5th Conference on Cereal Biotechnology and Breeding

jointly organized by EUCARPIA Cereal Section

November 4–7, 2019 • Budapest, Hungary

BOOK OF ABSTRACTS

AKJournals AKCongress

ISBN 978-963-454-489-0

Contents

Invited Speakers

Svalbard Global Seed Vault – securing gene bank collections in the Arctic

Åsmund Asdal[*]

Nordic Genetic Resource Centre, Alnarp, Sweden

[*]E-mail: asmund.asdal@nordgen.org

Keywords: plant genetic resources, gene bank, seed, Svalbaard Global Seed Vault

The Svalbard Global Seed Vault was opened in 2008, as a storage facility for duplicates of seed samples that are conserved in gene bank collections. Many gene banks have faced threats that often caused loss of plant genetic resources due to war and conflicts, natural disasters or lack of human or economic resources.

By mid-2019 76 depositor institutes have deposited more than one million seed samples in the Svalbard Global Seed Vault. The seeds in the Vault are the property of the depositor gene banks, and the owner gene bank can have the seeds back whenever they might need them.

ICARDA (International Centre for Agricultural Research in the Dry Areas), formerly having its headquarter in Aleppo, Syria, is the only institute that so far has requested seeds to be returned. In 2015, ICARDA launched an intensive regeneration program to reconstruct its active base collection in Lebanon and Morocco based on material secured in the Seed Vault. Major parts of ICARDAs initial deposits have been returned and significant amounts of new regenerated material have already been re-deposited in the Seed Vault.

Adjusted for withdrawals the current holdings of the Seed Vault (August 2019) is 985,085 seed samples.

The Seed Vault is built, funded and owned by the Norwegian government, which also guarantee for the long term maintenance of the facility and secure conservation of the seeds. The Seed Vault is managed through an agreement between the Norwegian Ministry of Agriculture and Food, the Global Crop Diversity Trust and the Nordic Genetic Resource Centre (NordGen). Crop Trust is partly funding the Seed Vault operations as part of the global conservation system. NordGen is responsible for the management and operation of the Vault.

During 2018 and 2019, a significant technical upgrade of the Seed Vault has been carried out. This includes construction of a new watertight entrance tunnel, new cooling systems and a new technical building adjacent to the Seed Vault.

The Global Durum Panel: a platform for leveraging tetraploid wheat diversity

Luigi Cattivelli[1], Elisabetta Mazzucotelli[1], Marco Maccaferri[2], Anna Maria Mastrangelo[3], Steven Xu[4], Justin Faris[4], Matthew Hayden[5], Penny Tricker[6], Hakan Ozkan[7], Viviana Echeenique[8], Brian Steffenson[9], Ron Knox[10], Sripada Udupa[11], Friedrich Longin[12], Daniela Marone[13], Giuseppe Petruzzino[13], Abdul Aziz Niane[11], Pablo Roncallo[8], Ahmed Amri[11], Hans Braun[14], Karim Ammar[14], Michael Baum[11], Roberto Tuberosa[2], Filippo M Bassi[11]*

[1]CREA-Research Centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, Italy
[2]Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy
[3]CREA-Research Centre for Cereal and Industrial Crops, Bergamo, Italy
[4]United States Department of Agriculture, Agricultural Research Service, Cereal Crops Research Unit, Red River Valley Agricultural Research Center, Fargo, ND, USA
[5]Department of Economic Development, Jobs, Transport and Resources, Agribio Centre, La Trobe Research and Development Park, Bundoora, Vic 3083, Australia
[6]School of Agriculture, Food and Wine, University of Adelaide, Australia
[7]Çukurova University, Faculty of Agriculture, Department of Field Crops, Adana, Turkey
[8]Centro Cientifico Tecnologico CONICET, Bahia Blanca, Argentina
[9]Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA
[10]Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, Swift Current Saskatchewan, Canada
[11]International Center for Agricultural Research in the Dry Areas (ICARDA), P.O. Box 6299, Rabat, Morocco
[12]LSA – Research Group Wheat, University of Hohenheim, Germany
[13]CREA-Research Centre for Cereal and Industrial Crops, Foggia, Italy
[14]International Maize and Wheat Improvement Center (CIMMYT), Texcoco de Mora, Mexico

Genetic diversity is of paramount importance as a source of novel alleles at loci controlling traits of agronomic importance. In durum wheat a global platform to analyse the genetic diversity has been established in the frame of the Expert working group on durum wheat genetics and breeding, part of Wheat Initiative. First, a large evolutionary panel (the Global Tetraploid wheat Collection) made of more than 1800 accessions was developed and characterized after Maccaferri et al. (Nature Genetics 2019). Then, the Global Durum Panel (GDP) made of 1,056 genotypes was established through a cooperative effort of many durum wheat scientists worldwide. GDP consists of 4 subpanels: modern cultivars, landraces including *T. turgidum* subspecies, emmer (cultivated and wild), and lines from the evolution composite cross program from INRA. Following an extensive genotypization through the wheat iSelect 90K SNP assay and a process of selection, nearly 20,000 unique, non-redundant, single Mendelian SNP markers that were both genetically and physically mapped were used to characterise the GDP for genetic diversity, population structure and genetic relationships. The analysis with Admixture revealed a population structure which mirrored the geographic origin of landraces and the breeding programs the durum wheat cultivars belong to. A more refined analysis focused on durum wheat cultivars with respect to the breeding program and

to the year of release. Following the AMOVA analysis, the highest proportion of molecular variance was observed within groups (>88%), when both breeding groups and year of release were considered. Moderate level of genetic diversity was observed for the 12 breeding programs considered, ranging from 0.26 for the Australian group to 0.35 for the Italian modern germplasm. A moderate but continuous decrease of genetic diversity was observed with respect to the year of release, across groups of cultivars released in decades from 1970 to 2018 (from 0.38 to 0.34). Finally, chromosome regions subjected to selection were identified through *Fst*, *XP-CLR*, *XP-EHH*, *hapFLK* metrics, and their gene content studied through marker projection to the recently released sequence of the Svevo durum wheat genome. The combination of genotyping and phenotypic information collected across the Globe will provide a strong tool to identify and exchange useful alleles among breeders.

Chromosome genomics in cereals: looking back and forward

Jaroslav Doležel[*]

Institute of Experimental Botany of the Czech Academy of Sciences,
Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic
[*]E-mail: dolezel@ueb.cas.cz

Keywords: chromosome sorting, flow cytometry, genome sequencing, gene cloning, mitotic chromosomes

Chromosome genomics has been developed to simplify genome sequencing and speed up gene mapping and cloning in plants with complex and polyploid genomes. This approach is based on dissecting the full nuclear genome to its individual chromosomes using flow cytometric sorting. Purification of individual chromosomes provides a lossless DNA complexity reduction and avoids problems due to DNA sequence redundancy, including the presence of homoeologs in polyploids – the situation common in cereals. Flow cytometry requires suspensions of intact mitotic metaphase chromosomes, which are most conveniently prepared from synchronized meristem root tip cells. As flow cytometry classifies chromosomes primarily according to DNA content, chromosomes that do not differ in size cannot be easily resolved and sorted. This obstacle has been overcome by means of chromosome translocation, deletion and alien addition lines from which chromosomes with changed size can be sorted. An alternative approach is to label chromosome DNA repeats by fluorescence *in situ* hybridization in suspension (FISHIS). If none of these approached can be employed, anonymous single chromosomes are sorted, their DNA amplified and DNA samples thus obtained are assigned to chromosomes after sequencing.

As the DNA of flow-sorted chromosomes is intact, they are suitable for a wide range of molecular techniques and sequencing technologies. Thus, chromosome genomics facilitated rapid production of draft genome assemblies for wheat, barley and rye. However, the recent progress in DNA sequencing technologies and bioinformatics makes it possible to avoid complexity reduction and produce high quality reference genomes. The use of flow-sorted chromosomes thus shifted accordingly to the validation of whole genome shotgun assemblies. Other important applications include identification of chromosomes with integrated transgenes, characterization of alien chromatin in introgression lines and targeted development of molecular markers. As the genomes of cereals are large, chromosome sorting is becoming a popular strategy to clone genes. The ability to target particular genome regions brings a significant reduction of costs and allows analyzing a chromosome of interest isolated from multiple genotypes. Recently, two chromosome-based gene cloning approaches, namely MutChromSeq and TACCA (targeted chromosome-based cloning via long-range assembly) have been developed and used to clone important genes. In MutChromSeq, chromosomes isolated from several independent mutants are sequenced to identify induced mutations by comparison to parental chromosomes; beside the chromosome location, no additional genetic mapping is required. TACCA uses a combination of short-read Illumina sequencing and chromosome contact maps of *in vitro* reconstituted chromosomes to generate megabase-sized scaffolds spanning the region harboring the cloned gene.

Acknowledgments
The author has been supported by the ERDF project "Plants as a tool for sustainable global development" (No. CZ.02.1.01/0.0/0.0/16_019/0000827).

Understanding changes in the genetics of bread wheat adaptation over time and between environments

Simon Griffiths[*]

John Innes Centre
[*]E-mail: simon.griffiths@jic.ac.uk

The global spread of agriculture has driven fascinating changes in the developmental profile of our crops. The main driving force for these changes has been adaptation and performance. In this presentation I will describe major wheat genes and QTL from which allelic variation has been used to generate these differences. These genes can mostly be through of height and flowering time effects but the range of phenotypes they influence is so broad that this does not do them justice. Their impact in terms of crop survival, optimal adaptation, stress tolerance, and grain yield is the motivation for this research. In the presentation I will describe how we are trying to capture this variation from elite and historic germplasm for systematic deployment in plant breeding programmes and new crop designs for adaptation in diverse mega-environments.

Crop phenomics: Diving into the „private life of crops”

Andreas Hund[*], *Lukas Kronenberg, Jonas Anderegg, Lukas Roth, Norbert Kirchgessner, Helge Aasen, Achim Walter*

Crop Science, ETH Zurich, Switzerland
[*]E-mail: andreas.hund@usys.ethz.ch

Keywords: phenotyping, abiotic stress, genotype–environment–interaction, crop models

In his BBC documentation „The Private Life of Plants”, David Attenborough presented the growth of plants in time-lapse sequences. Crop phenomics aims to bring this spirit to the breeders. The term “phenomics” relates to high-dimensional observable characteristics of an organism (*1*). Breeders stack multiple small-effect alleles in a recurrent selection process. As the effect of such alleles is often environment-dependent, breeders need to test candidate varieties under a wide range of field conditions. Optimally, these conditions should resemble all potential stress scenarios occurring in the production environment to identify varieties with high and stable yield. Unfortunately, yield testing is expensive, only possible towards the end of the breeding cycle and never covers all possible environmental extremes. For this reason, breeding research aims to identify crop ideotypes consisting of a combination of morphological and physiological traits conferring a satisfying adaptation to the target environment (*2*).

This is where phenomics or more specifically high-throughput phenotyping comes into play. The technology combines automation, new sensors, artificial intelligence and crop models, developed in the past decades. The development has started with fully automated indoor platforms. These platforms are often used to follow the stress-response of individual plants using repeated, non-destructive measurements. As indoor platforms cannot fully mimic all relevant pedoclimatic conditions, field testing is indispensable to prove the relevance of the measured traits. Since about 10 years, high-throughput field phenotyping (HTFP) is rapidly advancing (*3*). We believe that this technology will play an important role to understand genotype-by-environment interaction, cross-validate results of controlled environment experiments and improve selection efficiency. HTFP allows monitoring thousands of plots repeatedly to quantify, for example, the development of canopy cover, height, senescence, canopy temperature and the timing of critical developmental stages. In combination with environmental covariates, the response of these traits to changes in weather patterns can be followed throughout the season. Thus, a combination of envirotyping and continuous phenotyping helps to dissect the cumulative effects of small stresses during the growing season on crop development. Often the effect of these traits on yield are not immediately obvious, as their effect of responsiveness on yield is small and environment-dependent. It will be difficult for breeders to integrate them into a “binary, yes/no decision on which candidates to keep and which candidates to discard” as pointed out by Bernardo (*4*). Thus, the traits need to be included into crop models predicting environment-specific yield. However, the development of appropriate phenotyping technologies, assessing relevant traits to feed crop ideotype models is still in its infancies. We will give an overview on the development of crop phenomics as a discipline and will highlight its potential with examples from the field phenotyping platform FIP and from the phenofly platform of ETH Zurich.

References

1. D. Houle, D. R. Govindaraju, S. Omholt, *Nat. Rev. Genet.* **11**, 855–866 (2010).
2. P. Martre *et al.*, *Model-assisted phenotyping and ideotype design* (Academic Press Ltd-Elsevier Science Ltd, London, 2015).
3. A. Hund, L. Kronenberg, J. Anderegg, K. Yu, A. Walter, in *Advances in breeding techniques for cereal crops*, F. Ordon, W. Friedt, Eds. (Burleigh Dodds Science Publishing, Cambridge, UK, 2019; http://dx.doi.org/10.19103/AS.2019.0051.13), p. in press.
4. R. Bernardo, *Theor. Appl. Genet.* **129**, 2323–2332 (2016).

The *in planta* Particle Bombardment (iPB) method for cereal transformation and genome editing

Ryozo Imai[1], Haruyasu Hamada[2], Yuelin Liu[1], Qianyan Linghu[1], Yuya Kumagai[1], Ryuji Miki[2], Yozo Nagira[2], Naoaki Taoka[2]*

[1]Institute of Agrobiological Sciences, NARO, Tsukuba, Japan
[2]Biotechnology Development Laboratories, KANEKA Corp, Takasago, Japan
[*]E-mail: rzi@affrc.go.jp

Keywords: CRISPR/Cas9, genome editing, particle bombardment, SAM, transformation, wheat

Transformation is a key element for new breeding technology including genome editing. In many crop species, requirement of *in vitro* culture and regeneration processes hampers application of the transformation methods to commercial important varieties. It is therefore ideal to develop a novel transformation method that is independent of tissue culture and regeneration. To this end, we have developed a simple and reproducible *in planta* transformation method in wheat, using biolistic DNA delivery. Shoot apical meristems (SAMs) contain a subepidermal cell layer called L2, from which germ cells later develop during floral organogenesis. Therefore, the L2 cells can be an excellent target for the introduction of heritable genome modifications. SAMs were exposed from embryos of imbibed mature seeds and bombarded with the *GFP* gene and grown to fifth leaf stage, where DNA integration was tested by PCR. Out of 577 bombarded plants, five showed transgene integration and one showed inheritance to T1 generation. We successfully transformed the model wheat cultivar 'Fielder', as well as the recalcitrant Japanese elite cultivar 'Haruyokoi'. Gold particles coated with plasmids expressing CRISPR/Cas9 components designed to target to the *TaGASR7* gene were bombarded into SAM-exposed embryos of imbibed mature seeds. Mutations in the target gene were assessed in fifth-leaf tissue by cleaved amplified polymorphic sequence (CAPS) analysis. Eleven (5.2%) of the 210 bombarded plants carried mutant alleles, and the mutations of three (1.4%) of these were inherited in the next generation (T1). Genotype analysis of T1 plants identified plants homozygous for the three homeologous genes. These plants showed no detectable integration of the Cas9 and guide RNA genes, indicating that transient expression of CRISPR/Cas9 introduced the mutations. As gold particles can bind proteins as well as DNA/RNA, we next tried to introduce CRISPR/Cas9 ribonucleoprotein (RNP) directly into SAM for genome editing. Together, our current method can be used to achieve *in planta* genome editing in wheat using CRISPR/Cas9 and suggests possible applications of genome editing to other recalcitrant plant species and variations.

References

1. Hamada, H, Linghu, Q, Nagira, Y, Miki, R, Taoka, N and Imai, R (2017) An in planta biolistic method for stable wheat transformation. Sci Rep 7: 11443.
2. Hamada, H, Liu, Y, Nagira, Y, Miki, R, Taoka, N and Imai, R (2018) Biolistic-delivery-based transient CRISPR/Cas9 expression enables in planta genome editing in wheat. Sci Rep 8: 14422.

New breeding strategies and bioinformatic tools in cereals

Viktor Korzun[*]

KWS SAAT SE & Co. KGaA, Grimsehlstr. 31, 37574 Einbeck, Germany
[*]E-mail: https://www.kws.com/corp/en/

The growing population of the 21st century is placing increasing demand on our food production, with added pressures from diminishing resources and a more variable growing climate. In this context, genomics and associated molecular marker technology must play an important role in developing new varieties better adapted to address these key challenges.

Conventional breeding is time-consuming and strongly 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 and together with the rapid accumulation of genomics tools and the emergence of high throughput technologies and powerful statistic and bioinformatic tools has remarkable facilitated practical implementation into cereal breeding programmes.

The availability of new molecular tools and technologies is beginning to filter through the breeding programmes to have a significant impact on plant variety development and is proving to be the essential element required to accelerate this process and increase the genetic gain. The results of specific applications of molecular markers, genomic selection and genomics in cereals will be demonstrated and discussed.

Wheat genotypes and their interaction with plant-beneficial bacteria

Jordan Valente[1], Cécile Gruet[1], Florence Gerin[1], Agathe Mini[2], Rohan Richard[2], Séverine Rougeol[2], Jacques Le Gouis[2], Andreas Börner[3], Daniel Muller[1], Claire Prigent-Combaret[1], Yvan Moënne-Loccoz[1]*

[1]UMR CNRS 5557 Ecologie Microbienne, Université Lyon 1, 69622 Villeurbanne, France
[2]GDEC, INRA, UCA, 63000 Clermont-Ferrand, France
[3]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 06466 Gatersleben, Germany
[*]E-mail: yvan.moenne-loccoz@univ-lyon1.fr

Keywords: Plant Growth-Promoting Rhizobacteria (PGPR), rhizosphere, symbiosis, QTL, wheat history

Wheat roots interact with the soil microbial community, and a fraction of this root microbiome can have positive effects on plant growth, health and stress tolerance. However, wheat genotypes may differ from one another in their ability to interact with bacteria, including with Plant Growth-Promoting Rhizobacteria (PGPR). Within bread wheat (*Triticum aestivum*), the accessions most effective at promoting bacterial proliferation on roots and functioning of the phloroglucinol-producing PGPR *Pseudomonas kilonensis* F113 were not the same as the accessions interacting best with the indole-3-acetic acid-producing PGPR *Azospirillum brasilense* Sp245. Genome-wide association pointed to different genomic regions potentially involved in the interaction with these PGPR strains, and in the case of *A. brasilense* Sp245 it resulted in the identification of 22 regions associated with the expression of indole-3-acetic acid biosynthetic gene *ppdC*. In addition, the range of bread wheat accessions stimulated by *P. kilonensis* F113 or *A. brasilense* Sp245 differed. When considering PGPR interactions at a larger scale across the evolutionary history of wheat, it appears that durum wheat *(Triticum durum)* differed from its ancestors *Triticum urartu* and *Aegilops speltoides* based on higher recruitment of ACC deaminase bacteria or nitrogen-fixing bacteria by roots. Bread wheat differed from durum wheat but not from its other ancestor *Triticum tauschii*. A better understanding of wheat traits involved in the interactions with plant-beneficial bacteria would be useful for the development of varieties maximizing the added-value of these bacteria.

Acknowledgments
Support from the ANR projects BacterBlé (ANR-14-CE19-0017) and BreedWheat (ANR-10-BTBR-03) is acknowledged.

References
Valente, J., F. Gerin, J. Le Gouis, Y. Moënne-Loccoz, C. Prigent-Combaret. 2019. Ancient wheat varieties have a higher ability to interact with plant growth-promoting rhizobacteria. Plant, Cell & Environment https://doi.org/10.1111/pce.13652.

Use of cytoplasm of relative wild species for wheat breeding against gloval climate change

*Murai Koji**

Fukui Prefectural University
*E-mail: murai@fpu.ac.jp

Alloplasmic lines or cytoplasmic substitution lines having alien cytoplasm are created through recurrent backcrossing, for example *Aegilops* species as a female parent and bread wheat (*Triticum aestivum*) lines as a recurrent male parent. The alloplasmic lines have alien cytoplasm, resulting in a mismatch of the nuclear and cytoplasmic genomes which have co-evolved together in normal cytoplasm (euplasmic) lines. The alloplasmic lines often show the phenotypic alterations compared with the euplasmic lines because of the genome mismatch. The most well known phenotype in alloplasmic lines is cytoplasmic male sterility (CMS), which is useful for hybrid breeding. In wheat, the cytoplasms of *T. timopheevii*, *Ae. kotschyi* and *Ae. crassa* have been reported as the CMS inducing cytoplasm. Here I report that the alloplasmic lines having the cytoplasm of *Ae. geniculata* or *Ae. mutica* show delayed flowering compared with the euplasmic lines. In the wheat flowering pathway, *VERNALIZATION 1* (*VRN1*) encoding an APETALA1 /FRUITFULL-like MADS box transcription factor plays a central role in the activation of the florigen genes that induce floral meristems in the shoot apex. The alloplasmic wheat line showed a lower level of *VRN1* expression even after vernalization compared with the euplasmic line through the epigenetic regulation. I developed alloplasmic lines of several Japanese bread wheat cultivars with *Ae. mutica* cytoplasm to examine the effects of cytoplasm on flowering. All alloplasmic lines showed delayed heading time (5 to 15 days in the field), and the degree of heading delay depended on genotype. In central to southwestern Japan, autumn-sown early-heading spring wheat cultivars with *VRN-D1* are cultivated. In warm winter, the spring wheat cultivars transit reproductive growth phase at very early spring, leading to decleased tiller/ear numbers and low yeild performance. The alloplasmic lines with *Ae. mutica* cytoplasm can suppress the decrease in ear number during warm winter due to the delaied flowering. The alloplasmic lines should be useful for the development of varieties adapted to global warming.

Harnessing genetic resources – The key for improving disease resistance in cereals

Frank Ordon

Julius Kühn-Institute (JKI), Erwin-Baur-Str. 27, 06484 Quedlinburg, Germany

Wheat and barley are of prime importance for feeding the earth´s growing population. However, both are hit by many pathogens causing severe yield losses. Therefore, in order to ensure a sufficient cereal production today and in the future, improving resistance to biotic stress is an important task. In this respect, genetic resources are a valuable treasure trove. For using this genetic diversity in breeding, it is of prime importance (i) to screen genetic resources for resistance, (ii) develop molecular markers for these resistances, and (iii) use these for an enhanced introgression of resistances into adapted cultivars. Today, many molecular tools are available facilitating an efficient use of these genetic resources in breeding. While in the past marker development was time consuming and laborious, genomic resources like the Infinium iSelect genotyping bead chips, genotyping by sequencing (GBS) and the availability of reference sequences in barley and wheat facilitate efficient marker development for major genes and quantitative trait loci (QTL) up to enhanced gene isolation, today. The isolation of genes involved in resistance will transfer breeding to the allele level and will facilitate the sequenced based identification of novel alleles in large gene bank collections and their directed use in wheat and barley breeding as well as site directed mutagenesis. Examples of genomics based harnessing of genetic resources up to gene insolation for improving resistance to fungal and viral pathogens as well as to insects in wheat and barley are given.

Genetic regulation of barley development under abiotic stress and its impact on yield in a world-wide field study

Prof. Dr. Klaus Pillen

University of Halle

Since the dawn of agriculture, crop yield has always been impaired through abiotic stresses. In a field trial across five locations worldwide, we tested three abiotic stresses, nitrogen deficiency, drought and salinity, using HEB-YIELD, a selected subset of the wild barley nested association mapping population HEB-25.

We showed that barley flowering time genes *Ppd-H1*, *Sdw1*, *Vrn-H1* and *Vrn-H3* exert pleiotropic effects on traits related to plant development and grain yield. Under field conditions, these effects are strongly influenced by environmental cues like day length and temperature. For example, in Al-Karak, Jordan, the day length-sensitive wild barley allele of *Ppd-H1* was associated with an increase of grain yield by up to 30% compared to the insensitive elite barley allele. The observed yield increase is accompanied by pleiotropic effects of *Ppd-H1* resulting in shorter life cycle, extended grain filling period and increased grain size.

Our study indicates that the adequate timing of plant development is crucial to maximize yield formation under harsh environmental conditions. We provide evidence that wild barley germplasm, introgressed into elite barley cultivars, can be utilized to secure and increase grain yield under abiotic stress conditions. The presented knowledge may be transferred to related crop species like wheat and rice to secure the rising global food demand for cereals.

Big data strategies for predicting hybrid performance in wheat

*Jochen C. Reif**

Department of Breeding Research, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Corrensstraße 3, 06466 Stadt Seeland, Germany
*E-mail: reif@ipk-gatersleben.de

The development of affordable high-throughput 'omics'-platforms increased the speed, the velocity and the variety of data generated in wheat breeding programs. Breeders aim to exploit this wealth of 'omics' data in order to boost selection gain. Increasing wheat yield is a key global challenge to produce sufficient food for a growing human population.
One potential breeding strategy to enhance wheat yield and yield stability is through the exploiting of heterosis by breeding hybrid instead of line varieties. Wheat hybrids are currently cultivated on only <1% of the global acreage. One reason of the lack of success in hybrid wheat breeding so far is the challenge to efficiently select superior hybrids out of millions of potential single cross-combinations. Genomic selection has been suggested as a promising approach to resolve these limitations.

In my presentation, I will discuss the potential and limits of genome-wide prediction of the hybrid performance. Starting from a comprehensive hybrid wheat data set representing one breeding cycle (Zhao et al. 2015 PNAS 112:15624-15629), the driving forces of the prediction accuracies will be highlighted for genome-wide prediction models based on additive, dominance, and epistatic effects. Prediction accuracies will be studied then across breeding cycles making use of a further comprehensive hybrid wheat data set. In particular, we will explore the opportunity to forecast the prediction accuracy of individual hybrids. Finally, opportunities to integrate genome-wide prediction approaches in hybrid wheat breeding programs are discussed.

From genome to pan-genome in barley and wheat

Nils Stein[*]

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
[*]E-mail: stein@ipk-gatersleben.de

Genomics based-breeding and research in molecular genetics and developmental biology was lagging behind in barley and wheat, two major crops in Europe and the world, because high quality genome sequences were missing. This has changed now, as for both species annotated high-quality reference chromosome-scale sequence assemblies were made available recently. In both cases the first reference sequence assemblies are representing a big leap forward, however, these assemblies are by far not representative for all domesticated genome diversity in both species. Thus in order to efficiently unlock genomic diversity for research and breeding, international efforts were initiated to describe the pan-genomes of barley and bread wheat by producing multiple high quality chromosome-scale assemblies. In addition, entire diversity collections of wheat and barley comprising several ten thousands of accessions are currently being genotyped by sequence-based methods. The presentation will report on the status of this research in the small grain cereals and provide detailed insights into the level of genome diversity of these major crop species.

Oral Presentations

Exhibitor's talks

Artificial intelligence for genomics: history and current state

Sergii Shelpuk[*], *Ruslana Radchuk*

DeepTrait, Lublin, Poland
[*]E-mail: sergii.shelpuk@deeptrait.ai

Keywords: deep learning, artificial intelligence, genomic data, phenomic data, crop plants, genetic markers

The modern, deep learning-based artificial intelligence has revolutionized advertisement, retail, healthcare, manufacturing, call center, and many other industries. The research in novel AI algorithms and technologies grows exponentially, the number of AI papers published since 2010 exceeds 60 000 among which 17 000 were presented in 2018 only. However, very few of them deal with genomic data. To the day, there are only about 300 papers dedicated to applying modern AI techniques to genomes.

In 2012, the victory of AlexNet model at the image recognition competition ImageNet Large Scale Visual Recognition Challenge marked the turning point in AI development. In this competition, a novel, deep learning neural network outperformed the most advanced previous generation machine learning (ML) models with 10% accuracy. Since then, deep learning became a standard for AI applications for computer vision, natural language understanding, and speech recognition completely replacing ML in these areas.

ML methods work not with the data directly but with data representations. ML does not work with pixels, sound frequencies or base pairs, it works with features, hand-designed descriptors of the raw data. For images, these descriptors are SIFT, SURF, HOG, and others, a highly complex, expert-built algorithms aimed to represent the picture it a way understandable for ML. These descriptors were very hard to invent and build; SIFT, for example, was developed for about 10 years. Some of them are protected by patents and require licensing for commercial use. In genomics, the same descriptors are genetic markers: SNPs, indels, known genes. They share the same problems as the computer vision descriptors: are hard to build, expensive, protected by IP law, and require human expertise.

In contrast, modern deep learning methods do not require descriptors, they work with images at the pixel level and provide better performance than descriptor based ML. Today, virtually all state of the art in AI for computer vision is based on deep learning.

Among all available AI applications for genomics, the vast majority use ML and descriptors: SNP markers, genes, sometimes indels and k-meres. Very few of them apply deep learning to the genome at the basepair level. And even they analyze only short subsequences up to 20 thousand base pairs.

DeepTrait presents the first available deep learning technology which can work with a full genome of hundreds of millions basepair long and find perfect markers causing a specific trait directly out of the sequence data. The publically available results of DeepTrait technology testing were obtained with open *E.coli* and *Arabidopsis thaliana* genomic datasets. In these experiments, our goal was to determine genetic determinants of antibiotics resistance in *E.coli* and genetic mechanisms controlling flowering time of *A. thaliana*. The results confirm that by having only few hundred sequenced and phenotyped genomes, our technology can find majority of already known phenotype-associated genes and genomic elements causing a particular trait along with currently unknown or understudied genome elements tied to this trait.

The advantages of a highly relational database for plant breeding programs

Dieter K Mulitze[1*]

[1]Agronomix Software, Inc, Winnipeg, MB, Canada
[*]E-mail: mulitze@agronomix.com

Keywords: plant breeding, databases, information technology

Plant breeding programs typically generate considerable amounts of data, especially when there are many crosses and descendants over generations. Aside from managing the records, the more complex the linkage or relation of all the data, then the greater the possibilities for the breeder in developing superior hybrids or varieties. An obvious linkage would be finished marker and related data to genotypes and phenotypic data, whether in experiments or nurseries. The relational links as a 'history' from parents to crosses and down all generations with data and the breeding events would help to monitor the response to selection and enable more optimal breeding decisions, especially if using more complex data queries and index selection in any or all generations. For any nursery or experiment, the ability to instantly link data from either or both parents to each population, plant or cross in a open data view would also help in selections. When assessing any parent or inbred, the ability to easily view all crosses involving that genotype, its descendants and performance with other geneotypes can help in planning future crosses. With trials data across years, data queries for any genotype or a group of genotypes with any or all data from all years and locations will help in the final stages of advancement towards releasing a variety. The goal is to mine the database for the most optimal decisions. This should improve genomic prediction by utilizing the most complete dataset. But experiments and locations have environmental and management factors that ideally should be linked as well, even for all experiments or nurseries across an entire year with a few clicks. This in turn facilitates more advanced meta analyses. The advantages become obvious when phenotypic, genetic and environmental data can all be relationally linked in one data view.

Search tools are enhanced by a more relational database. You can only find all experiments where an entry has been tested with a few clicks if all the data is linked. Or, find all the experiments or nurseries that have data for a given trait. Perhaps a new disease has appeared and a breeder wants to see all data taken for that trait. Breeders typically rename a genotype from some internal breeder's code to a new name for the released variety. If the database is linked with 'keys', then one can make the name change in one place in the database and this is then instantly replicated through the entire database. Open any experiment or nursery, and the new name will appear. The same is true for the name of a trait. If 'keys' are indeed used consistently in the entire database, then when you change the name of the trait in one place, all experiments, nurseries, germplasm info and more all works perfectly with the new trait name. This all saves time and effort for the breeder.

The above advantages of a highly relational database for plant breeding will be presented using the new Genovix software for plant breeding and variety testing. The software has a Microsoft SQL back-end database and a modern .NET interface.

T1: Plant Genetic Resources for Future Breeding

The genetic basis of grain shape along barley spike

Ahmad M. Alqudah[*]

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)
[*]E-mail: alqudah@ipk-gatersleben.de

Grain shape and weight are important components of barley yield. Grain homogeneity in shape and weight is also crucial for improving grain quality. In general, two-rowed (*VRS1*) barley has more uniform grain than six-rowed (*vrs1*) which makes it preferable for malt production. Natural variation in grain shape uniformity along the spike in diverse collections could be valuable sources for crop improvement. We, therefore, studied the uniformity in grain shape and weight along the spike (basal *vs.* apical and central *vs.* lateral) in a natural barley collection to detect desirable uniformity and yield allele(s). We discovered a clear basal-to-apical gradient along the spike in grain shape and weight. Regardless of row-type, basal part of barley spike has more uniform grain than the apical part. The wild-type (*VRS1*) grains had significantly wider grains and increased thousand kernel weight (TKW) while *vrs1* grains had longer grains with increased grain area. Grain area and grain length showed more natural variation than other traits especially in the apical part of the spike and in six-rowed accessions. Moreover, the central grain in six-rowed showed high variation in grain area and length, while the natural variation in grain width and TKW was much clearer in the basal part and two-rowed barley. The observed significant differences in grain shape imply a genetic link between the grain traits, which was validated from the association of *vrs1* locus with longer grain and grain area. We used state-of-the-art genome-wide association bioinformatics tools to suggest candidate genes underlying the natural variation of the uniformity in grain shape along the spike. Interestingly, genes encoding SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) proteins appeared highly associated with grain uniformity along the spike. These findings provide an important foundation for uncovering the biological functions of *HvSPLs* suggesting their pivotal role in regulating agronomic traits. Exploitation of natural variation in grain shape and weight will be valuable sources of natural trait-enhancing alleles for crop breeding.

Insights into influence of domestication and selection on the rye *(Secale L.)* genome based on GBS genotyping of diverse accessions with different origin and improvement status

Anna Hawliczek[1], Ewa Borzęcka[1], Nikolaos Alachiotis[2], Katarzyna Tofil[1], Piotr Gawroński[1], Dörthe Siekmann[3], Bernd Hackauf[4], Roman Dušinský[5], Miroslav Švec[5], Hanna Bolibok-Brągoszewska[1]*

[1]Department of Plant Genetics Breeding and Biotechnology, Warsaw University of Life Sciences-SGGW, Warsaw, Poland
[2]Institute of Computer Science, Foundation for Research and Technology-Hellas, Heraklion, Crete, Greece
[3]HYBRO Saatzucht GmbH and Co. KG, Schenkenberg, Germany
[4]Julius Kühn-Institut, Groß Lüsewitz, Germany
[5]Department of Genetics, Comenius University in Bratislava, Bratislava, Slovakia
[*]E-mail: hanna_bolibok_bragoszewska@sggw.pl

Keywords: genetic diversity, population structure, domestication, selective sweeps, GBS, Secale, rye

During domestication processes and subsequent breeding practices crop plants were subjected to intensive selection for certain desirable traits, such as high and stable yield. Selection pressure causes changes in polymorphism level and in consequence the chromosome segments containing genes subjected to positive selection exhibit lower polymorphism in more advanced germplasm. The combined effects of the domestication bottleneck, recurrent use of adapted germplasm, and avoidance of wide genetic recombination in breeding schemes resulted in an overall reduction of genetic diversity of the elite
germplasm in major crops in comparison to the wild forms. Identification of genome regions targeted by selection and therefore displaying low diversity, coupled with a detailed characterization of the genetic diversity structure of less adapted germplasm, has the potential to facilitate the use of unadapted germplasm in breeding programs by providing a basis for targeted broadening of diversity in defined genome regions.

The aim of this study is to identify and to characterize genome regions targeted by selection during domestication and breeding in rye (*Secale cereale* L.). We assembled a diverse set of 480 rye accessions representing different geographic origins and improvement status, and performed an in-depth analysis of genetic diversity patterns, population structure and phylogenetic relationships based on ca. 13 000 high quality SNP (DArTseq) markers, selected from almost 80 000 SNPs differentiating the accessions. A clear separation into three groups was observed: (i) the most divergent *S. sylvestre* group, (ii) *S. strictum* group, and (iii) *S. cereale* (including all cultivated accessions) + *S. vavilovii* group. According to expectations, modern cultivars and breeding materials turned out to occupy a small area of diversity space, while historical varieties and landraces were much more diverse. Comparative analysis of MAF and PIC values' distribution along chromosomes revealed several regions characterised by contrasting values in germplasm groups with distinct improvement status. We will also present the results of selective sweep detection obtained with OmegaPlus, SweeD, and RAiSD.

In parallel, we pursue also a sequence-homology based approach of gene identification and to date we identified putative rye orthologs of domestication genes *Q*, *GIF1*, *BTR2*, *Rht-B1*, *VRN1*, and *VRN2*. Currently, we focus on obtaining full genomic sequences for these genes using a BAC library of rye inbred line L318 for future use during analysis of their allelic variation in diverse rye accessions.

Acknowledgments
This research was founded by the Polish National Science Centre grant No. DEC-2014/14/E/NZ9/00285

The discovery of the tandem kinase-pseudokinase protein family inspired by the cloning of the wheat stripe rust resistance gene *Yr15*

Tzion Fahima[1,2], *Valentina Klymiuk*[1,2], *Andrii Fatiukha*[1,2]

[1]Institute of Evolution, University of Haifa, Haifa, Israel
[2]The Department of Evolutionary and Environmental Biology, University of Haifa, Haifa, Israel
*E-mail: tfahima@evo.haifa.ac.il

Keywords: Yellow rust resistance, tandem kinase-pseudokinase, wild emmer wheat.

Wheat is one of the most important food crops for human consumption globally. Breeders are making efforts to produce high yielding modern varieties, however, the productivity of a particular cultivar can be severely restricted by its susceptibility to diseases, such as stipe rust, caused by the fungus *Puccinia striiformis* f. sp. *tritici* (*Pst*). To overcome this problem, disease resistance genes from wheat wild relatives can be introgressed into elite cultivars. *Yr15*, is a wild emmer wheat (*Triticum dicoccoides*) gene, known to carry high resistance to a wide range of *Pst* isolates. Comparative genomics, chromosome walking, BAC libraries (wild emmer and bread wheat), whole genome assemblies, EMS mutagenesis and transgenic approaches enabled us to clone *Yr15* and validate its function. Analysis of the protein encoded by *Yr15* revealed that it has a putative kinase-pseudokinase structure, designated as Wheat Tandem Kinase 1 (*WTK1*) (Klymiuk et al. 2018; Nature Communications). The available reference wheat genomes, Chinese Spring, Svevo and Zavitan, have paved the way for the discovery of a unique protein family. *WTK1* orthologs and paralogs are found in all group 1 and 6 wheat chromosomes. The exon-intron structure of orthologues copies is similar to that of *Wtk1* from *Yr15* donor accession G25, but differ in numerous SNPs and indels that cause changes in reading frames. Although exon-intron structure of the paralogues copies (6A, 6B and 6D) is similar to *Wtk1,* the total number of exons was increased from six to seven due to the split of exon 4 into two. The unique gene architecture of *WTK1* was found in 92 putative proteins across the plant kingdom, including the barley RPG1 and a candidate for *Un8*, suggesting that they are members of a distinct family of plant proteins, termed here tandem kinase-pseudokinases (TKPs). We found that 175 out of 184 kinase/pseudokinase domains of these TKPs were associated with receptor-like kinases (RLKs), suggesting that TKPs are involved in plant defense mechanisms. The decoy role can be proposed as one of the potential model of function of the pseudokinases of TKP family members in immune response. A further phylogenetic analysis indicated that TKP family members originated from either gene duplication or gene fusion events, suggesting a polyphyletic origin of the TKPs. The presence of kinase-pseudokinase structure in plant TKPs and animal Janus kinases (JAKs), is suggesting convergent molecular evolution of proteins involved in immunity in both of the kingdoms.

Current achievements in study of black-spiked barley

Anastasiia Yu. Glagoleva[1], Sergey R. Mursalimov[1], Natalya V. Gracheva[2], Nickolay A. Shmakov[1], Natalya M. Levanova[1], Andreas Börner[3], Dmitry A. Afonnikov[1], Olesya Yu. Shoeva[1], Elena K. Khlestkina[1,4]*

[1]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia
[2]Volgograd State Technical University, Volgograd, Russia
[3]Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
[4]N. I. Vavilov All-Russian Research Institute of Plant Genetic Resources, Saint-Petersburg, Russia
[*]E-mail: glagoleva@bionet.nsc.ru

Keywords: *Hordeum vulgare*, melanin, black seed, near-isogenic lines, chloroplasts, albinism

In barley (*Hordeum vulgare* L.), several types of grain pigmentation caused by phenolic compounds were described. The well-known ones are purple and blue, associated with the anthocyanins biosynthesis in grain pericarp and aleurone layer, respectively. Black pigmentation of hulls and grain pericarp is much less studied. The pigment responsible for the black spike has long been suspected to be a melanin. The involvement of flavonoid biosynthesis pathway in barley melanin formation was previously excluded. Melanin synthesis in barley spike is under monogenic control of the *Blp* locus mapped on chromosome 1HL. Recently, the locus was narrowed down to 21 genes, but the candidate genes have not been identified yet.

The aim of the current study was to find out chemical nature of black pigments, mechanisms underlying their synthesis and to reveal the genes responsible for black spike trait. The study was performed using the precise genetic model, the near-isogenic barley lines (NILs): black-colored i:Bw*Blp* carrying the *Blp* locus and the uncolored recurrent parent (cv. Bowman).

We extracted black pigments from grains of the NIL demonstrated their melanic nature. The positive identification was supported by an FT-IR spectroscopy analysis, which revealed the presence of phenolic fragments, quinone and an aromatic carbon backbone characteristic of melanin. The comparative microscopy analysis of grains at early, soft and hard dough developmental stages revealed the intracellular dark structures in cells of pericarp and hulls of the i:Bw*Blp* line starting from early dough stage. Co-localization of the dark structures and red autofluorescence signal of chlorophyll was observed in the same organelles. Thus, the melanin pigments are formed and accumulated in chloroplasts. The comparative RNA-seq analysis of NILs in hulls and grain pericarp revealed the influence of *Blp* locus to expression more than thousand genes. Among them, the genes belonging phenylpropanoid and fatty acids biosynthesis pathways were the most represented and upregulated in black-colored NIL. To study the relationships between chlorophyll and melanin biosynthesis we developed the hybrid line i:*Blpalm* characterized simultaneously by partial chlorophyll insufficiency and accumulation of melanins in spike. The fact of obtaining such line allows suggesting chlorophyll/photosynthesis-independent melanin synthesis.

We hypothesized that the polyphenol oxidase (PPO) could have a potential role in melanin formation in intact barley spike. Investigation of PPO gene family in barley revealed four functionally active *Ppo* genes. In addition to previously described *Ppo1* and *Ppo2* mapped

to chromosome 2H, we identified the *Ppo3* and the *Ppo4* genes located on chromosomes 3H and 4H, respectively. The comparative analysis of the *Ppo* genes expression in hulls and grain pericarp at the different stages of spike development demonstrated the relationship between the *Ppo2* gene expression and the stage of melanin pigment appearance.

Acknowledgments
The comparative RNA-seq, microscopy analysis and study of the barley *Ppo* gene family were supported by RSF grant №16-04-00086; development and comparative study of the hybrid NIL with partial albinism and melanin in spike was supported by RSF grant №19-76-00018.

Leveraging phenotypic diversity to boost barley genetic resources utilization

Maria Y. Gonzalez[1], Stephan Weise[2], Yusheng Zhao[1], Norman Philipp[1], Daniel Arend[1], Andreas Börner[2], Markus Oppermann[2], Andreas Graner[2], Jochen C. Reif[1], Albert W. Schulthess[1]*

[1]Department of Breeding Research, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), D-06466, Gatersleben, Germany
[2]Department of Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), D-06466, Gatersleben, Germany
[*]Email: reif@ipk-gatersleben.dereif@ipk-gatersleben.de

Keywords: unbalanced data, genetic resources, data quality assessment

Genebanks host oustanding genetic diversity for research and plant improvement. However, restricted resources limit the systematic trait performance assessment. In this context, historical non-orthogonal data of seed regeneration trials is valuable to leverage phenotypic diversity at no extra cost. This study includes records for 12,872 accessions of the IPK barley collection involving phenotypic data for relevant agronomic traits such as: flowering time, plant height, and thousand-grain weight. This information was gathered for seven decades during the seed regeneration routine. The data was analyzed using linear mixed models with a rigorous quality assessment based on plausibility checks, outlier correction, and studying the potential bias on estimating first- and second-degree statistics. Heritability estimates ranged from 0.83 to 0.92, and precision in computing the best linear unbiased estimations (BLUEs) exceeded 0.85. The data is publicly available following the FAIR principles referring to: Findability, Accessibility, Interoperability, and Reusability. In the future, the phenotypic information will be involved in a genomic prediction approach to boost the utilization of genetic resources.

Acknowledgments
The Federal Ministry of Education and Research of Germany is acknowledged for funding (grant FKZ-031B0184A (AWS) and FKZ031B0190A (MYG)).

References
Gonzalez, M.Y. et al. Unbalanced historical phenotypic data from seed regeneration of a barley *ex situ* collection. Scientific Data 5, 180278 (2018).

Location of recessive genes associated with abnormal wax formation in rye

Magdalena Góralska[1*], *Anna Bienias*[1], *Natalia Lenarczyk*[1], *Ilona Czyczyło-Mysza*[2], *Beata Myśków*[1]

[1]Department of Genetics, Plant Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Poland
[2]The Franciszek Górski Institute of Plant Physiology, Polish Academy of Science, ul. Niezapominajek 21, 30-239, Cracow, Poland
[*]E-mail: magdalena.goralska@zut.edu.pl

Keywords: transcriptome analysis, wax layer, inbred line, rye

Rye is a species enabling the development of poor quality agricultural land on which other cereals fail to grow. This is due to the low requirements for soil richness and resistance to both biotic and abiotic stress. Drought and disease tolerance may be due in part to the presence of a coat of wax that gives rye a bluish color. The genetic background of rye wax production is poorly understood. One gene controlling wax formation on leaves, stems and ears (wa1) whose mutated allele determined the plant without wax, was mapped on chromosome 7R.

The purpose of this study was to determine the chromosomal location of recessive genes associated with the formation of an abnormal wax coating on genetic maps of three mapping populations. Maps were constructed using DArTseq, RAPD, SCAR and SSR markers. The research material were F2 generations of three interline cross of rye: K916 × KZbw (BK2), M12bw × 2050 (BM2) and S32N / 07 × RXL10bw (BSR). In each case, one parent component with a wax-disrupting mutation was used to derive hybrids, while the other was inbred with a typical appearance.

The results of the segregation of DArTseq markers obtained from Diversity Arrays Technology and other molecular markers were assessed for compliance of segregation with the single-gene model (Chi-square test). Correct segregations and phenotypic segregations describing the presence of wax layer in each population were subjected to linkage analysis using the JoinMap 5.0 program. Wax-disrupting mutations have been localized on chromosome 2R, for the BK2 population, and on 7R, for the BM2 and BSR populations. The genotype number of mapping populations BK2, BM2 and BSR was 186, 250 and 266, respectively.

In the next stage, based on the literature data, molecular markers associated with the wax inhibitor gene in wheat were examined. The resulting amplicons in the parental lines were sequenced. The sequences were compared with scafolds published by a team of German scientists. The scafolds selected in this way were used to design primers and identify polymorphisms correlating with a gene that disrupts wax production in the BSR and BM2 populations. Additional information on the relationship between markers linked to identified genes was provided by random forest algorithm analyzes.

Acknowledgments
The research was financed from the NCN project no. 2015/17/B/NZ9/01694.

Feasibility of long-term storage of short-lived wheat pollen

Daniela Impe[1*], *Daniel Ballesteros*[2], *Till Ischebeck*[3], *Hardy Rolletschek*[1], *Michael Melzer*[1], *Andreas Börner*[1], *Manuela Nagel*[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
[2]Royal Botanic Gardens Kew, Wellcome Trust Millenium Building, Wakehurst Place, United Kingdom
[3]Department of Plant Biochemistry, Georg-August University, Göttingen, Germany
[*]E-mail: impe@ipk-gatersleben.de

Keywords: wheat breeding, hybrid breeding, wheat pollen, viability, pollen storage, cryopreservation

Pollen, the haploid male gametophyte in flowering plants, is a potential organ to preserve plant genetic resources. Short and long-term storage of pollen can support efforts to widen the genetic diversity in breeding programs and facilitate hybrid seed production, especially in spatially and temporally isolated parents. However, in wheat, one of the most important crops of the world, pollen are known to be short-lived and viable pollen can not be stored so far. The storage conditions, especially relative humidity (RH) and temperature are key factors affecting the survival of stored pollen. Therefore, the present project aims to elucidate physiological, biochemical and genetic factors influencing pollen viability and longevity under different storage conditions to work towards an efficient wheat pollen preservation.

At room temperature (20°C) at both, low and high RH, wheat pollen lose rapidly germination within one hour. In contrast, under cold temperatures (8°C) and at both, low and high RH, pollen viability was extended. To gain a comprehensive overview of processes contributing to viability loss, a metabolomic and microscopic study have been initiated. Transmission electrone microscopy illustrates ultra-structural differences in cellular compartments (nucleus) between fresh and stored pollen. Interestingly, in wheat pollen stored for one hour under ambient and cold conditions, endogenous levels of soluble sugars, (fructose, glucose and sucrose) increased by two- to threefold assuming that sugars are actively mobilized during pollen storage. Furthermore, in stored pollen, we found elevated concentrations of metabolites and amino acids which are involved in the Krebs cycle.

Finally, due to the high desiccation-sensitivity and short longevity of wheat pollen its cryopreservation was attempted. We investigated the effects of partial and rapid desiccation following exposure to liquid nitrogen (–196°C). To rapidly (within minutes) reduce the water content of wheat pollen we used a self-constructed 'flash drier' designed previously for maize pollen (Buitink et al. 1996). The biophysical changes of wheat pollen during drying and cooling were investigated using differential scanning calorimetry and provided new information about the feasibility of cryopreservation of viable wheat pollen.

References

Buitink, J., Walters-Vertucci, C., Hoekstra, F. A., Leprince, O. (1996). Calorimetric properties of dehydrating pollen (analysis of a dessication-tolerant and an intolerant species). Plant Physiology, 111(1), 235-242. DOI: https://doi.org/10.1104/pp.111.1.235

From genetic diversity to crop improvement: The Wild Cereal Gene Bank at the Institute of Evolution, University of Haifa, Israel

Tamar Krugman[*] *and Tzion Faima*

Department of Evolutionary and Environmental Biology, and Institute of Evolution,
University of Haifa, Haifa, Israel
[*]E-mail: tkrugman@univ.haifa.ac.il

Keywords: wild emmer wheat, wild barley, genetic diversity

Crop wild relatives (CWRs) offer a rich repertoire of beneficial alleles that can be deployed for crop improvement. Ex-situ and in-situ conservation and preservation of the extensive genetic diversity of crop progenitors can contribute to food security, especially in view of climate change and increased food demand caused by the continuous growth of world population. The Wild Cereal Gene Bank (ICGB) at the Institute of Evolution, University of Haifa, Israel, harbors extensive collections (~ 15,600 accessions) of the prime wild progenitors of wheat, Triticum dicoccoides, (6,025 accessions) and barley (Hordeum spontaneum (5,925 acc.), and the secondary progenitors of cereals Aegilops spp., (10 species, total of 1,910 acc.) and the grass model plant Brachypodium spp. (1,750 acc) (Krugman et al., 2018). These collections originated from diverse ecologies in Israel and neighboring countries in the Near East Fertile Crescent. This region is the center of origin and diversity of wild emmer wheat and wild barley, especially northern Israel. The ICGB aims to collect and conserve the unique and rich genepools of these wild cereals, to study their genome organization, diversity and evolution and exploit their rich genetic diversity for crop improvement.

The ICGB collections represent genetic resources from diverse natural habitats and eco-geographical conditions. Our studies show that they harbor high genetic diversity, which is partly adaptive. In addition, we have identified high diversity in important agronomic traits, such as tolerance to biotic (pathogens) and abiotic (heat, drought and salinity) stresses, and increased grain protein and mineral contents. Hence, these collections were used as the basis for identification and cloning of novel genes and alleles that were shown to contribute to improvement of agronomically important traits, after introgression into bread wheat, such as the high grain protein QTL (Gpc-B1) (Uauy et al., 2006) and the stripe rust resistance genes Yr36 (Fu et al., 2009) and Yr15 (Klymiuk et al., 2018). In addition, we applied QTL mapping and marker assisted selection to introgress QTLs for drought resistance and increased GPC from wild emmer wheat into bread wheat cultivars. Therefore, these studies demonstrate that these unique CWR genetic resources, together with modern genomic tools, are particularly important for advancing agriculture and food production, and provide solutions for the decline in crop genetic diversity and their increased susceptibility to environmental stresses.

References

1. Uauy, C. et al., 2006. Science 314:1298–1301.
2. Fu, D. et al., 2009. Science 323:1357–1360.
3. Klymiuk, V. et al., 2018. Nature communications, 9: 3735.
4. Krugman, T. et al., 2018. *Israel Journal of Plant Sciences*, 65(3-4), pp.129–146.

Development of prebreeding forms of triticale (× *Triticosecale* Wittmack) through chromosome manipulations

Michał T. Kwiatek[1*], *Jerzy Nawracała*[1]

[1]Department of Genetics and Plant Breeding, Poznan University of Life Sciences, Dojazd 11; 60-632 Poznań, Poland
[*]E-mail: michal.kwiatek@up.poznan.pl

Keywords: breeding, chromosome additions, chromosome substitutions, chromosome translocations, triticale

In this lecture, I will take an opportunity to present the current state of knowledge and directions of future prebreeding studies of hexaploid triticale concerning chromosome manipulations. I am going to specify the main goals for creating chromosome aberrations in this artificially generated crop, which are referred to as introgression of genes that are responsible for quality traits, biotic stresses resistance, and heterosis. I'll discuss the breeding methods, supported by cytomolecular analyses, which are based on development of chromosome aberrations induced by meiotic restitution, chromosome elimination, chromosome fragmentation or random fusion of chromatin fragments into chromosome structures. What is more, this kind of chromosome manipulations can be generated through induced cross – hybridizations, which are alternatives to genome editing technologies, associated with the production of genetically modified organisms (GMOs). I will also present the newest modifications and improvements in triticale breeding strategies, which involve recent achievements in cytogenetics and genomics. At last, I will try to discuss how the new methods, such as gametocidal factor system or induced homoeologous recombination, can be exploited to accelerate the breeding considering particular end – use properties of triticale.

Transcriptome analysis revealing differentially expressed genes involved in the formation of a wax layer in rye inbred lines

Magdalena Góralska[1], Katarzyna Molik[1] Natalia Lenarczyk[1], Anna Bienias[1], Kamila Kapłoniak[2], Beata Myśków[1]*

[1]Department of Genetics, Plant Breeding and Biotechnology, West Pomeranian University of Technology, Szczecin, Poland
[2]The Franciszek Górski Institute of Plant Physiology, Polish Academy of Science, ul. Niezapominajek 21, 30-239, Cracow, Poland.
[*]E-mail: Katarzyna.Sobiech@zut.edu.pl

Keywords: transcriptome analysis, wax layer, differential gene expression, rye

The leaf and stem surfaces of many plants are covered with cuticular wax, which confers a glaucous phenotype. Cuticular wax consists mainly of saturated very long-chain fatty acids (VLCFAs), alkanes, primary and secondary alcohols, aldehydes, ketones, esters and sterols. Wax coating protects plants against ultraviolet radiation, reduces water retention on the plant surface and plays an important role in plant defense against pathogens.

The molecular basis for the formation of wax cover in rye is not fully understood. To clarify this mechanism, we analyzed the transcriptomes of 3 pairs of near-isogenic inbred lines of rye (Ds2wl-Ds2w, M12wl-M12w, Rxl10wl-Rxl10w). These NILs differed in the presence / absence of wax cover. We identified several hundred genes with different expression (DEG) for each pair of NILs. Most DEGs were revealed in a pair of M12wl / M12w lines – 1456 of which 489 were up regulated and 967 were down regulated. 1000 DEGs (571 regulated up and 429 regulated down) were determined in Rxl10wl / Rxl10w lines and 681 DEGs (425 regulated up and 256 regulated down) in lines Ds2wl / Ds2w.

Sequence analysis of all DEGs was performed to assign annotations. The sequences were also compared to scaffolds published by a team of German scientists to identify the location on the chromosome. For DEGs that showed a high level of expression difference and or had annotations potentially associated with wax formation, primers were designed to detect intra-gene polymorphism. The primers were tested on a set of 4 pairs of inbred rye lines from which mapping populations were derived. 430 pairs of primers were tested, of which 44% generated a polymorphic product for a minimum of 1 pair of line lines.

Acknowledgments
The research was financed from the NCN project no. 2015/17/B/NZ9/01694

The genetic analysis of root size in bread wheat under contrasting water regimes

Tatyana Pshenichnikova[1*], *Alexander Simonov*[1], *Evgeniya Morozova*[1], *Ludmila Shchukina*[1], *Olga Smirnova*[1], *Andreas Börner*[2]

[1]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia
[2]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), 06466, Gatersleben, Germany
[*]E-mail: wheatpsh@bionet.nsc.ru

Keywords: bread wheat, roots, drought, substitution lines, monosomic analysis, QTL analysis

Root length and weight were studied in the set of inter-varietal single chromosome substitution lines of bread wheat Chinese Spring (Synthetic 6x), CS (Syn), under normal and restricted water supply The donor of separate chromosome pairs was a synthetic hexaploid AABBDD (*Triticum dicoccoides* × *Aegilops tauschii)* having a winter growth habit. It was found that 1A substitution resulted in a substantial reduction of root size in the wheat plant. The substitution of 5D chromosome, on contrary, led to its significant increase comparing to the recipient and donor. Additionally, the interrelation between growth habit and root size was studied with the use of cv. S29 - carrier if the two dominant alleles of *Vrn-A1* and *Vrn-B1* genes. The obtained monosomic populations for chromosomes of 5th homoeological group were studied under 40-days vernalization. The largest root size was found in 5A monosomic population from which the dominant allele of *Vrn-A1* gene was excluded. However, the root size in the two other populations was also significantly higher than in S29. On the next stage of investigation, a set of genotyped introgression lines CS (Syn 5D) (Pestsova et al. 2001) was studied under the contrasting water conditions. The lines were studied after 30-days vernalization. The greatest root weight and length as well as a number of days till flowering comparable to 5D substitution line was detected in the introgression lines 5D-5, 5D-6 и 5D-10. These lines has a common introgression fragment in the long arm of 5D chromosome marked with the molecular marker *Xgwm-292* and the gene *Vrn-D1* (Pestsova et al. 2006). The lines were additionally studied under 45- and 60-day vernalization. Three significant QTLs (LOD>3,0) for the date of flowering, length and weight of roots were co-localized in chromosome 5D in the locus *Xgwm292* under vernalization of 30 days. With a longer vernalization, the connection of this molecular marker with three characters completely disappeared. Generally, drought effected depressingly on root development in all lines but the greatest root weight and length was detected in the substitution line for 5D chromosome and in the introgression lines 5D-5, 5D-6 и 5D-10. The data obtained may prove the existence of the locus responsible for the traits under study in this region of 5D chromosome.

Acknowledgments
The work was financially supported by the grant #p-54_NSO (RFBR #19-416-540001 p_a) .The greenhouse experiments were carried out at the Common Use Center – Laboratory of Artificial Plant Growth of the ICG SB RAS with the support of the budget project of the ICG SB RAS No. 0324-2019-0039.

References

1. Pestsova EG, Börner A, Röder MS (2001) Development of a set of *Triticum aestivum–Aegilops tauschii* introgression lines. Hereditas 135:139–143
2. Pestsova EC, Börner A, RöderMS (2006) Development and QTL assessment of *Triticum aestivum-Aegilops tauschii* introgression lines. Theor Appl Genet 112:634–647

Natural variation of grain architecture-related traits in a winter wheat population

Matías Schierenbeck[1,2,3*], *Rasha Tarawneh*[1], *Ahmad M. Alqudah*[1], *Ulrike Lohwasser*[1], *María Rosa Simón*[2,4], *Andreas Börner*[1]

[1]Genebank Department, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
[2]Cereals, Faculty of Agricultural Sciences and Forestry, National University of La Plata, La Plata, Argentina
[3]CONICET CCT La Plata. La Plata, Buenos Aires, Argentina
[4]CICBA. La Plata, Buenos Aires, Argentina
[*]E-mail: m_schierenbeck@hotmail.com

Keywords: Wheat, Thousand kernel weight, Grain architecture, GWAS

The future productivity of wheat (*Triticum aestivum* L.) will be of utmost importance for global food security since it is the most widely grown crop worldwide. It is known that thousand kernel weight (TKW) is closely associated with kernel size traits, such as kernel length (KL), kernel width (KW) and kernel diameter ratio (KDR) in wheat. Kernel size traits usually contribute to yield by affecting the TKW and can also be associated with milling and processing. Therefore, improving kernel weight and size-related traits is a prime breeding target for wheat yield potential and end-use quality. In order to study the genetic variation and detect quantitative trait loci affecting grain architecture traits, TKW, KL, KW, KDR and kernel area (KA) were measured in a winter wheat panel "FROWHEAT" consisting of 265 accessions from 28 countries selected for association mapping. Measurements were performed at the Leibniz Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany) using material harvest during three years (2016, 2017 and 2018). All the variables showed highly significant differences between *Years*, *Genotypes* and *Years* × *Genotypes* interaction. In general terms, TKW ranged from 29.08 g to 63.05 g, KL fluctuated between 5.54 mm to 7.70 mm, KW between 3.05 mm to 4.06 mm, KA from 12.81 mm^2 to 22.23 mm^2 and KDR between 1.579 to 2.155. Highly significant correlations were detected between most of the variables measured. A genome-wide association analysis was performed using the Wheat 90K Illumina iSelect SNP array consisting of 81,587 SNPs. A mixed linear model (MLM) using the Q + Kinship matrix was employed to calculate the associations between the markers and the estimated means of each accessions (BLUEs). Markers linked to the loci obtained through this project could be used for marker-assisted selection in wheat breeding programs for improving yield and quality.

Transcriptomic analysis of barley partial albinism

Nickolay Shmakov[1*], *Anastasiya Glagoleva*[1], *Gennadiy Vasiliev*[1], *Dmitry Afonnikov*[1], *Elena Khlestkina*[2]

[1]Institute of Cytology and Genetics, SB RAS, Russia
[2]Vavilov Institute of Plant Genetic Resources, RAS, St. Petersburg, Russia
[*]E-mail: shmakov@bionet.nsc.ru

Keywords: barley, plant albinism, RNA-seq, plastid genome

Plastids are semi-autonomous organoids that give higher plants the ability to photosynthesize. Despite that plastids retain their own genomes, most photosynthesis-associated proteins are encoded by the nuclear genes. Thus, a precise coordination between the two genomes is required in order to maintain a working photosynthetic apparatus, and many aspects of such coordination are not know yet. Malfunctioning of this coordination can lead to plant albinism or chlorophyll deficiency. Studying such cases can shed light on specifics of plastid-nucleus crosstalk. An example of plant partial albinism is barley near isogenic line i:BwAlm. This line contains a mutant allele of Alm gene located on the short arm of 3H chromosome. However, neither seqeunce of the gene nor its role in partial albinism formation are known.

Total mRNA from lemma and pericarps of i:Bw*Alm* NIL plants and isogenic line Bowman plants was sequenced, and RNA-seq analysis was performed to identify changes in plant transcriptome leading to the partial albinism. Differential expression search was performed. A significantly larger amount of genes down-regulated in the i:Bwi*Alm* NIL was observed than of genes up-regulated in that line. Expression of all plastid genes is lower in i:Bw*Alm* line; however, different functional groups of plastid genes showed different levels of expression change. Enrichment analysis was performed for GO terms and metabolic pathways that DEGs participate in. Consistency of expression levels changes for DEGs participating in some of the pathways was observed, including both nuclear and plastid genes. Differential expression for a specified set of genes was verified through quantitative RT-PCR.

De novo transcriptome reconstruction was performed, and a contig specific to i:Bw*Alm* line transcriptome and absent in current barley genome assembly was identified. SPFH-prohibitin domain was identified in the contig. Resequencing of the contig confirmed its presence in the i:Bw*Alm* NIL genome, but not in Bowman line genome. Using wheat-barley additional lines, it was localized to the short arm of 3H barley chromosome.

Acknowledgments
This work was supported by RSF project 18-14-00293

Regulation of duplicated genes expression coding TFs involved in flavonoid biosynthesis in Triticeae tribe

Strygina K.V.[1, 2*], *Khlestkina E.K.*[1, 2]

[1]Federal Research Center N. I. Vavilov All-Russian Institute of Plant Genetic Resources, Saint-Petersburg, Russia
[2]Federal Research Center Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia
[*]E-mail: k.strygina@vir.nw.ru

Keywords: *Aegilops*, *Hordeum*, *Triticum*, anthocyanins, MBW, transcription factors, DNA methylation

Gene duplication is the main evolution mechanism leading to the emergence of new gene functions and new species. Genomes of polyploid plants have an increased number of genes copies. Thereby, the studying of polyploid genomes is of interest to determine the functional and evolutionary features of the duplicated genes. Here, we report results of study on paralogous and homeologous copies of regulatory *Myb*, *bHLH* and *WD40* genes (members of the regulatory MBW complex) involved in flavonoid biosynthesis in allohexaploid bread wheat *Triticum aestivum* L., diploid barley *Hordeum vulgare* L. and their relatives. In addition, we studied the methylation patterns of promoters of duplicated flavonoid biosynthesis genes in wheat genome. In this study, we identified and characterized *bHLH* family TFs. The members of this family were found in chromosomes 2 and 4. Copies of another TFs family member (*Myb*-like TFs) were found in chromosomes 4 and 7. The *WD40* family members were revealed on chromosome 6. Investigation of the structure organisation and transcriptional activity of these genes revealed the full range of regulatory MBW genes controlling the synthesis of anthocyanins in the pericarp and aleurone layer of wheat and barley. We demonstrated that bHLH-coding gene *HvMyc2* is the main regulator of the appearance of blue colour in barley grain. The bHLH-coding candidate gene *TaMyc-B1* determining the colour of the wheat coleoptile was also detected. In addition, analysis of methylation patterns of promoters of flavonoid biosynthesis genes in wheat showed that the methylation does not make a significant contribution in the specific expression pattern of the studied genes. In general, the results of the comparison of the flavonoid biosynthesis genes copies demonstrated that the maintenance of the duplications in genomes of Triticeae tribe members is due to their functional diversification and tissue-specific activity.

Acknowledgments
This study was partially supported by RFBR (№ 18-416-543007).

Candidate genes for drought stress tolerance in spring barley collection – A genome wide association study

Rasha A. Tarawneh[*] *and Andreas Börner*

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben),
Seeland / OT Gatersleben, Germany
[*]E-mail: tarawneh@ipk-gaterslebne.de

Keywords: drought stress, GWAS, candidate genes

Drought is one of the main abiotic stresses limiting barley (*Hordeum vulgare*. L) growth and productivity around the world. Climate change has become one of the biggest issues of the twenty-first century where many climate-based simulations have predicted an increase in the intensity and frequency of abiotic stresses. Understanding the genetic bases of response to drought is a requirement to obtain high yielding and stress tolerant barley cultivars. A set of 184 spring barley genebank accessions from 23 countries, genotyped with a 9k chip was evaluated in the field in two consecutive years (2016 and 2017). Post anthesis drought stress was simulated by using chemical desiccation technique. Potassium iodide (KI, 1% w/v) was applied 10 days after anthesis. Different morphological and agronomical traits were evaluated such as plant height, spike length, kernel number/spike, kernel weight/spike and thousand-kernel weight. Statistical analysis showed that drought stress had strong impact on different measured traits and significant differences between the accessions were shown.

Genome-wide association study (GWAS) was performed to reveal significant marker-trait associations (MTAs) and to identify candidate genes under drought and control treatments based on the estimated means of both growing seasons (Best Linear Unbiased Estimators (BLUEs)). After applying the False Discovery Rate (FDR) test at $p<0.05$, 412 MTAs were detected on the seven barley chromosomes. Highly significant associated SNPs identified under drought were co-localized according to their physical position as stated in the latest barley reference genome sequence in order to identify potential candidate genes. Ten candidate genes were identified, distributed on chromosomes 1H and 2H with five genes for each. The identified candidate genes, particularly genes involved in defense and drought tolerance could be considered as potential genes for drought tolerance. These genes can be further explored to be used in marker-assisted selection and molecular characterization.

New concept of the evolution of polyploid plants – the example of hexaploid wheat

Natalia Tikhenko[1,2], Ahmad Muhammad Alqudah[1], Ljudmilla Borisjuk[1], Stefan Ortleb[1], Twan Rutten[1], Dandan Wu[1], Manuela Nagel[1], Axel Himmelbach[1], Marion Röder[1], Stefanie Thumm[1], Andreas Houben[1], Andreas Börner[1], Martin Mascher[1]*

[1]Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
[2]SPb Branch Vavilov Institute of General Genetics, RAS, St. Petersburg, Russia
[*]E-mail: tikhenko@ipk-gatersleben.de

Keywords: distant hybridization**,** endosperm development, reproductive isolation, evolution of wheat

Wheat is the second most important food crop in the world after maize and provides more than 20% of the daily protein and of the food calories for 7.66 billion people. World wheat production for 2017 was a record 759.4 Mt. Hexaploid wheat (*Triticum aestivum* L.) contributes approximately 95% to the total wheat production, while another 4% comes from durum wheat and 1% from dicoccum wheat (FAO 2019). The high productivity and adaptability of bread wheat to various growing conditions is due to the polyploid nature of this, evolutionarily speaking, 'young' species. Hexaploid wheat is the product of a stepwise natural hybridization of three different diploid species: (i) a yet-undiscovered extant or extinct *Aegilops* species (genome B) closely related to *Ae. speltoides*, (ii) *T. urartu* (genome A) and (iii) *Ae. tauschii* (genome D) followed by genome doubling. The speciation process has been associated with ecogeographical expansion and domestication and resulted in a unique species *T. aestivum*. However, the true relationship between individual genomes is still not well understood. The availability of nullisomic-tetrasomic and deletion lines make it possible to identify the contribution of individual chromosomes and loci in the formation of the most important features that determine the productivity and adaptability of wheat.

In crosses between Chinese Spring (CS) nullisomic-tetrasomic lines (CSNTs) and rye inbred lines we observed that chromosome 1D had a large effect on hybrid seeds development. Notably, in all 376 hybrid caryopses examined after the cross N1D/T1A with rye the endosperm was missing, showing that development had been aborted at an early stage. This provided an indication that chromosome 1D carries a specific locus (loci) essential for seed development both in the rye-pollinated and, as we show here, in the self-pollinated situations as well. Tetrasomy for chromosome 1B partially compensated for the absence of 1D, since 64% of the N1DT1B x rye hybrid caryopses contained a differentiated embryo. It thus seems that chromosome 1B carries a homoeoallele(s) of the 1D locus (or loci), which influences the development of the hybrid wheat-rye embryo and endosperm, but to a lesser degree.

The physical mapping of this unknown locus (loci) located on chromosome 1D responsible for embryo and endosperm development in wheat-rye hybrids via interaction with the rye genome and for the spike morphology has been investigated by analyzing seed set and 1000 grain weight in hexaploid wheat. The new locus (loci) was classified as a speciation locus with a positive effect on the function of genes in the hybrid genome. The role of this

locus (loci) in the evolution of hexaploid wheat and the changes in the genome structure of the deletion lines for chromosome 1D under strong cytological selection for nearly 30 years will be discussed.

This study was funded by the German Research Foundation (No. BO 1423/17-1/603175).

References:

1. FAO. Crop prospects and food situation – Quarterly Global Report – March 2019 (Rome, 2019). License: CC BY-NC-SA 3.0 IGO.

Impact of ionic liquids on induction of wheat microspore embryogenesis

Dorota Weigt[1*], *Magdalena Magaj*[1], *Janetta Nimann*[1], *Sylwia Mikołajczyk*[1], *Jerzy Nawracała*[1], *Idzi Siatkowski*[2]

[1]Department of Genetics and Plant Breeding, University of Life Sciences, Poznań, Poland
[2]Department of Mathematical and Statistical Method, University of Life Sciences, Poznań, Poland
[*]E-mail: dorota.weigt@up.poznan.pl

Keywords: microspore, embryogenesis, wheat, induction, ionic liquids

Doubled haploids obtained by embryogenesis of microspores are fully homozygous. Due to that, they are valuable plant material for research and breeding. To start embryogenesis, microspores requires genetic changes, because embryogenic program of their genes is under epigenetic repression. Modifications in their transcription and translation profiles lead to chromatin reorganisation. Only severe changes can make microspores divide symmetrically, which is believed to be beneficial to formation of embryogenic calli, from which plantlents then regenerate. The most widely applied inducer of microspores embryogenesis is an auxinic herbicide, 2,4-D (2,4-dichlorophenoxyacetic acid). This synthetic hormone is a stress factor when added to a medium, necessary in reprogramming the development of microspores from gametophytic to sporophytic pathway. Moreover, 2,4-D has auxin-like effects essential to proliferate of microspores. Unfortunately, numerous wheat genotypes are recalcitrant and do not undergo induction under the influence of common growth regulators. For that reason, III generation ion liquids of molecular structure similar to auxins, were used in this study: C2 – (2-chloroethyl ammonium 2,4-dichlorophenoxy) acetate; TM – Trimethylvinylammonium (2,4-dichlorophenoxy) acetate; CD - 2-Chloroethyl trimethylammonium 2,6-dichloro-2-methoxybenzoate.

These compounds were added to the first medium, in ten different experimental combinations, aiming for a total inductors concentration of 2 ml/l. Medium containing only 2,4-D was used as a control.

Four cultivars of spring wheat were examinated: two embryogenic (DC356/08-4-5/09, Ac Abbey) and two recalcitrant (HN ROD 513750, CLTR 7027). The methodology used had been described by Weigt i in. (2016). A total of 12 000 anthers were placed in the experiment – 300 anthers/genotype/combination.

The investigated ion liquids successfully increased the effectiveness of microspores' embryogenesis induction in most used experimental combinations in comparison to the control. Moreover their addition to the media initiated micropores' embriogenesis even in the recalcitrant genotypes. Three media containing a mixed composition of auxin-like compounds: CD/C2; CD/2,4-D; TM/CD, turned out to be the most favorable. Embryogenesis induction on those media was over two times more efficient in comparison to the control. The effect of breaking the genotype recalcitrance manifesting by regenerated green plantlets, was observed in media containing: C2; TM/CD; TM/C2; CD/2,4-D. Medium CD/C2 efficiently stimulated microspores to embriogenesis induction, however the obtained plants were mainly albinotic.

Summing up, the effect of ion liquids on microspores' embryogenesis induction turned out to be beneficial and had positive impact on embryogenic cells genotypes, as well as on resistant genotypes, increasing the effectiveness of embryogenesis induction and green plant regeneration from microspores of all analysed genotypes.

References

Weigt D., Kiel A., Nawracała J, Pluta M., Łacka A. 2016. Solid-stemmed spring wheat cultivars give better androgenic response than hollow-stemmed cultivars in anther culture. In Vitro Cellular & Developmental Biology – Plant. 52: 619-625. DOI:10.1007/s11627-016-9793-2.

T2: Adaptation to Changing Environments and Plant Microbes Interactions

Temperature dependent hormonal and metabolomics changes during supplementary far-red light induced pre-hardening process in barley

Mohamed Ahres[1,2*], *Áron H. Kamiran*[2], *Krisztián Gierczik*[1,2], *Ákos Boldizsár*[2], *Radomíra Vanková*[3] *and Gábor Galiba*[1,2]

[1]Festetics Doctoral School, Georgikon Faculty, University of Pannonia, 8360 Keszthely, Hungary
[2]Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 2462 Martonvásár, Hungary
[3]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany, Czech Academy of Sciences, 165 02 Prague 6, Czech Republic
[*]E-mail: ahres.mohamed@agrar.mta.hu

Keywords: barley, frost-tolerance, metabolites, LED lighting, far-red light, low temperature

The ability of plant species to adapt to cold and to develop frost tolerance is a genetically determined physiological trait. This adaptation process, so-called "cold-hardening", takes a relatively long time – at least a few weeks – for overwintering plants at temperate climate zone. During autumns, environmental changes (e.g. temperature, light intensity, spectra) are required for cereals to prepare physiologically to winter frost. The most important environmental factors are shortening of day length and alteration in the light spectrum, which are assumed to have a pivotal role in the induction of the cold hardening process. It has been proven both by the study of the model plant Arabidopsis and cereals that low red to far-red ratio in white light induces cold acclimation in temperature dependent way. It was also well elaborated that this pre-hardening process is regulated by phytochromes through the induction of CBF-regulon. However, the physiologic mechanism of the effect of the modulating light on the induction of cold acclimation process has not been elucidated yet. In this experiment barley plantlets were grown under different light conditions (white light and white light supplemented with far-red light) at 5°C and 15°C and the total hormone platform was measured by HPLC MS/MS while the metabolic profile was measured by GC-MS after one day and after a cold acclimation period. Remarkable changes in abscisic acid, phaseic acid, jasmonic acid concentration were observed as a result of the far-red light supplementation of the incident light. In terms of active cytokinins, trans-zeatin was unchanged, while cis-zeatin was reduced by the far-red supplementation of the incident white light at both temperatures at all sampling times. Many from the 63 investigated metabolites showed significant alterations caused by the cold temperature, furthermore, some of them were induced by the supplemental light treatment as well. From these tetronic acid, glycylglycine, propanoic acid and some hypothetical sugar derivatives showed notable changes, especially under the supplementary far-red light. These results clearly show that the modification of the light spectra may influence the metabolite composition of the plants, as well as hormone signalling pathways, which have great importance in regulation of plant development and in frost tolerance, too.

This work was supported by the National Research, Development and Innovation Office 'OTKA' K 128575 and by the EFOP-3.6.3-VEKOP-16-2017-00008 projects. The project was co-financed by the European Union and the European Social Fund.

Two-season (2S) cereals as a solution for subduing the negative effects of the changing climate in Southeast Europe

Borislav Kobiljski[1*], *Gojko Mladenović*[1], *Zorica Jestrović*[1]

[1]Biogranum d.o.o., Novi Sad, Serbia
[*]E-mail: borislav.kobiljski@biogranum.com

Keywords: breeding, cereals, climate, 2S

Today, even the greatest skeptics cannot deny that the climate patterns around the globe are changing dramatically. Over previous years the yield of the leading cereals varieties grown in Southeast Europe ranged from poor to outstanding. In addition, recent research papers and media articles from Southeast Europe report the local and regional weather extremes have had a detrimental effect on cereals production. The latest official EU and FAO reports show a twenty-year trend of average wheat yield stagnation in most regions across Europe, regardless of all technological and breeding advances made. Evidently, cereals breeders must reconsider their breeding objectives in order to subdue the negative effects of the changing climate. Top priority of cereals breeding should be maintaining or increasing yield, with emphasis on long-term yield stability and quality over different environments. This requires a paradigm shift of the ideotypes the breeders are selecting for.

One of the opportunities for Southeast Europe is breeding for two-season cereals. 2S varieties are not real winter or real spring types. Furthermore, they are not so-called facultative (alternative) types, which are essentially winter cereals which can be also grown in the spring and vice-versa. Real 2S cereals are harboring balanced genepools from both winter and spring types, and have a more flexible planting and harvesting time, thus being able to accommodate to different environments and varying weather conditions.

Regarding the breeding targets in creation of 2S cereals many objectives should be considered. High tillering ability of most cereals grown today is a huge disadvantage during drought and heat stress, so breeding for optimal tillers number per plant is paramount. Numerous environmental negative effects can be successfully mitigated by adjustments of the plant architecture, in respect to root and leaves characteristics. For example, extremely narrow wheat plant stature could also be a valuable option for countering diverse environmental negative impacts on yield potential. Also, breeding varieties which possess high technological quality will indirectly play a significant role in stabilizing yield, mostly due to better tolerance to both pre-harvest sprouting and yield loss due to frequent rainfall at harvest time.

We will present and discuss these and other breeding objectives we implemented in our work, that have the potential to subdue the effects of the changing climate in Southeast Europe, as well as the results obtained so far.

Determination of redox-responsive miRNAs, their targets and network in wheat

Gábor Kocsy[1], Zsolt Gulyás[1], Jie Cao[2], Balázs Kalapos[1,3], Magda Pál[1], Yingyin Yao[2], Gábor Galiba[1,3]*

[1]Department of Plant Molecular Biology, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary
[2]State Key Laboratory for Agrobiotechnology and Key Laboratory of Crop Heterosis and Utilization, China Agricultural University, Beijing, China
[3]Festetics Doctoral School, Georgikon Faculty, University of Pannonia, Keszthely, Hungary
[*]E-mail: kocsy.gabor@agrar.mta.hu

Keywords: glutathione, miRNA, redox, target gene, wheat

Involvement of miRNAs in the redox-dependent regulatory processes was studied in wheat (Cao et al., 2019). Treatment with H_2O_2 (10 mM, 1 day) affected the redox environment in leaf tissues as shown by the lower glutathione content and greater half-cell reduction potential of the glutathione disulphide/glutathione redox pair and increased ascorbate peroxidase activity. Simultaneously the miRNA transcript profile also changed, since 70 miRNAs with a minimum 1.5-fold difference in their expression between control and treated (0, 3, 6 h) seedlings were determined. Based on degradome sequencing, they regulate the transcription of 86 genes. Bioinformatics analysis identified 6808 possible additional target genes. The transcriptome analysis corroborated the H_2O_2-responsiveness (24 h treatment) of 1647 targets which are mainly involved in the control of redox processes, transcription and protein phosphorylation and degradation. In a time-course experiment (0, 1, 3, 6, 9, 12, 24 h treatment) a correlation was observed between the amounts of glutathione, other antioxidants and the expression of the H_2O_2-responsive miRNAs and their target mRNAs. This relationship indicates the glutathione-dependent redox regulation of miRNAs and their targets, which contributes to the adjustment of the metabolism to the alterations in the environmental conditions. In this control miRNAs and their target genes form a regulatory network which was revealed by bioinformatics modelling.

Acknowledgments
This work was funded by the National Research, Development and Innovation Office (Hungary, TÉT_12_CN-1-2012-0002 and ANN 117949) and the National Key Research and Development Program of China (2016YFD0101004).

Reference
Cao J., Gulyás Z., Kalapos B., Boldizsár Á., Liu X.Y., Pál M., Yao Y.Y., Galiba G., Kocsy G. (2019): Identification of a redox-dependent regulatory network of miRNAs and their targets in wheat. Journal of Experimental Botany, 70: 85–99.

Temperature-response QTLs in wheat affects final height and the timing of key developmental stages

Lukas Kronenberg[1], Steven Yates[2], Martin Boer[3], Norbert Kirchgessner[1], Achim Walter[1], Andreas Hund[1]*

[1]Crop Science, ETH Zurich, Switzerland
[2]Molecular Plant Breeding, ETH Zurich, Switzerland
[3]Biometrics, Wageningen University & Research, The Netherlands
[*]E-mail: lukas.kronenberg@usys.ethz.chlukas.kronenberg@usys.ethz.ch

Keywords: ETH FIP, field phenotyping, wheat, physiology, temperature-response, development, plant height

Stem elongation (SE) is a critical phase for yield formation in wheat. Temperature is a major abiotic factor affecting the timing and dynamics of SE. It is yet unclear, if genetic differences in temperature-response can be utilized to adjust phenology and improve yield. High throughput phenotyping tools help to shed light on this question as they enable the assessment of dynamic traits in the field (Hund *et al.*, 2019).

We used a terrestrial laser scanner to measure canopy height in a set of >330 winter wheat genotypes of the GABI wheat panel (Kollers *et al.*, 2013). Bi-weekly measurements were performed in the field phenotyping platform FIP of ETH Zurich, from 2015 to 2017. Genotypes differed for start and end of SE (Kronenberg *et al.*, 2017). To quantify genotype-specific temperature-response, the rates of SE during the different intervals were regressed over the mean temperatures during these intervals. The obtained parameters were used to map temperature-responsive (regression slope) and temperature irresponsive (regression intercept) QTLs. The QTLs were mapped by means of a genome-wide association study (GWAS) using an Illumina 90K marker chip.

The temperature-response was highly heritable ($H^2 = 0.81$) and a strong response was related to a later start and end of SE as well as an increased final height. Three temperature-responsive and four temperature-irresponsive QTLs were detected. Putative candidate genes for temperature-response QTLs were related to the flowering pathway in *A. thaliana* while temperature–irresponsive QTLs were related to growth and reduced height genes. These loci, together with the loci for start and end of stem elongation accounted for 49% of the variability in final height. We conclude that the analysis of crop development in response to environmental covariates in the field provides new insight into physiological processes.

References

1. Hund A, Kronenberg L, Anderegg J, Yu K, Walter A. 2019. Non-invasive field phenotyping of cereal development. In: Ordon F, Friedt W, eds. Advances in breeding techniques for cereal crops. Cambridge UK: Buleigh Dodds Science Publishing.
2. Kollers S, Rodemann B, Ling J, Korzun V, Ebmeyer E, Argillier O, Hinze M, Plieske J, Kulosa D, Ganal MW, *et al.* 2013. Whole Genome Association Mapping of Fusarium Head Blight Resistance in European Winter Wheat (Triticum aestivum L.). *PLOS ONE* 8: e57500.
3. Kronenberg L, Yu K, Walter A, Hund A. 2017. Monitoring the dynamics of wheat stem elongation: genotypes differ at critical stages. *Euphytica* 213: 157.

Combining ability and heterotic grouping of early generation tropical maize (*Zea mays* L.) under low nitrogen conditions

Oluwafemi Oluwatosin Lawal[1*]

[1]Crop Production Department, Kwara State University, Malete, Nigeria
[*]E-mail: oluwatosin.lawal@kwasu.edu.ngoluwatosin.lawal@kwasu.edu.ng

Keywords: combining ability, heterotic grouping, food security, early generation, maize, hybrid

Low soil nitrogen is a major abiotic constraint limiting maize in filling its role as food security crop in Nigeria and sub Saharan Africa (SSA). Hybrid maize is more efficient in utilizing soil nutrients and hence out-yield open pollinated varieties (OPVs). Thus, making hybrid maize capable of guaranteeing food security in Africa. However, in producing hybrid maize, large number of lines and segregants are handled wherein inferior lines could mar the selection of superior lines at later generation. Hence, early generation testing becomes imperative in hybrid production in order to screen out poor lines. Also, important is heterotic grouping, a match mating set for hybrid production. To this end, this study was conducted to (i) assess the combining ability of S_3 lines (ii) classify them into heterotic groups based on grain yield. Eighty two 'DMRSR-W-Syn' S_3 lines were crossed to two testers 1368 and 9071 (parents of Oba super1, a commercial hybrid in Nigeria) in a line x tester design to generate 164 testcross hybrids evaluated under low (30kgN/ha) condition in Mokwa (9°18'N, 5°04'E, Southern Guinea Savannah) and Zaria (12°00'N, 8°22'E, Northern Guinea Savannah). The field was laid out in alpha lattice with a single row plot of 3m long at 25 x 75cm intra and inter row spacing respectively in two replicates. Data collected were analysed using SAS 9.4 version. The result revealed that 41 (50.00%) 'DMRSR-W-Syn' S_3 had positive general combining ability (GCA) effect estimates for grain yield. Lines with negative specific combining ability (SCA) effects with 1368 and mean grain yield higher than "9071 x 1368" were grouped into 1368 heterotic group and vice versa into 9071 group. Hence, only 27 (32.93%) lines were classified into heterotic groups. Sixteen (59.26%) were grouped into 1368 while 11 (40.74%) were classified into 9071 heterotic group. Crossing lines in the two groups are expected to maximize heterosis in hybrid production while lines in any of the group when crossed give good synthetic variety. Also, 18 (31.95%) lines had positive GCA effects and significantly higher grain yield than “9071 x 1368” and were classified into 1368;9071 heterotic groups. These lines form good male parents in hybrid production or good testers in combining ability studies.

Hormone responses to cold acclimation and frost treatment in monocots

Radomira Vankova[1], Sylva Prerostova[1], Barbara Kramna[1,2], Jan Simura[3], Alena Gaudinova[1], Vojtech Knirsch[1], Ondrej Novak[3]*

[1]Laboratory of Hormonal Regulations in Plants, Institute of Experimental Botany, The Czech Academy of Sciences, Prague, Czech Republic
[2]Department of Experimental Plant Biology, Faculty of Science, Charles University, Prague, Czech Republic
[3]Laboratory of Growth Regulators, Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University and Institute of Experimental Botany Czech Acad Sci, Olomouc, Czech Republic
[*]E-mail: vankova@ueb.cas.cz

Keywords: cold acclimation, frost, phytohormone, *Lolium perenne*

Plants in temperate zone had to adapt to the changing temperatures during year seasons. Plant frost tolerance could be improved by exposure to low, non-freezing temperatures. Plant responses to environmental changes are regulated by phytohormones. Cold responses were followed in frost-sensitive and frost-tolerant clones of ryegrass *Lolium perenne* in order to describe changes associated with higher stress tolerance. Cold acclimation (3°C for 7 d) resulted in elevation of abscisic acid (ABA) in leaves, to higher extent in the more tolerant clone. This finding is in accordance with the hormonal changes observed in winter and spring wheat and barley. ABA was enhanced also in crowns. Salicylic acid (SA) was elevated in all tested organs, to the highest extent in roots of the more tolerant clone. In contrast, jasmonic acid (JA) was increased in leaves, but decreased in crowns and roots. Only minor changes were observed in the case of auxin (indole-3-acetic acid). After cold acclimation, moderate increase of active cytokinins was found. The most profound up-regulation of gene expression was found in the case of transcription of Ice recrystallization inhibition protein-like proteins *LpIRI3* and *LpIRI4* (in all tested organs and genotypes).

Ryegrass plants were also exposed to frost treatment (-7°C for 4 d). Increase of ABA and SA was observed in all tested organs, the former one especially in leaves, the latter one in roots. In both cases, this strong up-regulated was considerably diminished by cold acclimation. Direct frost treatment resulted in JA increase in leaves, while opposite trend was found in roots and crowns. Pre-acclimation abolished these changes. Frost led to moderate increase of active cytokinins in crowns and roots, much more distinct in the sensitive clone. Transcription of *LpIRI3* and *LpIRI4* was relatively low after direct frost treatment, while high expression level, achieved during cold acclimation, was preserved after subsequent frost.
Intensive cross-talk among plant hormones during low temperature responses will be discussed.

Acknowledgments
The work was supported by the Czech Science Foundation, project no. 17-06613S.

T3: New Breeding Strategies and Bioinformatic Tools

New breeding strategies for mixed cropping in a barley (*H. vulgare* L.) pea (*P. sativum* L.) model system

Benedikt Haug[1*], *Monika M. Messmer*[1], *Emma Forst*[2], *Tristan Mary-Huard*[2,3], *Jérôme Enjalbert*[2], *Isabelle Goldringer*[2], *Pierre Hohmann*[1]

[1]FiBL Research Institute of Organic Agriculture, Frick, Switzerland
[2]GQE Le Moulon, INRA, Univ. Paris Sud, CNRS, AgroParisTech, Université Paris-Saclay, Gif sur Yvette, France
[3]MIA-Paris, AgroParisTech, Paris (France)
[*]E-mail: benedikt.haug@fibl.org

Keywords: plant breeding, mixed cropping, pea, barley, intercropping

Crop mixtures consisting of cereals and legumes have proven as a well-adapted arrangement due to their complementarity towards important resources, especially nitrogen. Crop mixtures combine high yield performance and yield stability. They can contribute to a diversified cropping landscape and adaptation to climate change. The search for alternatives to protein imports from overseas and investments in post-harvest separation technologies are currently fostering their adoption by farmers in Western-Europe, especially under organic and low-input farming conditions. However, screening and breeding for mixed cropping has hardly been explored for arable crops. Thus, the objective was to develop novel breeding strategies and tools specifically for mixed cropping systems.

We tested mixtures and pure stands of a morphologically diverse panel of 32 spring pea (*Pisum sativum* L.) and eight spring barley (*Hordeum vulgare* L.) cultivars in replicated field trials at two locations in Switzerland over two years with pea as the focal species. In an incomplete factorial design (Fig. 1) we determined general and specific mixing ability (GMA and SMA, respectively) of pea and barley in analogy to GCA and SCA (general and specific combining ability) in hybrid breeding. Key traits, such as early vigour, canopy height and leaf morphology parameters were measured, due to their potential use as covariates or indirect selection criteria for mixing ability. Our results show that total yield of mixtures can only partly be explained by pea pure stand yields ($R^2 = 0.35$), making the latter a weak predictor for mixture yield. Pea GMA variance was predominant over SMA variance which underlines the potential for breeding for mixing ability using a tester. Key traits, such as pea stipule area were correlated ($R^2 = 0.56$) with total mixture yield and merit further investigation as indirect selection criteria. The separated yield fractions of pea and barley in mixtures allow to decompose GMA of pea into the producer effect of pea cultivar on pea fraction yield and the associate effect of pea on barley fraction yield. This novel concept allows to elucidate key trait effects on fraction yields of pea and barley which might otherwise be masked when solely using a GMA approach.

barleys \ peas	P01	P02	P03	P04	P05	P06	P07	P08	P09	P10	P11	P12	P13	P14	P15	P16	P17	P18	P19	P20	P21	P22	P23	P24	P25	P26	P27	P28	P29	P30	P31	P32
No barley (pure stand pea)	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
No pea (pure stand barley)	1																															
B1	1	1				1					1				1					1			1				1			1		
B2	1	1					1			1						1		1					1					1			1	
B3	1				1	1				1					1					1	1				1						1	
B4	1			1					1				1				1						1					1			1	
B5	1				1			1				1		1				1					1				1					1
B6	1		1				1					1		1						1	1					1				1		
B7	1		1					1			1					1		1						1			1					1
B8	1			1					1				1							1					1			1				

Figure 1. Incomplete factorial design of 8 barley and 32 pea cultivars

Acknowledgments

The project ReMIX "Redesigning European cropping systems based on species mixtures" is funded by the EU's Horizon 2020 Research and Innovation Programme (grant agreement No 727217) and the Swiss State Secretariat for Education, Research and Innovation (SERI, contract number 17.00091). We thank Getreidezüchtung Peter Kunz, Agroscope Reckenholz and Stefan Rindisbacher for their contribution.

Ten years of genomic selection in an applied wheat breeding program – from expectations to experience

Sebastian Michel[1*], *Franziska Löschenberger*[2], *Christian Ametz*[2], *Hermann Bürstmayr*[1]

[1]Department of Agrobiotechnology, IFA-Tulln, University of Natural Resources and Life Sciences Vienna, Konrad-Lorenz-Str. 20, 3430 Tulln, Austria
[2]Saatzucht Donau GesmbH. & CoKG, Saatzuchtstrasse 11, 2301 Probstdorf, Austria
[*]E-mail: sebastian.michel@boku.ac.at

Keywords: wheat, genomic prediction, baking quality, fusarium head blight, stripe rust, frost tolerance

The advent of cost-efficient genotyping techniques enabled the routine fingerprinting of breeding populations with thousands of genome-wide distributed markers. The efficient usage of these fingerprints to genomically predict breeding values of early and advanced generation breeding material has been one of the major plant breeding topics in recent years. Using the example of an applied winter wheat breeding program an overview about genomic selection for line variety development will be giving starting with the initial expectations when a pilot study was initiated in 2009–2012. Results and experiences from the first validation experiment in 2013–2014 will be reported as well as further prospects, progresses and challenges during its routine application in the program until 2019. Several case studies will be presented for this purpose, including the integration of genomic and phenotypic information for predicting grain yield and baking quality as well as the application of genomic index selection to combine these negatively correlated traits in bread wheat. Furthermore, some insight will be given into genomic selection for disease resistance on the example of Fusarium head blight, specifically addressing the frequently observed confounding effects with other agronomic traits like flowering biology and plant height. Challenges in the application of genomic selection with respect to strong genotype-by-environment interactions and an appropriate training population design will be discussed for biotic stresses in the light of stripe rust race dynamics and for abiotic stresses on the difficult and laborious to phenotype frost and drought tolerance. Lastly, a brief overview will be given on genomic cross prediction based on recent empirical and simulation studies, emphasizing the large importance of an appropriate diversity management when genomically planning crosses within a single program as well as across different breeding programs in the framework of the breeders' exemption.

References

1. Michel S, Löschenberger F, Ametz C et al (2019) Combining grain yield, protein content and protein quality by multi-trait genomic selection in bread wheat. Theor Appl Genet doi: 10.1007/s00122-019-03386-1
2. Michel S, Löschenberger F, Ametz C et al (2019) Simultaneous selection for grain yield and protein content in genomics-assisted wheat breeding. Theor Appl Genet doi: 10.1007/s00122-019-03312-5
3. Michel S, Kummer C, Gallee M et al (2018) Improving the baking quality of bread wheat by genomic selection in early generations. Theor Appl Genet doi: 10.1007/s00122-017-2998-x
4. Michel S, Ametz C, Gungor H et al (2017) Genomic assisted selection for enhancing line breeding: merging genomic and phenotypic selection in winter wheat breeding programs with preliminary yield trials. Theor Appl Genet doi: 10.1007/s00122-016-2818-8
5. Michel S, Ametz C, Gungor H et al (2016) Genomic selection across multiple breeding cycles in applied bread wheat breeding. Theor Appl Genet doi: 10.1007/s00122-016-2694-2

Phenotype-genotype association studies of Siberian barley germplasm for seedling and adult plant resistance to spot blotch

Irina Rozanova[1,2*], *Nina Lashina*[3], *Vadim Efimov*[2], *Olga Afanasenko*[3], *Elena Khlestkina*[1,2]

[1]N.I.Vavilov All-Russian Research Institute of Plant Genetic Resources (VIR), Sant-Petesburg, Russia
[2]Institute of Cytology and Genetics, Siberian Branch of the Russian, Novosibirsk, Russia
[3]All-Russian Research Institute for Plant Protection, St. Petersburg, 196608, Russia
[*]E-mail: i.rozanova@vir.nw.ru

Keywords: barley, GWAS, PLS-analysis, SNP, spot blotch

Siberian barley germplasm has not been widely involved in phenotype-genotype association studies. In the current study, we used association mapping approach for identification of genomic regions related to spot blotch resistance (both at the seedling and adult plant stages) using Siberian spring barley collection. A total of 94 spring barley accessions were genotyped using the 50K SNP iSelect array containing 44,040 SNPs, 40,703 of which were scorable. A total of 39,140 SNPs (89%) were polymorphic. After quality control filtering of the genotyping dataset, 27,319 SNPs (62,0 %) were selected for GWAS. A total of 94 spring barley accessions were phenotyped at seedling stage with three *Cocliobus sativus* isolates (Kr2, Ch3 and O18.2) in the laboratory and also at the adult stage with one isolate (Ch3) in the field. According to the Fetch-Steffenson rating scale 17%/20%/18% of genotypes were resistant and 22%/26%/34% were moderate-resistant to the Kr2/Ch3/O18.2 isolates at the seedling stage respectively and 5% were resistant and 18% were moderate-resistant to Ch3 isolate at the adult stage.

The data were assessed using GLM model with accounting for population structure. The programe packages PAST, Tassel 5 and R were used for data analysis.

The 97 significant SNPs (26 on the 1H, 36 on the 2H and 35 on the 3H chromosome) were found associated with seedling resistance and 2 SNPs were associated with adult plant resistance. Principal component analysis (PCA) was performed using phenotype evaluation data as well as genotyping data. Both sets of principal components were taken as two blocks least squares (2B-PLS) analysis to identify the promising genotypes resistant to spot blotch. Correlation of phenotype-genotype bicomponents showed that all varieties that have resistance to every isolate at every stage collected in one cluster, and ones that showed the susceptibility to every isolate at every stage were in another opposite cluster. Thus, the promising genotypes (25 resistant varieties) as well as 8 susceptible genotypes were identified. It was found that the first bicomponent has good correlation with responsibility to all isolates/ stages. In total, 45 SNPs were associated with overall responsibility to spot blotch for every isolates. Eight SNPs were selected for further conversion to convenient PCR markers for marker-assisted selection of plants resistant to all isolates at all stages.

Acknowledgments
The study was supported by the Russian Science Foundation (Project No 16-04-00086).

T4: Modern Technologies From Phenomics to Genome Editing

Application of delayed luminescence in seed testing

Kehinde Adeboye[*], *Andreas Boerner*[2]

Department of Genebank, Leibniz Instittute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
[*]E-mail: adeboye@ipk-gatersleben.de

Keywords: delayed luminescence, seeds, seed testing, genebank

Delayed Luminescence describes a phenomenon whereby biological materials emit very weak lights for a relatively long time (seconds and more) after illumination. It began to gain wide scientific interest upon the discovery that the intensity of the weak light could be modified by stressors. Numerous scientific findings have since then proposed its usage in analytical studies. Basic analytical studies have been carried out in agriculture, medicine etc. correlating the physiological status of a biosystem with various parameters of delayed luminescence. In agriculture and seed science in particular, researchers correlated seed life with the intensity, spectral or time of delayed emitted light and proposed the usage of delayed luminescence as a fast-noninvasive method of monitoring seed viability where known procedures are destructive and time consuming. However, till date, this claim only remains within the academic confines and there are no known reports of its advancement for use, particularly in seed testing and Genebank Management. The gap in knowledge and application of delayed luminescence in seed testing will be extensively discussed at the conference.

Acknowledgments
DAAD and IPK Gatersleben

Characterization of late development in diverse wheat germplasm using time-resolved spectral reflectance measurements

Jonas Anderegg[1], Kang Yu[1,2], Helge Aasen[1], Achim Walter[1], Frank Liebisch[1], Andreas Hund[1]*

[1]Crop Science, ETH Zurich, Switzerland
[2]Division of Forest, Nature and Landscape, Department of Earth and Environmental Sciences, 3001 Leuven, Belgium
[*]E-mail: jonas.anderegg@usys.ethz.ch

Keywords: high-throughput phenotyping, canopy reflectance, hyperspectral remote sensing, field-based phenotyping, wheat

The ability of a genotype to stay green affects the primary breeding target traits grain yield (GY) and grain protein concentration (GPC) in wheat. The dynamics of senescence *per se* as well as related effects on GY and GPC are under complex genetic and environmental control. This greatly complicates the exploitation of existing genetic variation in stay-green for the improvement of bread wheat, as intense field-testing in target environments is required. High throughput methods to assess senescence dynamics in large field trials will allow for i) indirect selection in early breeding generations, when yield is not yet measurable and ii) mapping of the genomic regions controlling the trait. We evaluated the potential of repeated hyperspectral canopy reflectance measurements to track canopy senescence across years with strongly contrasting environmental conditions, using a set of >330 winter wheat genotypes. We compared the potential of spectral indices and multivariate modelling techniques to infer visually observed senescence dynamics from hyperspectral time series. Spectra- and scoring-derived parameters describing the dynamics of senescence were used to predict GY and GPC and a feature selection algorithm was used to identify the most predictive features. The three-band plant senescence reflectance index (PSRI) approximated the visually observed senescence dynamics best, whereas multivariate models suffered from a strong year-specificity. Feature selection identified visual scorings as most predictive for GY but also PSRI ranked among the most predictive features. In contrast, adding additional spectral features had little effect. Thus, it appears that visual scoring remains the gold standard to quantify leaf senescence in moderately large trials. The possible reason is that spectra from point sensors are affected by genotype-specific changes in canopy structure during senescence. As a solution, image-based techniques may enable to track leaf senescence in a similar manner as done by visual scoring. Parallel work also suggested that using complementary spectral features may allow to delineate disease-related effects from physiological apical senescence, improving the precision of estimates of both traits. The index-based parameterization of the canopy reflectance dynamics offers the advantage of upscaling to very large breeding trials using appropriate phenotyping platforms.

Extending the toolbox for targeted mutagenesis and base editing using improved guide RNAs, modular vectors and protoplasts

Stefan Hiekel[1], Nagaveni Budhagatapalli[1], Robert Hoffie[1], Iris Koeppel[1], Jochen Kumlehn[1]*

[1]Plant Reproductive Biology, Department of Physiology and Cell Biology,
Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
[*]E-mail: hiekel@ipk-gatersleben.de

Keywords: CRISPR/Cas, Adenosine/Cytidine-Deaminases, gRNA structure, cleavage efficiency

The substantial growth of the human population creates an ever-increasing demand for food production which is to be satisfied under changing climatic conditions. In order to cope with these enormous challenges, we need to breed superbly adapted varieties with high yield, excellent stress tolerance and as little nutritional needs as possible. The time available to develop such cultivars is dramatically limited and therefore we need faster and more precise techniques than in the past. One of the most impressive developments in this area over the last few years has been the use of bacteria-derived RNA-guided Cas endonucleases for targeted mutagenesis in many crop plant species. This biotechnological tool consists of two components, a Cas nuclease protein and a guide RNA (gRNA) which directs the nuclease to its specific target in the plant genome. Double-strand breaks induced by the nuclease can be repaired by cellular mechanisms which are however prone to failure, resulting in mutations in the corresponding genomic site. Although this technology works mostly well, in a considerable portion of cases, the target sequence gets either not mutated or at only very low frequency. To address this problem, we used several prediction tools and considered various criteria for target site selection, including features of the corresponding gRNA's secondary structure. In addition, protoplast-based transformation systems for barley, wheat, maize and Arabidopsis were established, which allows us to test vectors and to assess the mutagenesis efficiency of selected gRNAs *in vivo*. To increase the expression of gRNAs and to improve their interaction with the Cas9 protein, a cryptic termination signal was removed from the gRNA scaffold and a structurally essential stem was elongated. Both changes together improved the mutagenesis efficiency in barley by up to 30%. In a further approach, a catalytically impaired Cas9 was used in fusion with either an adenosine or a cytidine deaminase to specifically induce single base pair substitutions, which was exemplified in barley.

Acknowledgments
We thank Ingrid Otto and Carola Bollmann for excellent technical assistance.

TraitSpotting: Development of field phenotyping methods for early growth monitoring of winter wheat breeding experiments

Lukas Roth[1], Helge Aasen[1], Christoph Barendregt[2], Karl-Heinz Camp[2], Andreas Hund[1]*

[1]ETH Zurich, Institute of Agricultural Sciences, Universitätstrasse 2, 8092 Zurich, Switzerland
[2]Delley Samen und Pflanzen AG, Route de Portalban 40, 1567 Delley, Switzerland
[*]E-mail: lukas.roth@usys.ethz.ch

Keywords: high-troughput phenotyping, UAV, heritability, winter wheat

Unmanned aerial vehicles (UAVs) are increasingly used for field observations in science, but applications in breeding are yet sparse. The reason for the lack of established UAV-based phenotyping methods may be threefold: (1) Proper flight planning and adjustment of camera parameter is difficult, but essential to get images of sufficient quality; (2) Extraction and management of plot-based data from images is challenging, as breeding experiments consists of hundreds and thousands of small plots; (3) Reliable methods to extract the relevant traits are yet rare.

The main aim of the project TraitSpotting (www.ethz.ch/traitspotting) is to bring drone-based phenotyping into the breeding nursery: In a first part, we developed a tool-set to support users in planning of UAV based remote sensing missions (Roth et al. 2018a). In a second part, we integrated standard remote sensing products such as orthophotos, and developed a new method to evaluate canopy cover. The method takes advantage of different viewing angles at which the individual plots are imaged during a flight campaign (Roth et al. 2018b).

In a third, ongoing part, replicated multi-location field experiments serve as base to develop reliable and relevant phenotyping methods. For winter wheat, new methods based on segmented multi-view RGB images revealed high potentials to determine plant density, tiller count and the begin of stem elongation for a set of elite cultivars of Agroscope/ Delley Semences et Plantes SA (Switzerland). Multi-year-location heritability values for plant density, tiller count and the start of stem elongation were at 0.88, 0.74, and 0.72, respectively. Corresponding manual measurement heritability values were lower for plant density and tiller count (0.43 and 0.58), but higher for begin stem of elongation (0.90).

In this conference contribution, we like to present the above mentioned three parts as base for an integrated phenotyping strategy. We will describe our approach that includes flight planning, plot identification, feature-extraction from UAV campaigns, correction for design factors and spatial effects, as well as the modeling of developmental processes to predict tiller numbers and the beginning of stem elongation.

References

1. Roth, L., Hund, A., & Aasen, H. (2018a). PhenoFly Planning Tool: flight planning for high-resolution optical remote sensing with unmanned areal systems. Plant Methods, 14(116). https://doi.org/10.1186/s13007-018-0376-6
2. Roth, L., Aasen, H., Walter, A., & Liebisch, F. (2018b). Extracting leaf area index using viewing geometry effects—A new perspective on high-resolution unmanned aerial system photography. ISPRS Journal of Photogrammetry and Remote Sensing, 141, 161–175. https://doi.org/10.1016/j.isprsjprs.2018.04.012

Fine tuning of anthocyanin synthesis in barley grain by targeted mutagenesis

Alexandr Vikhorev[1,2*], *Ksenia Strygina*[3], *Sofia Gerasimova*[1,2], *Elena Khlestkina*[1,3]

[1]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia
[2]Novosibirsk State University, Novosibirsk, Russia
[3]N. I. Vavilov Research Institute of Plant Industry, Saint-Petersburg, Russia
[*]E-mail: vikhorev@bionet.nsc.ru

Keywords: anthocyanin biosynthesis, CYP75, flavonoid pigments, *Hordeum*, P450, genome editing

Nowadays functional nutrition attracts considerable attention, therefore the study of healthy beneficial biologically active compounds is an important task. Anthocyanins are natural plant pigments known for their health benefit as well as for a range of functions important for regulation of plant growth and development, protection from pathogens and unfavorable environment conditions. Among structural anthocyanin biosynthesis genes, the genes coding for flavonoid 3'-hydroxylase (*F3'H*, CYP75B, EC 1.14.13.21) and flavonoid 3',5'-hydroxylase (*F3'5'H*, CYP75A, EC 1.14.13.88) are the proper targets for fine tuning of synthesis in order to get desired composition of anthocyanins. These genes catalyze the hydroxylation of B-ring of dihydrokaempferol and direct the pathway of biosynthesis towards the formation of pink pigments cyanidins and blue pigments delphinidins, respectively.

We have suggested that the loss-of-function mutation in the *F3'5'H* gene can switch the biosynthesis of blue pigments to the pink pigments in the aleurone layer of barley grain. However, before this study there were no complete data on structural and functional organization of the *F3'H* and *F3'5'H* gene families in barley genome. The aim of the present study was to identify and investigate all copies of the *F3'H* and *F3'5'H* genes in barley and to reveal proper targets for modification using RNA-guided Cas9 endonuclease.

In barley, the near-isogenic lines (NILs) PLP (purple lemma and pericarp, NGB22213) and BA (intense blue aleurone, NGB20651) characterized by tissue-specific accumulation of pigments in grain pericarp and aleurone layer were previously developed based on Bowman cultivar (NGB22812) background. These precise model was used in the current study.

Based on sequences of previously revealed genes *F3'H-1* and *F3'5'H-1*, one homologous copy belonging to the family F3'H (chromosome 6HS) and three homologous copies belonging to the family *F3'5'H-1* (chromosomes 6HL, 6HS and 7HS) were identified in barley genome. All genes have a cytochrome P450 domain, with the exeption of the *F3'5'H-3* gene. This copy carries a frame shift mutation disrupting the domain. Analysis of gene expression and evaluation of the anthocyanin content in coloured and uncoloured tissues and organs (such as aleurone layer, lemma, pericarp and stem) of the NILs have revealed that the accumulation of pink pigments in the pericarp is under control of the *F3'H-1* gene, the accumulation of pink pigments in the stem – the *F3'H-2* gene, and the accumulation of blue pigments in the aleurone layer – the *F3'5'H-1* gene. The genes *F3'5'H-2*, *F3'5'H-3* and *F3'5'H-4* probably do not participate in the biosynthesis of anthocyanins in the studied tissues. According to these findings, the *F3'5'H-1* gene was selected as a target for modification. sgRNAs for this gene were designed and their activity was predicted *in silico*. These sgRNAs can be used to obtain barley mutants accumulating pink instead of blue pigments in aleurone layer.

Acknowledgments
This study was partially supported by the RFBR (№ 18-416-543007).

Poster Presentations

T1: Plant Genetic Resources for Future Breeding

Characterization of Georgian Soft Wheat Germplasm Diversity

Mariam Betsiashvili[1*], *Tsotne Samadashvili*[12], *Nino Silagava*[1], *Natia Simonishvili*[1], *Ulrike Lohwasser*[3]

[1]Bank of Plant Genetic Resources, Agricultural University of Georgia, Tbilisi, Georgia
[2]Research Division of Cereal Crops, Scientific-Research Center of Agriculture, Tbilisi, Georgia
[3]Genebank Department, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
[*]E-mail: m.betsiashvili@agruni.edu.ge

Keywords: soft wheat, *Triticum aestivum*, bio-morphological characterization, breeding, application

Georgia is one of the centers of wheat origin (Vavilov, 1935) and wheat is one of the ancient and characteristic crops of Georgia. Its cultivation began in the V–VI century BC and continued through the whole history of the country. So far, 27 cultural and wild species of wheat have been described around the world, out of which 14 species are native to Georgia, and five of them are narrow endemic and found nowhere else in the world.

Georgia is also distinguished by its diversity of local soft wheat varieties. There are 40 subspecies of soft wheat in Georgia and among them the most widespread are - *var. aestivum*, *var. ferrugineum*, *var. lutescens*, *var. milturum* (Samadashvili et al., 2019). All these mostly endemic forms are diverse according to their ecological, biological and morphological properties and diversity of genetic variations was established during the centuries in very different natural-historical conditions.

Widening the genetic bases available for future genetic progress is one of the main goals of plant breeders. Thus, conservation and characterization of genetic diversity in regional breeding pools are very important.

In this study, we characterized and evaluated Georgian soft wheat endemic species. Local Genebank accessions and samples from the foreign genebanks (IPK, USDA-ARS, CIMMYT) were characterized in two-years replicated agricultural experiments based on traits that are indicated in the international descriptors of the crop.

Overall, 28 traits were measured in the 260 wheat accessions and each was described according to their bio-morphological and agronomic traits and evaluated on grain yield and quality. In addition to phenotypic measurements in the field, we examined – spike and grain morphology traits at the physiological maturity stage. Ten plants from each sample were randomly selected for trait measurements.

Selected accessions also were studied on seed quality traits – protein, minerals, sugars, carbohydrates, fiber. Well-characterized accessions are classified into groups according to unique traits and assembled in PGR core-collections.

According to bio-morphological characterization and evaluation data, we revealed the real value of conserved samples and increased possibilities to improve their use in research and breeding to facilitate variety improvement, as well as in phylogenetic studies and educational programs.

Acknowledgments
This work is supported by the Basic Research State Grant #218050 "Acquisition, Regeneration and Characterization of Unique Georgian Plant Genetic Resources Conserved in Foreign Genebanks" funded by Shota Rustaveli National Science Foundation of Georgia.

References
1. Vavilov, N. I.; Löve, Doris (trans.) (1992) Origin and Geography of Cultivated Plants. Cambridge University Press. p. xxi. ISBN 978-0521404273.
2. Samadashvili T., Chkhutiashvili., Betsiashvili M. (2019) Georgian wheat Germplasm and its genetic and breeding value. LAP Lambert Academic Publishing ISBN: 978-613-9-47609-1, 60 p.

Detection of significant loci associated with agronomical important traits in Serbian barley core collection

Ljiljana Brbaklic[1], Milan Mirosavljevic[1], Dragana Trkulja[1], Ankica Kondic Spika[1], Vladimir Acin[1], Sanja Mikic[1], Verica Takac[1], Vojislava Momčilovic[1]*

[1]Institute of Field and Vegetable Crops, Novi Sad, Serbia
[*]E-mail: ljiljana.brbaklic@ifvcns.ns.ac.rs

Keywords: *Hordeum vulgare* L., yield components, population structure, microsatellites, QTLs, genetic variability, marker-trait association

Barley (*Hordeum vulgare* L. subsp. *vulgare*) is the second most important cereal crop widely grown in a range of various climate and environmental conditions, primarily used for livestock feed, in alcohol production and human consumption. The main objective of this study was to assess genetic diversity and to reveal the genomic regions, which control the most valuable breeder`s traits, important for Serbian barley breeding programme. A set of 71 diverse elite lines and well-adapted commercial barley cultivars was used for genotypic and phenotypic evaluation. The field trial was conducted near Novi Sad, at Rimski šančevi, Serbia, while the following agronomic traits were measured during the three growing seasons: heading and flowering time, stem height, yield, thousand grain weight, hectolitre mass and spike length. A set of 15 microsatellites, covering all linkage groups, was used to detect 83 polymorphic alleles with an average of 5.53 alleles per locus. A satisfying level of differentiation and informativeness in almost all genomic loci were found. The PIC values ranged from 0.108 (GMS001) to 0.740 (Bmac0067). Population structure distributed the genotypes into two clusters primarily corresponding with row type. A total number of 18 marker-trait associations was detected in two and/or three years by applying general linear model, whereas mixed linear model confirmed presence of stable associations for 5 QTLs. Flowering time and spike length were associated with 5 analysed markers, whereas hectolitre weight did not show significant associations with the analysed markers. The stable and significant QTLs could be of the great importance for further improvement of barley varieties during the selection of the parents, which carry desirable alleles and could be valuable preliminary groundwork for further genomic selection with fine-tuning refinement.

Acknowledgments
The Ministry of Education, Science and Technological Development of the Republic of Serbia (TR31066)

The use of molecular markers in the breeding of bread wheat (Triticum aestivum L.) for the conditions of North Kazakhstan

Valentina Lemesh[1], Adylkhan Babkenov[2**], Aryna Burakova[1], Oksana Zaitseva[1], Sandugash Babkenova[2]*

[1]Institute of Genetics and Cytology of the National Academy of Sciences, Minsk, Republic of Belarus
[2]Scientific and Production Center of Grain Farming after A. I. Barayev, Shortandy-1, Republic of Kazakhstan
[*]E-mail: V.Lemesh@igc.by
[**]E-mail: babkenov64@mail.ru

Keywords: wheat, marker assisted selection, molecular markers, *Glu-1*, *Vrn-1*, *Ppd-1*

The combination of different genes in the same variety of wheat is challenging due to the polyploid nature of the breeding forms. Marker assisted selection (MAS) or molecular breeding offers on to accelerate the traditional breeding and allows to simplify the process of selecting parental forms for crossing, to significantly reduce the amount of analyzed material, to eliminate the influence of the environmental conditions and to reduce the terms to create new varieties.

In this work in order to control the transfer of selectively valuable alleles of genes as well as the selection of homozygous forms of wheat molecular-genetic analysis of allelic composition of high molecular weight glutenin genes *Glu-1*, vernalization genes *Vrn-1* and photoperiod sensitive genes *Ppd-1* in 53 hybrids and 45 parental varieties were concluded.

It was shown that 23 hybrids were homozygotes and inherited alleles of parental varieties. However in some cases there was a discrepancy between the identified and expected alleles. These discrepancies may be due to to heterogeneous of the original forms as well as cross pollination during stabilization of hybrids.

When determining the allelic composition of HMW glutenin genes 13 combination of genes were identified. The assessment of quality of HMW glutenin subunits encoded by identified alleles of genes varied within 5–12 points. For hybrids with combinations *Glu-A1b Glu-B1c Glu-D1d* and *Glu-A1a Glu-B1c Glu-D1d* were given a high score for bread (9 points). These hybrids are recommended for use in the selection of a bread-making quality of grain.

In the analysis of the allelic composition of genes controlling the reaction to vernalization and sensitivity to photoperiod 20 combinations were identified. For all plants *Ppd-A1b* and *Ppd-B1b* were revealed. 28 hybrids with combined of genes *Vrn-A1a Vrn-B1a vrn-D1 Ppd-D1b* and *Vrn-A1a Vrn-B1c vrn-D1 Ppd-D1b* are promising for breeding on earliness.

The most breeding interest are ten hybrids characterized by a balanced allelic composition for all studied genes.

Acknowledgments
This investigation was carried out within the framework of the agreement of Institute of genetics and cytology of NAS of Belarus and Scientific and production center of grain farming after A. I. Barayev, Republic of Kazakhstan in 2016–2017.

References
List of References here, if applicable.

Resistance of European winter wheat cultivars to *Zymoseptoria tritici* isolate IPO86036

Paweł Cz. Czembor[*], *Dominika Piaskowska*

Plant Breeding & Acclimatization Institute – National Research Institute,
Department of Plant Breeding and Genetics, Radzików, 05-870 Błonie, Poland
[*]E-mail: p.czembor@ihar.edu.pl

Keywords: adult plant resistance, Septoria tritici blotch, wheat

Septoria tritici blotch (STB) of wheat (*Triticum aestivum*), caused by the fungal pathogen *Mycosphaerella graminicola* (anamorph: *Zymoseptoria tritici*, syn. *Septoria tritici*), is present in most wheat-growing areas worldwide. Host resistance is the most economical and safest method of controlling the disease and information on resistance loci is crucial for effective breeding for resistance programs. In the study we used a set of 83 wheat cultivars registered in the Descriptive List of Agricultural Plant Varieties (COBORU 2012), 92 cultivars from other European countries and 25 cultivars/lines with identified STB resistance loci. The wheat genotypes were tested on adult plant stage under polytunnel conditions with watering system. Fully expanded flag leaves were sprayed with spore suspension of IPO86036 *Z. tritici* isolate. After incubation period, the percentage leaf area covered by necrosis (NEC) and covered by pycnidia (PYC) were measured on flag leaf (computer assisted image analysis) of each wheat cultivar/line. Large variation was detected for both disease parameters: NEC 19.67–94.7% and PYC 0.15–50.29%. These parameters were used in agglomerative hierarchical clustering (AHC) analysis with UPGA algorithm (unweighted pair-group average) to dissect groups of wheat cultivars/lines with different resistance levels. Six groups of wheat cultivars/lines were identified and the largest group comprised 130 genotypes with NEC ranged from 19.67% to 70.71% and PYC ranged from 0.15% to 20.9%. Within this group, a set of 11 highly resistant wheat cultivars were identified (NEC<30% and PYC<10%): Kranich, Desamo, Lear, Tuareg, Jenga, Zappa, Intro, Glaucus, Erasmus, M3 synthetic (W-7976) [identified STB resistance loci *Stb16q*(3DL) + *Stb17*(5AL)] and Solitar (QTLs on 5A, 6D and 7D). The broad range of disease resistance parameters values may suggest that resistance to STB in European cultivars is contributed mainly by quantitative loci and those with main effects. Presented work (phenotyping data) is a part of larger project aiming at identification of resistance genes (*Stb*) to Septoria tritici blotch in winter wheat and will be used in near future in association mapping approach.

Acknowledgments
This research was supported financially by the Polish Ministry of Agriculture and Rural Development, program Fundamental Research for Biological Progress in Crop Production (years 2014–2020), task 4.

Resistance to fusarium ear rot in maize: heritability and trait associations

Seweryn Frasinki[1*], *Elzbieta Czembor*[1], *Krzysztof Wojcik*[2], *Jozef Adamczyk*[3]

[1]Forage Grasses and Legumes Dept., Plant Breeding and Acclimatization Institute-NRI, Radzikow, Poland
[2]Plant Breeding Smolice Ltd., Co., Kobylin, Poland
[2]Malopolska Plant Breeding Raising HBP Ldt., Poland
[*]E-mail: s.frasinski@ihar.edu.pl

Keywords: *Fusarium*, ear rot, mycotoxin, maize, heritability

Maize is one of the most important crops, widely used not only for food and animal feed, but also as biofuel and bioproduct source. Poland, over the past decade, became fifth producing country in Europe, with total area planted over 1M ha (silage and grain combined). Ear rot caused by *Fusarium* spp. are most significant fungal disease, causing reduction in yield and affecting its quality. Changing environmental conditions, among conservations tillage techniques and maize/wheat dominated crop rotating systems influence the incidence of the disease. These factors may also affect the structure of the pathogen population, disease severity and associated mycotoxin contamination levels. In Poland an increase in the level of grain contamination by toxins produced by *Fusarium* has been observed in recent years. Among with appropriate agronomic practice, the use of highly resistant hybrids is an important part of the integrated plant protection method.

Because of this, the aim of this study was to determine heritability of resistance to ear rot and traits that are related to this disease resistance.

Forty-two inbred lines, which belong to flint (23) and dent (19) group and different KOB and SH gene pools, were crossed. Based on phenotypic ear rot assessment using scale 1 to 7 after inoculation with *F. graminearum* and under natural infection, they were divided into 3 groups: highly resistant (14 flint and 5 dent), moderately susceptible and susceptible (5 flint and 9 dent). For the next step of our study, parental lines from the first and last group and their F_1 hybrids were included (19 flint and 30 dent). As a control, hybrids which parental lines were included into moderate susceptible and highly resistant groups were evaluated. They were characterized under field condition taking into account such traits as: time of tassel anthesis and silk emergence, silk length and anthocyanin content, cob morphology, height. Ear rot resistance level of selected hybrids after inoculation has been confirmed by determining the content of DON. After cob harvest by hand their grain was grounded and tested by RIDA QUICK SCAN immunochromatographic test reader. Heritability and correlations between disease severity, DON contamination and selected traits were determined.

Acknowledgments
This work was funded by Polish Ministry of Agriculture and Rural Development No. 4-1-06- 3-01 (33)

Take two: Viability tests for wheat pollen

Daniela Impe[1*], *Janka Reiz, Claudia Köpnick*[1], *Hardy Rolletschek*[1], *Manuela Nagel*[1]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany
[*]E-mail: impe@ipk-gatersleben.de

Keywords: wheat pollen, viability, *in vitro* pollen germination, raffinose, stigmatic germination, impedance flow cytometry, FDA staining

Hybrid breeding is a promising method to overcome yield stagnation in wheat (*Triticum aestivum* L.) in the past years. High hybrid seed production relies on high pollen quality and an accurate identification of viable pollen. Wheat shed tricellular short-lived pollen at maturity. This study aims to evaluate, compare and improve viability tests for wheat pollen. The analysis of 157 liquid and solid media revealed that a solid medium with raffinose showed highest pollen germination (up to 87%). In fresh wheat pollen, raffinose is present only in minor amounts, whereas sucrose is the main sugar. In the germination medium, partly or complete substitution of raffinose with other sugars (sucrose, maltose) or sugar alcohols (mannitol, sorbitol) resulted in reduction of pollen tube growth assuming a higher metabolic efficiency or antioxidant activity of raffinose. *In vitro* pollen germination of viable fresh pollen and non-viable pollen inactivated by storage at ambient conditions (23°C an 50% relative humidity) for >60min was compared with stigmatic germination, fluorescein diacetate (FDA) staining, and impedance flow (IF) cytometry. Results of FDA staining and IF cytometry were higher than *in vitro* pollen germintaion but correlated significantly. Only Alexander staining failed to discriminate between viable, fresh pollen and inactivated pollen and hence was excluded as viability test for pollen. The comparison of 26 different wheat lines showed a high variability in germination. Significant difference associated with annuity could be found between spring types and winter types, but germination was neither associated with biotype (landrace, variety) nor with the sets grown at different time points. Pollen germination of 25 rye (*Secale cereale* L.), 11 barley (*Hordeum vulgare* L.) and 4 maize (*Zea mays* L.) were lower compared to wheat, concluding that media for *in vitro* pollen germination require specific modifications for different plant species and lines. FDA staining and IF cytometry are simple and fast techniques, but may overesimate pollen tube growth, whereas stigmatic germination is more laborious. Hence, as the exact potential of pollen to germinate remains elusive, a combination of pollen germination test with viability assessed by FDA staining and/or IF cytometry may provide reasonable indications of the ability of pollen to germinate and grow.

Comparison of organic and conventional planting of modern and traditional varieties

P. Parchanska[1], M. Vohradnikova[1], T. Blaha[1], I. Bizova[1], Daskova L.[2], Jiraskova K.[1]

Selgen a.s. Plant Breeding Station Stupice[1]
Selton. Researche Centrum[2]

Recently, the discussion on agriculture is mainly devoted to the issue of sustainability of yield levels while minimizing the impact on the environment. The rising population and the demand for nutritionally balanced meals contradict the desired reduction in agricultural productivity. The breeding goal of each breeder is to increase resistance to diseases and pests with the possibility of reducing chemical treatment.

We tested ourselves by discussing whether modern varieties can be grown without treatment and whether they can be grown under organic farming. We wanted to compare the characteristics of old and new varieties, yield, agronomic characteristics and their quality. We compared traditional varieties and newly registered varieties. Our aim was to test the suitability of growing modern varieties in various farming regimes. In any case, critics often attribute to modern varieties almost harmful effects on human health. Another aim was to put light on the quality composition of the original and modern varieties.

We tested a set of varieties in the years 2016–2017 in the regime of untreated and treated variants. In the year 2018 was added also the vertebrate in organic farming.

winter wheat variety	year of registration	obs.field score								
		frost test		heading	height		lodging		yield t/ha	
		I	II		untr.	tr.	untr.	tr.	untr.	tr.
CHLUMECKÁ 12	1919	8	6	30.5.	135	130	1	4	4,90	7,51
STUPICKÁ BASTARD	1927	8	7	2.6.	140	140	1	4	5,65	8,25
HANÁCKÁ OSINATÁ	1939	6	5	30.5.	145	140	2	5	4,78	7,56
PYŠELKA	1940	6	4	12.6.	120	120	2	5	4,39	9,60
RUBIOTA (Tr.spelta)	2001	5	3	1.6.	140	140	7	8	6,27	8,94
BOHEMIA	2007	9	8	29.5.	120	95	9	9	10,76	12,02
SKORPION	2011	8	6	1.6.	105	90	8	9	7,14	8,73
ANNIE	2014	9	7	30.5.	110	90	9	9	9,66	10,10
JULIE	2014	9	7	28.5.	115	95	8	9	11,23	12,53
RUMONA (Tr.monococcum)	2017	7	7	14.6.	130	125	1	2	NESKL	2,88

NAZV - QK1710302 název projektu „Zvýšení odolnosti pšenic vůči suchu, mrazu, padlí a fuzariózám klasu pomocí metod genomiky a proteomiky. “

Funded by European Union
Horizon 2020
Grant agreement No 771367

ECOBREED project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 771367. The content of this paper reflects only the author's view and the European Union Agency is not responsible for any use that may be made of the information it contains.

Mapping of the Lr55 brown rust resistance gene in common wheat (*Triticum aestivum* L.)

Aleksandra Pietrusińska[1*], *Mirosław Tyrka*[2**]

[1]National Centre for Plant Genetic Resources, Plant Breeding and Acclimatization Institute, National Research Institute at Radzikow, 05-870 Blonie, Poland,
[*]E-mail: a.pietrusinska@ihar.edu.pl
[2]Department of Biotechnology and Bioinformatics, Rzeszow University of Technology, 35-959 Rzeszow, Poland
[**]E-mail: mtyrka@prz.edu.pl

Wheat leaf rust is one of the most serious diseases of spring and winter wheat. The greatest losses are recorded in winter wheat. The symptoms are observed at all stages of plant development. In case of severe paralysis, the losses caused by this disease range from 40 to 50%. In Poland, the average yield loss is estimated at about 5 to 10%.

The main objective of today's plant production is to achieve the highest possible yield while minimizing the use of plant protection products. The cultivation of varieties with favourable economic features, including high yielding potential, is closely related to their resistance to fungal and viral diseases. An important role is played by the resistance breeding of cereals, which makes use of many tools of classical and molecular genetics. Molecular markers, commonly used for selection of gene(s) and genetic mapping for resistance to fungal diseases of cereals, including brown rust of cereals and grasses, play an important role.

Up yo now, there are no reports on molecular markers that can be successfully used to select a gene for the resistance to brown rust of cereals and grasses in plant breeding material. Therefore, the aim of this study is to determine molecular marker(s) in bread wheat, coupled with the gene of resistance to leaf rust *Lr55*. The plant material consisted of two mapping populations: F_2 (*Lr55*×Bogatka) and F_2 (*Lr55*×Nadobna). In total, 15 DArTs and 68 SSR markers were used to map the *Lr55* gene. On the basis of genetic analyses, the following conclusions can be drawn:

– It was confirmed that the *Lr55* gene is located on the 1BS chromosome.
– The mapped fragment is associated with the region syntenic to *Elymus trachycaulis.*
– Based on the obtained segregations for the mapping population (Bogatka × *Lr55*), molecular markers were determined, which can be used for selection of the *Lr55* gene in plant material.

Detailed data are in the process of publication.

Biological and economical characteristics of the allotetraploid from cereal family with genomic formula DDA^{u}A^{u}

Kishtili Kurkiev[1], Irina Adonina[2], Mina Gadjimagomedova[1], Ludmila Shchukina[2], Tatayana Pshenichnikova[2]

[1]Dagestan Experimental Station – department of VIR, Derbent, Russia
[2]Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

Keywords: *Triticum urartu*, *Aegilops tauschii*, growth habit, flavonoid pigmentation, leaf and spike morphology, yield components, technological properties of grain and flour

The synthesis of new allopolyploid cereal genotypes is an important task aimed at involving new genetic resources in breeding programs. Diploid species of the genera *Triticum* and *Aegilops* – bread wheat relatives – are the important source of agronomically valuable traits. Tetraploid synthetic with genomic formula DDA^{u}A^{u} was obtained by N.A. Navruzbekov through crossing *Aegilops tauschii* Coss. and *Triticum urartu* Thum. ex. Gandil. The purpose of this work was to study the chromosomal composition and biological and economically important traits of the tetraploid. Cytogenetic analysis using fluorescent in situ hybridization showed the presence of all chromosomes of the D genome in the synthetic's chromosomal complement. With the help of a stepwise vernalization, the winter habit of tetraploid synthetic was established with the optimum vernalization requirement of 45 days. In the greenhouse conditions, two groups of genotypes were found with a difference in flowering date of 6.5 days, which may indicate an allelism at the *Vrn-3* locus. The coloring of various organs of the tetraploid plant such as, coleoptile, stem, anthers and glumes of the spike was revealed. The coloration of the aleurone layer of the grain may indicate that the donor species T. urartu is a carrier of the *Ba* gene that controls its blue color. The new morphotype of leaf pubescence was found. In terms of productivity, tetraploid is comparable to the bread wheat. Grains are characterized by a super-soft structure and high wet gluten content – from 39–45 to 65%, in field and greenhouse conditions, respectively. Thus, tetraploid can be used to create the new genotypes in wheat breeding as a source of untapped genetic diversity, as well as a new genetic model for studying the patterns of evolution of polyploid plants.

Acknowledgments
The work on fluorescent *in situ* hybridization (FISH) and microphotography of leaf pubescence were performed on the basis of the Common Use Center for Microscopy of Biologic Objects of the ICG SB RAS. Greenhouse experiments were carried out at the Common Use Center – Laboratory of Artificial Plant Growth of the ICG SB RAS with the support of the budget project of the ICG SB RAS No. 0324-2019-0039. Field studies were conducted on the Dagestan ES of VIR in the framework of the topic of research and development #0662-2019-0006.

Validation of KASP markers associated with pre-harvest sprouting tolerance in a panel of European winter wheat cultivars

Rajković Bruno[1,2*], *Šarčević Hrvoje*[1,2], *Lovrić Ana*[1], *Maričević Marko*[3], *Novoselović Dario*[2,4]

[1]University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia
[2]Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia
[3]Bc Institute for Breeding and Production of Field Crops, Rugvica, Dugoselska 7, 10370 Dugo Selo, Croatia
[4]Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia
[*]E-mail: brajkovic@agr.hr

Keywords: Pre-harvest sprouting, molecular markers, wheat, dormancy

Pre-harvest sprouting (PHS) can reduce grain yield as well as bread-making quality of wheat resulting in economic losses for farmers and millers. Tolerance to PHS is therefore a desirable trait of a cultivar when prolonged rainy periods occur before harvest. Although the tolerance to PHS is known to be associated with various physiological, morphological and developmental characteristics of the seed and the spike, seed dormancy has been considered most critical factor for PHS tolerance. The level of seed dormancy as well as other components of PHS tolerance are controlled by genotype (G), environment (E) as well as G × E interaction. A number of studies reported various types of DNA markers to be significantly associated with quantitative trait loci (QTL) controlling PHS resistance, offering the possibility of their use in marker- assisted selection (MAS).

In the present study, we validated 40 kompetitive allele-specific PCR (KASP) markers in a panel of 178 European winter wheat cultivars. All KASP markers have been previously reported to be significantly associated with the PHS tolerance in the cultivar panels or mapping populations, thus revealing potential application in wheat breeding programs. The PHS tolerance of 178 wheat genotypes, which was grown in a replicated field trial at location Zagreb, Croatia in 2016/2017 and 2017/2018 growing seasons was assessed using germination tests with hand threshed grains at harvest maturity and was expressed as weighted germination index (WGI) as described by Reddy et al. (1985). Forty KASP assays were selected from published reports and leaf samples from 178 genotypes were collected using LGC's Plant Sample Collection Kit™ and sent to LGC Genomics for genotyping.

Single-marker linear regression analysis was used to determine the association between KASP marker and genotype mean WGI across years. The magnitude of the marker-associated phenotypic effect was described by the coefficient of determination (r^2), which is a fraction of the total phenotypic variance accounted by the marker genotype. Combined analysis of variance across years revealed significant effect of genotype (G), year (Y) and G×Y for WGI. Out of 40 used KASP markers, 12 markers were included in the regression analysis, while other 28 markers were discarded because of being monomorphic, heterozygous on too many loci or they gave no signals. The regression of WGI on KASP markers was significant only for three markers, TaPHS1-646, TaPHS-666 (chromosome 3A) and TamKK3-A (chromosome 4A) with coefficients of determination (r^2) being 0.0238, 0.0412 and 0.0825,

respectively. Regression of WGI on the combinations of alleles (haplotypes) with the above mentioned three markers resulted in a considerably larger determination coefficient (r^2 = 0.098) suggesting a greater efficiency of the marker combination than individual markers in MAS implementation.

References

Reddy, L.V., Metzger, R.J. and Ching, T.M. (1985) Effect of Temperature on Seed Dormancy of Wheat. Crop Science Vol. 25: 455–458

Characterization of the allelic variation at the *VRN-1* locus of common wheat (*Triticum aestivum* L.) genotypes from South-Eastern Europe

Karolina Różaniecka[1*], *Michał Nowak*[1], *Justyna Leśniowska-Nowak*[1], *Krzysztof Kowalczyk*[1]

[1]Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences, Lublin, Poland
[*]E-mail: karolina.r@onet.com.pl

Keywords: vernalization, *Vrn* genes, common wheat, molecular markers

The effect of low temperature activity at certain stages of cereal development is very important for the transition of the plant from vegetative to generative phase (Chouard, 1960). On the genetic level of common wheat, the dominant alleles (*Vrn-A1*, *Vrn-B1*, *Vrn-D1*) determine the development of spring growth habit, whereas the recessive alleles determine the development of winter growth habit. The allelic variation at the *VRN-A1* locus is related to mutations within the promoter sequence (Yan et al. 2004), or deletions within the first intron (Fu et al. 2005). For the *VRN-B1* and *VRN-D1* loci, alterations in the promoter sequence were not observed, and the allelic variation is determined only by deletion within the sequence of the first intron (Fu et al. 2005). The aim of presented study was complex characterization of the allelic variation at the *VRN-1* locus of common wheat genotypes of South-Eastern European origin.

The plant research material of the presented study consisted of 94 spring and 153 winter accessions of common wheat (*Triticum aestivum* L.) originated from 17 countries of South-Eastern Europe area, including Austria, Belarus, Bulgaria, Croatia, Czech Republic, Estonia, Greece, Hungary, Latvia, Lithuania, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, and Ukraine.

The identification of the *VRN-1* alleles was carried out by means of the molecular markers based on the analysis of the *Vrn-A1* promoter region and the first intron of the *Vrn-1* gene (Yan et al. 2004, Fu et al. 2005).

The dominant *Vrn-A1* allele was observed in 79 analyzed genotypes – for a majority of them the *Vrn-A1a* form of the allele was recorded, only for 4 accessions, the *Vrn-A1b* allele was observed. The dominant *Vrn-B1* allele was found in 59 and *Vrn-D1* in 28 of analyzed genotypes. In the case of the 140 tested accessions, recessive alleles were present for all three analyzed loci.

The results of our research showed that the dominant *Vrn-A1* allele determines is the major genetic factor determining the spring growth habit of common wheat. This dominant allele was present in 76 out of 94 analyzed spring wheat accessions. For the remaining 18 genotypes spring growth habit is determined by dominant *Vrn-B1*, *Vrn-D1* or *Vrn-B1+Vrn-D1* alleles presence (5, 3, and 5 accessions, respectively). The exception was 5 genotypes showing spring growth habit despite 3 recessive alleles occurrence (*vrn-A1*, *vrn-B1*, *vrn-D1*) originating from Bulgaria and Ukraine.

The presence of recessive allele *vrn-A1* determines the winter growth habit of the majority of analyzed common wheat genotypes (150 out of 153). The exception was 3 accessions originated from Latvia, Lithuania, and Serbia.

Presented results shed new light on the geographic distribution of *Vrn-1* alleles on the area of South-Eastern Europe. Moreover, information about *VRN* genotype could be useful for subsequent studies concerning frost tolerance and response to low temperature in common wheat, as well as for breeding purposes.

References

1. Chouard P. 1960. Vernalization and its relations to dormancy. Annu. Rev. Plant. Physiol. 11: 191–238.
2. Yan L, Helguera M, Kato K, Fukuyama S, Sherman J, Dubcovsky J. 2004. Allelic variation at the *VRN-1* promoter region in polyploid wheat. Theor. Appl. Genet. 109: 1677–1686.
3. Fu D, Szűcs P, Yan L, Helguera M, Skinner JS, Von Zitzewitz J, Hayes PM, Dubcovsky J. 2005. Large deletions within the first intron in *VRN-1* are associated with spring growth habit in barley and wheat. Mol. Genet. Genomics 273: 54–65.

Crossability of genetically diverse triticale genotypes selected on the basis of single chromosomes localized DArTseq markers

Karolina Różaniecka[1], Michał Nowak[1], Justyna Leśniowska-Nowak[1], Magdalena Sozoniuk[1], Magdalena Kawęcka[1], Piotr T. Bednarek[2]*

[1]Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences, Lublin, Poland
[2]Plant Breeding and Acclimatization Institute, National Research Institute, Radzików, Poland
[*]E-mail: karolina.r@onet.com.pl

Keywords: DArTseq markers, genetic diversity, crossability, triticale

Triticale (×Triticosecale Wittmack) is a relatively new, artificially produced genus. It combines in one form the high quality and yield of wheat and resistance of rye. Because of the fact, that triticale did not pass the natural process of evolution, it has a narrow gene pool in comparison to another species.

High throughput molecular marker systems are currently one of the most important tools for genetic diversity analysis. In polyploid plant species distribution of the markers is not equal for all regions of the genome. The objective of the study was the determination of the crossability of genetically diverse Polish triticale genotypes selected on the basis of single chromosomes localized DArTseq markers.

The plant material consisted of 470 winter triticale breeding lines. Genotyping of these lines was performed by means of DArTseq technique (Diversity Array Technology, Canberra, Australia). As a result of DArTseq analysis 87 493 markers were obtained for each of the tested genotypes. After preliminary analysis 24 353 markers were selected for subsequent analyses. Obtained results showed that maximal genetic distances between analyzed triticale genotypes were different and dependent on chromosome localization of the markers. For maximal genetic distance, the highest value was noticed for 3R chromosome markers (0.9838) and the lowest for 5A chromosome (0.8382). The obtained values of the coefficient of maximum genetic distance were the basis for the selection of parental components for crossing. A pair of genotypes which were characterized by the highest genetic diversity within each chromosome were selected and used as parental forms for crossing.

Obtained results revealed, that the level of crossability was dependent on chromosome localization of markers on the basis of which the genetic distance between parental forms used for crossing was determined. This observation can be valuable for the design of crossing in triticale breeding programs, especially focused on hybrid breeding.

Acknowledgement
The results of the study were obtained within the framework of the project funded by Polish Ministry of Agriculture and Rural Development entitled ‘Identification of the genome regions and DNA markers linked to heterosis in hexaploid winter triticale’.

Investigation on the structure of 1RS chromosome arm in a wheat-*Secale cereanum* T1BL.1RS translocation conferring stripe rust resistance to wheat

Kitti Szőke-Pázsi[1], *István Molnár*[1,2], *Balázs Kalapos*[1], *László Ivanizs*[1], *Márta Molnár-Láng*[1], *Éva Szakács*[1*]

[1]Agricultural Institute, Centre of Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary
[2]Institute of Experimental Botany, Centre of the Region Haná for Biotechnological and Agricultural Research, Olomouc, Czech Republic
[*]E-mail: szakacs.eva@agrar.mta.hu

Keywords: DArTseq, *Secale cereanum,* recombination, T1BL.1RS, stripe rust, resistance

Chromosome arm 1RS of 'Petkus' rye, in the form of T1BL.1RS translocation, has long been present in European wheat cultivars owing to its positive effect on yield, and genes providing resistance against leaf rust (*Lr26*), stem rust (*Sr31*), stripe or yellow rust (*Yr9*) and powdery mildew (*Pm8*). These resistance genes except for *Sr31* are no longer effective, consequently, broadening the genetic diversity on the 1RS arm is a pressing issue in wheat breeding.

'Kriszta' is a Hungarian perennial rye cultivar deriving from a cross between *Secale cereale* (cv. Várda) and *S. strictum* (ssp. *anatolicum*), having high dietary fiber and protein content, being tolerant to frost and drought, and resistant to foliar diseases. Earlier, the wheat line Martonvásári 9 kr1 (Mv9kr1) carrying recessive crossability genes *kr1kr1kr2kr2* was crossed with 'Kriszta', and recently a stripe and leaf rust resistant T1BL.1RS wheat line (line '179') has been selected in field conditions. Its yield, as well as protein and arabinoxylan content was significantly higher than those of the parental wheat genotype. Fluorescence *in situ* hybridization pattern with DNA repeat probe pSc119.2 suggests that the 1RS chromosome arm in '179' derives from a recombination between *S. cereale* and *S. strictum* ssp. *anatolicum*.

Allelic composition of the line '179' together with 'Mv9kr1, 'Mv Magdaléna' (having 1RS of 'Petkus' origin) and Mv9kr1/Kriszta 1R disomic addition sensitive to foliar diseases was investigated by DArTseq technology (Rye DArTseq v. 1.0). Of the generated 258,090 Silico- and 71,177 SNP-DArTseq markers we selected 5,312 putative 'Kriszta' 1R-specific Silico- and 1,755 SNP-DArTseq markers. In order to confirm that the selected markers are specific for the rye 1R chromosome, we used trimmed marker sequences for BLASTn against the rye Lo7 genome sequences. 679 Silico and 168 SNP (a total of 847) markers were aligned to the rye Lo7 WGS 1R contigs. We defined 331 Silico- and 110 SNP-DArTseq markers associated with genes, which were functionally annotated by the HMM (Hidden Markov Model) based Pfam database or Gene Ontology terms. Using the Rye Genome Zipper database, from the 847 markers we identified 233 (203 Silico + 30 SNP) markers locating on the 1RS arm (0–60.72 cM region). From these 1RS-specific markers we displayed the resistant ones on a genetic map (111 Silico + 18 SNP), from which 8 Silico- and 2 SNP-DArTseq markers were associated with the resistance related LRR (Leucine Rich Repeat) domains. Comparison of allelic compositions revealed about 16 % (27 Silico and 10 SNP markers) difference between 1RS of line '179' and that of 'Mv Magdaléna' ('Petkus').

Acknowledgments
This research was funded by the National Research, Development and Innovation Office – NKFIH, K119387 and K116277, and Marie Curie Fellowship grant award 'AEGILWHEAT' (H2020-MSCA-IF-2016-746253).

T2: Adaptation to Changing Environments and Plant Microbes Interactions

A search for genetic determinants of phosphorus deficiency tolerance in rye (*Secale cereale* L.)

Anna Hawliczek[1], Leszek Bolibok[2], Ewa Borzęcka[1], Katarzyna Tofil[1], Piotr Gawroński[1], Magdalena Święcicka[1], Hanna Bolibok-Brągoszewska[1]*

[1]Department of Plant Genetics Breeding and Biotechnology, Warsaw University of Life Sciences-SGGW, Warsaw, Poland
[2]Department of Silviculture, Warsaw University of Life Sciences-SGGW, Warsaw, Poland
[*]E-mail: hanna_bolibok_bragoszewska@sggw.pl

Keywords: Phosphorus deficiency tolerance, nutrient deficiency tolerance, *Secale*, rye, hydroponics, gene expression

Phosphorus (P) is one of macronutrients, indispensable for growth and development of plants, especially for the development of roots. On the cellular level P is involved in various key processes, such as synthesis of nucleic acids. Plant utilize a relative small proportion of applied P fertilizers (up to 30%), because of conversion of P in the soil into plant-inaccessible forms. Therefore, it is crucial to understand the mechanism of P use efficiency (PUE) and tolerance of low P content, and to apply this knowledge in breeding of new crop varieties, producing satisfactory yields under reduced or none P fertilizing, to successfully meet the challenge of feeding the growing human population.

Rye is closely related to wheat and barley and an important source of variation for wheat breeding. It is considered to have the highest tolerance of abiotic stresses among *Triticeae*, including the highest tolerance of nutrient deficiency. Our aim is to gain knowledge concerning genetic determinants of PUE in rye, with the special emphasis on the identification of rye's unique solutions of coping with P-deficiency.

We established a hydroponic based method of assessing P-deficiency tolerance in rye inbred lines at the early vegetative stage of growth. Using a modified Hoagland's nutrient solution, with 0.2 mM KH_2PO_4 in control conditions and 0.02 mM KH_2PO_4 (with additional 0.18 mM KCl) in low P solution, we identified so far two pairs of inbred lines: K3 and L310, and L9 and L318, which performance did not significantly differ in P normal conditions, but differed significantly in P-low conditions (Kruskal-Wallis test, $p = 0{,}05$).

In parallel, we started a synteny-based identification of putative rye orthologs of known genes involved in PUE. We searched in the genome sequence of rye inbred line Lo7 and in publicly available rye transcriptome data for sequences exhibiting similarity to several genes involved in PUE, that were previously identified in rice. So far rye sequences exhibiting significant similarity to bHLH transcription factor *PTF1*, high affinity phosphate transporter *PT6* (three putative family members), and *PHO1;2* were found. Next, we carried out qRT-PCR analyses using root samples collected from inbred lines K3 and L310 on day 17 of the hydroponic trial. We observed a significantly higher expression in roots of plants grown

in low P conditions for two putative rye orthologs of *PT6* (preliminary named *ScPTG_6* and *ScPTG_6*) in both genotypes. Additionally, in the inbred line L310 only we observed a significantly higher expression of putative rye ortholog of *PHO1;2* and of putative rye ortholog of wheat phosphate transporter *TaPHT1.10*.

In conclusion, we succeeded in establishing a hydroponics-based screening method for P-deficiency tolerance in rye and confirmed that there is genetic variability with respect to P-deficiency in the rye inbred lines. This genetic variability is sufficient to identify inbred lines with contrasting response to P-deficiency, suitable for further research involving transcriptomic approaches of candidate gene identification.

Development of waxy wheat genotypes for Northern Europe

Kristina Jaškūnė, Rita Armonienė, Gražina Statkevičiūtė, Jurgita Cesevičienė, Andrii Gorash, Žilvinas Liatukas, Vytautas Ruzgas, Gintaras Brazauskas[*]

[1]Lithuanian Research Centre for Agriculture and Forestry, Akademija, Lithuania
[*]E-mail: gintaras.brazauskas@lammc.lt

Keywords: waxy wheat, amylopectin starch, biorefinery, winter wheat breeding

Common wheat starch is composed of a mixture of amylose and amylopectin (approximately 25 to 75 ratio, respectively), while waxy wheat starch contains almost solely amylopectin. The waxy starch is desired by processing industries due to improved qualities of the products produced from waxy wheat flour, such as extended freshness time and improved palatability, as well as other industrial applications. The waxy trait is determined by the absence of functional Wx proteins in wheat because of the mutations in all three homeologous waxy genes, Wx-A1, Wx-B1 and Wx-D1. These null mutations have been transferred to a number of wheat genotypes however none of these are adapted to climatic conditions of Northern Europe, characterized by cold winters and cool long-day summers.

Here we present a project aiming to develop freezing resistant winter wheat waxy varieties, using traditional and innovative breeding methods. The project objective will be achieved by implementing specific project tasks: i) to develop high-throughput selection protocols suitable for wheat line screening for increased freezing tolerance, ii) to screen TILLING population for novel mutations in GBSS, SUS and SBE genes, iii) to develop waxy winter wheat breeding lines by introgressing the waxy trait into advanced breeding material.

Authors will present first results of TILLING screening in starch biosynthesis genes and relationship between waxy trait and freezing tolerance in winter wheat.

Acknowledgments
The project is funded by Research Council of Lithuania, grant no. 01.2.2.–LMT–K–718-01-0065

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

Tomasz Góral[1*], *Halina Wiśniewska*[2], *Piotr Ochodzki*[1], *Dorota Walentyn-Góral*[1], *Maciej Majka*[2]

[1]Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, Poland
[2]Institute of Plant Genetics of the Polish Academy of Sciences, Poznań, Poland
[*]E-mail: t.goral@ihar.edu.pl

Keywords: type your, keywords here, separated, by commas

Fusarium head blight (FHB) is a disease of cereals caused by fungi of genus *Fusarium*. The main causing species are *F. culmorum* and *F. graminearum*. These fungi produce toxic metabolites – mycotoxins, with phyto-and zootoxic potential. Mycotoxins are accumulated in cereal grains and can contaminate food and feed products. One of the ways to control FHB and reduce the negative impact of toxins on human and animal health is cultivation of FHB resistant cultivars.

Winter wheat breeding lines (54) and 19 check cultivars/lines were evaluated in field experiments in two locations (Poznań, Radzików). Check cultivars/lines were: five resistant checks: '20828[Fhb1–]', 'A40-19-1-2', 'Arina', 'Fregata', 'UNG 136.6.1.1[Fhb1+]'; six lines with *Fhb1* gene from crosses of winter wheat cultivars with 'Sumai 3'; five susceptible checks; three high yielding cultivars 'Artist', 'Patras' and 'RGT Kilimanjaro'. Wheat heads were inoculated with the spore suspension of *F. culmorum* isolates producing deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEN). Fusarium head blight index (FHBi) was evaluated. The proportion of *Fusarium* damaged kernels (FDK) was determined visually by dividing the sample on healthy looking kernels and with symptoms of *Fusarium* damage. Using the technique of gas chromatography, HPLC and immunoenzymatic tests the contents of ergosterol (ERG), DON and acetyl derivatives, NIV and ZEN in the grain were analysed.

Average FHBi amounted to 5.5%, ranging from 0.5 ('S 13 [Fhb1+]') to 31.5% ('KBP 14 16 (S)'). Proportion of *Fusarium* damaged kernels was on average 12.7%. FDK ranged from 3.0 ('S 12 [Fhb1+]') to 40.4% ('KBP 14 16 (S)'). The contents of the ERG in grain was an average of 10.8 mg/kg, ranging from 3.7 ('A40-19-1-2') to 27.5 mg/kg ('RGT Kilimanjaro'). In the samples from Poznań on average 15.5 mg/kg ERG was found, and in samples from Radzików the contents of the ERG was lower at 6.2 mg/kg.

The average content of DON in grain was 1.495 mg/kg. The range of variation from 0.058 to 7.726 mg/kg. In the samples from Poznań, the content of DON was very low and amounted to 0.595 mg/kg (0.023 – 8.300 mg/kg). In Radzików, it was 4 times higher at 2.395 mg/kg (0-13.825 mg/kg). DON acetyl derivatives (3AcDON and 15AcDON) were found in the grain of wheat. The contents of these toxins were very low. The average content of nivalenol (NIV) in the grain was higher than the content of DON at 2.778 mg/kg. The range of variation from 0.353 to 8.173 mg/kg. In the samples of Radzików contents of the NIV was very low and was 0.067 mg/kg (0-0.375 mg/kg). In Poznań was very high at 5.488 mg/kg (0.680-16.295 mg/kg). Average total content of the trichothecenes B in the grain of wheat lines was 4.338 mg/kg. The range of variation from 0.840 ('S 10 [Fhb1 +]') to 14.465 mg/kg ('POB

0416'). Content of ZEN in the grain was low and amounted to an average of 45 □g/kg. The range of variation from 0 ('S 12 [Fhb1+]' and 11 other lines) to 454 □g/kg ('KBP 14 16 (S)'). In the samples from Poznań, only an average of 17 □g/kg of ZEN was found, and from Radzików content of ZEN was 73 mg/kg.

Lines with low accumulation of ergosterol and specific mycotoxins has been found. Multivariate PCA analysis allowed the identification of genotypes with low accumulation of ergosterol and different groups of mycotoxins as well as low FHBi and FDK values.

Sources of powdery mildew resistance in modern bread wheat cultivars

Bulat Islamov[*]

Estonian Crop Research Institute, Jõgeva, Estonia
[*]E-mail: bulat.islamov@etki.ee

Keywords: powdery mildew, wheat, resistance

Powdery mildew caused by a fungus *Blumeria graminis* f. sp. *tritici* (Bgt) is a common disease of wheat. Severe powdery mildew epidemics, if left uncontrolled, can significantly decrease yield. Breeding wheat cultivars for disease resistance is economically justified and environmentaly safe alternative to fungicide treatments. The important prerequisite for a resistance breeding is the knowledge of virulence structure of a local pathogen population and the availability of breeding lines and cultivars that could serve as resistance sources. This study has two aims: (1) to identify virulences present in local powdery mildew population and (2) to assess resistance in modern wheat cultivars to be used as breeding material.

Three sets of wheat genotypes were used: (1) 45 spring wheat cultivars, (2) 16 winter wheat cultivars and (3) wheat cultivars and breeding lines with known Pm genes, i.e. differential set. Seedlings were scored after challenge with 15 Bgt isolates collected in Estonia in 2015-2019.The powdery mildew infection score was also assessed under natural infection in the field.

In a seedling test spring wheat cultivars Amulett, Cricket, Flippen, Levels, Sonett showed resistance to all Bgt isolates. Spring wheat cultivars Arabella, Berlock, Boett, Cricket, Daugana, Diskett, Hamlet, Happy, Hewilla, KWS Collada, Tybalt, Vinjett and winter wheat cultivar Bonanza despite having low median infection score, were susceptible to at least one Bgt isolate. Generally, field resistance of tested cultivars conformed with the seedling test infection score. However, field infection scores of cultivars Buddy, Hamlet, Hiie, KWS Collada and Quintus significantly exceeded seedling test scores. In a set of Bgt differential lines breeding line BRG 3N having *Pm16* gene showed seedling resistance to most Bgt isolates.

The reason for unexpectedly high infection scores in the field will be further investigated. As a result of this study sources of resistance to Bgt infection were identified and can be used in a breeding programme.

Acknowledgments
Help of Andrii Gorash (LAMMC), Anne Ingver (ECRI), Reine Koppel (ECRI) and Gene Bank Dept., CRI Prague – Ruzině in obtaining seeds for experiments with seedlings and organizing field experiments is acknowledged.

Correlations between agronomical traits, yield and grain filling parameters of barley genotypes in control and terminal drought conditions

Vesna Kandić[1], *Dejan Dodig*[1]

[1]Maize Research Institute „Zemun Polje“, Slobodana Bajića 1, Belgrade, Serbia
*E-mail: vesna.kandic@gmail.com

Keywords: barley, terminal drought, correlatins, agronomical traits, yield

The aim of this study was determine correlations among investigated traits of two- and six- rowed barley genotypes in order to define criteria for indirect selection for resistance to drought in the period after flowering. 25 barley genotypes were grown in a randomised complete block design trial with two replications, two treatments at two locations, in the period 2010–2011 and again in 2011–2012. One treatment was control (C), while in the other treatment mechanical defoliation was performed (D), 7 days after flowering of each genotype. Through the inhibition of current photosynthesis (as result of defoliation), the treatment simulated drought conditions during grain filling. Of all investigated traits the strongest highly significant correlations were obtained between yield and biomass. For the group of two-rowed genotypes (0.841***) in C and (0.581*) in D, and for six-rowed (0.968***) in C and (0.883***) in D treatment. Selection for the yield in drought conditions, should be focused on getting the biggest biomass, because when ongoing assimilation is decreased, the translocation of assimilates from vegetative parts significantly contributes to the yield. Correlation coefficients between the yield and the harvest index, in both treatments were more significant for the six- than two- rowed genotypes.

The spike index could be used as a reliable selection criterion in drought conditions, due to its very significant correlation with the yield (0.689 **) and (0.802 **), for two- and six rowed genotypes respectively. The average production per spike had statistically significant correlation with the yield in both treatments just for the group of the six- rowed genotypes. On the other side, in drought treatment this trait was in negative correlation with the length of the uppermost internode (–0.791 **), the length of the exposed part of the internode (–0.847 **) and the plant height (–0.638 *), so breeders should focus on lower 6-rowed genotypes in which the translocation into the spikes is faster, but so as to keep as much biomass as possible. Very strong correlations were obtained between the 1000 kernel weight and the average intensity of grain filling, for two- rowed genotypes (0.930 ***) and (0.826 ***), in (C) and (D) treatment respectively, as well as for six- rowed (0.937 ***) and (0.933 ***), in (C) and (D), respectively. The average intensity of grain filling in six-rowed genotypes was in negative correlation with the plant height (–0.832 **), the spike length (–0.814 **) and the number of grains per spike (–0.717 *) in (C) and (D) treatment (–0.637 *), (–0.875 ***) and (–0.779 **). Highly significant correlation coefficients obtained between yield in (C) and (D) treatment, (0.730 ***), indicating that the selection under optimal conditions would lead to an increase in yield in drought conditions, and vice versa.

Acknowledgements
This work was supported by the Serbian Ministry of Education, Science and Technological Development under Grant TR 31005

Increased proportion of far-red in the incident white light modify membrane lipid composition by temperature dependent manner in winter barley

Kovacs T.[1*], *Ahres M.*[2,3], *Gombos Z.*[1], *Galiba G.*[2,3]

[1]Institute of Plant Biology, Biological Research Centre, Hungarian Academy of Sciences, H-6701 Szeged, Hungary
[2]Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 2462 Martonvásár, Hungary
[3]Festetics Doctoral School, Georgikon Faculty, University of Pannonia, 8360 Keszthely, Hungary
[*]E-mail: kovacs.terezia90@gmail.com

Keywords: barley, frost-tolerance, lipid composition, far-red light, low temperature

It is well elaborated that *CBF*-regulon (*C-repeat binding factor*) plays a central role in the modulated light spectrum induced cold acclimation process both in Arabidopsis and cereals. The freezing tolerance was determined by electrolyte leakage, from the leaves treated by far-red supplemented white incident light, by conductometer showing the degree of the cell membrane integrity. Consequently, the changes in cell membrane lipid and protein composition have a crucial role during this hardening process. Our goal in this study was to elucidate the differences in the lipid composition extracted from leaves treated by modulated light at two different temperatures. In this experiment barley plantlets were grown under different light conditions [white light and white light supplemented with far-red (735 nm) light (FR)] at 5°C and 15°C and the different lipid molecular species were determined by MS spectroscopy. We could observe some changes in LPG and LPE lipid groups, and in PC/PE and MGDG/DGDG ratio during the supplemented FR light. By calculating the double-bond index, we noticed major changes in the model at 15°C. The overall lipid content, change at PE and PS under increased FR light ratio at 15°C. In several PS species we found potent changes in cooler growth conditions. After the 1st day of the treatment the amount of PG species increased as a result of FR treatment at 15°C. These changes can be experienced in PG 34:4, 34:3, 34:2 and 34:1. Our results clearly show that the increased FR ratio in the incident light affects the lipid composition in barley leaves at temperature dependent manner.

Acknowledgements
The project was supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project and by the Hungarian Scientific Research Fund 'OTKA' K128575.

*Zoltán Gombos one of our author has depart this life

Triticale dihaploid and somaclonal lines screening for resistance to *P. nodorum*

Lidia Kowalska[1*], *Edward Arseniuk*[1]

[1]Department of Plant Pathology, Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, 05-870 Blonie, Poland
[*]E-mail: l.kowalska@ihar.edu.pl

Keywords: *Parastagonospora nodorum*, somaclonal, androgenic variation, triticale, resistance breeding

One of the destructive pathogens of triticale worldwide is *Parastagonospora nodorum*, a causal agent of Stagonospora nodorum blotch (SNB). SNB on average causes yield losses of 15–20% largely attributable to kernel weight reduction. To control this disease conventional methods of breeding for resistance need to be supported by biotechnological ones, like somatic embryogenesis and androgenesis. Therefore, an effort was undertaken to compare variation to *P. nodorum* response among winter triticale somaclones, dihaploids and conventional cultivars.

A population of 16 somaclonal and twenty one dihaploid triticale lines from seven crosses were used to test their resistance to *P. nodorum* under field conditions. Lines were grown in disease free (fungicide sprayed) and inoculated with the pathogen microplots in 2 replications of a split-plot design in a single environment. Inoculation of plant leaves was done three times with a mixture of *P. nodorum* isolates of high pathogenicity, which originated from different geographic regions of Poland. Spore concentrations were adjusted to 4×10^6 of viable pycnidiospores per one millilitre. The disease severity was rated on a scale, where >90% – susceptible, <10% – resistant.

The differences between SNB response of plant leaves of somaclones and dihaploids for all components were statistically significant. Higher resistance to *P. nodorum* was observed more often on leaves of somaclonal lines than on dihaploid ones. On average disease severity reached 16.4% on leaves of somaclones and 17.2% on leaves of dihaploids. Some of genotypes were showing low leaf infection, e.g. dihaploid D-41 (infection was 4%) and a somaclone S-49 (2%).

The results from this study suggest that dihaploid and somaclonal variation might be considered as an additional source of triticale natural resistance to the pathogen and it could be recommended to use in commercial breeding programs. Resistance breeding shows farmers, and plant breeders how to use a long-neglected technique to develop new cereal varieties with elevated natural resistance to pests and diseases.

Acknowledgments
This work was financially supported by Ministry of Agriculture and Rural Development

Improving and maintaining winter hardiness and frost tolerance in bread wheat by genomic selection

Sebastian Michel[1], Franziska Löschenberger[2], Christian Ametz[2], Ellen Sparry[3], Hermann Bürstmayr[1]*

[1]Department of Agrobiotechnology, IFA-Tulln, University of Natural Resources and Life Sciences Vienna, Konrad-Lorenz-Str. 20, 3430 Tulln, Austria
[2]Saatzucht Donau GesmbH. & CoKG, Saatzuchtstrasse 11, 2301 Probstdorf, Austria
[3]C&M Seeds, 6180 5th Line, Palmerston, ON, Canada N0G 2P0
[*]E-mail: sebastian.michel@boku.ac.at

Keywords: bread wheat, cold tolerance, copy number variation, genomic prediction, winter survival

Winter hardiness is a major constraint for autumn sown crops in temperate regions, and thus an important breeding goal in the development of new winter wheat varieties. Winter hardiness is though influenced by many environmental factors rendering phenotypic selection under field conditions a difficult task due to irregular occurrence or absence of winter damage in field trials. Controlled frost tolerance tests in growth chamber experiments are on the other hand even with few genotypes often costly and laborious, which makes a genomic breeding strategy for early generation selection an attractive alternative. The aims of this study were thus to compare the merit of marker-assisted selection using the major frost tolerance QTL Fr-A2 with genomic prediction for winter hardiness and frost tolerance, and to assess the potential of combining both measures with a genomic selection index using a high-density marker map or a reduced set of pre-selected markers. Cross-validation within two training populations phenotyped for frost tolerance and winter hardiness underpinned the importance of Fr-A2 for frost tolerance especially when upweighting its effect in genomic prediction models, while a combined genomic selection index increased the prediction accuracy for an independent validation population in comparison to training with winter hardiness data alone. The prediction accuracy could moreover be maintained with pre-selected marker sets, which is highly relevant when employing cost reducing fingerprinting techniques such as targeted genotyping-by-sequencing. Genomic selection showed thus large potential to improve or maintain the performance of winter wheat for these difficult, costly and laborious to phenotype traits.

References

Michel S, Hellinger J, Löschenberger F et al (2019) Improving and maintaining winter hardiness and frost tolerance in bread wheat by genomic selection. Submitted to Front Plant Sci

Genetic variation, heritability and genotype *x* moisture conditions interaction of morpho-physiological traits in durum wheat

*Reza Mohammadi**

Dryland Agricultural Research Institute, Sararood Branch, AREEO, Kermanshah, Iran
*E-mail: r.mohammadi@areeo.ac.ir

Keywords: durum wheat, drought stress, agro-physiological traits, heritability, genotype *x* moisture conditions

The field trials were conducted on selected durum genotypes with known performance under rainfed conditions to: (i) investigate for the morpho-physiological traits that could most explain drought tolerance and (ii) identify those that would produce high and stable grain yield across drought conditions from mild to severe conditions. Combined analysis of variance on grain yield and the studied traits indicated significant effects ($P < 0.01$) for genotype, year, moisture condition, year x moisture condition, genotype *x* year, and genotype *x* moisture condition interactions. There were differences in trait associations across the years and moisture conditions showing that the traits were significantly influenced by the year and moisture conditions effects. The heading date, plant height, spike length and SPAD-reading with low ratio of σ^2ge/σ^2g showed a high value for heritability. Drought stress reduced grain yield by 76%. Specific adaptation to drought conditions showed significant crossover interaction between well-performing genotypes. The traits heading date, peduncle extrusion, plant height, days to maturity and 1000-kernel weight with lower genotype *x* moisture interaction than grain yield, were most contributed to drought tolerance. Using the GGE biplot analysis the breeding lines G16, G12, G15 and G10 were identified as the most stable and high yielding genotypes across different moisture stress conditions that can be used in durum breeding program to develop drought tolerant cultivars. The breeding lines out-yielded the check cultivars are the stand out as the best promising materials but present different sets of favorable traits under stressed or favorable conditions.

NUE variation within a panel of selected winter wheat cultivars under South-Eastern European conditions

Novoselović Dario[2,4], Ivić Marko[4], Plavšin Ivana[2,4], Lovrić Ana[1], Černe Marko[2,5], Maričević Marko[3], Rajković Bruno[1,2], Šarčević Hrvoje[1,2]*

[1]University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia
[2]Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), University of Zagreb, Faculty of Agriculture, Svetošimunska 25, 10000 Zagreb, Croatia
[3]Bc Institute for Breeding and Production of Field Crops, Rugvica, Dugoselska 7, 10370 Dugo Selo, Croatia
[4]Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia
[5]Institute for Agriculture and Tourism Poreč, C. Huguesa 8, 52440 Poreč, Croatia
[*]E-mail: dario.novoselovic@poljinos.hr

Keywords: NUE, genetic variation, correlations, wheat, yield

Nitrogen use efficiency (NUE) in wheat presents one of the most important agro-ecological concerns that affects our environment and levels of grain yield as well as bread-making quality resulting in possible economic gains or losses for both farmers and millers. Having cultivars with high NUE is therefore a desirable trait of a cultivar when prices of nitrogen inputs and environmental risks are rising. NUE by its nature is known to be associated with various genetic, physiological, morphological and developmental characteristics of wheat plant. The objective of this study was to assess and characterize the components of variation controlled by genotype (G), environment (E) and G × E interaction and relationship among analysed traits. In the present study, we tested 48 winter wheat cultivars from different breeding programs in region in a replicated field trials at three locations (Osijek, Poreč and Zagreb), Croatia in 2016/2017 and 2017/2018 year at two nitrogen levels applied in top-dressing (0 and 100 kg of N/ha). For grain yield and grain protein content all sources of variation were significant except for Genotype × N level with almost undetectable contribution. Other contributions were estimated 4.29 % for ENV × NLEV (Environment × N level), 18.6 % for GEN (genotype) effects, 5.28 % for GEN × ENV (genotype × environment effects) and 55.73 % for ENV (environmental effects). In similar way, for grain protein content (GPC) contribution from these sources of variation were: 10.66 % for ENV × NLEV, 33.47 % for GEN, 7.29 % for GEN × ENV and 38.27 % for ENV. For NUE and its components nitrogen uptake efficiency (NUPE) and nitrogen utilization efficiency (NUTE), among different sources of variation, the most important were the effects of ENV (NUE-38.04 %, NUPE-60.60 % and NUTE-45.89 %), ENV × NLEV effects (NUE-32.07 %, NUPE-24.27 % and NUTE-8.78 %), GEN effects ((NUE-11.87 %, NUPE-2.52 % and NUTE-23.56 %). Less pronounced effects were found GEN × ENV (NUE-3.82 %, NUPE-1.77 % and NUTE-6.97 %) and Genotype × NLEV (NUE-3.67 %, NUPE-0 % and NUTE-0.29 ^{NS} %).

Significant phenotypic correlations were found between grain yield with NUTE (r_P = 0.32*) and GPC (r_P = –0.30*), GPC and NUPE (r_P = 0.45***) and between NUTE (r_P = –0.39*) with NUPE (r_P = 0.59***) and NUTE (r_P = 0.50***). Estimated genetic correlations between two N levels were for grain yield (r_G = 1.04 ± 0.02), GPC (r_G = 0.99 ± 0.01), NUE (r_G = 0.99 ± 0.01), NUPE (r_G = 1.32 ± 0.21) and NUTE (r_G = 1.01 ± 0.021).

References

R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria.
ISBN 3-900051-07-0, URL http://www.R-project.org.

Biosynthesis of benzoxazinoids in rye (*Secale cereale* L.) – where this story begins?

Magdalena Święcicka[1], Beata Bakera[1], Wojciech Burza[1], Mariusz Kowalczyk[2], Anna Stochmal[2], Monika Rakoczy-Trojanowska[1]*

[1]Department of Plant Genetics, Breeding and Biotechnology Warsaw University of Life Sciences (SGGW), Nowoursynowska 166 St. 02-787 Warsaw, Poland
[2]Department of Biochemistry and Crop Quality, Institute of Soil Science and Plant Cultivation – State Research Institute, Czartoryskich 8 St., 24-100 Pulawy, Poland
[*]E-mail: monika_rakoczy_trojanowska@sggw.pl

Keywords: *ScBx* gene, *ScIgl* gene, biotic stress, abiotic stress

Benzoxazinoids (BXs) are secondary metabolites synthesized by many species of the Poaceae family, including rye, that play an important role in biotic and abiotic stress resistance, and in allelopathy. Up to day, 10 orthologues of maize genes - *Bx1-Bx7*; *Igl*, *glu* and *GT* controlling BX biosynthesis have been discovered in rye. According to the general opinion, the first step in BX biosynthesis (conversion of indole-3-glycerolphosphate to indole, controlled by *Bx1* gene and its orthologues, encoding for indole-3-glycerol phosphate lyase – IGL), takes place in chloroplasts. The results of our multifaceted experiments on rye BXs clearly showed that this statement is only partly true. In all the experiments comprising such stresses as allelocompounds secreted by Berseem clover and low temperature during vernalization, we have found that the both the production of BXs and the expression of genes controlling their biosynthesis are induced both in the aerial parts (AP) and roots (R). You could assume that BX produced in response to stress are transported from AP to R (and oppositely), but such a scenario is rather impossible due to the immediacy of the induction of gene expression and metabolite production in parts of plants not subjected to stress. Moreover, BXs and the expression of genes controlling their biosynthesis were detected not only in induced, but also in the native conditions. The final proof was the detection of BX in roots of germinating seeds devoid of coleoptile and in root primordia produced *in vitro*. Therefore, we hypothesize that the story "BX biosynthesis" may start in aerial parts of plants and in roots (most probably in leucoplasts), both under native and stress conditions.

Acknowledgments
The research has been financed by the National Science Centre (Poland) – No UMO-2015/19/B/NZ9/00921.

Effect of brown rust infection on the expression of genes putatively related to resistance response

Magdalena Święcicka[1], Ewa Siedlecka[1] and Monika Rakoczy-Trojanowska[1]*

[1]Warsaw University of Life Sciences, Nowoursynowska 166, 02-787 Warszawa, Poland
[*]E-mail: magdalena_swiecicka@sggw.pl

Keywords: brown rust, rye R genes, benzoxazinoids, resistance response.

Brown rust (BR) of rye, caused by an obligate biotrophic basidiomycete fungus *Puccinia recondite* f.sp. *secalis* (Roberge ex Desmaz) (*Prs*) is one of the most important diseases of rye in Central and Eastern Europe including Poland. Up to date, several rye R genes (designated as *Pr*) connected with the defence reaction against *Prs* have been described. However, none of them has been isolated physically and, consequently, their expression profile in plants infected by rust is unknown.

The aim of the presented study was to examine the expression changes of two genes: *ScBx4* and *Lr1* in rye seedlings infected with *Prs*. *ScBx4* controls one of the steps of benzoxazinoids biosynthesis (which are main protective secondary metabolites in rye) and *Lr1* is an orthologue of wheat resistance gene *Lr1*. Previously, we have found, that the SNP ScBx4_1583 in gene *ScBx4* is associated with the response of rye to BR, but the in-depth studies of its function have not been carried out. Recently, in our laboratory, the putative orthologue of wheat gene *Lr1*, has been identified in rye genome bioinformatically and experimentally. Nevertheless, its function as resistance-associated genes is still unknown.

The expression profiles of *Lr1* and *ScBx4* genes were studied using qPCR. The aerial parts of three inbred rye lines (L318, D33, D39) were inoculated with *Prs* and after 8, 17, 24, 48 hours post inoculation material was collected. The tested time points were experimentally chosen: 8 h – the first appressoria are observed, 17 h – haustorium mother cells (HMC) appear, 24 h – the number of HMC increase, 48 h – the first micronecroses are observed. Our analysis indicates that *Lr1* and *ScBx4* were differentially expressed during *Prs* infection. *Lr1* gene was especially upregulated 17 hpi both in more susceptible (L318) and more resistant (D39) line. During the *Prs* pathogenesis the expression of *Lr1* gene increased in L318 and decreased in D39, what suggests the important role of *Lr1* in this process. In opposite *ScBx4* was upregulated 8 hpi in all tested lines what may indicate plant unspecific resistance response.

This work was financially supported by the National Centre Science, project UMO-2018/31/B/NZ9/00439

Effects of different polyamine treatments on salicylic acid-deficient *Arabidopsis* plants

Judit Tajti[*], *Kamirán Áron Hamow*[1,2], *Edit Németh*[1], *Magda Pál*[1]

[1]Department of Plant Physiology, Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, 2462 Martonvásár, Hungary
[2]Plant Protection Institute, Centre for Agricultural Research of the Hungarian Academy of Sciences, Budapest, Hungary
[*]E-mail: tajti.judit@agrar.mta.hutajti.judit@agrar.mta.hu

Keywords: *Arabidopsis*, polyamine metabolism, plant hormones

Polyamines are small, aliphatic molecules that can be found in all living organisms. They are involved in direct interactions with other metabolic routes and hormonal cross-talk, and have an important role in plant stress responses and in signalling processes. The most abundant polyamines are putrescine (PUT), spermidin (SPD) and spermine (SPM). Although they have a similar structure, their effects on plants can be very different.

There are two ways to change the endogenous level of plant compounds such as polyamines and plant hormons. One of them is the exogenous application of the compound or it is also possible to use synthesis mutant or transgenic plants. In order to reveal the relationship between the polyamines and plant hormones and to have an insight into their role as signalling molecules, exogenous polyamine treatments (0,5 mM PUT, SPD and SPM for 24 hour) were applied on two types of salicylic acid-deficient *Arabidopsis* plants (*sid2*: salicylic acid synthesis mutant; *eds5*: unable to transport salicylic acid from chloroplast) grown hidroponically. Col-0 ecotype was used as control.

To follow up the actual physiological status of the plants, chlorophyll-*a* fluorescence induction measurements were conducted. UHPLC-MS/MS analyses were carried out to investigate the effects of different polyamines on endogenous hormone levels (abscisic acid, gibberilic acid, jasmonic acid and salicylic acid). In order to compare the common and different points of signalling pathways and regulatory mechanism induced by the individual polyamines, gene expression studies were also performed. Our main interests were the key enzymes playing role in polyamine metabolism and in the biosynthesis of the above mentioned plant hormones.

Our results revealed that mutants with deficient salicylic acid synthesis or transport responded partly differently to the polyamine treatments, in addition there were significant differences between the pattern of the changes induced by the PUT, SPD or SPM treatments. These findings will may contribute to better understand the role of polyamines and their connection with other hormones.

Acknowledgments
This work was supported by the grant K124472.

T3: New Breeding Strategies and Bioinformatic Tools

Common bunt: study of predominant virulences in France and developement of a resistance test for registration in the French Catalogue of common wheat varieties for organic farming

Valérie Cadot[1], Geoffrey Orgeur[1], Thomas Baldwin[1], Julie Gombert[2] Laurence Fontaine[3], Philippe du Cheyron[4], Julien Bruyère[5], Sandrine Oste[5], Jean Champion[6], Jean-Philippe Maigniel[7], Aurélie Mailliard[7], Valérie Grimault[1]*

[1]GEVES Beaucouzé – 25 rue Georges Morel, 49071 Beaucouzé, France
[2]FNAMS Impasse du Verger, 49800 Brain sur l'Authion, France
[3]ITAB 9, rue André Brouard - CS 70510, 49105 Angers Cedex 02, France
[4]Arvalis Institut du Végétal Route de Châteaufort, ZA des graviers 91190 – Villiers le Bâcle, France
[5]FREDON Nord Pas-de-Calais 265, rue Becquerel BP 74, 62750 Loos-en-Gohelle, France
[6]Chambre d'Agriculture de la Drôme, Ferme expérimentale d'Etoile - 2485 route des Pécollets, 26800 Etoile sur Rhône, France
[7]GEVES, Unité expérimentale de l'Anjouère, 49370 La Pouëze, France
[*]E-mail: valerie.cadot@geves.fr

Keywords: *Tilletia,* Common bunt, virulence, resistance test, organic farming

Common bunt is a re-emerging seed-borne disease affecting particularly organic cereal farming. *Tilletia caries* was identified as the predominant species in 26 isolates collected from 15 French departments with 100% occurrence, compared with *T. foetida,* at 15.4%. Twenty strains of *T. caries* were selected to identify their virulences, from their behaviour on differential host range (Goates, 2012), revealing a predominant virulence on Bt-7 resistance gene, with a frequency of 48%, followed by Bt-2 (24%) and Bt-15 (19%). An early varietal resistance test by qPCR at the 3- leaf stage, quantifying *Tilletia sp*. DNA, was developed. This early test was found to be well correlated with the adult field test, based on visual notation of the bunted ear rate from a panel of varieties grown using organic practises ($R_{Spearman} = 0.89$). A resistance test using the predominant virulences will be taken into account for the registration in the French Catalogue of soft wheat varieties for organic farming.

Acknowledgments
The partners in the CASDAR Carie ABBLE project would like to thank the Ministry of Agriculture, Agri-Food and Forestry for its financial contribution to the "Agriculture and Rural Development" Trust Fund Account.
FREDON Nord Pas-de-Calais also thanks the Hauts-de-France Region for the financial support provided in co-financing.

References
Goates, B. J. 2012. Identification of new pathogenic races of common bunt and dwarf bunt fungi, and evaluation of known races using an expanded set of differential lines. Plant Dis. 96:361–369. doi:10.1094/PDIS-04-11-0339

Breeding for FHB Resistance in Czech Republic – New Resistant Varieties

Veskrna O.[1], Horcicka P.[1], Jezek S.[2], Zrckova M.[1], Pospisilova V.[1], Schmiedlova I.[2], Matyk J.[2]

Selgen a.s. Plant Breeding Station Stupice[1]
Selton. Researche Centrum[2]

Fusarium head blight (FHB) and following mycotoxin production is important problem of safety wheat production in Czech Republic. Protection against this disease is possible only by keeping the whole complex of measures, where the variety resistance represents an important role. Effect of variety resistance was found to be more stable than fungicide treatment.

There were described many FHB resistance resources but due to poor agronomical character without any significant success in conventional wheat production. It can be stated that the introgression of FHB resistance QTL into elite high-yielding cultivars often brings a potential risk of worsening of important agronomical traits.

Poster described recently registered winter wheat variety Viki which keep improved FHB tolerance as well as good agronomical traits and have good potential for safety cultivation in fusarium favorable areas.

NAZV – QK1710302 název projektu „Zvýšení odolnosti pšenic vůči suchu, mrazu, padlí a fuzariózám klasu pomocí metod genomiky a proteomiky. “

ecobreed
IMPROVING CROPS

Funded by European Union
Horizon 2020
Grant agreement No 771367

ECOBREED project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 771367. The content of this paper reflects only the author's view and the European Union Agency is not responsible for any use that may be made of the information it contains.

Identification of putative Phytochrome Interacting Factors (PIFs) in domesticated barley (*Hordeum vulgare* L.)

Krisztián Gierczik[1,2,], Balázs Kalapos[1,2], Attila Vágújfalvi[1], Gábor Galiba[1,2]*

[1]Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, H2462 Hungary
[2]Festetics Doctoral School, Georgikon Faculty, University of Pannonia, Keszthely, H-8360 Hungary
[*]E-mail: gierczik.krisztian@agrar.mta.hu

Keywords: barley, in silico analysis, phylogenetic analysis, bHLH proteins, PIFs

Phytochrome Interacting Factors (PIFs) are belongs to the plant basic helix-loop-helix (bHLH) protein superfamily, and they have regulatory role as transcription factors as well. PIFs interact with the red and farred light absorbing phytochrome photoreceptors, thus they play crucial regulatory role in different light associated signaling pathways. Their functional analysis are insufficient, only a few studies focus on the identification of PIFs – mostly in Arabidopsis – until now.

In this study, we identified 163 bHLH proteins in barley, and classified them into 25 subfamilies by in silico methods. To reveal the putative barley PIF sequences, we included few known PIF amino acid sequences: 7 from Arabidopsis, 3 from rice (Oryza sativa L.) and 1 from soy bean (Glycine max L.). All of the studied known PIFs were classified into one subfamily – as it could be predicted – thus confirming the accuracy of the phylogenetic analyses. Beside the 11 known PIFs, this subfamily contains 9 unique barley sequences out of the 163 bHLH hits. To find out which one of these are members of the barley PIF protein family, *in vivo* functional analyses are needed in the future.

Acknowledgment
This work is supported by the National Research, Development and Innovation Office 'OTKA' K128575 project and by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-financed by the European Union and the European Social Fund.

The *SERK* gene expression vs the androgenetic capacity in the rye (*Secale cereale* L.) anther culture

Sylwia Mikołajczyk[1*], *Agnieszka Tomkowiak*[1], *Dorota Weigt*[1], *Zbigniew Broda*[1]

[1]Department of Genetics and Plant Breeding, Poznan University of Life Sciences, Poznan, Poland
[*]E-mail: sylwia.mikolajczyk@up.poznan.pl

Keywords: rye, androgenesis, *SERK* gene

Due to problems with the production of haploidic rye plants by androgenesis it is necessary to search for genes responsible for the expression of this trait. The *SERK* (*Somatic Embryogenesis Receptor-like Kinase*) gene is considered to be the molecular marker of somatic embryogenesis. So far the compound has been described in species such as *Dactylis glomerata*, *Arabidopsis thaliana*, and *Medicago truncatula*. The *SERK* gene expression level is a key factor responsible for cellular differentiation, auxin-induced somatic embryogenesis as well as biotic and abiotic stress reactions. The aim of the study was to analyse the *SERK* gene expression by means of the PCR and RT-PCR technique and determine its usefulness for the selection of rye with high embryogenicity of microspores and high efficiency of androgenesis in the anther culture.

The research was conducted on fragments of different tissues of rye plants: the somatic tissue of leaves, anthers unexposed to low temperature, anthers treated with a temperature of 4 °C for 5 days and tissues obtained in the anther culture (callus, roots, fragments of green leaves and albino regenerants. The donor plants came from winter rye population cultivars and pre-breeding materials.

The PCR reaction was conducted in the same conditions for the identified SERK gene. The profile of the PCR reaction differed in only a temperature of linking starters established according to the temperature of their melting: preliminary denaturation – 3 min. in 94 °C, 40 cycles (denaturation – 30 s. in 94 °C, linking starters – 1 min. in 58 °C, the synthesis – 1 min. in 72 °C), final synthesis – 5 min. in 72 °C, the max storage. 24 h in 4 °C. The PCR products were run on 2.5% agarose gels in 1x TBE Buffer. Expected size of the generated product – 234 bp, sequences of starters: F5 'TTGCTGGAGGTGTTGCTG3' and R5 'TACACCTTTC-CAAAGCCAC3'. On the basis of an analysis of received products in the reaction with the starters peculiar to the SERK gene they stated, that presence single products of polymorphic examined objects not allowing for diversifying of the rye genotypes.

RNA was isolated from the samples by means of a GeneMATRIX Universal RNA Purification Kit. cDNA was isolated by means of an NG dART RT Kit. The reverse transcription was carried out in a volume of 15µl with 300 ng of the RNA preparation. The Real Time PCR was carried out in a LIghtCycler 480 II with SensiFast SYBR® No-ROX Kit.

The results of the gene expression analysis with the RT-PCR method showed diversified *SERK* gene expression. The tissues analysed according to the *SERK* gene expression level were divided into 4 groups. The highest *SERK* gene expression was observed in the somatic tissue of rye leaves from the pre-breeding materials. The second highest *SERK* gene expression was observed in the green and albino leaves of regenerants from anther cultures and in

roots obtained by rhizogenesis. The third group consisted of the somatic tissue of leaves from the following rye population cultivars: Antonińskie, Dankowskie Nowe and Słowiańskie. The lowest *SERK* gene expression was observed in the callus tissue obtained in the anther cultures of the rye cultivars and pre-breeding materials.

Acknowledgments
The research was supported by the Ministry of Agriculture and Rural Development project HOR. hn.802.9.2019, research task No. 86

Heterosis prediction in triticale based upon single chromosomes genetic diversity assessment

Michał Nowak[1], Justyna Leśniowska-Nowak[1], Karolina Dudziak[2], Magdalena Sozoniuk[1], Piotr T. Bednarek[3]*

[1]Institute of Plant Genetics, Breeding and Biotechnology, University of Life Sciences, Lublin, Poland
[2]Chair and Department of Biochemistry and Molecular Biology, Medical University, Lublin, Poland
[3]Plant Breeding and Acclimatization Institute, National Research Institute, Radzików, Poland
[*]E-mail: michal.nowak@up.lublin.pl

Keywords: heterosis, DArTseq markers, genetic diversity, triticale

The phenomenon of heterosis in plants, despite wide practical use, has not been fully recognized and characterized so far. Particular emphasis is placed on the analysis and identification of molecular bases of heterosis occurrence. Studies conducted for various plant species indicate that one of the most important elements determining the occurrence of this phenomenon is the appropriate level of genetic diversity between parental forms. Previous research is often based on the assumption that the probability of heterosis occurrence increases with the increase in genetic diversity of parental forms. However, the research conducted so far for triticale has not provided an answer to the question on the relationship between heterosis level and genetic diversity of parental forms. Our approach allowed to confirm the assumed working hypothesis that the analysis based on markers located on specific chromosomes will allow much more precise prediction of heterosis in the F_1 generation than the analysis based on the total marker pool.

The plant material consisted of 470 winter triticale breeding lines. Genotyping of these lines was performed by means of DArTseq technique (Diversity Array Technology, Canberra, Australia). The aim of the presented research was to evaluate the yield of the F_1 triticale hybrids obtained by crossing of genotypes with the highest genetic diversity and to estimate the effect of heterosis. The cross combinations were designed on the basis of the results obtained by means of the analysis of DNA markers located on individual triticale chromosomes. The effect of heterosis was estimated on the basis of 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 analysis of obtained results showed that chromosome localization of markers on the basis of which the genetic distance between parental forms used for crossing was determined had a significant influence on the heterosis effect. MPH values for the studied forms ranged from -28% using markers located on chromosome 6R to +129% using markers located on chromosome 5A. For BPH effect the values were -38% and +111%, respectively. Additionally, the obtained results were compared with the result obtained using the total marker pool for estimating the genetic distance, regardless of the chromosome location, and it was shown that the selection of forms based on the total pool of markers did not allow to obtain the effect of heterosis, and the yield of the hybrid form was 11% lower than the average yield of parental forms and yield of the form with better parameters.

Our results suggest that the determination of the genetic diversity based on the selected chromosome(s) could be a better approach than the use of total marker pool. This information could be especially useful for heterosis effect prediction in breeding programs.

Acknowledgement
The results of the study were obtained within the framework of the project funded by Polish Ministry of Agriculture and Rural Development entitled 'Identification of the genome regions and DNA markers linked to heterosis in hexaploid winter triticale'.

A new assignment for PCR-based molecular markers of wheat *Glu-B1* locus

*Marina Tikhonova**

Estonian Crop Research Institute, Jogeva, Estonia
*E-mail: marina.tikhonova@etki.ee

Keywords: wheat, HMW-GS, Glu-B1, DNA markers

Wheat is one of the most important cereals worldwide. A large portion of wheat is used in bread baking.

High molecular weight glutenin subunits (HMW-GS) are strictly related to bread-making quality and are responsible for elasticity and polymer formation of wheat dough. Using of DNA markers for the identification of HMW-GS loci allelic composition increases the efficiency and speed of the development of cultivars with higher baking quality. A large number of DNA markers for the HMW-GS loci have been developed. Recent studies revealed new banding patterns, which were not reported earlier, for many of the previously known markers for the HMW-GS at the *Glu-B1* locus (Janni et al., 2017). In this study 3 previously developed PCR-based molecular markers for *Glu-B1* alleles were tested on 12 test cultivars with variable composition of HMW alleles at the *Glu-B1* locus.

A new specific amplification was revealed for the CauBx642 (Xua et al., 2008), MAR (Butow et al., 2004) and By01F/By02R (Frank et al., 2017) molecular markers. The specificity of CauBx642 for By14 (~630 bp) and Bx17 (~534 bp) was shown. The same PCR fragment (~642 bp) was also observed in cultivars with Bx7, Bx7OE, Bx13, Bx20. The ~520-bp amplicon was obtained in cultivars with Bx7, Bx6, Bx13, Bx14, Bx17 using marker MAR, whereas the size of the marker fragment for By20 was ~700 bp and for Bx7+By0, Bx7OE was 563 bp . Marker By01F/By02R produces ~841-bp amplicon specifically for By9 and no bands for By15, By20, while cultivars with the other By subunits had a ~902-bp fragment. Obtained results can be used for the selection of DNA markers for wheat *Glu-B1* HMW-GS.

Acknowledgments
I acknowledge Maksim Makarenko (SfeDU), Külli Annamaa (ECRI) and Andres Mäe (ECRI) for help in preparing this research.

References

1. Janni M., Cadonici S., Pignone D., Marmiroli N. (2017). Survey and new insights in the application of PCR-based molecular markers for identification of HMW-GS at the Glu-B1 locus in durum and bread wheat. Plant Breeding. 00:1–7.of References here, if applicable.
2. Xua Q., Xua J., Liua C.L., Changa C., Wanga C.P., Youa M.S., Lia B.Y., Liua G.T. (2008). PCR-based markers for identification of HMW-GS at Glu-B1x loci in common wheat. Journal of Cereal Science. 47: 394–398.
3. Butow B. J., Gale K. R., Ikea J., Juhász A., Bedö Z., Tamás L., Gianibelli M. C. (2004). Dissemination of the highly expressed Bx7 glutenin subunit (Glu-B1al allele) in wheat as revealed by novel PCR markers and RP-HPLC. Theor. Appl. Genet. 109: 1525–1535.
4. Frank K., Miró K., Nagy T., Marincs F. (2017). Development of a PCR-based DNA marker for Glu-1By alleles in the old Hungarian Bánkúti wheat. Mol. Breeding. 37:120.

T4: Modern Technologies From Phenomics to Genome Editing

Optimization of Triticale ABA8'OH Crispr/Cas9 mutagenesis – construct design and validation by protoplast transfection.

*Krzysztof Michalski and Anna M. Linkiewicz**

Plant Breeding and Acclimatization Institute – National Research Institute, Radzików, 05-870 Błonie, Poland
*E-mail: corresponding.a.linkiewicz@ihar.edu.pl

Keywords: Triticale, genome editing, CRISPR/Cas9, protoplasts, targeted mutagenesis, construct validation

The CRISPR/Cas9 genome editing technique provided long-awaited molecular biology tool to edit specific DNA sequences in plants. Despite the fact that CRISPR/Cas9 constructs are relatively easy to assemble and can be designed to target almost any location in the plant genome, examples of successful improvement of agronomic important traits by CRISPR/Cas9 are still limited. The individual sequence editing tools exhibit large variations in efficiency. Estimation of CRISPR/Cas9 activity by computational methods may not be accurate for all applications as the criteria are derived from partial, context specific data (Gagnon et al. 2014). A convenient procedure to assay the cleavage activity of CRISPR/Cas9 endonucleases in plants should be considered before carrying out a designed plant genome editing project. We have designed a number of constructs to examine the potential for CRISPR/Cas9 editing of the ABA8'OH gene in Triticale. Here we report the study on CRISPR/Cas9 construct design and validation *via* protoplast transfection assay. Methodology previously optimized for the wheat protoplasts transfection (Shan et al., 2014) was adapted for Triticale. To increase the efficiency of the deletion mediated by CRISPR/Cas9 vectors we have used the additional TREX2 exonuclease, which was reported to enhance heritable mutations by 2,5-fold (Cermak et al., 2017).

References

1. Gagnon J.A. Valen E. Thyme S.B. Huang P. Ahkmetova L. Pauli A. Montague T.G. Zimmerman S. Richter C. Schier A.F. 2014. Efficient mutagenesis by Cas9 protein-mediated oligonucleotide insertion and large-scale assessment of single-guide RNAs. PLoS One 9 e98186
2. Cermak T, Curtin SJ, Gil-Humanes J, Cegan R, Kono TJY, Konecna E, Belanto JJ, Starker CG, Mathre JW, Greenstein RL, Voytas DF. A multi-purpose toolkit to enable advanced genome engineering in plants. Plant Cell. 2017 May 18. pii: tpc.00922.2016. doi: 10.1105/tpc.16.00922.
3. Shan Q, Wang Y, Li J, Gao C. Genome editing in rice and wheat using the CRISPR/Cas system. Nat Protoc. 2014;9:2395–410.

AUTHOR INDEX