment interaction (GGE) analysis has shown improved prediction accuracy, computational efficiency, and the ability to predict unobserved environments with higher accuracy (Jighly et al. 2021). However, 3GS requires balanced reference data which limits its applications in practical multi-environmental breeding programs. In this research, we improved 3GS model to handle unbalanced data without affecting prediction accuracy. We applied 3GS on a large population of 2,542 bread wheat lines planted in 20 unbalanced and diverse field trials across five Australian locations (Narrabri, Merredin, Horsham, Cadoux, and Geraldton) in two times of sowing with number of lines per trial ranging from 179 to 1,924. The model was compared to standard genomic best linear unbiased prediction (GBLUP) that considers GEI. The results showed that 3GS had an average accuracy for grain yield across environments of 0.737 which was much higher than GBLUP average accuracy of 0.467 (about 57.7% increase). Lines were clustered based on their relatedness (two major clusters were revealed) and one of cluster was used as a reference to predict the performance of the other cluster. 3GS showed higher prediction advantage compared to GBLUP in this scenario with 0.572 vs. 0.266, respectively (about 114.8% increase). Modern breeding programs can benefit from 3GS to improve their prediction accuracies especially for deviated environments and unrelated genotypes.

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# Identification of factors influencing predictive ability of phenomic selection and comparison to genomic selection in wheat breeding programs

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**Keywords**: bread wheat, genomic selection (GS), genomic-like omics-based (GLOB) prediction, near infrared spectroscopy (NIRS), phenomic selection (PS), plant breeding

In plant breeding, the selection of the best individuals is mainly based on phenotyping records. Because phenotyping is costly and time consuming, predictive tools such as Genomic selection (GS) have been developed in order to select among unphenotyped candidates. GS allows predicting the target traits for the selection candidates using the phenotypes of a training set and genotypic information collected on the training set and the selection candidates. Despite a good potential of the method to assist breeders in their selection choices, the cost of the genotyping still remains expensive, as GS requires to genotype each year the new selection candidates. In 2018, Rincent et al. developed a new, low cost, and high throughput method to predict the target trait of unobserved selection candidates. This method called phenomic selection (PS) is similar to GS, but genotyping is replaced by near infrared spectroscopy (NIRS). NIRS has the main advantage of being affordable, and already routinely applied on the selection candidates for many species such as wheat. GS has been well studied for twenty years, and many factors influencing its predictive ability are well understood. In PS, little is known about the factors influencing the predictive abilities, and about its performance relative to GS. We conducted the analyses on several datasets, corresponding to breeding lines drawn from the first or second years of trial evaluation from two breeding companies and one research institute in France. We evaluated several factors affecting PS predictive abilities including the possibility of combining spectra collected in different environments or at different steps of the breeding program. Contrary to genotypic data, near infrared spectra are indeed influenced by both the genotype and the environment. Thus, a selection candidate can be characterised by a multitude of spectra measured in different environments. The statistical model used was a simple H-BLUP model, reaching prediction ability from 0.26 to 0.62. Our results showed that the environment in which the NIR spectra was collected had an impor-

tant impact on predictive ability and this impact was specific to the trait considered. Among all the models tested, combining NIR spectra from different environments were the best PS models and were at least as accurate as GS in most of the datasets. We finally tested a model which gathered NIRS and molecular marker effects. This model, GH-BLUP, was the best model of all, regardless of the trait or dataset, with prediction abilities reaching 0.31 to 0.73. In this study we showed that PS could be a great support tool for breeders to improve wheat breeding programs and could efficiently replace or complement GS.

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## Prediction, classification and annotation of long noncoding RNAs

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**Keywords**: maize, lncRNA, lncRNA structure and function, computational pipeline

Long non-coding RNAs (lncRNAs) typically defined as transcripts of more than 200 nucleotides in length and without any protein coding potential. These RNAs are involved in important plant development processes such as phosphate homeostasis, flowering, photomorphogenesis and stress response. However, their structural and functional properties are not clear. Information about lncRNA sequences and their expression typically obtained from transcriptome sequencing. To process these data relevant tools for automated lncRNA are required.

Here, we propose a pipeline for automatic identification, classification and annotation of plant lncRNAs based on their localization in the genome. This pipeline was applied to the analysis of transcriptome sequences from ~800 *Zea mays* RNA-seq libraries.

The pipeline includes: (1) identification of lncRNAs by lncFinder and CPC2; (2) transmembrane potential prediction by TMHMM; (3) Mapping lncRNA to reference genome by GMAP; lncRNAs classification by its localization in the genome using gffcompare; (5) analysis of the lncRNAs structural features; (6) search for conservative lncRNA with homology to sequences from other organisms. The pipeline implemented using the Snakemake [1] workflow management system language.

More than 45 million transcripts from *Z. mays* were analyzed by this pipeline. LncFinder and CPC analysis allowed to identify ~33 million lncRNAs. For ~12 million (26%) lncRNAs the significant transmembrane potential was predicted and these transcripts were removed from analysis. More than 21 million (47%) lncRNAs were aligned to the reference genome. We identified 3.5 million exon antisense, 21900 intron antisense, 1 million exon, 117342 intron, 2.5 million intergenic lncRNAs. The analysis of the structural and functional features of lncRNAs demonstrated that majority of lncRNAs have a single exon structure (~60%), approximately 70% of multi-exonic lncRNAs have an intron length of 1 to 500 nt, approximately 68% of lncRNAs have an exon size of 2 to 300 nt. Of the 7 million lncRNAs in maize, 215.930 lncRNA have homologs among known lncRNAs from other plants. The highest proportion of conserved lncRNAs was identified by comparison with sorghum and rice sequences. Tissue-specificity analysis for three groups (conservative lncRNAs (403376), non-conservative lncRNAs (4925564) and protein coding (3 millions) RNAs) showed that the vast majority of lncRNAs are expressed in ovary tissues. Antisense lncRNAs alignment (exon, intron) with the structure of the target gene, showed that the predominant amount of lncRNA is aligned on exon 1 of the target gene.

The proposed automatic pipeline made it possible to identify 1164345 new lncRNAs in maize genome, classified into classes depending on their localization in the genome, annotate them and evaluate their structural features.

The work was funded by the Kurchatov Genome Center of the Federal Research Center IC&G SB RAS, agreement with the Ministry of Education and Science of the Russian Federation № 075-15-2019-1662.

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## Extensive QTL analysis of rice grain size using a convenient and flexible microfluidics platform

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Quantitative trait loci (QTL) mapping has provided a better way to understand the genetic regulation of yield traits in various plants and cereals, including rice. While hundreds of QTLs associated with rice grain shape traits have been identified, an efficient and easily implementable molecular marker system is crucial in order to assist with molecular assisted breeding and genetic analysis. Single-nucleotide polymporhisms (SNPs) have been proven to be an abundant and effective DNA marker for molecular assisted breeding and genetic analysis. By using an integrated microfluidic approach, based on Fluidigm's technology, there is now the possibility to perform fast, cost-effective and high-throughput SNP genotyping of tens to hundreds of SNPs simultaneously on respectively tens hundreds of samples at the same time. Here we will present a study using Fluidigm's microfluidic system in which this high-throughput SNP profiling approach was used to associate QTLs to different traits in a rice breeding study.

## Session VI – Genomic and Genetic Manipulation

### Site-directed genetic engineering in cereals - principles and applications

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**Keywords**: CRISPR-associated endonucleases, genome editing, plant performance, vector system

To utilize the entire potential of CRISPR-associated (Cas) endonuclease technology and to keep pace with its rapid development, a suite of modular and versatile vectors has been developed. This so-called CAScade system relies on the use of type IIS restriction enzymes, which enables us to implement complex molecular cloning procedures in just a few comparatively simple steps. Not only can multiple guide RNAs be expressed simultaneously, also newly emerging system components such as Cas derivatives with improved or novel functionality can be readily tested and utilized. The increasing interest in customizable endonuclease technology has also stimulated a resurgence of protoplast culture and transfection, which is of great utility when taking novel methodical approaches as well as for the functional prevalidation of constructs before it comes to the exceedingly laborious targeted genetic modification at the whole-plant level. In addition, options to couple site-directed genetic engineering with haploid technology have been implemented, be it for the efficient genetic fixation of modified alleles, or for *cas9*/gRNA-transgenic, haploidy-inducing lines used as pollen parent. The latter approach provides the opportunity of provoking target site-specific modifications with much reduced genotype dependency. Recent results of translational research in barley, wheat and maize include the improvement of plant resistance to viral and fungal pathogens and the modification of further yield- and product quality-determining features.

# Chromosome genomics of *Aegilops* supports marker development and introgression breeding of wheat

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**Keywords**: aegilops, introgression breeding, chromosome sorting, sequencing, biparental genetic map, single-gene FISH map, PCR marker, wheat-Aegilops translocations

Goatgrasses (*Aegilops*) were not domesticated and provide a huge, untapped source of genetic diversity that could be exploited in wheat improvement through chromosome mediated introgression of desirable traits. However, the introgression of favorable traits from wild relatives to wheat is hampered by the poor knowledge of their genomes and scarcity of molecular tools. The analysis of complex Triticeae genomes can be simplified by the application of genomic tools to chromosomes. Chromosome genomics relies on the ability to dissect nuclear genomes to chromosomes by flow-sorting and facilitates development of markers, identification and cloning agronomically important genes.

The natural populations of allotetraploid *Aegilops biuncialis* ($U^bU^bM^bM^b$) exhibit a high genetic diversity and together with their diploid progenitors, *Ae. umbellulata* (UU) and *Ae. comosa* (MM), they represent valuable source of new genes for resistance to diseases, abiotic stresses and grain quality.

Differential labelling of DAPI-stained chromosomes by fluorescent oligonucleotides enabled to flow sort complete set of U- and M-genome chromosomes from diploid *Aegilops*. DNA sequence assemblies of U and M chromosomes facilitated the assigment of genetic linkage groups to chromosomes in a biparental genetic map of *Ae. biuncialis*. The comparative analysis of genome structure based on single-gene FISH mapping of 43 cDNA clones, DArTseq genetic mapping and the gene content of the chromosomes revealed that most of the M-genome chromosomes preserved a collinearity with those of wheat, while multiple structural reorganizations were identified in U-genome chromosomes.

The predicted gene models as well as the FISH-mapped cDNA sequences allowed to design intron targeting PCR markers for a marker-assisted selection system suitable for development of new wheat-*Aegilops* chromosome addition and translocation lines.

**Acknowledgments**
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# Generation of haploidy inducers for Cas endonuclease-mediated mutagenesis in barley

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**Keywords**: haploidy-inducing lines, *PHOSPHOLIPASE A1*, site-directed mutagenesis

The production and utilization of doubled haploid (DH) lines is one of the most effective biotechnological measures in modern plant breeding. Each DH line is a unique result of meiotic recombination and yet is itself entirely true-breeding (i.e. homozygous across the wholegenome). One way to produce DH lines is to employ haploidy-inducing lines as pollen parents. To some extent, the progeny of such crosses include haploid plants that carry only the genome derived from egg cells of the pollinated mother plant. In maize, the capability of inducing haploid progeny proved to be largely due to loss-of-function of the sperm cell-specific *PHOSPHOLIPASE A1* (*PLA1*) gene. With the aim to produce haploidy-inducing lines for barley, we identified the barley *PLA1* orthologue that was then subjected to site-directed mutagenesis using Cas endonuclease technology. Among the generated transgenic plants carrying *cas9* and *HvPLA1*-specific guide RNA expression units, nine proved mutated in their target motifs. Selfing of these mutants resulted in haploid progeny with an efficiency of about 5%. The haploidy-inducing capacity of these mutants was then confirmed and quantified by employing them to pollinate wild-type barley, which resulted in a proportion of 5.8% haploid progeny. Using such *pla1* knockout lines, we are further about to establish a method of targeted mutagenesis that may be applicable to any barley genotypes of choice. This concept involves the delivery of Cas9 and target gene-specific guide RNA from sperm cells of *cas9*/guide RNA-transgenic haploidy inducer lines to the zygote via fertilization, so that the wild-type genomes derived from the maternal parents become accessible to site-directed mutagenesis, whereas the paternal, transgene-carrying genome is lost during early embryogenesis. Colchicine-induced genome duplication may then give rise to doubled haploid, transgene-free plants, whose exclusively maternally derived genetic makeup is carrying homozygous mutations in a target gene-of-interest.

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# Engineering of cereal spike architecture by site-directed mutagenesis

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**Keywords:** genome editing, Cas9, barley, wheat, yield potential

The yield potential of temperate cereals depends on number and size of the grains. However, the grain number is limited by the number of spikelet sites present in the spike. In the present study, two genes involved in spike formation were modified by the genome engineering tool RNA-guided Cas9 endonuclease to illuminate their function.

The *BRANCHED HEAD (BH)* gene of wheat (*Triticum aestivum*, L.) plays a remarkable role in the shaping of spike architecture. By expressing specified guide RNAs (gRNAs) and Cas9 endonuclease via biolistic DNA transfer to immature embryos followed by plant regeneration, suitable target motifs were addressed within the *BRANCHED HEAD (BH)* coding sequences present in the three subgenomes of common wheat. To increase the efficiency of targeted mutagenesis, two regions conserved among the three *BH* homeoalleles were targeted simultaneously. Mutated target motifs were detected in stably transgenic plants, and, intriguingly, also in transgene-free ones, where transient expression of gRNA and Cas9 right after DNA transfer must have been sufficient to provoke gene-specific alterations. Target region-specific PCR amplification and Sanger sequencing identified single, double and triple mutant plants. Additional combinations of loss-of-function homeoalleles were generated by crossing respective single mutant plants. Haploid technology was employed to generate a variety of lines with individual or combined mutant alleles being instantly genetically fixed. A phenotypic evaluation revealed excessive formation of supernumarary spikelets as well as branching of spikes in cases of double and triple homeoallele knockout plants, which was, however, associated with a high rate of floret infertility. Astonishingly, an analysis of some single *bh* allele knockout plants also revealed alterations in root development.

The transcription factor *SQUAMOSA PROMOTOR BINDING PROTEIN-LIKE 14 (SPL14)* in barley (*Hordeum vulgare*, L.) is involved in early spike development. After *Agrobacterium*-mediated DNA transfer of specified gRNAs and Cas9 endonuclease into immature embryos and following plant regeneration, two addressed target motifs in the second exon of *SPL14* were analysed for presence of mutations. Sequencing of the target regions in regenerated plants revealed homozygous mutations which cause loss-of-function of *SPL14* owing to translational frameshift. Progeny of primary mutated plants were analyzed for phenotypic alterations, which included changes in plant height, length and number of internodes as well as spike length and grain production. Our findings revealed the role of *SPL14* for the phase change in the shoot apical meristem.

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# Towards rye genome editing – establishment of adventitious shoot formation *in vitro* and a genetic engineering platform

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Rye (*Secale cereale* L.) is a diploid winter or spring cereal plant which proved highly recalcitrant to plant regeneration *in vitro* as well as to genetic transformation. It is genetically and phenotypically highly versatile and the second most used crop to produce bread worldwide, while green plants are used for livestock pasture. Rye has been widely used as a gene pool for improving wheat because of its high tolerance to harsh conditions such as drought, low nutrient availability, and cold temperatures, which makes it a particularly useful crop where other species fail to be productive. In addition, rye can be grown as a cover plant to prevent soil erosion. In contrast to its high value, rye plants suffer considerable damage by several diseases such as ergot, while biotechnologies such as haploid technology and genetic engineering have, as yet, not been playing a role in breeding programs. To provide a technological basis for contemporary genetic engineering approaches, the principle of adventitious shoot formation from immature embryo explants was established by conducting multi-factorial experiments involving a panel of rye genotypes cultivated under several conditions for callus induction and plant regeneration.

Callus formation, callus type, and regeneration were highly affected by genotype and culture conditions. Inbred line Lo7, which represents the sequenced reference genome, showed the highest success rates of callus induction and plant regeneration. Taking advantage of this preliminary progress achieved, transformation experiments were conducted to express a chimeric protein consisting of wheat GRF4 (GROWTH-REGULATING FACTOR 4) and its cofactor GIF1 (GRF-INTERACTING FACTOR 1) to further improve regeneration and thus to allow for genetic transformation. Based upon these results, genetic engineering using *Agrobacterium*-mediated DNA delivery is in progress to produce stable transgenic and genome-edited rye plants.

# Developing strong restorer-of-fertility genes for hybrid breeding in wheat

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**Keywords**: wheat, hybrid breeding, restorer-of-fertility genes, pentatricopeptide repeat proteins, cytoplasmic male sterility, mitochondria, *orf279*

Hybrid wheat varieties better adapted to unpredictable weather conditions could yield 10-15% more than conventional lines. A cost-effective system to produce hybrid seeds on commercial scale in wheat is missing. In many other crops, including maize, rice and brassicas, cytoplasmic male sterility (CMS) and restorer-of-fertility (Rf) genes are successfully used for this purpose. However, wheat lacks effective Rf genes that can ensure maximal seed set of F1 hybrids. One promising system involves the application of Triticum timopheevii-type cytoplasmic male sterility (T-CMS) and nuclear restorer genes. Recently, we have cloned the sequences of Rf1 and Rf3 restorer genes and determined the genetic cause of sterility in wheat T-CMS to be orf279. However, it has been observed that the level of fertility restoration conferred by these genes strongly varies between genotypes. Currently, we are using genomics and genetics approaches to understand why fertility restoration of Rf1 and Rf3 in elite lines of bread wheat is inconsistent and why T. timopheevii - the cytoplasm donor - is fully fertile. The obtained knowledge will be used to develop strong restorer genes that can be used in hybrid breeding programmes. The outcomes of this project could boost the stagnated wheat yield gains in Australia and worldwide. Higher and more stable yields will contribute to higher food security for the growing human population.

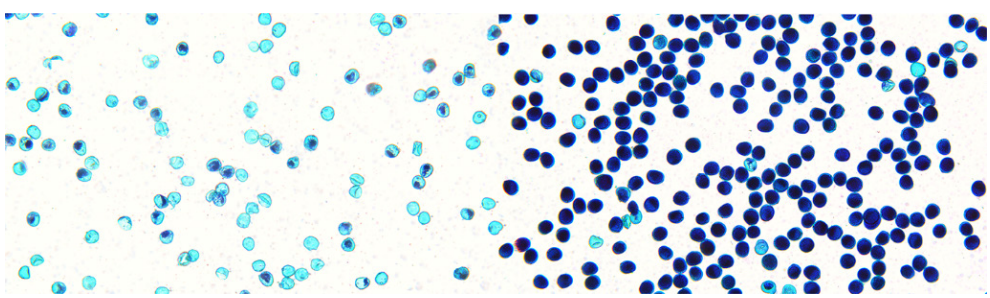

**Figure.** Alexander's stain of sterile (light blue) and fertile (dark blue) pollen grains.

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# CRISPR/Cas9 mediated targeted mutagenesis of *Liguleless1* in sorghum provides a rapidly scorable phenotype

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**Keywords**: genome editing, CRISPR-Cas9, sorghum, transgenic, targeted mutagenesis, leaf inclination angle, biolistic

Sorghum (*Sorghum bicolor* L. Moench) is one of the global top five most cultivated cereal crops. Although biotechnology approaches have great potential to complement sorghum crop improvement, their application has remained limited. To allow optimization of genome editing protocols, visual identification of edited events in tissue culture is desired. Here, CRISPR/Cas9 was utilized for targeted mutagenesis of *Liguleless1* (*lg1*), a gene responsible for leaf inclination angle. Genome editing reagents were co-delivered into immature embryos of sorghum (var. Tx430) alongside the *nptII* selectable marker via particle bombardment. Transgenic lines were regenerated, and sanger sequencing confirmed a single nucleotide insertion at the *lg1* target site. When in tissue culture, monoallelic edited lines displayed an upright leaf phenotype, which persisted after transfer to soil. T1 progeny of an event carrying the insertion were analyzed for comparison between biallelic-, monoallelic-, and non-edited lines. Biallelic *lg1* knockouts resulted in a complete lack of ligules and a more severe reduction in leaf inclination angle than observed in monoallelic lines. This work highlights *lg1* knockout as an ideal strategy for creating a rapidly scorable phenotype in tissue culture and will facilitate optimization of genome editing reagents and their delivery. *Lg1* mutants lacking the genome editing reagents were also recovered, which hold potential for enhancing canopy level photosynthesis in the field.

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*Friday, 5 November*

## Session VII – Phenotyping Technologies

### Phenomic infrastructure at IPK and applications

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**Keywords**: phenomics, non-invasive, crop plants

The IPK holds state-of-the-art phenomics infrastructure. The world's only plant cultuvation hall (PCH) allows full control over environmental conditions by simulating field-like growing conditions and harbors two phenotyping platforms. In the container/PhenoCrane system, the yield potential and growth dynamics of plant stands can be studied under field-like climate simulations. In this system, the environmental conditions are particularly variable (e.g. temperatures and light levels corresponding to winter or midsummer conditions, rapid changes in conditions as also occurs in the field, wind simulation, variable $CO_2$ concentrations), so that research can be conducted under relevant future climate scenarios. The plant stands in large-volume containers are phenotyped with a multi-sensor imaging platform, the PhenoCrane. The second system in the PCH is the rhizotron system, which is equipped with cameras for root and shoot imaging and an automatic irrigation station to study the dynamics of root growth and architecture.

Furthermore, there are modern high-throughput systems (HTP) at the IPK, which automatically irrigate and record plants via cameras in the visible and near-infrared range or detect the total fluorescence of the plants. In addition, there is a FluorCam for the automated recording of photosynthesis parameters.

The systems installed at the IPK are used for a wide range of research purposes, depending on the biological question, either more basic or more application-oriented. The non-invasive phenotyping of plants enables research on plant growth under a wide range of environmental influences. The data obtained can be used to establish relationships (associations) between phenotype, genotype and environmental factors (as well as their interaction) and thus, among other things, to investgate the effect of genetic and epigenetic variation or to elucidate the underlying molecular processes and mechanisms. This infrastructure for plant phenotyping is of international importance. The IPK is therefore embedded in networks at national (DPPN), European (EPPN) and international (IPPN) level.

# Precision phenotyping for shoot development under contrasting water regimes to further characterize wild emmer (*Triticum turgidum* ssp. *dicoccoides*) QTL that improve grain yield under drought in durum (*T.turgidum* ssp. *durum*) and bread wheat (*T.aestivum*)

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**Keywords**: high throughput phenotyping, wild emmer, near-isogenic lines, drought

With global warming, droughts or dry and hot conditions are likely to become frequent by mid-century, and wheat yields risk to fall below their long-term average (Leng et al. 2019; Toreti et al., 2019).

One option in transforming modern varieties into climate-resilient varieties is to identify beneficial alleles from landraces and wild relatives and incorporate them into high-yielding varieties (Mascher et al., 2019). In the present study, the effect of two wild emmer QTLs for yield under drought (Merchuck-Ovnat et al. 2016a; Merchuck-Ovnat et al. 2016b) introgressed in three different isogenic wheat lines was investigated in comparison to their corresponding elite variety parents under well-watered conditions and drought stress using non-destructive high-throughput phenotyping (HTP). For the first time, HTP was applied over the whole plant life cycle. The yield beneficial effect under drought conditions of the introgressed QTL segments had already been tested under field conditions in Israel, and could be confirmed in the HTP trial, where the QTL effects could be investigated throughout the life cycle. Daily phenotyping explained how the QTLs work in the different genetic backgrounds and which traits were important at which developmental stage of the entire life cycle.

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# Wheat spike morphometric characteristics analysis using 2D image processing

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**Keywords**: wheat spike, plant phenotyping, 2D image analysis, morphometry

The shape and structure of the spike are one of the most important characteristics of cereals associated with their economically valuable qualities such as productivity, lack of fragility of the spike and ease of threshing. Studying the genes that control these characters will allow to purposefully create new varieties with improved characteristics in terms of yield, ease of threshing and resistance to environmental factors One of the major problems in this area is a need to perform thousands morphometric measurements of spike during genetic experiments.

We proposed protocols to obtain spike 2D images in the lab conditions and the method for spike morphometry. Digital images are acquired in two variants: a spike on a table (one projection) or fixed with a clip (four projections). The method for spike automatic morphometry is based on the image digital processing [1]. The method identifies spike and awns in the image and estimates their quantitative characteristics (area in image, length, width, circularity, etc.). The geometric model of two quadrangles is used to describe spike shape. Parameters of this model are used to predict spike shape type (spelt, normal, or compact), spike density [1] and classification of plants into teraploid/hexaploid species by machine learning [2].

We applied our algorithm also for analysis of the 14 genotypes of five species of wheat: *Triticum aestivum*, *Triticum spelta*, *Triticum compactum*, *Triticum sphaerococcum*, *Triticum antiquorum*. The results allowed us to identify the features of the spike that differ significantly for individual genotypes: the size of the central part, the length of the spike, the width of the spike base, the area of the spike central segment and the base of the spike. The data obtained are completely consistent with the existing classification and confirm it.

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# Estimation of stem-solidness in spring wheat genotypes: 50 varieties and hybrids of $F_1$ and $F_2$ generations

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**Keywords**: spring wheat, solid stean, hollow steam, segregation distribution

Solid-stemmed wheat genotypes are better protected from damage caused by wheat stem sawfly (*Cephus pygmaeus* L.) larvae and at lower risk of lodging, as they are additionally strengthened. Most wheat cultivars grown in Europe have a hollow stem. The aim of this study was to determine the genetic determination of stem solidness in spring wheat. To this purpose, fifty varieties with different levels of stem solidness were collected. After a three-year field experiment the collection was evaluated for agronomic traits and stem solidness. As a result of the analysis, the stems were divided into three stem solidness classes (hollow stem, intermediate, and solid stem). The parental genotypes for crossing in a hollow stem x solid stem were selected on the basis of the assessment of stem solidness in the first year of research. The parental varieties, hybrids of $F_1$ and $F_2$ generations were analyzed. The data thus obtained was used to statistically grouping of phenotype classes. The method of dividing the assessed plants into phenotypic classes was dependent on the grouping method adopted. Data grouping using frequency histogram analysis was found to be more favorable than grouping using the k-means method. The significant influence of atmospheric factors led to difficulties in analyzing the evaluation of stem solidness.

On the basis of the obtained results, two hypotheses were made concerning the inheritance of the stem solidness: two genes and full domination (9: 3: 3: 1 segregation), two genes and dominant epistasis of hollow stem trait (12:3:1 segregation). Testing the segregation ratio of stem solidness using the chi-square test confirm dominant epistasis epistasis, where the epistatic gene is the one determining the hollow stem. This hypothesis was confirmed for the statistical division of the Bombona x CItr7027 cross combination, the real division of the Bombona x Lillian and Ostka Smolicka x Tybalt conbinations, and for the actual distribution in data sets where the fathers were the Lillian and Tybalt varieties.

# Advances in the structural characterization of wheat flour arabinoxylan by high-resolution NMR technologies

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**Keywords**: arabinoxylan, dietary fiber, DOSY NMR, solid-state NMR

In many countries, the consumption of dietary fiber falls below the recommended daily intake of 25 g/day defined by the European Food Safety Authority (EFSA). As a staple crop, wheat (*Triticum aestivum* L.) is a major source of dietary fiber in the European diet. The predominant dietary fiber in wheat is arabinoxylan (AX). It consists of a linear backbone of β-(1,4)-linked xylose residues that can be unsubstituted, mono-substituted or di-substituted with arabinose residues, some of which carry a phenolic acid residue (in essence ferulic acid) [1]. Part of the wheat flour AX (20-30%) is water-extractable (WE-AX), whereas the major part (70-80%) is water-unextractable (WU-AX). AX molecules differ in molecular weight and degree of arabinose and ferulic acid substitution[2,3]. AX structural features determine their solubility, viscosity, water binding capacity and therefore also its overall functionality during product making, but potentially also its health effects[4]. The genetic variation and high heritability of AX levels and extractability in wheat provide opportunities for producing new lines with enhanced technological and potentially also nutritional properties. To this end, high-resolution Nuclear Magnetic Resonance (NMR) technologies were used as a tool to characterize the AX structural heterogeneity in both conventional and high dietary fiber wheat flours. WE-AX and WU-AX were isolated from wheat flours prior to analysis with high-resolution liquid- and solid-state NMR. $^{1}$H Diffusion-Ordered NMR Spectroscopy (DOSY NMR) allowed in depth elucidation of WE-AX subpopulations based on WE-AX diffusive properties. Such properties differed between different wheat lines. Between fractions, WE-AX molecules precipitating at higher ethanol concentrations (and having a higher proportion of di-substitution) exhibited a higher diffusivity than molecules precipitating at lower ethanol concentrations (and having a high proportion of mono-substitution). Within each fraction, WE-AX structures with a high proportion of di-substitution had slightly lower diffusivities than structures with a high proportion of mono-substitution[5]. Solid-state $^{13}$C High-Power Decoupling with Magic Angle Spinning (HPDEC MAS) NMR was performed to structurally characterize isolated WU-AX. To the best of our knowledge, this was the first time wheat WU-AX could be analyzed with sufficient resolution by solid-state NMR without prior solubilization. Further peak identifications will allow in depth structural characterization of WU-AX substitution patterns. In-depth knowledge of the structural heterogeneity of WE-AX and WU-AX in white flour as provided by high resolution NMR will enhance insights in the technological and nutritional of wheat AX, which depend on genotype, environmental factors, and their interaction[6].

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# A simple and cost-effective method to estimate arabinoxylan contents in wheat, rye and triticale flours

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**Keywords**: dietary fibre, wheat breeding, arabinoxylan quantification, colorimetry, gas chromatography

Modern wheat breeding focusses mainly on increasing yield and resistances to biotic and abiotic stresses. However, there is now increasing interest in improving the health benefits of wheat including increasing their dietary fibre content. Dietary fibre is associated with many health benefits and EFSA recommends daily intakes of 25 g for women and 38 g for men. However, the actual intakes are usually lower with an average European citizen consuming only 18 g/day. Whole wheat grain is a major source of dietary fibre but fibre is depleted in its refined white flour which is used to produce most types of bread and other wheat-based foods. FIBRAXFUN is a multidisciplinary consortium which is developing a knowledge base to exploit novel types of wheat whereof white flours are high in arabinoxylan (AX) dietary fibre. The program includes the identification of novel quantitative trait loci for high AX in white flour (Lovegrove et al., 2020) and the identification of genetic variation in AX content in rye and triticale. To facilitate these studies, a colorimetric method for pentose determination (Douglas, 1981; Kiszonas et al., 2012) was optimised to efficiently determine the AX contents of wheat, rye and triticale flours. Flour samples or water extracts of flours are initially dispersed in a reagent containing acetic acid, hydrochloric acid and phloroglucinol to hydrolyse the polysaccharides present into its monosaccharides. The pentose monosaccharides (xylose and arabinose) are then converted into furfural which reacts with phloroglucinol to form a pink coloured complex. The method can be used to determine total (TOT) and water-extractable (WE) AX in wholemeal and white flours from wheat, rye and triticale. The analyses showed good correlations with the AX levels determined by gas chromatography following hydrolysis, reduction of the monosaccharides and conversion of the resulting alditols into their peracetates. The colorimetric method is ideally suited for screening large sample sets in a fast, simple and cost-effective fashion and is being used to identify and exploit of novel types of wheat rich in AX fibre as a first step towards the development of high-fibre white wheat flour products.

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# Session VIII – Future Challenges and Innovations

## Future challenges and innovations

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The growing population of the 21st century is placing increasing demands on our food production, this is compounded by pressures from diminishing resources and more variable growing conditions. In Europe farmers and the agricultural industry also have to navigate the clearly defined targets from the EU Green Deal Strategy. In this context, genomics and associated molecular marker technology, and genome editing, must play an important role in developing new varieties that are better adapted to address these key challenges.

Conventional breeding is time-consuming and heavily depends on environmental conditions. Therefore, breeders are extremely interested in new technologies that improve, accelerate and optimise breeding processes. During the last decade, molecular marker technology has provided a wide range of novel approaches to improve selection strategies. Together with the rapid development of genomics tools and the emergence of high throughput technologies, as well as impressive statistic and bioinformatic tools, there has been a remarkable practical implementation into cereal breeding programmes. Such new opportunities in phenomics, genomics and bioinformatics make it feasible to explore the vast untapped collections of crop genetic resources to create novel trait combinations. More recently, genome editing was discovered and adapted for plant research and plant breeding. This technology has powerful potential to be a new breakthrough technique for improving speed and precision in plant breeding.

Consensus among public and private crop scientists represents, if nothing else, a useful platform to begin discussing some of the obvious asymmetries in crop research that are currently holding back genetic gains and yield gaps in a wide range of crops and environments. This aligns with society's expectations that the academic, crop improvement and farmer communities ensure future food security in a generally less predictable, if not harsher, climate. And this also leads to a more stable foundation for crop science to embrace increasingly realistic research scenarios.

In the presented work, the results of specific applications of molecular markers, genomic selection, genomics and genome editing in cereals, and the strength of public-private collaborations for future innovation are demonstrated and discussed.

# Combined effect of ploughing, minimal tillage and green manure managements in a temperate agro-ecosystem

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There is a need to mitigate climate change by sequestrating organic C on agricultural lands. But, this challenge must be met while agricultural productivity should be improved to keep up with the increasing global demand for more and safer food and feed supplies. The application of green manure is a traditional method in Western Europe because it enriches the soil with C and nutrients, improves its water storage capacity and protects the surface from erosion. The advantage of using catch crop cover is not evident and justified in Hungary, owing to the fact that production is mainly precipitation limited. A new maize-spring cereal bicultural field trial was established in 2020 under ploughing and minimal tillage managements combined with four different type of fallow treatments: unsown fallow, phacelia, canola and winter mix to investigate the role of green fallow in the Hungarian agro-ecosystem at Martonvásár. In our research three main questions are arising, i) which fallow treatment (sown or unsown fallow systems under cultivation or minimum tillage) provides the best initial circumstances for the main crop, ii) how these alternative agricultural systems will respond to climate change in short, middle and long term period, and iii) how could models support decision making in this regard? To get more information about the intercrop periods, the effect of different catch crop covers and tillage managements on the development of the main crop is essential for crop breeding too, as different management options offer different circumstances for the main crops (e.g. different NPK levels, depth of soil layer, carbon and soil water content).

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# A multipart breeding strategy for introgression of exotic germplasm in elite breeding programs using genomic selection

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**Keywords**: genetic diversity, polygenic traits, introgression, multipart strategy

Profit in crop production is largely controlled by polygenic traits. Genetic variation of polygenic traits is a key determinant of genetic improvement in selective breeding programs. However, fast progress in genetic improvement comes at the cost of a rapid loss of genetic variation. Germplasm available through expired PVP (Plant Variety Protection) lines is a potential resource of variation previously lost in elite breeding programs [1]. Utilizing this variation provides opportunity for an additional genetic gain. However, reintroducing germplasm is challenging due to genetic lag, linkage drag, and adaptation of the germplasm to a different environment. Many have suggested the use of pre-breeding programs using recurrent selection to overcome these challenges. Yet, pre-breeding for polygenic traits poses another challenge, as generally most genes only have a small effect on the trait of interest. Here we show that we can overcome these challenges by creating a pre-breeding strategy with feedback pathways to optimise recurrent genomic selection. Stochastic simulations in AlphaSimR [2] were used to mimic an elite maize breeding program and quantify effects of introgression of exPVP germplasm into this population. A multipart breeding strategy was developed consisting of a bridging part, population improvement part, and product development part. The bridging part aimed to break down linkage drag and improve performance of exotic germplasm through rapid recurrent selection. The population improvement part aimed to produce the most desired parents for the product development part, which in turn produced commercial varieties. Parameters were optimised for the breeding strategy and the impact of each parameter was investigated. Haploblock origin of the final elite population was investigated to assess the success of subsequent introgression events. Results showed introgression of exPVP germplasm into an elite breeding program using a multipart breeding strategy resulted in higher genetic gain. The multipart breeding strategy was successful in closing the performance gap between the exotic and elite population and breaking down linkage drag between favourable and deleterious alleles. The rate at which exotic germplasm was introgressed, accuracy of genomic selection, and number of breeding cycles per year had the largest influence on genetic gain obtained. Both first and subsequent introgression events contributed to the composition of the genotypes present in the elite population, showing that subsequent introgression events were successful. We conclude that the multipart breeding strategy has potential to improve polygenic traits, giving us a tool to overcome the possible approaching plateau in crop yields, therefore, contributing to global food security.

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# Comparative study of powdery mildew resistance breeding strategies in barley

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**Keywords**: powdery mildew resistance, MLO, gene editing, barley, marker-assisted selection

Powdery mildew outbreaks often result in severe harvest loss due to reduced grain yield and quality in barley (*Hordeum vulgare*). Natural as well as chemically induced mutant lines carrying recessive *mlo* (*mildew resistance locus o*) alleles display resistance to the foliar powdery mildew disease-causing fungi. MLO marker-assisted selection has been successfully employed in agriculture over decades. Here we show a comparative study of powdery mildew resistance breeding strategies in barley. We are implementing both crossing-based conventional breeding techniques as well as CRISPR-Cas9 mediated precise breeding tools. Both approaches have their pros and cons, hence, we aim to compare among other factors the feasibility, time cost and adaptability of these tools in barley breeding.

For the traditional crossing-based breeding, we have chosen two donor cultivars (cv-s 'Selene', 'Spectra') with the natural allele *mlo-11* originating from Ethiopian landrace and one donor cultivar (cv 'Alexis') with EMS-derived allele *mlo-9*. As the acceptor we used Estonian cultivars 'Maali' and 'Tuuli' known to be susceptible to powdery mildew. Selection of plants with the desired alleles has been verified after each of the four back-crossing by PCR-based marker analysis. F4 plants obtained from five different crosses were self-pollinated and altogether 1824 plants of F5 were genotyped and phenotyped. From those F5 plant 354/1824 (19%) were selected for further analysis. Large-scale field-testing of promising breeding lines is planned for the next growth seasons.

For the precise breeding strategy, we aim to induce *mlo* loss-of-function allele by creating DNA double-stranded breaks (DSBs) with CRISPR-Cas9 tools followed by search for indels induced by the DNA repair system. *Agrobacterium*-mediated transformation was applied to cv 'Golden Promise'. We are going to apply the optimized precise breeding tools also to the Estonian elite cultivars.

In conclusion, the need for using less pesticides and increasing yield has strongly affected plant biotechnology and breeding in cereals. Here we show our first results from a comparative study between traditional breeding and precise gene editing in barley aiming to create new cultivars resistant to powdery mildew, which would need less pesticides and have increased yield.

# Resilience to environmental uncertanity relies on crop spacing via improved plant yield efficiency

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**Keywords**: aggressive ideotype, productive ideotype, competition

We face a substantial yield gap in maize and wheat because of environmental uncertainty. One root source is stagnation of yielding capacity *per se*, i.e., low plant yield efficiency. Improved plant yield efficiency is closely connected with the genotype of low intra-specific (inter-genotypic) competitiveness due to the inverse relationship of yielding with competitive ability. Thus, genotypes can be distinguished into two extreme ideotypes. The aggressive ideotype benefits when it develops in competition with other genotypes but cannot perform on its own. On the other edge, the productive ideotype suffers from inter-genotypic competition but stands out for yielding performance when it develops alone. Breeding under inter-genotypic competition favors the 'aggressive' ideotype at the expense of yielding capacity, resulting in density-dependent varieties that exhibit erratic optimum density (high and low optimum density at favorable and adverse environments, respectively). The necessity to stabilize the optimum density at low levels brings to the fore crop spacing via the 'productive' ideotype. From the agronomy perspective, crop spacing is imperative to: (i) moderate intra-species competition and inter-plant acquired differences, an essential condition to optimize the use of resources at the crop level; (ii) compensate for yield loss due to missing plants and promote stability; (iii) incorporate multi-genotypic varieties as a means to counteract unpredictable stresses and offer a buffer against environmental diversity; (iv) expand the adoption of the low-input agriculture to protect natural resources and prevent soil degradation; (v) lower and stabilize the threshold of optimum density without compromising the high yield levels of favorable environments. From the breeding perspective, the 'productive' ideotype is selectable only at an ultra-low density that precludes any plant-to-plant interference for inputs (nil-competition). The 'nil-competition' regime fully satisfies the three Falconer's rules for efficient single-plant selection: (i) wide inter-plant differences facilitate the single-plant selection from the an early segregating generation; (ii) moderated environmental influences on genotype expression, and systematic and even entry allocation across the entire experimental area to sample the spatial heterogeneity, improve heritability; (iii) plants of the targeted 'productive' ideotype are accumulated at the right edge of the yield distribution allowing the application of very high selection pressure; in addition, (iv) it is avoided potential biased selection due to acquired advantage or loss of desired genotypes due to proximity to empty hills, on the occasion of missing plants in the field trial.

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# Poster Sessions

*Wednesday, 3 November*

## Session I – Natural Diversity for Genetic Improvement

### Genetic dissection of grain architecture related traits in a winter wheat population

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**Keywords**: wheat, thousand kernel weight, grain architecture, GWAS

The future productivity of wheat (*T. aestivum* L.) as the most grown crop worldwide is of utmost importance for global food security. Thousand kernel weight (TKW) in wheat is closely associated with grain architecture-related traits, e.g. kernel length (KL), kernel width (KW), kernel area (KA), kernel diameter ratio (KDR), and factor form density (FFD). Discovering the genetic architecture of natural variation in these traits, identifying QTL and candidate genes are the main aims of this study. Therefore, grain architecture-related traits in 261 worldwide winter accessions over three field-year experiments were evaluated. Genome-wide association analysis using 90K SNP array in FarmCPU model revealed several interesting genomic regions including 17 significant SNPs passing false discovery rate threshold and strongly associated with the studied traits. Four of associated SNPs were physically located inside candidate genes within LD interval e.g. BobWhite_c5872_589 (602,710,399 bp) found to be inside TraesCS6A01G383800 (602,699,767–602,711,726 bp). Further analysis reveals the four novel candidate genes potentially involved in more than one grain architecture related traits with a pleiotropic effects e.g. TraesCS6A01G383800 gene on 6A encoding oxidoreductase activity was associated with TKW and KA. The allelic variation at the associated SNPs showed significant differences between the accessions carrying the wild and mutated alleles e.g. accessions carrying C allele of *BobWhite_c5872_589*, TraesCS6A01G383800 had significantly higher TKW than the accessions carrying T allele. Interestingly, these genes were highly expressed in the grain-tissues, demonstrating their pivotal role in controlling the grain architecture. These results are valuable for identifying regions associated with kernel weight and dimensions and potentially help breeders in improving kernel weight and architecture-related traits in order to increase wheat yield potential and end-use quality.

# GWAS analysis in a collection of Spanish bread wheat landraces

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**Keywords**: landraces, bread wheat, GWAS, breeding

Wheat landraces, specifically adapted to their region of origin and traditionally grown with less inputs, represent an important source of genetic variability (1), providing a wide range for adaptability, and quality-related traits (2). Thus, landraces might harbour variability useful for breeding programs, but their proper use requires a deep characterization of those traits and if possible, the availability of genetic markers linked with the different target traits. Genome-wide association study (GWAS) is a powerful statistical genetic method used to identify candidate genes effectively and efficiently for many traits in wheat, proving to be an important tool in breeding programs. A subset of 189 accessions from the collection of bread wheat landraces conserved at the Spanish National Plant Genetic Resources Centre (CRF-INIA, CSIC) characterized at genomic level, has shown the wide genetic diversity represented in this set compared to other germplasm collections (3).

The main objective of this study is to characterize this set of 189 landraces in relation to agromorphological and end-use quality traits, and to identify genomic regions linked with the traits in order to develop genetic markers that will facilitate the introduction of this variability in breeding programs.

Phenotyping has been conducted under field conditions over four years. The traits characterized were days to heading and to maturity, plant height, spike length, grains per spike, spikelets per spike, thousand kernel weight, grain size and shape (area, perimeter, and major and minor ellipse), grain protein content and gluten strength. 4856 polymorphic SNP markers in the collection (3) were used to perform GWAS employing a General Linear Model, using population structure (Q) as covariate. Bonferroni correction was applied, after calculating the number of independent markers with the function tagger from HAPLOVIEW (4). More than 300 marker-trait associations (MTAs) were found. The number of associations was highly variable depending on the trait ranging from 2 MTAs (e.g., spikelets per spike) to 139 MTAs (e.g., grain perimeter). Besides some regions were associated with several traits and might be key targets for breeding (like on chromosome 1B for traits days to maturity, area, perimeter, major ellipse, and spike length). We also found some new associations not previously described potentially interesting, like for gluten strength on chromosomes 2B, 3D, 4A and 5A. In conclusion, further studies of the MTAs detected in the present study could give valuable information to be used for marker-assisted selection in wheat breeding programs.

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# Phenotypic and genetic diversity in a Spanish barley landrace

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**Keywords**: barley, landrace, sowing dates, vernalisation, development, GWAS

Barley (*Hordeum vulgare* L.) landraces represent a large reservoir of genetic diversity for adaptation to local conditions. Genetic diversity between landraces has been widely studied. Studies carried out with a core collection of lines derived from landraces of Spanish origin showed differences in important adaptation features to biotic and abiotic stresses (Silvar et al. 2010, Yahiaoui et al. 2014, Contreras-Moreira et al. 2019). Intra-landrace diversity, however, has received less attention. Do landraces harbour genetic diversity useful for adaptation to shifting conditions occurring in their environments? To respond to this question, we have studied the local barley population H206, collected as independent plants in a location of Ávila (Spain). Previous studies on subsets of accessions from this population revealed substantial amounts of genetic (Comadran et al. 2009), and phenotypic diversity (García, 2002). The objective of the present work is to analyse the genetic diversity in the full H206 population, and its relationship with developmental responses when exposed to a range of climatic conditions.

To meet this objective, field and glasshouse experiments (Fig. 1) were carried out with the 99 accessions available (coming from SSD multiplications of spikes harvested from different plants of the original population), during growing season 2020/21 in the facilities of EEAD-CSIC, Zaragoza, Spain. Field experiments were planted at three sowing dates (16$^{th}$ Oct, 19$^{Th}$ Nov and 3$^{rd}$ Feb), to explore the full range of early, optimum and late sowings for the region. A glasshouse experiment was set with day/night temperatures of 20˚C/17˚C under long photoperiod (16 hours), with plants exposed to full (8 weeks) or suboptimal (4 weeks) vernalisation periods. Date of onset of stem elongation, flowering time, duration of the late reproductive phase, and plant height were determined in both experiments. The accessions were genotyped with the 50k barley SNP chip, and GWAS was performed. The existence of intra-population variation in landrace H206, and the identification of QTL for developmental traits will be discussed.

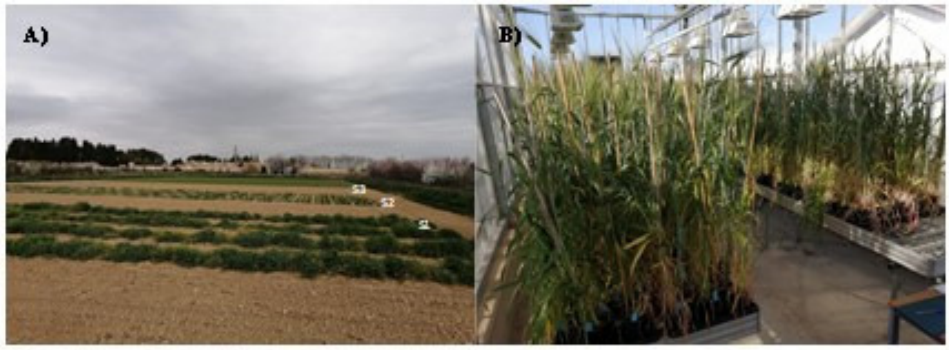


**Figure 1**. Local barley population H206 experiments under field conditions with three sowing dates (A) and under glasshouse conditions with two vernalisation treatments (B).

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# Durum wheat (*Triticum durum* L.) landraces reveal potential for the biofortification of carotenoid esters in grain

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**Keywords**: biofortification, carotenoids, durum wheat, esters, landraces, lutein

Carotenoids are lipophilic pigments and are essential in the human diet for their important functions for health. In addition, carotenoids determine the desired yellow color for industrial quality of durum wheat products such as pasta or couscous . Durum wheat (*Triticum durum* L.) has high amounts of carotenoids being lutein the main carotenoid in their endosperm. However, carotenoid esters have never been described in tetraploid wheats as durum wheat or emmer (Ziegler et al., 2015). The esterification of carotenoids allows greater accumulation in plant tissues and increases the stability of these pigments which leads to greater retention of carotenoids through the food chain (Rodríguez-Concepción et al., 2018). Therefore, the carotenoids esterification is being considered as a new target in breeding. In this work, we determined the carotenoid profile of 156 accessions of the Spanish durum wheat collection conserved at the National Plant Genetic Resources Centre (CRF-INIA, Alcalá de Henares, Spain) looking for varieties autochthonous with the ability to esterify. Four accessions were found that produce monoesters and diesters (BGE047507, BGE047520, BGE047535 and BGE047536), and eleven accessions that presented traces of monoesters (Requena-Ramírez et al., 2021). The identification of the first durum wheat accessions with esterification ability represents a significant advance for carotenoid biofortification of wheat grain.

**Acknowledgments**
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## Molecular marker analysis of gene bank tetraploid wheat accessions

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**Keywords**: tetraploid wheat, genetic diversity, RAPD, germplasm collection, molecular marker

Molecular marker methods based on the PCR technique make it possible to identify germplasm items and establish their relationship. No such study has been performed before at our institute, which has thousands of *Triticum* accessions. The present research work was included 24 germplasm accessions, from which 21 *T. turanicum* Jakubz., furthermore we used as a reference three wheat species (*T. durum* Desf., *T. dicoccon* (Schrank) Schübl. and *T. aestivum* L.). Several molecular marker methods are known, from which the most simple and quick to perform is the random amplified polymorphic DNA (RAPD). Our goal was to identify crop germplasm collection items, moreover, to recognize any duplicates in the collection. A total of 50 DNA bands were amplified with the 8 decamer primer out of which 41 (82%) were polymorphic. The fragment size ranged from 280 bp – 2kb. The items to be identified on the dendogram were well separated from the reference species. These results proved that RAPD markers could be a powerful method for grouping of wheat genotypes, which is extremely important at the germplasm collection.

**Acknowledgments**
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# Genomics of maize resistance to kernel contamination with fumonisins using a Multiparental Advanced Generation InterCross population (MAGIC)

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**Keywords**: maize kernel, fumonisin, resistance, MAGIC, GWAS

Maize kernel is exposed to several fungal genera, most notably *Fusarium verticillioides* which can contaminate maize kernels with fumonisins. In an effort to increase genetic gains and avoid the laborious tasks of conventional breeding, the use of marker-assisted selection or genomic selection programs was proposed. However, many studies have been focused on the detection of Quantitative Trait Locus (QTL) for resistance to Fusarium Ear Rot (FER) but fewer located QTL for resistance to fumonisin contamination. In the present study, a Genome Wide Association Study (GWAS) was performed on a Multiparental Advanced Generation InterCross (MAGIC) population that had previously been used to locate QTL for resistance to FER (Butrón *et al*., 2019). Six QTLs for fumonisin content were detected in the bins 3.08, 4.07, 4.10, 7.03-7.04, 9.04-9.05 and 10.04-10.5. Five of the six QTLs collocate in regions where QTLs for FER were also found. In general, kernel fumonisin content was highly correlated with FER, but there are studies in which high concentrations of fumonisins were found in visually FER resistant genotypes. In those cases, QTL conferring resistance/susceptibility to fumonisin contamination would not show any effect on FER as it has been observed in the current study because the most reliable QTL for fumonisin content was found in a region where no QTL for FER were previously detected. In conclusion, although a direct genomic selection approach to reduce fumonisin content could be suitable, improving resistance to fumonisin accumulation by genomic selection for FER could be easier and more cost efficient.

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## Session II – Environmental Adaptation

# Evaluating the impact of heat and drought co-stress on the chlorophyll content and gas exchange of two barley (*Hordeum vulgare* L.) genotypes

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**Keywords**: climate change, heat and drought, mid-uninucleate, chlorophyll content, photosynthetic performance

Increased temperature and drought due to the ongoing climate change phenomenon resulting from elevated atmospheric carbon dioxide are expected to be the two main yield decreasing factors. These abiotic conditions pose major threats to crop production as the incidences of their combined actions is expected to increase in the near future. Their combined effect on the yield of many crops is stronger than the effect of each stress alone. Thus, the growth, physiological and metabolic responses of plants to a combination of heat and drought (HD) stresses are unique and cannot be directly extrapolated from the responses to each of these stresses separately. Plant photosynthetic performance which has a positive relation with chlorophyll content is known to be reduced as a result of HD treatment.

Two two-rowed winter barley genotypes, Spinner and Lambada, with contrasting stress responses were used in the experiments. Until the mid-uninucleate (MU) stage of microspore development, the daily max/min temperature rose from 12.5/5.5 °C to 19/10.5 °C and irrigation was carried out regularly in the morning at a rate of 150 ml/day. Plants with main spikes at MU stage were identified each day in the morning and transferred into control or stress chambers. The MU stage of the microspores were checked by acetocarmine staining followed by light microscopy and based on the distance between the auricles of the flag leaf and the penultimate leaf. Heat and drought (HD) stress was generated by total water withholding at 30/20 °C max/min temperature for 5 days from MU stage until flowering under controlled conditions. The relative water content (RWC), gas exchange parameters [net photosynthesis ($A_{net}$), stomatal conductance ($g_s$), intracellular $CO_2$ concentration (Ci) and transpiration rate (E) measured from the 1$^{st}$ to the 5$^{th}$ day of treatment (S1-S5) and 5 days after regeneration (R1-R5) and the chlorophyll content of the flag leaves were determined.

Applied HD co-stress had no significant effect on the RWC of the flag leaves of the Lambada genotype but induced a significant 39% reduction in the flag leaves of the Spinner genotype. HD co-stress significantly reduced the chlorophyll a, b, and a+b contents in both genotypes compared to their respective controls. However, carotenoid content was signifi-

cantly reduced in Spinner whereas Lambada recorded no reduction. Combination of the two stressors induced a significant increase in the chlorophyll a/b ratio in the flag leaves of Lambada and Spinner (4% and 6%) respectively. S5 recorded a decrease in $A_{net}$, $g_s$, Ci, and E of both genotypes as compared to S1. Lambada had a relatively higher $A_{net,}$ $g_s$, E and water use efficiency (WUE) than Spinner at S5 whereas Spinner had a higher Ci at S5. However, WUE of both genotypes were higher in S5 as compared to S1. R1-R5 recorded an increase in $A_{net}$, $g_s$, Ci, and E of both Lambada and Spinner genotypes as compared to their respective S5. Lambada had a relatively higher $A_{net}$, $g_s$, and E during the days of regeneration (R1-R5) as compared to Spinner. Nonetheless, Spinner saw a higher Ci level in R1, R2, R3, and R4 than Lambada, which only recorded a higher Ci in R5. Both genotypes overall recorded lower WUE in R1-R5 as compared to S1-S5.

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# Meta-analysis of agronomic-genotypic associations in a European field trial network of spring barley

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**Keywords**: barley, GWAS, meta-analysis, grain yield, flowering time, plant height

Within the framework of EU projects ExBarDiv and ClimBar, 151 spring two-rowed barley cultivars were assayed in field trials. After curation of field data, 16 trials from Finland, Germany, Italy, Morocco, Spain and United Kingdom were kept. They were carried out over 2009, 2010, 2016 and 2017 seasons, in autumn and spring sowings. BLUEs for plant height, heading date and grain yield were calculated. Accessions were genotyped with the 50K Illumina Infinium SNP array. After imputation, markers with less than 0.05 minor allele frequency were discarded for next analyses. Single trial GWAS was performed using a mixed linear model (MLM) with the GAPIT suit [1], in R, using a kinship matrix as adjustment for population structure. Meta-analyses for each trait were carried out using the METAL software [2], for the entire set of trials, and for the partitioning of trials that best captured genotype by environment interaction after AMMI analyses (trials divided by sowing date for heading time, and by latitude for plant height). An association threshold was selected as the minimum -log10(*p*-value) found after 1000 meta-analyses of independent permutations of the actual *p*-values from each trial. Multiple marker associations were identified per trait. Some occurred for the entire set of trials, others were specific of the trial subsets defined by AMMI analyses. GWAS peaks confidence region limits were defined by finding the positions in which the LD decay around the peak reached the genome-wide background linkage disequilibrium threshold. The exploration of peak regions for the presence of putative candidate genes is in progress.

**Acknowledgments**
ClimBar and Exbardiv projects for phenotypic data, JHI for genotypic data. FMT was funded by a PhD contract from Gobierno de Aragón.

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# Transcriptome profiling of short-term low temperature-treated winter wheat

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**Keywords**: cold, freezing tolerance, RNA-seq, transcription factors, *Triticum aestivum* L.

Winter wheat has higher yield potential; however, a lack of sufficient freezing tolerance is still the main limiting factor to achieve maximum yields in most temperate regions. An exposure of low temperatures at autumn is a crucial environmental factor in overwintering crops. It triggers the expression of many genes determining the increase of freezing tolerance and winterhardiness of plants. The objective of the recently published study (Aleliūnas *et al.* 2020) was to evaluate transcriptome changes induced by a short-term low temperature stress using an RNA-seq approach in winter wheat. Results showed that organellar transcriptome was only slightly affected by a short-term low temperature stress. The effect on protein-coding gene expression profiles was more pronounced in wheat chloroplast than mitochondrion. The short-term low temperature treatment has a significant (FDR < 0.05) effect on nuclear transcriptome in winter wheat, significantly altering the expression of 15,042 genes out of 107,888 totally examined high-confidence (HC) genes. From this number, 2,466 genes had strongly (log2 FC > 2 or log2 FC < -2) affected expression profiles. The strongest upregulation was observed in the chromosomes from homoeologous group 5, followed by group 2. Differentially expressed genes with the most extreme upregulation encompassed stress-related transcription factors and a group of genes related to Jasmonate signalling pathway. The increased expression for a group of genes related to Jasmonate signalling pathway leads to the assumption that Jasmonate plays a crucial role in the early response to low temperature stress in winter wheat.

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## Session III – Biotic Stress Response

# Effect of heat and drought co-stress on functionality of generative organs and cells in wheat

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**Keywords**: anatomy, heat and drought co-stress, microspore, pollen cell, anatomy, antioxidant enzyme activity

The enhancement of yield stability even under unfavorable environmental conditions is one of the primary goals of wheat breeders. Among the extreme weather events, high temperature and drought are expected to be the main yield decreasing factors. It has been reported that more than 40% yield fluctuation of wheat can be attributed to heat waves and drought at the global, national and subnational scales. The growth, physiological and metabolic responses of plants to a combination of heat stress and water scarcity are unique and cannot be directly extrapolated from the responses to each of these stresses separately. Different stress combinations should be handled as a new state of stress in plants, requiring novel types of defense and acclimation responses. The sensitivity of a plant to environmental factors depends on the species, genotype and developmental stage, and on the duration and severity of the stress. Heat and drought stress during reproductive development may seriously affect crop yields, which can be attributed especially to the high sensitivity to stress shown by pollen development. Compared to pollen dysfunction, the significance of the damage sustained by the physically better protected female reproductive cells and organs is generally considered as a minor factor in yield loss. The sensitivity of reproductive tissues to simultaneous heat and drought (HD) stress is not well understood. Addressing the morphological, anatomical, physiological and molecular mechanisms conferring sensitivity and tolerance to HD co-stress will help to develop wheat genotypes capable of adapting to a changing climate.

Two winter wheat genotypes, the drought-tolerant Plainsman V and the drought-sensitive Cappelle Desprez were used in the experiments. Plants were grown under optimum environmental conditions until the mid-uninucleate stage of microspore development. Half of the plants were grown further under control conditions, the other plants were subjected to total water withholding at 32/24 °C max/min temperature for 5 days from mid uninucleate stage of microspore development until flowering.

Combined heat and drought stress applied for 5 days prior to anthesis induced a substantial reduction in the RWC of the flag leaves in both genotypes, altered the phenology of the

plants, reduced pollen viability and pollen germination, modified the morphology and the anatomy of the anthers, pistils and pollen cells, reduced the activity of antioxidant enzymes in the anthers and intensified lipid peroxidation, all leading to reduced fertility and to production loss in the sensitive genotype.

**Acknowledgments**
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# RustWatch: European rust surveillance supported by rust trap nurseries in wheat VCU trials

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**Keywords:** wheat rusts, early warning, stakeholders, VCU network, Yellow differentials

Three rust diseases have a strong impact worldwide on yield losses in soft wheat:
– *Puccinia striiformis* -> Yellow Rust,
– *Puccinia graminis tritici* -> Stem Rust,
– *Puccinia triticina* -> Leaf Rust

For yellow rust, worldwide annual losses are over US$979 million and their virulence has increased since the appearance of the Warrior races in 2011. Launched in May 2018, H2020 RustWatch is a European project on wheat rust prevention and control, based on a multi-stakeholder and multi-network approach, such as the Value for Cultivation and Use (VCU) registration network at European level, and the sharing of communication and research infrastructures.

This project led by the University of Aarhus in Denmark, for a duration of 4 years, involves 24 partners including 12 universities/research institutes, examination offices, agricultural advisory services, and breeders. Until now, RustWatch has contributed to rust surveys in many countries without national rust diagnostic labs, and rust sampling was intensified and diversified by utilizing existing stakeholder networks within plant breeding, variety testing (VCU) and agricultural advisory services.

GEVES, the French examination office for registration in the National list, is particularly involved in this project, as task leader of EU-VCU trap nurseries. In order to avoid a sampling bias of races from cultivated wheat, the rust sampling at VCU trial sites took advantage of a unique set of rust experimental lines (termed "differentials), which were deployed at more than 100 experimental sites across 18 countries. The EU-VCU trap nurseries have played an important role in providing information on races and genetic groups of yellow rust, leaf rust and stem rust. For instance, four new races of yellow rust, which may cause a shift in rust susceptibility of widely deployed wheat varieties, in case these races increase in frequency, were detected or confirmed in samples collected by VCU partners. Three of these races (all in the prevalent genetic group PstS10) were adapted to local wheat varieties, indicating that clonal evolution within existing Pst genetic groups is an important driver of the emergence of new Pst races within Europe. Currently SSR markers are not sufficient to distinguish these races inside the genetic group PstS10.

The detection of stem rust is expanding in the new cereal-growing areas in Southern Europe, France, Ireland and Norway, with at least 9 countries concerned in the last 3 years, including a first detection in France in 2020, and 75 new cases detected in 2021. Genetic groups and races are not always the same between the Western and East of Europe.

The University of Aarhus, in collaboration with GEVES, has expanded the Wheat Rust Toolbox database to manage the VCU trial surveillance results. Maps of races & genetic groups & disease pressure of yellow rust, leaf rust and stem rust, produced from VCU trap nurseries are also available on public website:

– https://agro.au.dk/forskning/internationale-platforme/wheatrust/yellow-rust-tools-maps-and-charts/genetic-groups-on-single-locations/
– https://agro.au.dk/forskning/projekter/rustwatch/wheat-rust-early-warning/vcu-surveillance/disease-severity-map/
– In the final phase of the project, different scenarios are discussed for the long-term sustainability of a new European early-warning system for wheat rust diseases.

*Thursday, 4 November*

## Session IV – Quality for Food and Industrial Use

### Phs-A1 confers pre-harvest sprouting resistance independent of phenology in European winter wheat and multiple genomes reveal structural variation

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**Keywords**: wheat, pre-harvest sprouting, GWAS, QTL, multiple genomes, structural variation

Pre-harvest sprouting, the germination of seeds within heads before harvest, is a major problem in global wheat (*Triticum aestivum L.*) production, manifested by the loss of bread-making quality in affected grain. Uncertainties in weather patterns, due to ongoing climate change are exacerbating this problem and therefore reducing farmers revenue and food availability as well as increasing price volatility. Breeding for pre-harvest sprouting resistant varieties is one of the most effective measures against this, but can be challenging since it is laborious to test especially in years lacking pre-harvest rain.

Marker-assisted selection can be a useful tool, since it allows selection for resistance based on molecular markers rather than phenotyping. To find such markers, association studies are required and therefore we tested a panel of 1000 wheat lines, representing a sizeable part of the European wheat germplasm, for pre-harvest sprouting resistance in four years. We used field and laboratory-based provocation methods and lines were genotyped using commercial SNP arrays and genotyping by sequencing assays. We performed genome-wide association in all years to find significant marker-trait associations and identified the most stable markers across years.

This approach revealed Phs-A1 as the most stable and effective locus, regulating pre-harvest sprouting independent of phenology or quality related traits, in European varieties under European conditions. Two genes have been proposed to modulate the effect of this locus, to identify the most effective gene in our population we aligned these genes and our markers to the newest available reference genome as well pan-genomes. Alignment confirmed that both PM19 and TaMKK3 might be involved in the regulation of pre-harvest sprouting, but also revealed structural variation within Phs-A1 across multiple genomes. Therefore, Phs-A1 might not only be influenced by allelic, but also by structural variation which can affect gene content and gene expression. Further analysis and long-read genomic data of multiple ge-

nomes are required to exclude the possibility that these might be assembly artefacts, however this study shows that recent advances in genome sequencing can aid classical marker-based strategies in improving complex traits.

**Acknowledgments**
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# Genome-wide association mapping of preharvest sprouting resistance in spring wheat (*Triticum aestivum* L.)

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**Keywords**: association mapping, preharvest sprouting, SNP marker

Wheat (*Triticum aestivum* L.) is an important cereal crop that provides ~20% of the daily global calorie and protein intake. However, wheat production is negatively affected by preharvest sprouting (PHS), which refers to the germination of seeds on the parent plant due to wet and moist conditions before harvest, and causes significant yield and quality loss. The incidence of PHS depends upon the level of dormancy, as an adaptive trait that blocks the germination of seeds under optimal environmental conditions, in which low level of seed dormancy leads to susceptibility to PHS. To identify genomic regions and genes that are important for PHS resistance, this study performed phenotypic and genotypic analyses of 192 diverse wheat lines in replicated field trials over two different environments. To this effect, germination index of the wheat line was determined as indicator of PHS trait while genotypic analysis was conducted using a 90K Illumina Infinium SNP genotyping array. A total of 20,205 SNP markers were derived and after filtering 18,793 markers remained for final analysis. STRUCTURE and TASSEL software were used to evaluate association between the genotype and the PHS trait. Subpopulations were determined using STRUCTURE and two sub-populations were generated. Significant markers were identified using general and mixed linear models, and these markers are located on Chromosome 4A, 5B and 3D. Further study of the association panel in more environments is in progress.

# A wheat-rye-triticale crossing platform to increase wheat white flour arabinoxylan content

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**Keywords**: wheat, triticale, rye, arabinoxylan, dietary fibre, breeding

Cereal dietary fibre (DF) content is a quantitative trait for which there is growing interest because of the health benefits associated with adequate DF intake. A promising target to enhance the DF content in refined flour of wheat (*Triticum aestivum*) is arabinoxylan (AX) as it constitutes the major DF component of its endosperm. A possible way to achieve this is by introgression of rye (*Secale cereale*) genomic regions determining high AX content. In fact, disomic addition of chromosomes 1R, 4R and 6R in wheat substantially increased its AX content (Schneider et al., 2016).

To this end, the FIBRAXFUN consortium is implementing a crossing platform to develop high-AX wheat-rye translocation lines from commercial West-European wheat cultivars featuring superior AX content. Within this crossing platform, two crossing schemes are being carried out in parallel. The first scheme involves developing wheat-rye translocation lines by means of wheat × rye crosses. First, the crossability loci *kr1/kr2/skr* are being introgressed into the elite wheat material. By introgression of the *kr1* recessive alleles only it is possible to already obtain up to 50% of crossability with rye (Molnár-Láng, 2015). At the same time, the rye material is being selfed to obtain near-homozygous rye parental lines. Once developed, these parental lines will be crossed, and the progeny screened for AX-associated translocation events. With this first crossing scheme the FIBRAXFUN consortium will be able to access and exploit the broad genetic diversity present in the rye diversity panel assembled in the context of the FIBRAXFUN activities (Piro et al., 2021). Finally, the second crossing scheme uses triticale (× *Triticosecale*) as alien bridge. This latter approach is limited by the narrow genetic diversity of the R genome of triticale. Nevertheless, it provides the significant advantage of an R chromatin source, triticale, which is readily crossable with wheat. Thus, triticale offers a faster way to deliver novel wheat cultivars with increased DF content.

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## Session V – Bioinformatics and Genomic Selection

# The prediction accuracy of genetic values is affected by imputation method within a wheat biparental population

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**Keywords**: wheat quality, genomic selection, RR-BLUP, marker density, imputation

Accelerated development of high-throughput genotyping methods in the last few decades has enabled the expansion of new breeding methods based on molecular markers, which has led to a significant acceleration of the breeding process. Genomic selection is one of the newly developed methods of marker-assisted selection in which molecular markers covering the whole genome are used. The main goal of genomic selection is to predict the genetic value of the individuals within validation population based on genomic estimated breeding value (GEBV).

In our study, RR-BLUP model (Endelman, 2011) was used to predict grain quality traits within biparental wheat population. The population is comprised of 139 recombinant inbred lines (RILs) derived from a cross between Bezostaya-1 and Klara cultivars. The experiment was conducted during 3 consecutive years at Osijek and Slavonski Brod (Croatia). Phenotypic data for quality traits grain protein content (GPC), wet gluten content (WGC) and test weight (TW) were collected. Population was genotyped using the DArTseq technology and a total of 1087 SNPs were used for genomic selection. To assess the effect of marker density on prediction accuracy maximum number of markers ($N_M = 1087$) and two subsets ($N_M = 544$ and $N_M = 272$) were used in predictions. Imputation of missing genotype data for each dataset was done using two approaches: mean imputation (MNI) and imputation within Beagle software (v. 5.1) (Browning et al., 2018), in order to assess the effect of the type of imputation of different marker densities on the prediction accuracy. Cross-validation was performed by randomly splitting the dataset into training and validation population in the ratio 80:20. Prediction accuracy was expressed as the mean value over the total number of cross-validation iterations which was set to 10 000.

Among the quality traits examined, the highest prediction accuracy was obtained for TW, regardless of the $N_M$ size and imputation method used. As expected, reducing the $N_M$ had a large negative effect on prediction accuracy for all examined quality traits. The decrease in prediction accuracy was even more pronounced when a dataset imputed by Beagle software was used. When using complete dataset ($N_M = 1087$), both imputation methods resulted in similar prediction accuracies. On the other hand, when $N_M = 272$ dataset was imputed using Beagle, prediction accuracy decreased by 33, 40 and 52%, for TW, GPC and WGC, respec-

tively. The reduction of prediction accuracy with reducing $N_M$ was less severe when MNI was applied, suggesting that MNI may be a method of choice for imputation within a biparental population when the size of the available marker data is limited.

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# Session VI – Genomic and Genetic Manipulation

## Establishing new tools for genetic studies in cereals

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**Keywords**: sorghum, barley, agro-infiltration, transient expression

Sorghum (*Sorghum bicolor*) and barley (*Hordeum vulgare*) are among the five most important cereal crops worldwide. Both are very resilient crops and an important source of food and feed. Additionally the fast growing, highly drought tolerant sorghum is an important source of bioenergy. Barley, a crop very well suited to cool climates, on the other hand is one of the most important raw materials for the brewing industry.

Even though both crops are very resilient, there are still challenges associated with their cultivation. Both crops face considerable yield reduction through for example fungal diseases or abiotic stresses. In the face of climate change, with more weather extremes, different stress factors are sure to increase. Therefore, a breeding effort is needed to adapt these crops to those new challenges. No matter which breeding method, conventional breeding, mutation breeding or novel breeding techniques, is used, knowledge of gene functions considerably speeds up the breeding process. This knowledge could also shed further light on why these crops are particularly hardy and subsequently be used for the breeding of other crops.

The established methods for such genetic studies are relatively limited, especially for sorghum. Sorghum is very recalcitrant to stable transformation and the traditional approach, the transformation of immature embryos, is very much seasonally dependant and restricted to subtropical and tropical climates. Recently however, a study, describing a simple yet promising method to study sorghum genes *in planta*, was published. This method uses agro-infiltration to transiently express genes in sorghum. We are currently in the process of developing this system further. So far we have demonstrated that it is far less genotype dependant than stable transformation. Furthermore, we have successfully applied the method in barley for the first time. Now we are ready to express genes of interest to help elucidate their function. Lastly, we want to establish this method as a tool for testing the efficiency of CRISPR/Cas constructs to be applied in the less robust and more time intensive stable transformation procedures.

## Session VII – Phenotyping Technologies

### Automated screening of wheat in environments, varying in nitrogen and water supply

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**Keywords:** drought, nutrition, phenotyping, photosynthesis, wheat

The two resources with greatest influence on wheat productivity are water and nitrogen (N). As an essential macronutrient, N amply contributes to biomass increase by affecting photosynthesis and other metabolic pathways, and has a direct impact on plant response to water stress. The contemporary automated phenotyping approaches render new possibilities for insight into the interplay between N limitation/excess and the alterations in the growth and photosynthetic responses in plants subjected to drought. An experiment was set up with two wheat genotypes, three levels of N supply (low, optimum and high) and two levels of water supply (well-watered and water deprivation, followed by re-hydration). The experiment was conducted in the Slovak Plant Screen Phenotyping Unit at Slovak University of Agriculture, Nitra, Slovakia. Vegetative phenotypic screens were performed using RGB, chlorophyll fluorescence and hyperspectral imaging units from tillering to flowering phenophase, collecting data on 200 growth and photosynthetic traits. The preliminary results showed that the estimated plant parameters could effectively differentiate the two genotypes (a modern semi-dwarf N-responsive variety and an old local N-non-responsive one) regarding their response to drought and re-hydration under various N nutrition. The forthcoming analysis of the comprehensive data set combining input from the automated phenotyping, manual measurements of photosynthetic traits, grain yield and quality traits will aid our understanding how wheat plants of different genetic constitution integrate water and nutrient exogenous signals. Such knowledge has the potential to help optimizing the resources use, while minimizing the adverse environmental impacts.

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# Diversity of root traits in seedlings of a barley RIL population

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**Keywords**: barley, root, seedling, linkage mapping, QTL

So far, root systems have not been thoroughly explored in breeding programs. Plant breeding will benefit from the study of root diversity and its relationship with resource acquisition. The roots of monocot and dicot crops develop in a different way, but both define their own root system architecture (RSA), which may be expressed as the spatial configuration of roots inside the soil (Lynch, 1995). Studying RSA and other derived parameters could lead to a better understanding of the patterns of root development in relation with soil exploration for the acquisition of water and nutrients.

The aim of this study is to evaluate root system diversity in a population of barley (*Hordeum vulgare* L.) recombinant inbred lines (RILs), and to identify quantitative trait loci (QTLs) for root traits potentially useful in breeding programs.

A population of 114 RILs from the Orria x Plaisant cross, an elite Spanish breeding population (Mansour et al., 2014), was tested for RSA traits under controlled and repeatable conditions. The RILs were previously genotyped with barley OPA1. The lines were evaluated at seedling stage, using a rhizoslides system, which is low-cost, medium-throughput method, amenable to breeding operations. A sandwich composed of a PVC plate, black cardboard sheet, filter paper, and a plastic sheet, A-4 size, with the lower end submerged in a container with distilled water, was used to grow the seeds (Fig. 1A). Six pre-germinated seedlings for each RIL were grown, one per sandwich, in a growth chamber for 7 days at 22/18 ºC and 12/12 h photoperiod (Fig. 1B). After that, roots were scanned using a flatbed scanner, at 330 ppi, and the following traits were measured using Smartroot software (Lobet et al., 2011): total root length, primary root length, mean length of the other seminal roots, total root surface area, mean root diameter, and root growth angle. Root number, shoot length, and shoot width were recorded by hand.

Results for the main traits characterizing the RSA of Orria x Plaisant population and associated QTLs will be reported.

**A)** **B)**

**Figure 1**. Rhizoslides system. A) 14 sandwiches of A-4 size inside growth chamber, with distilled water at the bottom of the box. B) Roots after 7 days growing in rhizoslides system.

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## Session VIII – Future Challenges and Innovations

# Identification of the genome regions associated with heterosis in hexaploid winter triticale

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**Keywords**: $F_1$ hybrids, genetic diversity, heterosis, hybrid breeding, triticale

The methods of hybrid breeding have been used for many crops to produce cultivars with superior performance. The major problem in hybrid breeding is the fact that so far, the genetic factors determining the occurrence of heterosis remain unknown. Studies of various plant species indicate that one of the most important factors determining the heterotic effect in the $F_1$ generation is the appropriate level of genetic diversity between parental forms. The purpose of this study was to answer the question of whether analysis of genetic variation based on molecular markers representing selected individual chromosomes would be more useful in predicting the effect of heterosis in triticale than using a general marker pool for whole genome.

The plant material consisted of 470 winter triticale breeding lines from Polish breeding companies. Genotyping was performed using the DArTseq technique (Diversity Array Technology, Canberra, Australia). In the first step, chromosomal localization of DNA markers identified for the tested lines was determined based on comparative mapping with genetic maps of wheat, triticale, and rye. The second part involved the analysis of the genetic similarity of the studied objects. This analysis was performed based on DNA markers located on individual chromosomes of triticale. Based on the obtained results forms with the highest degree of genetic diversity were selected for use as parental forms. The effect of heterosis was estimated based on the evaluation of yield per unit area, which was carried out under field experiment conditions. The yield of $F_1$ hybrids was evaluated both in comparison with the mean value for both parental forms (mid-parent, MPH) and the parental form with better parameters (best-parent, BPH). The experiment was repeated twice in subsequent years.

The results showed that chromosome localization of markers used to determine the genetic distance between parental forms used for crosses had a significant effect on the heterosis effect obtained in hybrids. Selection of the parental forms based on analysis of genetic diversity using the general marker pool did not allow to predict the occurrence of the heterosis effect in both years of the study, and the yield of hybrids was lower than that of the parental forms. The obtained results suggest the existence of regions in the triticale genome, whose genetic differentiation between the parental forms determines the predisposition to heterosis occurrence in $F_1$ hybrids. This finding may contribute to the development of hybrid breeding for cereal species where it has caused many problems so far.

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# Improving the phytosanitary efficiency of *Gliding Arc* plasma seed treatment by adding nitrogenous solutions to the process

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**Keywords**: cold plasma, *Gliding Arc*, wheat seed, nitrogen substances, aqueos solution, *Tilletia spores*

In this study we assessed the phytosanitary effect of *Gliding Arc* cold plasma on artificial infected wheat seeds by *Tilletia triticii* spores. The addition of aqueous solutions of nitrogenous substances (agricultural fertilizer calcium ammonium nitrate - CAN and Terra-Sorb® - TER) by spraying under the plasma nozzle during the treatment was used to increase the effectiveness of the treatment. Four plasma exposures were tested: plasma nozzle distance 6 cm, time 2 and 3 min and 10 cm distance time 2 and 4 min.

Overall, better results were recorded for the nitrogenous variants than for single plasma: for CAN by 12.7% and for TER by 8.6%. The germination of *Tilletia* spores was reduced on 47.4% in the best variant (6/3 CAN) compared to the control. Harder exposures did not show a statistical difference between the single plasma and nitrogenous addition variants. For less exposed variants, better results were recorded for variants with the addition of nitrogenous substances compared to single plasma. In the 6/2 TER variant, a decrease in the germination of *Tilletia* spores by 18.2% was recorded compared to the single 6/2, and in the 10/2 CAN variant by 32.5% compared to the single 10/2.

The work confirmed the effect of *Gliding Arc* plasma on reducing the germination of *Tilletia triticii* spores. The possibility of using liquid injection during the plasma process to increase the efficiency of the treatment was verified.

**Germination of *Tilletia triticii* spores (%)**

| Variant | Exposures (cm/min) | | | |
|---|---|---|---|---|
| | **10/2** | **10/4** | **6/2** | **6/3** |
| **CAN** | 53.5 | 71.8 | 67.9 | 47.4 |
| **TER** | 76.4 | 60.7 | 66.5 | 53.2 |
| **Single plasma** | 86 | 56.9 | 84.7 | 50.7 |
| **Control** | 100 | 100 | 100 | 100 |

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