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BOOK OF ABSTRACTS

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T1: PLANT GENETIC RESOURCES

Invited

Plant genetic resources in the global genebanks – conservation and exploitation

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Keywords: *ex situ* conservation, genetic mapping, germination monitoring, lipid oxidation, metabolite profiling, seed longevity

Plant genetic resources play a major role for global food security. The most significant and widespread mean of preserving plant genetic resources is *ex situ* conservation. Today about 1,750 *ex situ* genebanks world-wide maintain 7.4 million accessions (Table 1). One of the ten largest *ex situ* collections of our globe is located at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany, conserving 150,000 accessions from 3,200 plant species and 780 genera. Since the majority of genebank holdings globally is maintained as seed, seed storability is of exceptional importance for germplasm conservation.

At IPK research on seed longevity was initiated for a range of crops and wild relatives stored over decades. Historical germination data accumulated during 35 years of seed germination monitoring were analysed to predict species specific seed longevities. The study considered 75 species comprising 79,075 accessions and 157,402 observations. Beside interspecific differences variation was also detected within species and genetic analyses were initiated in barley, wheat, oilseed rape and tobacco.

In addition, mass spectrometry based untargeted metabolite profiling experiments were performed in order to detect biochemical changes coinciding with loss in seed germination. GC-MS analysis of the polar metabolome of wheat and barley identified glycerol and related intermediates as highly correlated to germination rate. Therefore, the lipidomic composition of a wheat panel was investigated using high-resolution liquid chromatography-mass spectrometry (LC-MS). A high proportion of tentative oxidized lipids was detected, suggesting lipid oxidation as the causal trigger for membrane degradation.

Table 1: The ten largest germplasm collections on earth (FAO 2010)

Institution	Country	Accessions
NPGS (National Plant Germplasm System)	USA	508,994
ICGR-CAAS (Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Science)	China	391,919
NBPGR (National Bureau of Plant Genetic Resources)	India	366,333
VIR (N. I. Vavilov Research Institute of Plant Industry)	Russia	322,238
NIAS (National Institute of Agrobiological Science)	Japan	243,463
CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo)	Mexico	173,571
IPK (Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung)	Germany	148,128
ICARDA (International Center for Agricultural Research in the Dry Areas)	Syria	132,793
ICRISAT (International Crops Research Institute for the Semi-Arid Tropics)	India	118,882
IRRI (International Rice Research Institute)	Philippines	109,161

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Oral presentations

Transcriptome comparative analysis of rye inbred lines, different in terms of wax layer

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

The genetic background of wax production in rye is poorly understood. So far, only one gene has been described to induce wax layer on leaves, stems and ears (*wa1*), whose mutant allele determined waxless plant and was mapped on chromosome 7R. The sequence of this and other genes that determine wax formation is not known. Until recently, rye was the species with a small number of genomic DNA and cDNA sequences, with respect to genome size. The first information on the results of the high-throughput sequencing of rye was provided by a team of German scientists who carried out a transcript of the five rye inbred lines using the Roche 454 platform, thus enriching the available rye sequence database.

The purpose of this study was to analyze transcripts of four pairs of inbred lines in order to find differences in expression between the genes involved in epicuticular wax layer. This work involved the use of rye inbred lines (*M12_wax* and *M12_waxless*, *Ds2_wax* and *Ds2_waxless*, *RXL10_wax* and *RXL10_waxless*, and *L35_wax* and *L35_waxless*). Each of the NIL lines was represented by a minimum of 2 biological replicates except *L35*. RNA isolated from leaves in a similar developmental phase was used to construct the TruSeq-RNA library, which was then sequenced using the Hi-Seq_2000 (Illumina) sequencer. Sequential data was subjected to bioinformatic analysis. Preliminary data processing included filtering of sequential readings with the minimum quality Q30, trimming of ends and removal of adapters with addresses. Next, a pool of reference sequences was created by performing a de novo assembly analysis using the TRINITY program.

The length of sequential readings was 101 nt. The number of read sequences was different for individual genotypes and ranged from 66 to 112 million readings. Proportionally, the number of nucleotides ranged from 6.6 to 11.2 billion nt. The quality of the sequences measured with Q30 was high and ranged from 96.9 to 97.7%. The constructed reference sequence pool counted 218,935 transcripts with a total nucleotide number of 183 million nt. The average contig was 500 nt, with a minimum of 200 and a maximum of 16,024 nt.

All sequences were analyzed for BLAST to detect functional annotations. Among reference transcripts 3215 were linked to biological processes, 25 998 to cellular components, 25 281 to molecular function and 135 503 to no function. Transcripts have been identified for the potential function of wax formation. Among the transcripts of all lines, 36 were associated with wax, 469 coding for fatty acids, 181 associated with sterols, 40 for acyl-CoA, 200 for glycerol, and 712 for lipids. Considering all the mentioned groups, most of the genes were

found in the DS2 line sequences (1370 genes) and the least in the M12 line sequences (1316 genes). Sequential data of the L35 line allowed for the selection of as many as 32 genes potentially wax coding. Significant differences in expression (fc-fold change of expression) of transcripts for individual NIL-i pairs were also observed. Significant levels were 2-fold increase / decrease in expression (fc > 2 and fc < 2). Out of the contigs for all pairs of NILs that showed significant differences in expression, only a dozen were associated with wax, sterols and acyl CoA, and several dozen fatty acids, glycerols and lipids. Confirmation of the potential function of selected wax genes is the subject of further work.

Acknowledgments

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Examination of genetic components determining the environmental adaptation of bread wheat (*Triticum aestivum* L.)

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Keywords: plant developmental phases, yield components, sowing time experiment, Genome-Wide Association Study (GWAS), wheat (*T. aestivum* L.)

Bread wheat is one of the most important objects of genetic examinations among agricultural plants. Due to its extraordinary genetic diversity this species has a wide environmental spreading. One of the most important components of the regional adaptation is plant development and flowering time, which is determined by gene groups that regulate vernalization requirement (*VRN*), i.e. the cold period that induces the transition from the vegetative to the generative phase, photoperiod sensitivity (*PPD*), earliness per se (*EPS*) and height-reducing genes (*RHT*) (Dubcovsky et al. 1998) to a great extent. This complex process is fundamentally influenced by environmental factors such as annual changes in photoperiod, high and low temperature periods, or intensity and spectral composition of light, which directly affect the plant developmental phases (Borras et al. 2009). The aim of our study was to investigate the flowering time of a wide range of wheat varieties using comprehensive phenological and molecular-genetics methods and to determine the association between plant developmental patterns and various yield components. The 188 wheat genotypes included in the analyses were obtained from the winter wheat gene bank of the MTA-ATK Agricultural Institute. The genotypes were chosen based on the two main allele types of the *PPD-D1* photoperiod sensitivity gene (94 photoperiod-insensitive and 94 photoperiod-sensitive). The GWA panel could be divided into four sub-groups, which showed close connection with the geographical origin. In general, one group contained American and Asian varieties, one included Central European, one included Hungarian and South European genotypes and one contained breeding materials from the western regions of Europe. The impacts of years and sowing time on

the developmental phases significantly changed as the plant development progressed; sowing time had a large effect on the early developmental phases, while years had a significant influence on the later developmental phases. Several chromosome regions were identified by GWAS, playing significant roles in overlapping regulation of the various developmental, morphological parameters and yield components. The most significant regions are on 1A, 1B, 2B, 2D, 4A, 4B, 4D, 5A, 6A and 6B. QTL effects connected to five known plant developmental genes were also identified by GWAS demonstrating the effectiveness of the method. These five genes are *PPD-B1* (2B) and *PPD-D1* (2D) photoperiod-sensitivity genes, *RHT-B1* (4B) and *RHT-D1* (4D) dwarfing genes, and *VRN-A1* (5A) vernalization response gene. Through their complex regulation mechanisms these genes have significant influence on several morphological traits and yield components in addition to plant development also under field grown conditions.

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Tritordeum: a man-made cereal

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Keywords: barley-wheat hybrids, tritordeum, *Hordeum chilense*

Many hybrids involving species of the genus *Triticum* and *Hordeum* (\times *Tritordeum* Ascherson et Graebner) are possible after the pioneering work of Anton Kruse in 1973. However, fertile amphiploids are much more difficult to obtain. We succeeded in producing such amphiploids by using *Hordeum chilense* as the female parent. As pollen donor we used the three genomic levels of *Triticum*: *Triticum aestivum* spp. (hexaploid), *Triticum turgidum* spp. (tetraploid) and *Aegilops tauschii* (diploid), obtaining fertile amphiploids in all three cases. Despite the short period since its creation, the hexaploid \times *Tritordeum*, *Hordeum chilense*-*Triticum turgidum* subsp. *durum*, ($2n = 6x = 42$, AABBH^{ch}H^{ch}) is already on the market. Recently, we have also obtained fertile amphiploids between *H. flexuosum* and *H. marinum* and durum wheat (unpublished).

In order to create the genetic variability needed to take this new species to farmers, we crossed 215 *Hordeum chilense* accessions, which we collected in Chile or obtained from the

USDA and the Nordic Germplasm Banks, with tetraploid and hexaploid wheats from CIM-MYT, ICARDA and the Spanish germplasm banks. Hexaploid tritordeum from *H. chilense* cross perfectly with tetraploid and hexaploid wheats as well as with triticale, producing self-fertile progenies. However, these crosses are difficult with tritordeum derived from *H. maritimum* and *H. flexuosum*. Indeed, all the hybrids obtained were sterile.

Early during the breeding process, we had to evaluate and decide which cytoplasm (*H. chilense* or wheat) was more favorable. Although the two cultivars already in the field, 'Aucan' and 'Bulel', are on wheat cytoplasm, this choice should be reevaluated using more advanced lines.

The primary hexaploid tritordeum shows some traits of wild wheats: prostrate growth habit, brittle rachis and tough glumes. Prostrate vegetative growth habit was the norm, but erect types emerge easily from the cross with the amphiploid *H. chilense-Triticum aestivum* subsp. *sphaerococcum*. The development of lines with good threshing was much more difficult. Chromosome substitution either of chromosome 2H^{ch} by 2D or 5H^{ch} by 5D resulted in good thresh ability, but yield was lower than complete H^{ch} genome tritordeum (Atienza et al. 2007a). We tried to obtain a non-brittle rachis line by mutation with EMS, and although we were not successful, an imidazole resistant tritordeum was produced by using this approach. However, free threshing is not fully accomplished yet.

The yield of cultivated tritordeum has already reached the level of old wheats, but the main goal of our program is to decrease the difference (20–30%) with the yield of modern wheat cultivars. A secondary objective of the breeding program is to improve the baking quality, as the dough of cultivars already released shows high extensibility but very low strength. A first approach was the introgression of chromosome 1D carrying HMW subunits 5x + 10y, either as a 1A or 1H^{ch} substitution. The reduction in yield of these lines made us reject this approach. The yield performance using the translocation 1AS/1DL or 1H^{ch}S/1DL was even lower (Ballesteros et al. 2003). However, despite the poor bread-making quality, tritordeum bread is much appreciated by consumers due to the high lutein content (Atienza et al. 2007b) and the difference in flavor compared to wheat.

The fact that in only 30 years, cultivated tritordeum has reached the yield level of some wheats, reveals the incredible potential of this new species.

Acknowledgments

In the memory of Juan Ballesteros the breeder of the first tritordeum cultivar. This presentation was funded by Grant AGL2013-43329-R, from the Ministerio de Economía y Competitividad, Spain (MINECO) including FEDER funding.

Mutational breeding for low-phytate barley grain

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Keywords: *Hordeum vulgare*, genetic mapping, GWAS, feed and food

Understanding the biosynthesis and accumulation of phytic acid (PhA) in seeds is of primary importance to minimize the release of phosphate to the environment caused by the inability of monogastric animals to hydrolyze PhA. Today, feed is either supplemented with inorganic phosphate or treated with phytase in order to provide sufficient phosphate for the animals. As phosphate is one of the limited and non-renewable resources on earth, and a shortage of quality phosphate is foreseen within this century, it is important to develop barley and rice varieties which accumulate less organic phosphate in the form of PhA thereby reducing the amount of non-metabolized phosphate that pass through the ecosystem. Another issue is that PhA as an anti-nutritional factor is related to the term “hidden hunger”, which is a result of living on a mineral-poor diet consisting solely of cereals as staple food. The minerals bound by PhA in barley and rice are immobilized and thus will not contribute as a source to retain a healthy diet. Breeding for low PhA level cereals will improve the health of many people around the world and as a feed improve the cereal based phosphate contribution.

PhA is a naturally occurring polyphosphorylated carbohydrate and has for a long time been known as a storage compound for phosphorus (P) in seeds but it is ubiquitous in eukaryotic organisms and is typically the most abundant inositol phosphate in cells. In plants, PhA is present in most tissues, including pollen, spores, and vegetative tissue such as roots, tubers, stems and leaves, and it has been implicated in many cellular processes. The negatively charged phosphate groups in PhA have a strong chelating effect on metal ions and in plants PhA is mainly found as salt-complexes with cations such as K⁺, Mg²⁺, Ca²⁺, Mn²⁺, Zn²⁺ and Fe³⁺. PhA accounts for 50–80% of the total phosphorus content of the seed and contributes up to 1.5% of the seed dry weight. However besides its function as a storage compound and signaling molecule, the regulatory network of inositol-phosphate metabolism is not well understood. The identification and functional elucidation of two new promising genes, mutation of which results in low phytic acid (*lpa*) mutant phenotype (*Hvlpa5-1*, *Hvlpa6-1*) in barley, have been mapped to barley chromosome 4H and differ from all known *lpa* genes previously reported. Importantly no negative impact on agronomics traits was observed for the two *lpa* mutants, which are thus in sharp contrast to most other reported *lpa* mutants, suggesting they are of great potential for breeding *lpa* varieties. Results from breeding and field trial in Latvia and Denmark and of GWAS studies will be presented.

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Discarding of the postzygotic reproductive barrier between hexaploid wheat (*Triticum aestivum* L.) and rye (*Secale cereale* L.)

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Postzygotic reproductive isolation in response to hybridization of plants is a major factor limiting production of the hybrids in breeding programs. This mechanism is manifested in the different stages of the ontogenesis of distant hybrids. In crosses between hexaploid wheat Chinese Spring and certain rye inbred lines abortion of shoot apical meristem (SAM) during the differentiation of the hybrid embryo leads to embryo lethality. Such kind of embryo lethality is the result of a negative complementary interaction between wheat and rye loci. These loci were named *Eml-R1* and *Eml-A1*. *Eml-R1* locus has two alleles which are either compatible (*Eml-R1a*) or incompatible (*Eml-R1b*) with the wheat genome. The postzygotic barrier that results from an interaction between incompatible wheat *Eml-A1* and rye *Eml-R1b* alleles can be easily overcome by the application of auxinic herbicide 2,4-D to tissue cultures of abnormal embryos at 14–16 days after pollination. Callus cultures of abnormal embryos show a high regenerative capacity, even without the application of exogenous cytokinin. This characteristic indicates that the reproductive barrier between hexaploid wheat and certain rye inbred lines may be mediated by epigenetics. After colchicine treatment during callus culture, amphidiploid plants were obtained. However, most of these plants did not produce seeds, due mainly to sterility of the pollen but also of the embryo sacs. These findings demonstrate that hybrid sterility affects both male and female gametophytes in plants obtained from abnormal embryos. The key roles of double fertilization and stress factors in the implementation of the apical meristem formation programme in embryos from incompatible intergeneric crosses

between hexaploid wheat and rye during *in vitro* culture are discussed. We also propose a hypothetical model for a wheat-rye lethality system involving differential expression of incompatible wheat *Eml-A1* and rye *Eml-R1b* alleles in an identical genetic background.

Acknowledgments

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Finding a black cat in a dark room – Identification of the *Mlo* gene in *Triticum monococcum* L.

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Keywords: *Mlo* gene, powdery mildew, *Triticum*

Most of the cultivated wheat species and varieties are sensitive to powdery mildew caused by the obligate pathogen fungus *Blumeria graminis* f.sp. *tritici*. The infection can result in up to 40% yield loss; therefore breeding resistant cultivars has a great importance. Because of the high adaptability of the fungus it is a great challenge for breeders as well. The identified dominant resistance genes provided only short-term and isolate-specific protection. The loss-of-function mutations of the *Mlo* gene represent an alternative protection in the co-evolutionary battle, because the presence of the functional wild type wheat MLO protein is essential for the successful fungal infection. This resistance mechanism, described first in wild barley (*Hordeum vulgare* L.) population, results in long-term, heritable powdery mildew resistance that is effective against all tested isolates. Orthologs of *Mlo* have been identified in numerous land plants including *Triticum aestivum*. Although more than 50 different mutations could be induced into these genes, only a few naturally evolved mutant *mlo*-alleles have been found so far. It is known for a long time that cultivars of einkorn (*T. monococcum* L.) have high level of resistance against diseases, including powdery mildew infection. In our work we investigate whether mutations occurring naturally in the *Mlo* gene/s provide this long-term protection. To identify such resistance genes would be useful for future breeding of resistant bread wheat cultivars.

Our research strategy was to amplify the entire *T. monococcum Mlo* gene (*TmMlo*) sequence in order to find SNPs or INDELS that may lead to loss-of-function mutations. We studied different *T. monococcum* accessions from seed banks and breeders, which showed elevated levels of resistance against powdery mildew infection under field conditions. To identify the *TmMlo* gene PCR primers designed for the *T. aestivum Mlo2* (*TaMlo2*) gene were used. First we amplified one of those sequence regions, which encodes conservative amino

acids essential for normal function of the MLO protein. The obtained ~ 700 bp long fragment corresponds to the 1285–1983 nucleotide region of *TaMlo2* gene. Sequence alignment resulted in three different types of sequences: the A-type showed 98% homology to the *TaMlo2* gene while the B- and C-types showed 80% and 78% homology to the barley *HvMlo-h1* gene, respectively. Although deletions and numerous SNPs were found in these groups, they were mainly localized in the predicted intron regions. To isolate the entire *TmMlo* gene, we designed primers for the 5'- and 3'-end based on sequences of *TaMlo2*, *HvMlo-h1* genes and *T. monococcum* and *T. urartu* contigs. We found two types of *TmMlo* sequences as suggested by our first results: one of them showed 94% homology to the *TaMlo2* gene and the other group showed 80% homology to the *HvMlo-h1* gene. Alignment of the predicted exon sequences resulted in two SNPs in the first group (showing similarity to *TaMlo2*) leading to one amino acid change only. INDELs and numerous SNPs were found in the second sequence group leading to significant differences in the predicted amino acid sequence. Interestingly one of the studied accessions showed 98% similarity to *Aegilops tauschii* subs. *tauschii* *Mlo* protein homolog 1 mRNA sequence. To confirm the predicted exon sequences, RNA was extracted from three Einkorn accessions. The cDNA sequences corresponded well to the predicted exon sequences.

Based on our results, we plan to identify SNPs and INDELs, which lead to loss-of-function mutation. Since no annotated *T. monococcum* *Mlo* gene sequence was found in databases, the identification of the *TmMlo* gene and finding a naturally evolved mutant *mlo*-allele would be important for basic research and plant improvement as well.

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Exploiting Natural Diversity for Genetic Improvement of Bread Wheat

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Keywords: wheat, diversity, genetic improvement, nested association mapping

Wheat is an important staple crop and most widely grown cereal in the world and Canada providing 20% of the total calories for the world's population. Global wheat demand is predicted to increase at least 60% by 2050 to feed over 9 billion population. In this context, it is necessary to accelerate genetic gains for yield potential and improvement for the adaptation to the increasing incidence of biotic and abiotic stresses due to climate change. However, loss of genetic diversity in elite wheat breeding populations has limited future genetic gain in wheat improvement. Introducing targeted traits from the synthetic hexaploid wheat (SHW) donors into adapted germplasm to widen the existing primary gene pool of bread wheat has proven to be a practical strategy to incorporate allelic diversity into modern wheat. In our study, 190 SHW lines were assessed along with elite lines for agronomic traits and leaf rust resistance at seedling stage. Genome wide association study (GWAS) was performed for identification of novel leaf rust resistance loci. On the basis of maximizing genotypic and phenotypic diversity, fifty genetically diverse founder lines including 25 SHW and 25 elite lines were selected to develop a nested association mapping (NAM) population. All founder lines were crossed to a common Canadian elite spring wheat line to allow the incorporation of novel genetic diversity and taking advantage of both linkage analysis and association mapping for dissection of complex quantitative traits. Preliminary field trial on selected sub-populations at BC1F3 showed broad variation for agronomic traits and spike morphology. The resulting NAM population of 4,700 recombinant inbred lines (RILs) from 50 sub-populations will provide a useful resource for not only dissection of genetic architecture of complex traits with higher statistical power and higher resolution but also identification of pre-breeding germplasm for wheat breeding programs.

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Poster presentations

Oat (*Avena sativa* L.) haploid embryo production by oat x maize (*Zea mays* L.) crosses

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Keywords: coconut water, cysteine, casein hydrolysate, haploid embryo conversion, DH lines

Haploidization is one of the methods of creating new cultivars that allows to shorten the production time of new cultivars. The aim of this study was to increase the efficiency of oat haploid embryos germination obtained by the wide crossing method. The experiments were performed on 40 oat (*Avena sativa* L.) F₁ progeny derived from Strzelce Plant Breeding Ltd. Oat emasculation was done 8 weeks after sowing. Plants were pollinated with maize and treated with a 2,4-dichlorophenoxyacetic acid (2,4-D). Three weeks after pollination, enlarged ovaries were collected and surface sterilized. Next embryos were isolated and placed on four regenerating media: (1) – 190-2 (Wang and Hu, 1984) with 0.5 mg dm⁻³ kinetin, 0.5 mg dm⁻³ 1-naphthaleneacetic acid (NAA), 9% maltose, 0.6% agar and pH 6.0, (2) – medium (1) with 50 mg dm⁻³ cysteine, (3) – medium (1) with 10% v/v coconut water and (4) – medium (1) with 50 mg dm⁻³ casein hydrolysate. Induction and regeneration of embryos were performed at 16 h photoperiod and light intensity of 130 μmol (photons) m² s⁻¹ and at 20/17 °C (day/night). Germinated embryos were transferred to MS medium (Murashige and Skoog, 1962) with 1.5% sucrose, solidified with 0.6% agar and pH 5.8. The obtained haploid plants were acclimated to *ex vitro* conditions. Well-rooted haploid plants were treated with colchicine in order to double the number of chromosomes. Ploidy level of plants was evaluated using a MACS Quant flow cytometer (MACS Quant, prod. Miltenyi Biotec). Of the 40 oats (F₁) genotypes, 974 panicles (26960 florets) were emasculated. Seven hundred forty five haploid embryos were obtained, while 211 germinated. The highest number of haploid embryos germinated on medium (1) – 0.40%, and the lowest on medium (4) with casein hydrolysate – 0.09%. On medium (2) with cysteine and on medium (3) with coconut water the percentage of germinating embryos was similar (0.24% and 0.20%, respectively). Addition of cysteine, coconut water and casein hydrolysate inhibited embryos germination. As a result of haploid plants acclimatization to natural conditions and chromosome doubling, 67 doubled haploid (DH) lines were obtained. The number of embryos and regenerated plants depended on the genotype. The embryos were induced in 39 of 40 examined genotypes. The embryos of eight genotypes: 5.8514, STH5.8529, STH4.403/1, STH5.451/1, 5.8422, STH5.8528, 5.8430 and STH5.5046 did not develop into haploid plants. DH lines were obtained from 28

genotypes. The highest number of embryos per emasculated florets (over 5%) was obtained from the genotypes 5.8508, 5.8525 and STH5.8518/1. The most DH lines generated 5.8507, STH5.8504/1 and STH5.8518/1 genotypes. On average, of all tested genotypes, 2.69% of embryos, 0.76% of germinating embryos, 0.43% of haploid plants and 0.24% of DH lines per one hundred emasculated florets were obtained.

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Development and QTL mapping in a 16 founder wheat MAGIC population

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Keywords: wheat, multi-founder populations, MAGIC, QTL mapping, community research resources

Development of experimental mapping populations enables dissection of complex traits in crops and underpins modern plant breeding. Plant Multi-parent Advanced Generation Inter-Cross (MAGIC) populations, as proposed by Mackay & Powell (2007), have now been constructed in many crop species. The advantages of MAGIC, including greater genetic diversity captured from the multiple parents as well as multiple rounds of intercrossing maximising recombination and minimising population structure, make them an ideal resource for QTL (Quantitative Trait Loci) mapping.

The ‘NIAB Diverse’ MAGIC wheat population has recently been developed from 16 founders, chosen to capture the greatest possible genetic diversity of UK adapted wheat varieties. These included both elite and historical varieties originating from several different northern European countries. Analysis based on SNPs genotyped using the Illumina iSelect 90k array show that over 90% of the genetic diversity in a panel of 519 UK wheat varieties is present in the 16 founders.

Using the wheat Affymetrix 35k axiom SNP array (Allen et al. 2016), 596 RILs (Recombinant Inbred Lines) have recently been genotyped at the F7 stage, and a genetic map is under development. Preliminary results from the first year of replicated yield trials indicated high power to detect QTLs in heritable traits such as flowering time and height, and further

analysis on a larger number of traits including yield and yield components is ongoing. These results confirm the NIAB Diverse MAGIC population as an excellent resource for genetic dissection of complex traits.

The population is being developed as an open access resource for the wheat research and breeding community. A replicated yield trial (2x6m plots) will be available for phenotyping by interested parties at NIAB-Cambridge in the 2018 season, and the population is available on request from NIAB. Basic phenotype data and the founder/progeny genotype data will also be available, along with the associated genetic map and QTL analysis pipeline under development at UCL. Please contact nick.fradgley@niab.com for further information.

Acknowledgments

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Unrevealing the secrets of aroma in Pakistani rice (*Oryza sativa*) based on biochemical and molecular association

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Keywords: Aroma, BADH2 gene, GABA, Rice, 2AP

Aroma is considered as one of the most important trait which is the key factor in determining the market price. The classic fragrance of aromatic rice is due to the compound 2-acetyl-1-pyrroline (2-AP) and its accumulation occurs as a result of loss of function mutation in *fgr* gene. This 8-base pair deletion and 3 single nucleotide polymorphisms in exon 7 of *fgr* gene causing a premature stop codon and a truncated BADH2 and prevents the enzyme to play its normal function. Hence, the catalysis of 4-amino butyraldehyde to 4-amino butyric acid (GABA) is interrupted which leads to the production of 2-AP conferring fragrance. Although, many varieties are reported to be fragrant without having these known mutations with intact *fgr* gene producing a functional BADH2 enzyme with elevated levels of 2AP, suggesting the involvement of other genes/mutations for controlling the development of 2 AP metabolism

pathways which are needed to explore. Chemical analysis of GABA and Proline revealed significantly low proline levels in aromatic rice varieties while GABA was not significantly different in both types of rice varieties. These findings suggest that GABA levels are maintained by *badh2* homologues or produced by an alternative pathway irrespective of BADH2 enzyme disruption. However, low amounts of Proline in aromatic rice may be the reason of general susceptibility of aromatic rice varieties against stress conditions. These findings will be helpful in understanding the factors contributing to rice aroma that could be implemented in rice breeding programs for the development of better aromatic varieties.

Identification of sources of resistance to *Zymoseptoria tritici* in durum wheat germplasm from predominantly North Africa region

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Keywords: Septoria tritici Blotch, durum wheat, sources of resistance

Septoria tritici Blotch (STB) caused by the fungus *Zymoseptoria tritici* poses a serious and persistent challenge to durum wheat production in North Africa Area such as in Tunisia. Under conducive weather conditions this threat infects wheat leaves causing important yield losses in fields cultivated with susceptible varieties. Host plant resistance is one of the main strategies in the management of this disease. To identify new resistant sources to STB, 148 wheat accessions from different geographic origins provided by ICARDA were evaluated for resistance to the Tunisian isolates of *Z. tritici* Tun6 and TM220 under field conditions at Béja, Tunisia. Forty five and 47 genotypes were respectively resistant to Tun06 and TM220 with 36 genotypes resistant to both isolates. Nine and 11 accessions showed specific resistance to Tun6 and TM220 respectively, indicating specific resistance of *Z. tritici* to durum wheat. The different sources of resistance identified in this study could be used by breeding programs aiming to raising the level of STB-resistance in new improved durum wheat cultivars.

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Developing wheat germplasm with durable resistance to leaf rust conferred by the partial resistance genes *Lr34*, *Lr46* and *Lr67*

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Keywords: wheat, *Puccinia triticina*, durable resistance, bread making quality

Development and release of wheat varieties resistant to leaf rust caused by *Puccinia triticina* Erikss. (*Pt.*), the most common and widespread disease of wheat worldwide, remain the main tool to control the damaging effects from epidemics. Although many of the leaf rust resistance genes already identified (>70, McIntosh et al., 2013), have been successfully used by wheat breeders to improve the race specific resistance to disease, a shift to adult-plant slow rusting resistance, quantitatively inherited, has been registered in many wheat breeding programs. This type of resistance expected to confer a higher durability than qualitative resistance, and became in the past years a more preferred breeding solution due to the identification of genes conditioning *slow-rusting* that show additive and pleiotropic effects on multiple diseases. Much progress has been achieved in searching and clarification for this type of resistance in wheat. Out of the most known four *slow-rusting* resistance genes, *Lr34* and *Lr67* have been cloned and better characterized.

With the aim to improve the durability of resistance to leaf rust in the wheat breeding program from NARDI Fundulea, have been developed six populations derived from crosses between the adapted cultivars *Glosa* (carrier of *Lr34*) and *Miranda* with the *slow-rusting* genes *Lr 34*, *Lr46* and *Lr67*. By molecular analysis with associated molecular markers: *cssfr5* (*Lr34*), *csLV46/TaqI* (*Lr46*) and *Lr67PLUSHSUTF/R*, *Lr67HSPSUTF/R* (*Lr67*), have been detected 574 RILs F₆ carriers of the single resistance genes *Lr34*, *Lr46* and *Lr67*, while combinations of two *Lr* resistance genes have been identified in 159 lines: *Lr34* and *Lr46* (n = 152) and *Lr34* and *Lr67* (n = 7), respectively. The evaluation of *slow-rusting*, APR resistance traits in recombinant lines, under artificial field inoculations and different environments (2014–2016), revealed low values of disease severity (DS, %) and AUDPC, expressed as % from the AUDPC values registered in the susceptible control (RAUDPC, %), relatively comparable with values registered at the donor parents for the *Lr 34*, *Lr46* and *Lr67* genes.

The recover of agronomic desirable traits have been analysed for: winter hardiness (1-9, where 1 = very resistant and 9 = very susceptible), heading (days from January 1st), plant height (cm) and some bread making characteristics (protein content, %; Zeleny value, ml; wet gluten, %),

According to experimental results, have been selected 15 new advanced pre-breeding lines carrying one or combination of two *slow-rusting* genes, associated with improved agronomic traits, as compared to the recurrent adapted parent(s). The selected lines are short straw type (95-115 cm), with a good level of winter hardiness (1-5), heading (119-127 days) and with

good level of the bread making quality traits: protein content (13.3-17.1%); wet gluten (30.9-44.6%) and Zeleny (37.3-67.4 ml).

This original germplasm combining *slow-rusting* resistance traits to leaf rust with valuable agronomic traits represents a step forward in diversification of wheat germplasm for breeding purposes.

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Do *TaCKX1* and *TaCKX2* genes regulate wheat productivity?

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Keywords: *TaCKX*, cytokinin, wheat, productivity

Multigene family of *CKX* genes encode cytokinin dehydrogenase proteins (CKX), which regulate cytokinin content in developing plants and substantially influence their development. The detailed biological function of most of the *TaCKX* genes in wheat is not known. The goal of this research is to characterize their functions and to select those, which might regulate yield-related traits in wheat. In our earlier research we showed that regulation patterns and expression level of *HvCKX* genes of barley indicated their roles in growth and reproductive development (Zalewski et al. 2010, 2012, 2014). Therefore the first step was to analyze expression pattern of 10 *TaCKX* genes in different tissues and organs of developing plants of 3 cultivars of wheat. Two genes: *TaCKX1* and *TaCKX2* showed high level of expression in developing kernels 0 DAP (days after pollination), 7 DAP and 14 DAP and were selected for further research and functional analysis. The selected genes were transcriptionally silenced in two wheat cultivars Kontesa and Ostka. This was achieved by *Agrobacterium*-mediated integration of RNAi silencing cassette. The relative expression of *TaCKX1* and *TaCKX2* in kernels 7 DAP and 14 DAP of selected transgenic lines was significantly decreased. In several lines decreased expression of the tested genes correlated with decreased activity of the CKX enzyme. The analysis of chlorophyll content in flag leaves and evaluation of selected phenotype traits in silenced T₁ lines will be presented and discussed.

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Polymorphism of rye inbred lines different in terms of wax layer

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Keywords: genetic diversity, molecular markers, *Secale cereale* (L.), wax

Rye (*Secale cereale* L.) is an important cereal crop, grown mainly in Europe, in temperate and cold climate. Its grain is used both as animal feed and for the production of alcohol and bread flour. Rye has many favorable qualities, such as exceptional cold hardiness, drought tolerance and disease resistance. Some of these attributes may be caused by the intense waxy cover on the green parts of the plants. The appearance of wax-free plants is frequently observed on the experimental or cultivated fields. The genetic basis for creating the waxy epidermal coating in rye is hardly known. Till now, only one gene responsible for the waxy film on straw, leaves and spikes has been detected and localized on the genetic map of rye. Having at our disposal several wax-less forms (inbred lines) we would like to explain genetic base of wax production. The goal of the research is to construct high-density genetics maps of a few mapping populations, created in order to localize different genes controlling wax coat production in rye. We purpose to use DArT-Seq technology and PCR-based markers with known chromosomal localization to realize this aim. This part of our research was aimed at detecting polymorphisms between 10 lines that were used to get 7 classical F₂ mapping populations. The idea was to appoint anchoring markers, helpful in determining linkage groups. As one of the applied PCR-based marker types, RAPDs known from a few rye genetic maps, among others from the map of population S120×S76, were used and these 2 lines were included into the set of analyzed lines as a standard. The electrophoretic separations in agarose gels following the PCRs with 152 selected primers allowed to reveal 1198 polymorphic zones, 110 of them defined as mapped on rye genetic maps before. Number of polymorphisms of 7 pairs of parental lines ranged from 310 to 493. NTSYSpc2.20 was applied to create dendrogram of genetic relationships between all 12 analyzed inbred lines. Genetic similarity (GS) values calculated with simple matching function ranged from 0.39 to 0.82. The greatest GS was demonstrated by 3 pairs of most closely related lines. This demonstrated that sufficiently large set of selected RAPD primers is a good tool for assessing pedigree relationships in rye and should be useful for genetic mapping purposes.

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The Watkins collection as a source for genetically versatile pre-breeding lines for NUE

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Keywords: Nitrogen Use Efficiency, landraces, Watkins, adaptation

Landraces are a great source of variation for the discovery and dissection of important traits and to develop pre-breeding lines. The Watkins collection comprises of phenotypically diverse landraces collected in the 1920s and 1930s from 32 countries, including India. We are utilising this diversity to investigate Nutrient Use Efficiency (NUE) within INEW, a Newton-Bhabha funded programme with six partner institutes in India and five organisations in the UK. Replicated field trials are essential to dissect biological NUE and to develop germplasm for pre-breeding, one of the main objectives of the INEW VJC. To put the “J” into the Virtual Joint Centre it is important that all the partners are using similar and related material. However, the field locations could not be more diverse, from irrigated trials at Pusa (25° 59' N) to rainfed fields in Nottingham (52° 82' N), requiring material adapted for very different environments. Early flowering material is needed for India to escape the heat and drought stress during grain filling whereas late flowering material can escape cold early in the UK season. A nested association mapping panel using 85 founders with Paragon (UK spring) as the common parent has been established at the JIC within the BBSRC WISP project. Families were selected that showed promising NUE traits in the UK and adapted subgroups of these families were chosen for field trialling in India and the UK.

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Localization of wax inhibitor markers on rye genetic maps

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Keywords: genetic map, EST markers, *Secale cereale* (L.)

The main objective of the study is to analyze three mutations that cause wax layer abnormalities on green parts of plants, including the location of chromosomal genes of the investigated genes on rye genetic maps. Plant material were: four pairs of NILs (near isogenic lines) (M12 and M12bw, DS2 and DS2bw, RXL10 and RXL10bw, L35 and L35bw), four pairs of inbred lines (Ds2 and RXL10, 541 and Ot1-3, 541 and 2020LM, S120 and S76) – parents for mapping population and four mapping populations (RIL-L, RIL-K, RIL-S, RIL-M).

EST markers linked with a wax inhibitor, mapped on chromosomes 2BS and 2DS of common wheat, were used. Twelve primer pairs (Wu et al., 2013) were tested, using standard PCR conditions. The electrophoresis of the amplification products was performed in 2% or 3% agarose gel. JoinMap4 was used for mapping.

There was no polymorphism detected between the near-isogenic lines using the primers tested. Marker CJ886319 demonstrated polymorphism in the case of three inbred line pairs (Ds2 and RXL10, 541 and Ot1-3, 541 and 2020LM), parental forms of the mapping populations. CJ519831 showed polymorphism between Ds2 and RXL10 and CA695634 differentiated lines S120 and S76. Differentiating primer pairs were used to determine segregation of wax inhibitor markers in four mapping populations. All polymorphic markers were mapped on the chromosome 7R. Earlier studies on rye wax-less mutant (Korzun et al. 1997) have also pointed chromosome 7R as the location of the *wal* gene.

Using the BAC library, the genome fragment including CJ886319 marker was detected and sequenced. 102 pairs of specific primers were designed for the resulting sequence. Moreover, 44 pairs of primers were designed to 22 sequences with the high degree of genetic similarity to the analyzed sequence. Research on the above mentioned rye inbred lines are in the testing phase.

Continuation of the research will be also carried out using classical F₂ mapping populations, derived as the crosses between different wax-less mutants and typical lines, in order to map genes responsible for wax coating production.

Acknowledgments

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Characterization of oat (*Avena sativa* L.) x maize (*Zea mays* L.) hybrids

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Keywords: genomic *in situ* hybridization, oat-maize addition lines (OMA)

Wide crossing of cereals with maize is often used to produce doubled haploids (DH). Usually in this method total elimination of maize chromosomes are commonly observed in most cereals, but oat can retain one or more maize chromosomes. The aim of the study was identification, morphological characterization and yield components analysis of the OMA (oat-maize addition) lines. In order to identify maize chromosomes retained in oat genome, oat genomic DNA was isolated from the flag leaf. DNA samples were analyzed by PCR method with using 5'-AAA GAC CTC ACG AAA GGC CCA AGG-3' and 5'-AAA TGG TTC ATG CCG ATT GCA CG-3' primers to detect the presence of maize chromatin. When the whole chromosomes or fragments of maize chromosomes were retained in oat genome, genomic DNA of 500 bp (specific maize retrotransposon fragment, Grande I) was amplified, and detected by electrophoresis. All OMA lines with detected Grande I were analyzed with genomic *in situ* hybridization (GISH) in order to detect and visualize maize chromosomes. To determine which maize chromosome was present in the OMA lines, DNA amplification using SSR-PCR with primers specific for maize chromosomes was performed. Of all the examined oat lines, in 47 OMA lines retrotransposon Grande I was detected. Twenty-six fertile OMA lines were analyzed with GISH. The whole maize chromosomes as well as a banding pattern probably colocalised with 25S rDNA sites in examined lines were found. Additional hybridization signals found in 2-3 pairs of chromosomes (depending on the analyzed line) might correspond 5S rDNA sequences (in the oat genome these sequences are located on 4 pairs of chromosomes). However, it can not be excluded that these signals came from other maize DNA sequences that have been incorporated into oat chromosomes. Studies based on SSR-PCR enabled the detection of 4, 5 and 6 maize chromosomes in three OMA lines. There were no significant morphological differences between OMA and DH lines. The obtained lines were varied mainly in number of grains and thousand grain weight.

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Multiple trait analysis of F5 and F6 data improve prediction accuracy of phenotype performance in barley and wheat breeding.

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Keywords: genomic prediction, multiple trait analysis, yield, barley, wheat

Genomic prediction allow breeders to predict breeding values (gEBVs) of unphenotyped new lines based on genotype and phenotype information from a training population (TP). Most relevant studies were conducted on the basis of single trait analysis. However, plant breeders usually record several phenotypes including yield, grain quality, and disease resistance. Benefitting from the genetic correlation of these traits, the multivariate analysis is likely to improve the predictive ability in comparison with univariate analysis. The objectives of this study were to (1) estimate the heritability of economically important traits in wheat and barley, (2) compare the prediction accuracy of several traits using multiple (MTGS) and single (STGS) trait genomic selection approach using pedigree (A), genomic relationship matrix (G), and both matrices (G + A), (3) apply F5 and F6 dataset as multiple training populations to predict the future yield performance in wheat and barley breeding lines.

1317 barley and 1325 wheat breeding lines were genotyped with the Illumina 9K (barley) and 15K (wheat) SNP chip, and phenotyped across multiple years and locations. In total, data from about 15K (barley) and 13K (wheat) individual plots were included in the analysis. After quality control steps, 4K and 11K SNPs for barley and wheat were retained for analysis. The models were cross-validated by a leave-one-set out strategy, where set is defined as the yearly sets of crosses.

Results showed that the MTGS increased the predictive ability for yield by 7% in barley and 57% in wheat compared with STGS alone. The analyses combining genomic and pedigree information increased the prediction accuracy by 4% (barley) and 3% (wheat) when compared with using the genomic relationship matrix only. Using MTGS the predictive ability was improved by 38% for powdery mildew resistance and 2% for ramularia resistance in barley, and 10% for protein content improvement when modelling protein content and Zeleny in wheat analysis.

In conclusion, MTGS can considerably increase the prediction accuracy for yield compared to STGS in wheat and barley breeding. This is especially the case when phenotypes are included from both F5 and F6 generations.

T2: NEW PLANT GENOME EDITING TECHNOLOGIES

Invited

CRISPR / Cas9 genome editing in wheat and barley

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Keywords: genome editing, CRISPR/Cas9, wheat, barley

Genome editing is revolutionizing biotechnology. For the first time we have the ability to produce targeted mutations in any gene of interest allowing rapid progress in many research areas. Mutant lines have been a valuable resource for plant breeders and for the plant research community however, the availability of mutants, especially in crop plants has to date been limited. RNA-guided Cas9 (CRISPR/Cas9) now allows very efficient targeted gene knock-outs in a range of crops (1). There are also opportunities for precise insertions or gene replacements (knock-ins) although these remain challenging.

In barley, a very efficient transformation system allows easy delivery of the components for CRISPR/Cas9 genome editing and we have shown that, on average, 40% of primary transgenic plants produced have active editing events. In the next generation, homozygous edited plants that no longer contain the transgenes can be easily identified.

In wheat production of targeted gene knock-outs is complicated by the complex genome and the presence of multiple copies of target genes. However, CRISPR/Cas9 based genome editing is still relatively efficient. Success depends on careful design of sgRNAs and also on have efficient screening strategies to detect mutation events.

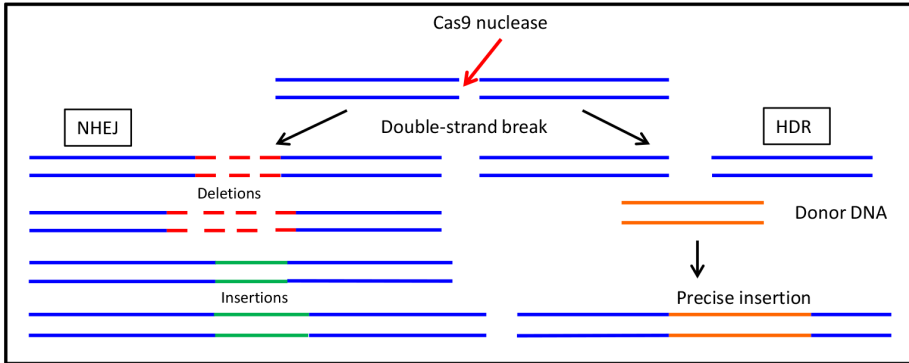


Figure 1. Cas9 guided to a specific genomic location causes a double strand break that is repaired by either non-homologous end-joining (NHEJ) or homology-directed repair (HDR). NHEJ can lead to insertions or deletions that disrupt gene function. HDR can also lead to point mutations or can allow insertion of DNA from a donor source.

Acknowledgments

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Oral presentations

Genome Editing in Barley: Targeting (1,3;1,4)- β -Glucan Synthases

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Keywords: CRISPR/Cas9, barley, cell walls, (1,3;1,4)- β -glucan

(1,3;1,4)- β -Glucan is one of the most abundant non-cellulosic polysaccharides of primary cell walls in grasses, and is found at high levels in barley endosperm compared with other cereals such as wheat and rice. Due to its solubility and viscosity in aqueous solutions, (1,3;1,4)- β -glucan in barley grain has a negative impact on brewing and distilling filtration processes. Conversely, (1,3;1,4)- β -glucan has beneficial effects on human health, where (1,3;1,4)- β -glucan acts as soluble dietary fibre in the small intestine, reducing the risk of developing cardiovascular disease, colorectal cancer and type II diabetes. The genes encoding (1,3;1,4)- β -glucan synthases, namely *HvCslF* and *HvCslH* (Cellulose synthase-like) gene super-family have been identified^{1,2}. *HvCslF6* has been shown to play a key role in the synthesis of grain (1,3;1,4)- β -glucan. However, other members of this gene family are expressed in different tissues and contribute to (1,3;1,4)- β -glucan synthesis: *HvCslF3* in roots; *HvCslF9* in early grain stages and *HvCslH* in leaves^{2,3}. These 4 genes were targeted using CRISPR/Cas9-based gene editing technology and a wide range of mutations (indels) leading to frameshift changes in T0 plants was obtained. A total of 44 T1 genotypes were confirmed to have CRISPR/Cas9-induced mutations: nine lines for *HvCslF3*, four lines for *HvCslF6*, 24 lines for *HvCslF9* and seven lines for *HvCslH*. For each gene, multiple unique independent CRISPR/Cas9 mutated lines were selected for analysis in subsequent generations. We isolated homozygous and *Cas9*-free (stable) genotypes carrying non-synonymous mutations likely to affect protein function: from single amino acid insertions to premature stop codons. Our work currently focuses on 1) Characterising stable lines using immunocytochemistry techniques to assess grain (1,3;1,4)- β -glucan content and other cell wall changes. 2) Generating double mutants by traditional crossing. A comprehensive phenotypic characterisation will complement this study, analysing cell wall composition in general. This will include quantification of (1,3;1,4)- β -glucan content in a range of tissues in addition to the mature grain. These mutants represent a valuable genetic resource for studying barley cell walls, and demonstrate the effectiveness of the CRISPR/Cas9-based gene editing technology.

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An application of CRISPR/Cas9 technique: modifying α -gliadin proteins in bread wheat to decrease the risk of Coeliac disease in humans

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Keywords: CRISPR/Cas9, gene editing, wheat, gluten, gliadin epitopes, coeliac disease

Hexaploid bread wheat (*Triticum aestivum*) is a staple crop in many parts of the world. A significant component of the wheat grain is gluten, a polymer of glutenin and gliadin proteins encoded by large gene families. These proteins harbour immunogenic epitopes which can trigger Coeliac disease in genetically predisposed humans (approximately 2 percent of the populations). The immunogenic properties of gliadins may be reduced by modification at specific positions or by their deletion. Therefore, mutagenesis technologies can be deployed to obtain hexaploid wheat plants producing grains containing fewer immunogenic gliadin epitopes, thereby increasing safety for Coeliac patients.

CRISPR/Cas9 technology is a site directed mutagenesis technique aiming at cutting DNA in a gene of interest. This system triggers the DNA repair mechanism, which is a highly error prone process in plant cells, often resulting in mutations of the gene of interest. We have designed different CRISPR/Cas9 constructs transformed hexaploid bread wheat to edit or to remove alpha-gliadin genes. In subsequent wheat grains, alpha-gliadin proteins should be altered or less abundant, which would avoid or decrease their recognition by the human immune system. Alpha-gliadins mutant lines are screened using Acid-PAGE protein separation methods. Further quantitative (ddPCR) and qualitative (amplicon sequencing) analyses will be performed on promising edited lines.

These results will enable conclusions to be drawn on the applicability of CRISPR/Cas9 as a targeted mutagenesis method for large gene families in polyploid plants. It should also provide valuable information about the relevance of using such an approach to create safer wheat varieties for Coeliac patients. Our novel lines will require further investigation including immunological tests using monoclonal antibodies to determine their immunogenicity level and rheological studies to evaluate the bread dough quality obtained using these generated “CD-safer wheat lines”.

Poster presentations

Efficient CRISPR/Cas9 based system for genome editing in barley (*Hordeum vulgare* L.)

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Keywords: CRISPR/Cas9, genome editing, barley, gateway cloning

Since its demonstration in 2012, CRISPR/Cas9 system has been applied as a break-through technology for precise genome editing in a broad range of organisms. The main advantages of this system are among others, the technical simplicity, easy multiplex gene targeting by single construct and the ability to cleave methylated DNA. The Cas9 nuclease can also be re-engineered or inactivated and used for other genetic engineering approaches, such as gene expression regulation or for labeling of specific chromosomal regions *in vivo*.

In this study we demonstrate the simple and efficient CRISPR/Cas9-mediated genome editing system for barley in which the target sequence is cloned into Gateway compatible vector for stable *Agrobacterium*-mediated transformation. To introduce site-directed mutations we chose two different barley genes, *HvCKX1* and *Nud*, both determining important agronomical traits. *HvCKX1* gene is a member of the *CKX* gene family encoding oxidase/dehydrogenase enzymes which metabolize cytokinins in plants and control developmental processes. *Nud* gene is a member of the gene family of ethylene-response transcription factor ERF. Mutations in this gene result in a phenotype with naked (hullless) caryopses. The crucial role of *CKX* genes in plant development and the possibility of direct detection of phenotypic changes in plants modified by site-directed mutagenesis of *Nud* gene were two main reasons for choosing these genes.

Immature embryos of barley were transformed with vectors containing codon-optimized gene of nuclease Cas9 and single guided RNA sequence (sgRNA) targeting either *HvCKX1* or *Nud* gene under wheat U6 RNA promoter. Induced mutations were detected by restriction analysis of amplified fragments of both genes. 71 transgenic plants were obtained after transformation with CRISPR/Cas9 construct for *HvCKX1* gene and 28 transgenic plants for *Nud* gene. The mutation frequency was 66% and 64% of *HvCKX1* and *Nud* genes respectively. In part of the plants the sequences carrying mutations were cloned and sequenced. Single nucleotide deletions or insertions were the most frequent types of mutations however, larger deletions

(up to 10 bp) were also detected. Moreover, in most of the plants with altered *Nud* gene, the naked caryopses with non-adherent hulls were observed. Our results show that the CRISPR/Cas9 technology is a simple and powerful tool for breeding improvement of crop plants.

Acknowledgments

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Editing net blotch susceptibility genes for disease resistance in barley using CRISPR/Cas9 through gene gun bombardment

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Keywords: net blotch, susceptibility, barley, CRISPR/Cas9, gene gun

Net blotch, a worldwide disease in barley (*Hordeum vulgare*), is caused by the necrotrophic fungal pathogen *Pyrenophora teres* f. *teres* (*Ptt*), resulting in a destructive foliar disease with up to 40% yield losses in susceptible barley cultivars. Distinct *Ptt* isolates can trigger differential disease responses depending on barley genotype. The cultivar Rika is resistant to *Ptt* isolate 15A but susceptible to *Ptt* isolate 6A. Conversely, the cultivar Kombar is susceptible to *Ptt* isolate 15A and resistant to *Ptt* isolate 6A. The centromeric region of barley chromosome 6H contains dominant susceptibility to 15A, 6A and multiple other isolates and a candidate gene with allelic specificity has been cloned from each of Kombar (*Spt.k*) and Rika (*Spt.r*). Further analysis of these *Spt1* alleles is necessary to confirm their function and eventually for exploring a novel resistance breeding strategy in barley based on knocking out genes for susceptibility to net blotch.

The CRISPR (clustered, regularly interspaced, short palindromic repeats)/Cas (CRISPR-associated) technique has recently been developed for targeted gene editing. Various transformation methods can be used to deliver CRISPR/Cas-containing plasmids into plant cells or tissues. Among them, the *Agrobacterium*-mediated transformation method is commonly used, especially for dicots. In the case of spring barley, *Agrobacterium*-mediated transformation is only efficient with the cultivar Golden promise. In our study, we are establishing an efficient particle bombardment transformation protocol for barley using CRISPR/Cas9 constructs for gene knock-out utilizing spring barley genotypes other than Golden Promise. We are targeting the *Spt* alleles in Rika and Kombar to confirm their function for net blotch susceptibility.

T3: BREEDING INFORMATICS

Invited

Optimization of breeding schemes using genomics, genomic predictions and simulations

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Keywords: breeding scheme, cross design, genomic predictions, simulation, haplotype inference, bread wheat

We will first illustrate how simulation of breeding schemes at constant cost and genomic predictions can help breeders choosing breeding schemes according to their either short or long term objectives. We will show results from the Virtual-GS pipeline, a decision tool developed in the R environment at INRA, GDEC, Clermont-Ferrand. It takes as input a vector including each activity's unit cost (crossing, double haploid production: DH, seed multiplication, cost per plot depending on the number of trials and replicates, genotyping cost). The second parameter is a matrix of parent genotypes. The third is a vector of marker effect estimates. It can be simulated or estimated using historical data. The fourth is the heritability of the trait. The fifth is the breeding strategy (PS: phenotypic selection, GS: genomic selection, GPS: genomic + phenotypic selection). The sixth is a vector of selection rate for each year ($\alpha_2 = 20\%$, $\alpha_3 = 15\%$, $\alpha_4 = 15\%$). The seventh is the number of cycles K you want to simulate. The eighth is the total cost CT of a breeding cycle. We illustrate it here with a 4 years' breeding cycle. For total cost CT = 1 000 000 euros, number of parents NP = 30 and crosses NC = 30, number of cycle K = 1, we compare the number of lines per cross at each generation (N2, N3 and N4) and final genetic gain using PS (phenotypic selection each year) or GPS (genomic + phenotypic selection) strategy, ie replacing phenotypic selection by genomic selection on Year 3. The pipeline can cross parents at random or optimize cross design based on genomic predictions.

We will then show how the Virtual-GS pipeline cross design module helped us designing a triple-round-robin multi-parental population in order to maximize genetic gain.

Finally, we will illustrate how haplotype inference can be useful to better describe germ-plasm diversity and increase power of association studies.

Oral presentations

Screening drought adaptive phenological traits in conventional and synthetic derived bread wheat germplasm and its implications for association mapping

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Keywords: drought stress, *Aegilops tauschii*, SSRs, Synthetic hexaploid wheats (SHWs), Association Mapping

Improving wheat yield under water limited conditions could be attained if more traits conferring better agronomic performance are brought together in the same genotype. This study was conducted to assess drought tolerance and diversity of a collection of conventional bread wheat (CBW) and synthetic-derived bread wheat (SBW) genotypes. Evaluation was carried out through phenological traits in the field under well-watered and drought stress conditions imposed at pre-anthesis stage for two successive growing seasons during 2010/11 and 2011/12. Association mapping (AM) analysis was used to dissect the genetic basis of drought-adaptive traits using mixed linear model (MLM). In total, 27 marker-trait associations (MTAs) were associated over 23 SSR loci for six phenological traits. There were 12 MTAs confined only to stress treatment, 2 MTAs confined to control environment and 7 MTAs were common in both treatments. The intra-chromosomal position of several of these MTAs coincided with those previously reported whereas there were some unique loci highlighted for drought-adaptive traits that had not been located to date. Opportunities for further wheat improvement are provided by these novel loci based on a marker approach.

Combining high density GWAS and grain ionomics to identify a major gene influencing sodium content

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Keywords: abiotic stress, GWAS

Plants that are more robust in terms of their tolerance towards abiotic stresses, such as salinity, can, in certain environments, produce higher yields than their less tolerant counterparts. There are multiple mechanisms that have been described as contributing to salt tolerance in crops, including exclusion or translocation of salt from roots and leaves, changing the architecture of roots, and partitioning sodium into certain leaf tissues ¹. However, little work to date has focused on the sodium content of the mature grain. We screened the grain sodium concentration of 131 contemporary UK barley accessions using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and observed a 4 fold variation in this trait within this collection. Pairing these data with 150,000 Single Nucleotide Polymorphisms (SNPs) derived from exome capture resequencing of these accessions enabled us to carry out a genome-wide association study (GWAS) to identify regions of the genome influencing grain sodium content. We identified a single highly significant association on 4HL ($-\log_{10}(p) = 11.9$) which spanned approximately 1.6Mb, and contained 94 gene models based on the new barley genome assembly ². Using accessions representing the phenotypic extremes of this collection a combination of Sanger sequencing and data mining of the exome capture dataset revealed one gene that contained 3 haplotypes segregating for grain sodium concentration. These haplotypes showed significant differences in terms of their grain sodium concentration, with one haplotype resulting in an average increase in grain sodium content of at least 1.8-fold from 28.59 mg/kg to 51.28 mg/kg. The expression profile of this gene for these three different haplotypes will be compared in a range of tissues, and its allelic diversity in a wider set of germplasm explored. The functional characterisation of this gene is ongoing, and these results will also be discussed.

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Genome-wide association study of nitrogen use efficiency in a Central European winter wheat collection

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Keywords: nitrogen use efficiency, association mapping, genotyping by sequencing, bread wheat

To satisfy future demands, the increase of wheat yield is inevitable. Simultaneously, maintaining high crop productivity and efficient use of nutrients, especially improving nitrogen use efficiency (NUE) is essential for sustainable agriculture. Previous studies revealed significant genetic variation in NUE in wheat, moreover, several loci, influencing NUE under various N supplies, have been also successfully mapped. However, the inheritance of NUE and its components are complex and they are highly influenced by environmental factors, nitrogen management practices and genotypic variation as well. Therefore, a better understanding of their genetic basis, their regulation is fundamental.

To investigate NUE related traits and their genetic and environmental regulation, field trials were evaluated in a Central European wheat collection containing 93 cultivars at two nitrogen input levels across three seasons. 19 agronomically important or nitrogen input related traits were measured or calculated. This elite germplasm collection was genotyped on DArTseq® genotypic platform to identify loci affecting complex agronomic traits. To conduct robust genome-wide association mapping the genetic diversity, population structure and linkage disequilibrium were examined.

Population structure was investigated by various methods and two subpopulations were identified. This differentiation is based on the origin and the breeding history of the cultivars, while analysis of linkage disequilibrium suggested that selective pressures had acted on chromosomes bearing loci with remarkable agronomic importance. Besides NUE, genetic basis for variation in agronomic traits indirectly affecting NUE and its components, moreover genetic loci underlying response to nitrogen fertilization were also determined. Altogether, 320 marker trait associations (MTA) were identified and clustered into 80 associated regions (AR) spreading over almost the entire genome. We found that most of the MTAs or ARs were environmental dependent, i.e. these environmental specific loci are associated with adaptation to specific environmental conditions. While some loci, significant for the same trait were identified under different environments. These regions are the best candidates for more detailed studies, because they may be involved in constitutive processes for agronomically important traits.

The present study identified several associated markers in those genomic regions where previous reports had found genes or quantitative trait loci that influencing the same traits, while most of the marker trait associations we describe herein identify novel genomic re-

gions. Our data provide an overview of the allele composition of bread wheat varieties anchored to DArTseq® markers, which will facilitate the understanding of the genetic basis of NUE and agronomically important traits. Since our cultivar collection is well adapted and commercially relevant in Central European area, therefore our study provides valuable results to local breeders.

Acknowledgments

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Association mapping studies on drought tolerance in spring barley gene bank accessions

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Keywords: barley, drought stress, chemical desiccation, genome-wide association

Barley is among the four most important crops globally with an annual production of 130 million tons (FAO, 2013). As other crops barley is subjected to different stresses. Thereby, drought is one of the most severe abiotic stresses affecting crop production.

In order to study the genetic variation and to detect quantitative trait loci for drought tolerance 184 spring barley accessions were genotyped with the 9k SNP chip and grown in the field in 2016 and 2017. To simulate drought stress chemical desiccation using potassium iodide (KI, 1% w/v) was applied 14 days after anthesis.

Morphological and agronomical traits were evaluated for identifying stress reactions. Recorded data were days to maturity, plant height, spike length, grain weight, grain number per spike and thousand grain weight. The drought treatment had a significant influence on many of the measured traits.

Analysis showed significant differences between genotypes. Chemical desiccation showed strong impacts on yield parameters. Here, thousand grain weight (TGW) decreased from 49.10 ± 6.7 to 39.79 ± 7.4 .

Results obtained from both years field experiments using Genome-wide association approach revealed major marker-trait associations after chemical desiccation.

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Poster presentations

Haplotypes of *Ppd-D1* gene and alleles of *Ppd-A1* and *Ppd-B1* in Ukrainian wheat varieties

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Keywords: photoperiod sensitivity, haplotypes, *Ppd*-genes, wheat varieties

Light period significantly influences on the growth and development of plants. Cereals, including bread wheat, react to change in the duration of light day by accelerating or slowing down the development. The degree of such reaction in bread wheat depends mainly on three genes that are localized in the second chromosome of each wheat subgenome ABD: *Ppd-A1* (2A), *Ppd-B1* (2B), *Ppd-D1* (2D). The dominant alleles (*a*) of *Ppd* genes reduce sensitivity to duration of the day, shorten the period to heading and genotypes with recessive alleles (*b*) have a strong reaction to the photoperiod.

The aim of the work was to determine alleles of genes *Ppd-A1*, *Ppd-B1*, *Ppd-D1* for modern winter and spring wheat varieties using molecular markers and to compare the genotypes according to *Ppd-I* genes with the sensitivity of varieties to photoperiod. As a material were used modern winter wheat varieties from Bilatserkovska Experimental Breeding Station (BEBS), Poltava State Agrarian Academy (PSAA), winter varieties and two-handed variety Zymoyarka from The V.M. Remeslo Myronivka Institute of wheat and spring wheat varieties from different breeding centers. Allele-specific PCR with primers (Beales et al., 2007; Seki et al., 2011; Nishida et al., 2013) to *Ppd-A1*, *Ppd-B1*, *Ppd-D1* genes were used.

Myronivka varieties have been differentiated into three groups, with different genotypes according to the system of genes *Ppd-I* and photoperiod sensitivity: *Ppd-A1a/Ppd-B1b/Ppd-D1a*, *Ppd-A1b/Ppd-B1b/Ppd-D1a*, *Ppd-A1b/Ppd-B1b/Ppd-D1b*. Within the group of varieties with alleles *Ppd-A1b/Ppd-B1b/Ppd-D1a* and *Ppd-A1b/Ppd-B1b/Ppd-D1b* variation in time to heading was observed.

Varieties from BEBS and PSAA were characterized by alleles *Ppd-A1b/Ppd-B1b/Ppd-D1a*. Duration of the "emergence-heading" period for BEBS varieties differ from 1 to 4.8 days.

Spring wheat varieties were divided into two groups: *Ppd-A1b/Ppd-B1b/Ppd-D1a* and *Ppd-A1b/Ppd-B1b/Ppd-D1b*.

For all investigated varieties were also investigated haplotypes of *Ppd-D1* gene – TE insertion from 1 intron, 5 bp deletion exon 7, 16 bp insertion exon 8 – according to Beales et al. (2007) and Guo et al. (2010). BEBS and PSAA varieties have not polymorphism in these loci, they have TE insertion from 1 intron, 5 bp deletion exon 7 and 16 bp insertion exon 8 were absent. In cv. Zymoyarka from Myronivka TE insertion from the first intron was absent, all other varieties from Myronivka have this insertion. The presence of TE insertion in the first intron reduces the expression level of *Ppd-D1b* and, as we assume, due to this,

these varieties had heading later for 11-28 days than Zymoyarka. Deletion in exon 7 (size 5 bp) was absent in tested varieties from Myronivka. In cv. Zymoyarka and Beregynya was detected 16 bp insertion in exon 8, that according to Guo et al. (2010) increases the rates of vegetation. Results of investigations of spring wheat varieties haplotypes of *Ppd-D1* gene will be presented in the poster.

Acknowledgments

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Genomic prediction of baking quality in winter wheat

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Keywords: genomic selection, wheat quality, alveograph, GWAS, GBLUP, cross-validations

Baking quality of wheat (*Triticum aestivum* L.) often has lower priority in breeding programs than high yield, because quality traits are expensive and slow to determine phenotypically. Predicting wheat quality traits using DNA markers can reduce the resources needed for phenotyping and make it possible to select lines based on their quality at earlier stages of breeding programs.

The aim of this study was to identify SNP markers with large effect on wheat quality traits and to develop models for genomic prediction of the traits.

In total, 635 F₆ winter wheat lines from two breeding cycles of the Danish breeding company Nordic Seed were genotyped with the 15K Illumina SNP chip. The lines were phenotyped for the quality traits flour yield, and Alveograph W, P, and L (*i.e.* dough strength, tenacity and extensibility).

GWAS were performed to identify SNPs with large effects on the traits. GBLUP (Genomic Best Linear Unbiased Prediction) was used for genomic predictions. The genomic predictions were evaluated using several types of cross-validations to study possible implementation strategies.

SNPs with large effects were identified for flour yield and for Alveograph W and P. Narrow sense heritabilities ranged from 0.38 for flour yield to 0.72 for Alveograph W. The genetic relationship between lines of the training and validation sets had a big effect on the genomic predictions compared to the effect of the training set size. Genomically estimated breeding values based on leave-one-out cross-validations ranged from 0.5 for flour yield to 0.79 for Alveograph W. Thus, genomic prediction of wheat quality traits appear promising for breeding of new high quality cultivars.

T4: PHENOTYPING & PHENOMICS

Invited

Integrated phenotyping concepts and infrastructures – technologies and concepts for phenotyping across scales

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Keywords: phenotyping, ESFRI, infrastructure

Plant phenotyping is an essential tool for many applications ranging from functional genomics, to (pre-)breeding, breeding and analysis of biodiversity. It develops rapidly into a bottleneck for progress in basic and applied research. Lack of adequate solutions for quantitative analysis of plant architecture and function as well as their interaction with the dynamic and heterogeneous environment hampers progress in basic sciences as well as in breeding-related research. In recent years significant interdisciplinary approaches have been started to overcome this “phenotyping bottleneck”. Techniques were developed to quantify the dynamics and the heterogeneity of plant structure and function as well as of environmental cues. In this presentation we will explain recent results from the phenotyping chain approach, by which we study the relevance of phenotyping technologies at various scales from the lab to the field in direct experimental approaches and from meta-analysis. We will give focus on novel technologies that might be very important for breeders and seed industry – including high-throughput, controlled-environment, seedbank-oriented, field phenotyping as well as remote sensing of plant performance. The integration of different scales is also a central element of EMPHASIS: the new pan-European for Multi-Site Plant Phenotyping And Simulation for Food Security in a Changing Climate, which is developing on the basis of the portfolio of existing national plant phenotyping centers in Europe. Here we will discuss the recent developments since EMPHASIS has been established as a ESFRI project as well as the role of the different phenotyping networks on the global scale like e.g. IPPN. We will also illustrate the importance of integration between phenotyping and precision agriculture (1).

Acknowledgments

We are grateful to all partners in DPPN, EPPN, EPPN2020, EMPHASIS and IPPN for their contributions to these fruitful networks.

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Oral presentations

Application of high-throughput phenotyping to understand the genetic architecture of biomass growth in cereals

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Keywords: biomass, growth, genome-wide association mapping, high-throughput phenotyping

Future yield improvement is hampered by biotic and abiotic stresses due to climate change and accordingly, wheat and barley yields in Germany showed a strong fluctuation during the last 15 years. One factor is the increased frequency and severity of drought stress events, especially in spring during the vegetative growth phase – affecting the formation of biomass. Understanding the genetics of biomass accumulation under well-watered and seasonal drought stress conditions can provide a basis for stabilizing or even increasing grain yield. To this end, an imaging system was used to evaluate daily biomass accumulation in spring barley and winter wheat collections under greenhouse conditions. Genome-wide association scans (GWAS) were applied to identify genetic markers associated with variation in biomass accumulation. Drought stress treatment started in the tillering phase and included a recovery period until experiments ended around tipping time. By daily non-invasive imaging dynamics of biomass accumulation could be resolved, both phenotypically and genetically. We obtained high heritabilities for biomass under both conditions and over the whole observation period. Early seedling biomass growth was marked by a set of QTL lasting only for a short time. These QTL were replaced by a different set that influenced vegetative biomass at later growth stages and that lasted for a longer period of time in both crop species (Figure 1). Distinct loci were identified for biomass under well-watered and stress conditions. In addition, candidate genes involved in plant growth hormone regulation and phenology were identified.

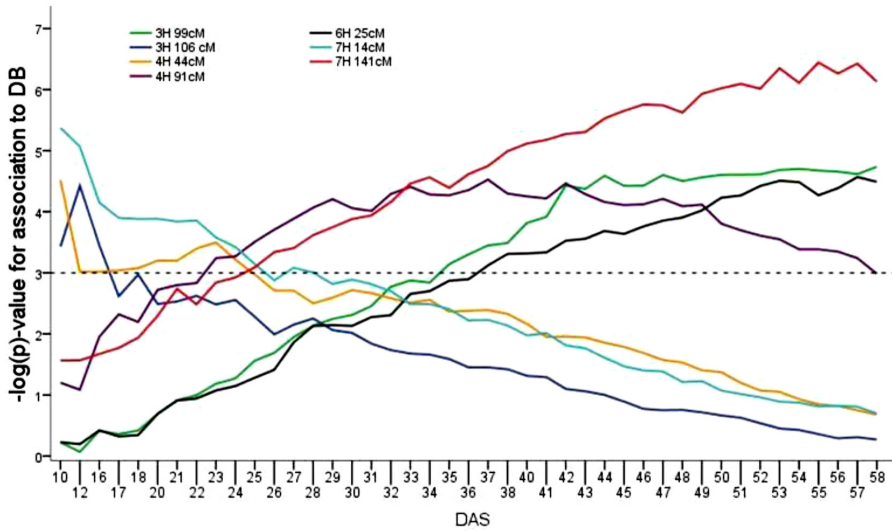


Figure 1: Summary of QTL identified for biomass over time exemplary shown for spring barley. Each colored line represents one SNP with its significance value ($-\log(p)$) for each day of the experiment expressed as days after sowing (DAS).

MAPEO, Drone based phenotyping for plant breeders

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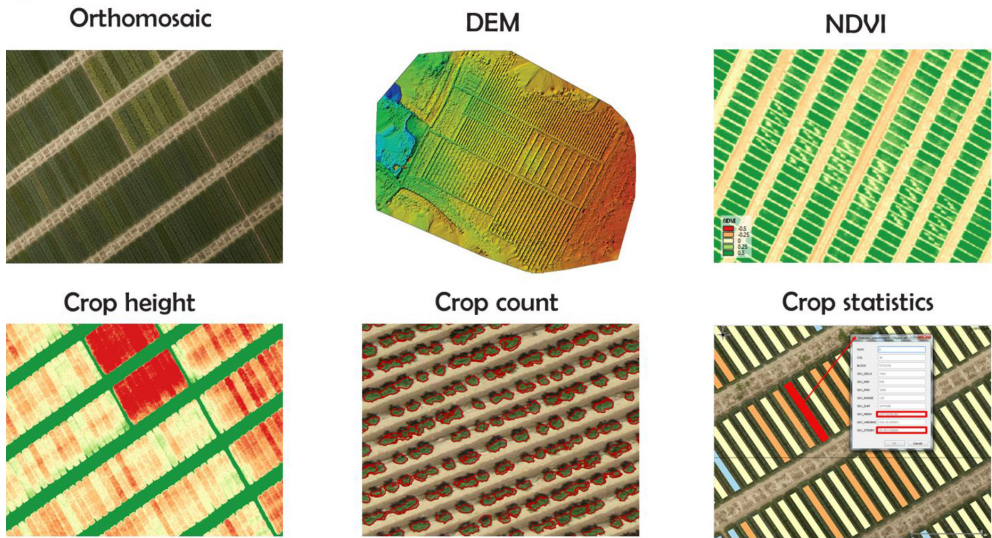
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Keywords: phenotyping, drone, image analytics, big data

Drone based, or drone assisted phenotyping has been described in the literature for many crops and traits with generally high accuracies in comparison to field based methods. Homan *et al* (2016) found for example consistently low RMSE values (0.07, 0.02 and 0.03 m for May, June and July, respectively) for drone based wheat height measurements, enabling crop growth rate to be derived from multi-temporal surface models. Also the drone industry has pointed towards agriculture as one of the leading industries for drone use. However, the practical implementation into the experimental fields of plant breeders or public research facilities has been limited to demonstrations and publicly funded research programs. The step towards fully exploiting the potential of drone based phenotyping requires (i) the simplification of data acquisition and data handling, (ii) data analytics which are focussed to extract relevant agrometrics on micro-plot level and (iii) the integration within day-to-day workflows. Over the past 2 years, we have collaborated with plant breeders and public research facilities to develop MAPEO, a drone image processing workflow dedicated to experimental fields. Plant height for wheat and corn plots, diseases in sugar beets and wheat trials, plant emergence

for potatoes and spinach are just a few traits for which image analytics were provided and which resulted in agrometrics which reaches or even surpassed field based measurements. Technologies like MAPEO are there to help make plant breeders to fully adopt and integrate drones into their day to day lives.

Figures



Example of drone based image products for experimental fields

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T5: BREEDING FOR INTENSIVE AND SEMI INTENSIVE CEREAL PRODUCTION

Invited

Winter wheat breeding ... the present and the future

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The world population is still increasing, but the birth rate is generally declining. By 2050, if moderately optimistic predictions are correct, it should stabilise at around 9.000 million compared to the current (2017) level of 7.000 million. If this is correct, breeders, agronomists and the plant protection industry have probably an achievable goal for increased production of wheat and other staple food crops. Currently, world wheat production is keeping pace with population growth and the carry-over from the old harvest to the new harvest should be enough to prevent spikes in the global wheat price and social unrest for the next 10-15 years assuming there are no unforeseen catastrophes. The current relatively low price of wheat, which is often related to low oil prices, is putting pressure on farmers to save money on inputs, including purchase of new seed.

Wheat breeding is carried out by organisations and companies with vastly different backgrounds, ranging from totally-owned subsidiaries of multi-national chemical companies, through multi-national breeders with no involvement in plant-protection, international non-profit organisations such as CIMMYT and ICARDA, national state-funded or semi-privatised organisations, to smaller national commercial breeding companies. In the last 40 years most former state breeding programmes in West and Central Europe have been fully or partially privatised and there has been a broad trend to internationalise these programmes.

Multi-national breeders are active wherever seed can be sold on a commercial basis. The priority is always on hybrid crops such as Maize, Sunflower and Oil Seed Rape, where an annual return on investment is guaranteed. As an inbred, wheat has always been a marginal crop regarding income and return on breeding investment. It is logical in this sense, that wheat breeders should look to hybrids as a way of stabilising income to fund further breeding work. Return on research investment comes from royalties on seed sales, sales of foundation seed or EPR (End Point Royalty) a system which is currently being highly successfully developed in Australia.

Most winter wheat programmes in Europe are Adaptation Area programmes which exploit climatic regions rather than stopping at national borders. This increases the efficiency of the programmes and lowers costs and increases income considerably.

A combination of improved/adapted varieties, progress in agronomy, fertiliser application and mechanisation together with integrated disease and insect control, and effective

extension services, with acceptance of new varieties in developing countries will be needed to achieve the goal of feeding the world ... no single factor alone can achieve this. There is some scope for use of current fallow in some regions, but this is set against increasing demands for urbanisation and problems of desertification and salinization.

In the short to medium term increased intensity of cultivation with higher fertiliser inputs and use of fungicides to control disease will deliver the required increase in yields. However, the danger is that resistant varieties could be ignored by farmers looking only for higher yield-potential under fungicide treated regimes leading to a huge potential disease inoculum on volunteer plants and disease epidemics on a large scale. The epidemics of Warrior Yellow Rust in Europe, North Africa and the Middle-East since 2014 have shown how dramatic this can be. The high pressure on the Yellow Rust pathogen caused by the wide cultivation of varieties with single major-gene resistances is possibly one of the causes of the frequent race changes seen in the Warrior group over the last few years. These rapid changes are underlined by the current advice from pathologists and advisory services to farmers regarding variety resistance: “Go to the field and look”.

Winter wheat varieties in West and Central Europe have to remain competitive in yield under intensive growing conditions with routine applications of fungicides and growth regulators. Apart from leading to a neglect of disease resistance in current programmes, it also leads to over-use of major-genes already available in high-yielding parent lines. The author estimates, that 80–85% of lines in current official trials (in Germany) are protected from Yellow Rust by two single major-genes or two major-genes in combination. Resistance in only the remaining 15–20% of lines cannot be explained by known major-genes and could be due to unknown major-genes or durable resistance from minor-genes. A similar situation can be found in Brown Rust resistance in West Europe where no single major-gene now found in high-yielding backgrounds is effective.

Although there are estimated to be around 65 known effective major-genes for resistance to Yellow Rust and Brown Rust the majority of these are in low-yielding backgrounds and often have deleterious linkage-drag effects. Most of the larger commercial breeding companies do have dedicated pre-breeding programmes to incorporate these genes, but progress can be very slow.

There is also the real danger that over-use of fungicides to achieve 100% clean crops could lead to problems in the long term with mutations of pathogens causing a build-up of resistance and reduced efficacy. This has already happened with Leaf Septoria and DTR/Tan Spot in Western Europe.

A single major-gene used widely under intensive growing conditions can be expected to be effective for an average of 7 years. Development of resistance to fungicides under these conditions also seems to occur at about the same rate. Given the cost of development of a new fungicide active-ingredient at €200 million compared to that of a new variety at €2 million it will be interesting to see in the future if the extra benefit of delayed senescence conferred on plants by fungicides is worthwhile.

Wheat breeding and selection methods have changed dramatically over the last five years since the deployment of cost-effective SNP markers and is likely to change even more in the near future with the development of genomic selection. Current developments in genomic

selection for certain fixed-traits such as frost tolerance or quality parameters are truly breathtaking.

SNP markers are not only useful for determination of known major-genes for disease resistance, but also for the discovery of possible durable minor-genes. However both markers and genomic selection require a large, reliable phenotypic data-base to be effective. In a country like Germany where all data from official trials is available to breeders and germ-plasm exchange between breeders by crossing agreements is usual, rapid progress is being made.

Current breeding methods employed by breeders include bulk-progeny breeding, pedigree selection, bulk-pedigree selection, single-seed descent, double haploids, chemical hybrids and genetic hybrids. Variety adaption (Lysenko) has been largely discredited, but may have some scientific basis in epigenetics. GMO breeding has largely come to a halt because of consumer resistance and high deregulation costs. CRISPR cas9 and gene-editing seem to offer possibilities that are only limited by the imagination of the breeder; but we have to wait and see if the technique falls under GMO legislation.

Among other topics of current interest for breeders are Nitrogen Use Efficiency (NUE) and grain protein content. The negative regression of protein content and yield has been a serious problem for many years. Although wheat protein is not an important component of western diets, in other parts of the world it can be beneficial. Also, although the baking industry is beginning to look more at functional protein in bread-making processes, protein content remains an important factor for export contracts. Finding the regression-breakers has proved extremely difficult in the past, but the author believes, that the introduction of simple multi-site on-combine NIR measurement of protein prior to selection decisions and progress in genomic selection for protein content will allow for considerable progress in this area. Furthermore, protein taken off the field in grain rather than left in the straw should improve the nitrogen balance at a time when nitrogen restrictions are being introduced in the EU.

Finally, breeding for insect resistance has been neglected in regions with intensive wheat cultivation, although much progress has been made in the USA by farmer-driven breeding for Hessian Fly, Stem Saw Fly and Russian Wheat Aphid (RWA) resistance. The re-emergence of the Orange Wheat Blossom Midge in Europe in the 1990's has given an impulse to renewed research on this subject. Climate change with global warming together with the removal of a wide range of insecticides and insecticide seed-treatments could make aphid-vectored Barley Yellow Dwarf Virus (BYDV) and dwarf leaf-hopper vectored Wheat Dwarf Virus (WDV) serious threats to wheat production in Europe.

Oral presentations

Breeding for resistance to aphids in wheat and barley

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Keywords: *Rhopalosiphum padi*, *Sitobion avenae*, *Schizaphis graminum*, *Diuraphis noxia*

Wheat and barley are very important food, beverage and feed crops worldwide. Breeding programs include resistance to various diseases as important selection criteria. However, selection for resistance to insects, such as aphids, is seldom applied. Yet aphids are among the most important pests wherever wheat and barley are grown, causing damage via direct feeding and indirectly via virus transmission. Species composition and pest status differ geographically; *Rhopalosiphum padi*, *Schizaphis graminum* and *Sitobion avenae* are very widely distributed since long, whereas *Diuraphis noxia* is considered an introduced pest in North and South America, South Africa and more recently in Australia. Breeding for resistance to *S. graminum* and *D. noxia* has resulted in resistant cultivars, both in wheat and barley. However, there are no deliberately bred cultivars with resistance to the other two aphid species. One reason for the success with *S. graminum* and *D. noxia* is the typical plant symptoms they cause, on which selections can be made. The resistance is typically of the classical gene-for-gene type, and there are cases where it has been overcome after resistance genes were deployed in cultivars. Here we review the known resistance loci, their origin and associated molecular markers. We also report on our own efforts to breed for resistance to aphids in wheat and barley, using traditional breeding methods combined with molecular markers and more recently also trying to knock out candidate aphid susceptibility genes in barley.

Changing the wheat breeding paradigm – examples from practice

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Keywords: breeding, wheat, change, paradigm

In order to overcome the problem of stagnating wheat yield and to secure further improvements regarding stress tolerance, technological quality, disease resistance etc., it is obvious that presently used wheat breeding approaches need immediate and innovative improvements. Since wheat breeding is very complex and related to numerous branches of science, like genetics, physiology, morphology, chemistry, etc., there is a multitude of opportunities to influence the breeding process. In addition, commonly used breeding practices need constant

improvement to cope with new challenges appearing over time. In this work we will discuss a few strategies and approaches addressing previously mentioned challenges, which are already implemented in our breeding program.

The first one is related to the strategy we are applying to creation of novel and desirable genetic variability to be exploited for significant improvements in wheat yield potential. We will present our non-standard approach to exploit DH line production opportunities, MAS implementation, and the breeding approach for improved technological quality.

For all mentioned approaches, presently achieved outcomes will also be presented.

Application of androgenesis in cereal breeding

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Keywords: *in vitro* androgenesis, anther culture, breeding, haploid, spelt wheat, rice and wheat

The application of biotechnology methods can effectively support the production of new varieties and hybrids in crop plants. One of these techniques is *in vitro* androgenesis – anther culture and isolated microspore culture – which can reduce the length of a breeding process. We focus on the improvement of these methods to support cereal breeding programmes like wheat, spelt wheat and rice.

Wheat anther culture is a well-established method in our laboratory which are applied for the production of DH lines in plant breeding programmes. Phenomenon of albinism was mitigated in anther culture, dominantly green plantlets were produced using our protocol. Thousands of DH lines was produced for breeding purposes. The most promising DH lines are under national test.

Our protocol was tested using four spelt wheat genotypes ('Franckenkorn', 'GK Fehér', 'Mv Martongold', 'Oberkulmer Rotkorn'). The seeds of 'Franckenkorn', 'Mv. Martongold' and 'Oberkulmer Rotkorn' varieties (cultivated genotypes in Hungary) were supplied by Dr. László Láng (Agricultural Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Martonvásár, Hungary). In anther culture, genotype dependency was observed, and cold pre-treatment enhanced the efficiency of the method. In isolated microspore culture of spelt wheat, the ovary co-culture supported the development of embryo-like structures. The low plant regeneration rate and high number of albinos hinder the practical application of isolated microspore culture while anther culture was efficient for *in vitro* green plantlets production in spelt wheat. The mean of green plantlets production was 41.45/100. The phenomenon of albinism was mitigated in anther culture (3.48 albinos/100 anthers).

In the last four years, we tested the efficiency of anther culture in Hungarian rice breeding programme. The effect of different media (N6, MS) and growth regulators and their com-

binations (2,4-D, kinetin, BAP, NAA) were compared for the induction of androgenesis and plant regeneration.

Hundreds of green plantlets were produced via anther culture which efficiency are close to the practical application of Hungarian rice breeding programme.

Acknowledgments

The authors thank the support of scientific projects (project code: OTKA-K_16-K119835; name of project: Improvement of spelt wheat lines with low fermentable carbohydrate content (FODMAP) using modern and classic research methods). The experiments were supported by the Hungarian Ministry of Agriculture (Project no. OD002) and the NARIC, Gödöllő, Hungary (code number: 0170). The experiments were interlocked with GINOP project (project number: GINOP-2.2.1-15-2016-00026). This project was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. The authors also thank the conscientious work of Csaba Ponta, Ferenc Markó and Sándor Vajasdi-Nagy.

“Improving Chilean durum wheat (*Triticum turgidum* L.var. durum) grain quality through breeding”

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Studying the carotenoid biosynthetic pathway to increase the grain yellow pigment content (GYPC) and the nutritional value of grains of durum wheat (DW), a tetraploid species (composed by genomes A and B) that is mainly used in the production of pasta, will considerably benefit the consumers and the industry. However, the key genetic constituents that regulate GYPC have been neither studied nor characterized in cultivars employed in Chile.

In this project, the genetic diversity was mainly obtained from the International Maize and Wheat Improvement Center (CIMMYT). In this context, the effects of phytoene synthase (PSY), the main candidate gene influencing GYPC of nine cultivars utilized in Chile, and of lycopene epsilon cyclase (LCYE) on the nutritional value of grains of DW were investigated.

To test the hypothesis of the association between the already identified PSY alleles related to high GYPC, PSY was studied using PCR for gene amplification followed by sequencing based upon the existing allelic variants of PSY-A1 and PSY-B1 obtained from the literature. In this way, the superior PSY allelic variants associated with high GYPC were identified and characterized. Regarding LCYE, Targeting-Induced Local Lesions IN Genomes (TILLING), a reverse-genetic strategy for the discovery and mapping of induced mutations was used. This technique combines traditional mutagenesis with high-throughput discovery of single-nucleotide changes, which will likely allow the identification of nutritionally-enhanced seeds with higher content of provitamin A through high-performance liquid chromatography (HPLC).

Overall, the main results of our study show medium-low GYPC levels of the current Chilean commercial varieties of DW, reflecting the need of improving this trait through breeding.

The allelic variant 1 (PSY1A-1) associated to higher GYPC. Using molecular markers associated to PSY1A (i.e., Psy1-A1_STS and YP7A-2 jointly) for marker-assisted selection can be sufficient and valuable to improve grain yellowness. In addition, PSY1A was 21-fold higher expressed in the high-yellowness relative to the low-yellowness genotypes at day 35 days after anthesis through q-PCR during grain filling. A major role for PSY1A in the genotypes associated to high GYPC was concluded. We are currently improving other grain quality traits such as gluten quality.

Acknowledgements: this work was supported by Conicyt [Chile's National Commission for Scientific and Technological Research], grant Fondecyt de Iniciación n°11110066 (2011-2014), and Fondecyt Regular n°11611298 (2016-2020).

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Towards breeding for higher photosynthetic efficiency under heat stress in bread wheat

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Keywords: chlorophyll fluorescence, candidate genes, heat stress, phenotyping, photosynthesis, QTL, wheat

The future climate is predicted to be more variable with higher frequency of heat waves and dry spells. Wheat is a heat sensitive crop but naturally existing genetic variation could be utilized for improving stress tolerance in wheat. In the present study, a *top-to-bottom* approach with physiological phenotyping by chlorophyll fluorescence combined with QTL analysis was carried out to identify potential quantitative trait locus (QTL) and underlying candidate

genes for improving photosynthetic efficiency under heat stress. The results showed that it was possible to use F_v/F_m (a chlorophyll fluorescence parameter that reflects maximum quantum efficiency of photosystem II) as a rapid and non-destructive phenotyping tool to identify wheat cultivars differing in overall photosynthesis during severe heat stress (40°C for 3 days, during flowering). Subsequently, using three bi-parental mapping populations generated with the three heat tolerant male (*originated in Afghanistan and Pakistan*) and the heat sensitive female parents (*originated in Germany*) selected from a pool of 1274 wheat cultivars based on their ability to maintain F_v/F_m as well as net photosynthesis, three significant QTLs and underlying candidate genes have been identified. Further, a set of flanking markers (DARtseq and SSRs) for each QTL have been identified that can be used for introgression to breed for heat tolerant wheat cultivars with improved photosynthetic efficiency.

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Integration of molecular markers and doubled haploids in wheat and barley breeding: A case study from Morocco

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Keywords: Marker-assisted breeding, doubled haploids, wheat, barley

Wheat and barley are the most important cereal crops in Morocco and in North Africa. Their production is constrained by several biotic and abiotic stresses. Climate change has further aggravated these problems. Since the landraces, old and newer varieties are reservoirs of variability and adaptive traits required for breeding for resistances to stresses and adaptation of climate change, we screened 400 bread wheat and 295 durum wheat accessions at Sidi El Aidi (Morocco) during 2015–16 and identified 4 lines (Kharouba, 2837, CWI77963 and CWI9941) highly resistant and 290 resistant/moderately resistant to yellow rust respectively. Similarly, Screening of 452 accessions of barley in the same location resulted in the identification of 116 moderately resistant accessions to net blotch disease. Some of the identified resistance sources are being used to make genetic crosses for studying genetics of resistance,

developing improved cultivars and to increase cultivar diversity in the country which can enhance 'life of the resistant cultivars' and adaptation to climate change.

As a first step and baseline information, we evaluated phenotypic and genotypic diversity of a set of 20 improved bread wheat cultivars released in Morocco since 1980s. The results revealed that many of the wheat cultivars were susceptible to prevailing biotypes of the Hessian fly and yellow rust. The genotyping of the 20 Moroccan wheat cultivars using 14 microsatellite markers revealed 59 alleles (Mean = 4.21), substantial genetic diversity (Range = 0 to 0.895, Mean = 0.576) and genetic distance (0.143 to 1.00). However, total number of alleles decreased (8.3%) in the cultivars developed during recent period (1990s) compared to that of 1980s. In order to enhance biotic and abiotic stress tolerance, end-use quality and allelic diversity of wheat cultivars in Morocco, 8 exotic wheat cultivars having various useful known genes were deployed and being used to make crosses with Moroccan cultivars. Further, these Moroccan cultivars and the exotic cultivars were characterized using 47 gene specific or functional markers and 7 linked random DNA markers associated with 21 loci of the most important traits for wheat breeding. The functional marker analysis revealed that 35 and 45% of the Moroccan cultivars, respectively have the rust resistance genes (*Lr34/Yr18/Pm38*) and dwarfing genes (*Rht1b* or *Rht2b* allele). The desired marker alleles for genes *Lr37/Yr17/Sr38*, *Sr24* and *Yr36* were present only in the exotic cultivars and absent in Moroccan cultivars. 25% of cultivars had *IBL. IRS* translocation. 70% of the wheat cultivars had *Ppo-D1a* and *Ppo-A1b* associated with low polyphenol oxidase activity. 10% of cultivars showed presence of a random DNA marker allele (175 bp) linked to Hessian fly resistance gene *H22*.

Integration of molecular markers within traditional breeding systems had enabled to select superior genotypes for traits that are difficult to select based solely on phenotype or to pyramid desirable combinations of genes into a single genetic background. The targeted crosses were made and subsequent generations were carried forward through traditional breeding systems and also in some cases through doubled haploids (DH) to speed up development of homozygous plants. Though marker-assisted breeding (MAB) can be applied to all segregating generations, we most commonly applied to early generations, including haploids, F_2 , BC_1F_1 , BC_1F_2 and the F_1 of complex crosses to enrich populations with favourable genes and their combinations. MAB also offered the opportunity to hasten transfer of desirable alleles from un-adapted exotic genetic backgrounds into a desirable germplasm through cross-breeding. Marker-linked loci responsible for traits such as resistance to diseases, quality and phenology were selected in the segregating populations and the desired genotypes were carried forward until they reach nearly homozygous. Once, the selected genotypes reach F_6 or later generations, they were validated for the selected traits under field conditions. Only lines expressing desired phenotypes were selected for seed increase and subsequent preliminary yield trials and multilocation trials. In conclusion, application of MAB and DH technologies greatly enhanced efficiency and effectiveness of utilization of the germplasm and enhanced genetic gains in the breeding programs.

Acknowledgments

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T6: BREEDING FOR ORGANIC AND LOW INPUT FOOD SYSTEMS

Oral presentations

Breeding of spelt wheat for organic and low-input agriculture

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Keywords: ancient cereal, genetic resources, hulled wheat, organic farming, *Triticum spelta*

Spelt wheat (*Triticum spelta* L.) was the predominant cereal for centuries in the Alps and Prealps of South-Western Germany, Northern Switzerland and Western Austria. In the 20th century, spelt was nearly totally replaced by common wheat because of higher yields and lower processing costs. Spelt became a niche crop, cultivated only in marginal areas due to its adaptability to low input and unfavorable growing conditions, and popular for its taste, aroma and digestibility. Recently the interest in ancient cereals such as spelt increased significantly, also in areas without traditional cultivation, due to the raise of organic farming and the awareness of wheat sensitivities. To compensate for low performances with respect to grain yield and lodging tolerance, spelt was partly crossed with common wheat resulting in heavy discussions about spelt’s specific health beneficial quality characteristics.

In the present project, a spelt wheat diversity panel including old and modern spelt varieties, landraces and genetic resources was evaluated since 2014 at four different European sites (i.e. Austria, Estonia, Germany, Switzerland) for morphological traits, growth and phenological traits, disease resistance, agronomic and grain quality traits. The trials were carried out under organic and/or low input conditions. A high variability in the investigated germplasm was observed especially for plant height and lodging tolerance, grain size and weight, resistance to yellow rust, and hulled grain yield. Multivariate analyses revealed three groups of spelt wheat: (i) old landraces, traditional varieties and genetic resources with medium to low yield, tall plant height and susceptibility to lodging, but individually high resistance to diseases; (ii) modern varieties with reduced plant height, improved lodging tolerance, medium to very high yield, but partly spelt atypical characteristics, e.g. higher percentage of free threshing grains, shorter and more plumb seeds; (iii) modern varieties and breeding lines from organic breeding programs with improved lodging tolerance despite tall plant height,

medium to high yield, and spelt typical characteristics, e.g. long, narrow and sharp-edged grains, culm coloring during ripening.

Without doubt spelt wheat needs genetic improvement to cope with changes in growing conditions due to global warming. However, it must be considered that despite the increased interest in ancient wheats, the market is highly volatile and sensible, and new varieties will be accepted by processors and consumers only if breeding will not change the typical morphology, taste, and product quality. Therefore, crosses with bread wheat should be minimized or avoided. Within the project new crosses were carried out especially with donors for yellow rust and common bunt resistance, lodging tolerance and winter hardness.

Acknowledgments

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Performance and selection of winter durum wheat genotypes in different European conventional and organic fields

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Keywords: durum wheat, GGE biplot, grain quality, low input agriculture, organic breeding, variety performance

Sustainability is a key factor for the future of agriculture. Productivity in agriculture has more than tripled in developed countries since the 1950s. Beyond the success of plant breeding, the increased use of inorganic fertilizers, application of pesticides, and spread of irrigation also contributed to this success. However, impressive yield increases started to decline in the 1980s because of the lack of sustainability. One of the most beneficial ways to increase sustainability is organic agriculture. In such agro-ecosystem-based holistic production systems the prerequisite of successful farming is the availability of crop genotypes that perform well. However, selection of winter durum wheat for sub-optimal growing conditions is still mainly neglected, and the organic seed market also lacks of information on credibly tested winter durum varieties suitable for organic agriculture.

Quality and agronomic performance of 14 diverse winter durum wheat genotypes were examined in Austria, France and Hungary for three years. Heading time, wet gluten content, semolina yield and grain protein content are traits that showed genotype-dependent significant differences between the two management systems examined (conventional and organic). Therefore, breeding for these traits could result in specifically adapted genotypes

for organic agriculture in different countries. Based on strong or moderately significant correlations between traits, gluten index and plant height could also be specifically selected in an indirect way. The need for environmentally specific selection for grain yield in later generations was also demonstrated. In general, varieties that had the highest performance in a given mega-environment originated from that mega-environment (except for yellow index). This finding provides evidence for the influence of the selection environment, whether it is the management system or the growing region. As the French site fell into a distinct mega-environment, it should be handled separately. The Hungarian site was found to be an ideal test environment for selecting genotypes with high adaptability for most of the quality traits, while the Austrian site could be used in selecting agronomic traits. This was also reflected in the breeding origin of the best winter durum genotypes for each trait. Based on these findings, a partly separate winter durum selection program is recommended for organic and low input agriculture in each country. Knowledge gained from durum wheat will be used to design efficient cultivar testing strategies for organic farming of other cereals in a European network. This will support farmers in their choice of cultivar with stable yield and high quality and thus support the emerging movement towards sustainable farming systems.

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Poster presentations

Ecologically clean production in the system of organic agriculture in Northern Kazakhstan

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Keywords: soil, organic farming, break crops, soil fertility

In recent years, biological farming, which excludes an application of mineral fertilizers and other chemicals, is becoming more important. Under such a system, soil fertility is maintained due to organic fertilizers, break crops, plant bypass, which are the resources of the farm itself. It determines the ecological and economic efficiency of the land use, since on the one hand it contributes to improving the quality of the soil, and on the other hand it ensures the production of environmentally friendly products [Lukin, 2007; Moiseev, 2006].

In Kazakhstan, the development and the use of organic farming are only gaining traction. At the moment, there is no data on the effect of organic farming on the quality of the soil organic matter and the carbon sequestration. For implementation of the above tasks, it is necessary to assess the present humus state and the degree of biological activity of soils with the compilation of an appropriate information system, which will also serve as a basis for monitoring the investigated territory. These factors play a major role in the transition to the system of “organic farming”, which is the core of the “green economy” noted in the President’s message to the people of Kazakhstan [The message of the President of the Republic of Kazakhstan, 2017].

The research has been started in 2015 at the field fixed-site laboratory of the Scientific and Production Center of the Grain Farming named after A.I. Baraev. The fixed-site laboratory is located on the territory of Damsa settlement, Shortandy district, Akmola region. When studying the content of organic matter in the soil with the use of annual crops as a green fertilizer, crops such as canola, mustard, and pea-oat mixture were plowed into the green-manured crop rotations. For the preservation of soil fertility, increase in productivity and quality of agricultural crops and products of their processing, the foundations of organic agriculture will be created for the production of ecologically clean food products. In the system of organic farming, with the green dressing of the pea-oat mixture, spring canola and mustard, the content of common humus on average for 2015–2016 changed from 2.55% on a crop rotation with a mustard plow up to 2.88% on a crop rotation with canola. Mineral fertilizers and herbicides were not used under organic farming system, but active mechanical tillage was carried out. Therefore, low content of humus can be explained by a rather greater mineralization of plant residues. The average yield for 2015 and 2016 was 24.6 dt/ha on the rotation with a canola plowing, 20.5 dt/ha with the mustard plowing, and 21.8 dt/ha with the pea-oat mixture plowing. In the crop rotation with canola, the humus content was higher comparing to other crop rotations, which also contributed to higher yields.

Acknowledgments

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Soil condition as a basis for the production of ecologically clean products in Northern Kazakhstan

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Keywords: soil, microelements, heavy metals, fertilizers

Nowadays, worldwide the production of ecologically clean products is increasing. Annual growth rates on average are 15–20%. The high demand for organic food allows the farmers of Kazakhstan to switch production to environmentally friendly products. To date, the total area of land certified for cultivation of organic products in Kazakhstan is 300 thousand hectares, or 1.2% of the arable land (Grigoruk, et al.). In order to perform this transition to organic food products, agricultural producers need to switch to organic farming, which requires a reduction in the content of pesticides and heavy metals in the soil and grain of plants. Studies show a high level of heavy metals (As, Cd, Co, Cr, Mn, Se, Pb and U) in wheat grain in the south of Kazakhstan. An increased content (As, Cd, Hg and Pb) was found in all regions of the country (Tattibayeva et al., 2016). In our studies, observations were made on the accumulation of trace elements and heavy metals when using mineral fertilizers in the north of Kazakhstan.

The long-term data were obtained on the dynamics of accumulation of heavy metals and microelements in soil and grain on sowings of flax and wheat crops in the fixed-site laboratory of agrochemistry on the territory of the Akmola region. The determination of microelements was carried out by atomic absorption spectrometry (AA – 140, Varian) in a 1M solution of nitric acid. Mineral fertilizers (superphosphate, nitroammophos and ammonium nitrate) were annually applied at a dose of $P_{20}N_{20}$.

The results of three-year studies show that the introduction of nitroammophos leads to a gradual accumulation of cadmium in the soil from 0.23 to 0.54 mg/kg and copper from 4.88 to 5.90 mg/kg. The cobalt content for this period increased from 6.61 to 9.01 mg/kg. The content of zinc in the soil increased from 3.61 to 6.26 mg/kg. The accumulation of these ele-

ments is due to the annual flow of mineral fertilizers into the soil, which, in addition to the macroelements, contain impurities of cadmium, copper and cobalt. It should be noted that in this region, there is a lack of zinc content. The content of cadmium in wheat grains varies from 0.06 to 0.19 mg/kg, in flax from 0.16 to 0.28 mg/kg in various embodiments.

Studies have shown that the introduction of nitroammophos leads to the accumulation of cobalt and cadmium in the soil. The application of superphosphate and ammonium nitrate increases the accumulation of zinc in the soil. The level of cadmium in the grain of flax is higher than in wheat at the same rate of fertilizer application, but there are no exceedances by permissible standards. Nevertheless, obtaining environmentally friendly products requires constant monitoring of the content of heavy metals in soil and grain.

Acknowledgments

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T7: PLANT ADAPTATION AND RESILIENCE

Invited

Resequencing the barley exome to identify genes controlling plant adaptation to environment

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Keywords: barley, exome-sequencing, CBF, Vrn, frost tolerance

Barley development and actual yield is strongly affected by a number of environmental stress factors that can prevent the plant from expressing its maximum genetic potential. Indeed abiotic stresses (temperatures, drought, anaerobiosis and soil salinity) are often responsible for severe grain losses although they have driven the evolution and the wide distribution of the genus *Hordeum* in temperate, subtropical and arctic areas.

We present a new resource to dissect natural allelic variation at candidate genes controlling key adaptive traits in barley, based on a collection of 500 accessions assembled in the context of the EU-FP7-WHEALBI (Wheat and Barley Legacy for Breeding Improvement) project. The collection comprises 227 old and elite cultivars, 238 landraces and 35 wild barleys (*H. spontaneum*) from a wide geographical range. Winter and spring nurseries (common garden trials) have been carried out in 4 contrasting environments (Scotland, North Italy, Hungary and Central Anatolia in Turkey, in this last site under both rainfed and irrigated conditions) and several phenological and morphological traits have been evaluated. Moreover, a comprehensive molecular variant analysis by exome sequencing (exome capture arrays) has been carried out, resulting in more than 2 million SNPs and InDels.

Plant growth habit and heading-date are the basic traits involved in barley adaptation to the environment since they allow synchronize life cycle with seasonal changes. Several

genes involved in these key adaptive traits have been characterized and they can serve as a proof of concept to validate the effectiveness of WHEALBI resource in mining new functional alleles. To this purpose allelic diversity of candidate genes has been evaluated in wild, landraces and cultivated barley to determine the extent of haplotype diversity and build haplotype networks.

The genetic diversity of barley *CBF* and *Vrn-H1*, two key loci responsible for frost tolerance, have been explored in 403 barley accessions using the exome sequencing information. This dataset along with curated annotation of the *CBF* genes provided the means to identify unique SNP variants in *HvCBF* genes and predict functional effects at the protein level. A large proportion of these SNPs were observed in the gene pool of barley landraces and wild relatives, only being fixed in the cultivated germplasm. Similarly, examining diversity at the winter allele of *Vrn-H1*, from *Hordeum vulgare* cv. Strider, as reference, several unknown deletions of this gene were identified as well as one accession with two copies of *Vrn-H1* gene.

To date, the genetic variants (SNPs and CNVs) of coding sequences developed and validated in this project are a snapshot of the existing genetic variability of barley, which will enable the research community to mine interesting alleles for other traits and exploit the untapped genetic diversity of barley.

Oral presentations

Identification and release of a salt tolerant wheat mutant variety for the saline areas of Bangladesh

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Salinity is a major abiotic stress limiting crop production in 20% of global cultivable land which also include 1.05 million ha (12.35%) in the coastal areas of Bangladesh (FAO, 2017; SRDI, 2016). The saline areas in Bangladesh remain mostly fallow during November to June due to soil salinity (2.0 to >16.0 dS/m) and scarcity of suitable irrigation water. Management of the saline soil for economic crop production is costly and sometimes impossible. Therefore, development of low water demanding crop variety with tolerance to salinity might be the best alternative to ensure food security of 160 million people of Bangladesh. As wheat needs only 2–3 irrigations to complete its life cycle, seeds of a salt tolerant segregating mutant population, L-880 of wheat were introduced from NIAB, Pakistan, screened and evaluated under saline field conditions during 2011–12 to 2014–15 growing seasons along with BARI Gom-25 (which can tolerate 8 dS/m salinity). Final yield trials were conducted at four locations each in saline and non-saline areas. The soil salinity of saline affected locations was recorded from 15 November 2015 to 20 February 2016 which showed gradual increase of salinity from 15 November and reached the highest (12.52 dS/m) at Khulna on 20 February (Fig.1). The mutant L-880-43 produced significantly higher grain yield than the check variety even under the highest salinity and in non saline soils as well (Fig.2). Considering all, National Seed Board of Bangladesh released it as Binagom-1 in 2016 for commercial cultivation in the saline and non-saline areas of Bangladesh.

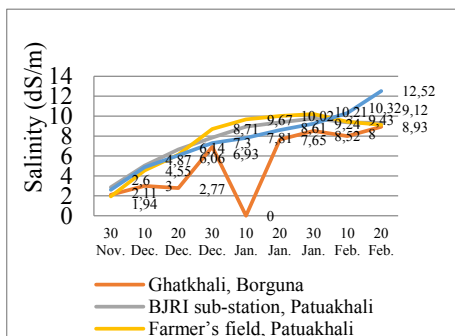


Fig. 1. Salinity records of the experimental plots during the growing season

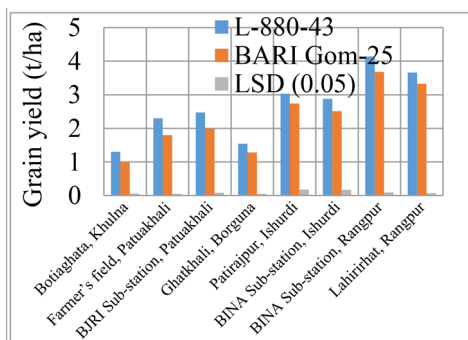


Fig. 2. Grain yield of the salt tolerant mutant and the check variety, BARI Gom-25 in the saline and non-saline areas (1st 4 locations in the saline areas)

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Is it possible to select wheat genotypes with improved tolerance to soil constraints?

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Keywords: plant available water capacity, sodium, potassium, chloride, north eastern Australia

The sub-tropical cropping region of north eastern Australia is characterised by summer dominant rainfall. Thus, wheat sown in autumn to avoid the hot wet summers must grow through the dry winter relying heavily on stored moisture in the deep clay soils that predominate [1]. Unfortunately over large areas, surface soil sodicity as well as subsoil salinity and phytotoxic concentrations of chloride (Cl) impose major soil constraints on wheat production [2–5]. These constraints inhibit soil moisture extraction, particularly from deep in the soil late in the season when marginal water use efficiency is high [6–8]. We aimed to determine whether genotypes tolerant to these constraints can be identified.

In 2007, 2008, 2009, 2015 and 2016, we evaluated the performance of 11, 17, 17, 36 and 38 wheat genotypes, respectively. From 2007 to 2015, lines were grown on two sites <0.5 km apart one with sodic soil predicted to reduce wheat production. Compared to the non-sodic site, the sodic site had significantly higher Cl concentration (>800 mg·Cl·kg⁻¹) in the subsoil (0.9–1.3 m soil depth) and higher exchangeable sodium percentage (ESP) (>6%) in

the surface and subsoil. In 2016, a similar non-sodic site was used but a new sodic site was chosen having greater surface soil ESP (12.5%) and greater Cl (1500–2000 mg/kg) concentration at depth.

Wheat yields were significantly lower at the sodic site than the non-sodic site in 2007 and 2008. They also varied significantly between genotypes. For example, in 2008 grain yield was reduced by between 8% and 33%. Soil moisture extraction, as measured by plant available water capacity (PAWC), was also between 3% and 37% lower [9]. The yield ranking for genotypes at the non-sodic site was not well correlated with that at the sodic site. Thus, selection for yield potential at non-sodic sites is not a good predictor of performance at sodic sites.

The difference in site mean yields between sodic and non-sodic sites varied greatly between seasons. The contrast between sites was reduced in 2015 and 2016 due to higher than normal in-crop rainfall which reduced crop reliance on stored soil moisture. In contrast in 2009, very low availability of rainfall and stored moisture severely reduced yields of all lines at both of the sites. This demonstrates the significant influence of seasonal water availability on the impact caused by soil constraints. However, yield rankings of genotypes continued to differ between sodic and non-sodic sites. Overall, certain genotypes were relatively susceptible to sodicity while others were relatively tolerant.

Most wheat genotypes grown at the sodic site in 2008 showed Ca deficiency symptoms on younger leaves having Ca concentration <0.2% in the youngest mature leaf (YML). Grain yields of wheat genotypes in 2008 increased significantly with increasing Ca and K in YML and decreased significantly with increasing Na and Cl. In step-wise regression, the PAWC of wheat genotypes was the principal determinant of variation in grain yield in 2008. Including the Ca concentration in the YML of wheat genotypes in the regression significantly improved the prediction of grain yield. This suggests that Ca concentration in YML could be a useful indicator of tolerance to soil constraints.

Thus, we successfully identified and quantified useful genetic variation in tolerance to soil sodicity between wheat genotypes suggesting that selection of more tolerant cultivars could improve productivity on sodic sites. As current cultivars have largely been selected for non-constrained soil conditions, these findings also suggest potential to breed new cultivars with superior tolerance to soil constraints.

Acknowledgments

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Circadian and light quality regulated expression of *CBF* genes influences the cold acclimation process in cereals

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Keywords: circadian, light quality, CBF, gene regulation, cereal

The successful overwintering is a major feature that affects crop quality and productivity in winter cereals. CBF transcription factors appear to act as master regulators of cold stress response. More and more information show that, apart from cold temperature, light intensity, day length and circadian clock also have influence on cold acclimation process, both in dicot and monocot plant species. It was also revealed that, among the several *CBFs*, *CBF14* seems to be one of the most potent one. In the case of barley, *HvCBF14* gene, member of the *HvCBF4* phylogenetic subgroup (which contains the *HvCBF2A*, *HvCBF4B* and *HvCBF9* genes as well) contributes to enhanced frost tolerance more efficiently than the others. We found that the *HvCBF4* subgroup genes were expressing in the late afternoon or early in the night. Moreover, we also showed that unlike the other subgroup genes, only these *CBF* genes showed circadian rhythms at relatively high temperature (22°C) in barley. Light-quality regulation of freezing tolerance was first described in *Arabidopsis*. By lowering the red/far-red ratio in the illuminating spectra at 15°C, we were able to induce *CBF14* expression and to

increase freezing tolerance in winter wheat and winter barley genotypes, but not in einkorn wheat, which has relatively low freezing tolerance (Novák *et al.*, 2016). As a part of the photo-transduction pathway, the expression profiles of *PHYA*, *PHYB* and *PHYC* were also compared. Based on these expression data, a model was proposed illustrating that, in response to the increased ratio of farred in the incident light, phyA induces, while phyB prevents the enhancement of freezing tolerance in cereals.

Acknowledgments

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Mass spectrometry imaging a new tool in plant pathology research: Visualisation of molecular wheat-*F. graminearum* interactions

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Keywords: MS imaging, wheat, *F. graminearum*, Fusarium root rot

Visualisation of molecular cereal-pathogen interactions is a new field in phytopathology. As a molecular camera, mass spectrometry (MS) imaging combines label-free and multiplex metabolite profiling with histopathology. This tool has been utilized to tissue sections of animals, insects, mammals [1]. We established MS imaging for all major plant organs based on the economically important crop plants, including wheat and oilseed rape [2]. Plant-pathogen association implicates a major shift in metabolic activity, comprising pathways and compounds that typically have a high functional diversity. This represents a challenge to the interpretation of global metabolic profiles. Here, the mapping of metabolites in disease-relevant tissues could be a tool to assess their relative contribution to resistance.

In the present study, atmospheric pressure high-resolution scanning microprobe matrix-assisted laser desorption/ionization (AP-SMALDI) MS imaging was used for the first time to describe the chemical distribution of wheat responses to *Fusarium graminearum*. Recently it was discovered that the fungus, typically a paradigm for Fusarium head blight (FHB), has a high ability to systemically colonize wheat via root infection, with severe consequences for plant fitness, soil health and food safety [3]. However, the molecular response to Fusarium root rot (FRR) infection and systemic spread is still unknown. The potentials of MS imaging for metabolomics in plant diseases and molecular histopathology are demonstrated by imaging the dynamic spatial-temporal response of stem tissues to FRR spread in the resistant cultivar Florence-Aurore; by uncovering conserved wheat responses to *F. graminearum* diseases that affect different plant organs; and by finding patterns of pathogenesis that coincide with microscopic observations.

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Effect of simultaneous heat-and-drought stress in wheat

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Keywords: heat-and-drought stress, pollen, reactive oxygen species, stigma, wheat, yield

Despite unfavourable climatic changes in many wheat growing areas, where unpredictable hot and dry weather episodes occur more and more frequently during wheat flowering, the interaction between these two significant stressors have received far too little attention. The main objective of our study is a complex characterization of the effect of simultaneous heat-and-drought (HeD) stress prior to and during fertilisation in wheat, with emphasis on changes in the morphology, physiology and molecular events.

Plants of winter wheat cultivars Plainsman V and Cappelle Desprez were grown in growth chambers and exposed to simultaneous heat stress (32°C/22°C max/min) and complete water withholding when the male reproductive cells were in the middle uninucleate stage of microspogenesis. The HeD treatment was continued for five days, until the volumetric water content of the soil dropped below 7%. Non-invasive physiological measurements were made

on the plants, and samples were taken from the flag-leaves, anthers and stigmas for invasive anatomical, physiological, biochemical, proteomic and gene expression analyses.

Statistical analysis of yield data revealed, that Plainsman V and Cappelle Desprez genotypes are HeD stress tolerant and HeD stress sensitive, respectively. The growth vigour, the number of productive tillers, fertility, grain weight per ear and harvest index of both genotypes declined in response to HeD stress, however, while the reduction of all parameters in Plainsman V were moderate, Cappelle Desprez suffered severe yield loss. In response to HeD stress significant reduction in the relative moisture content was observed in both cultivars. Both, osmotic adjustment and cell membrane stability of the tolerant genotype was significantly higher. The latter can be partially explained by the fact that the malondialdehyde (MDA) content of the sensitive genotype was higher than that of the tolerant variety. In response to stress there was a significant increase in the total reactive oxygen species (ROS) content of the stigmas in the upper half of the spikes in both genotypes. When individual ROS were analysed we was found that the quantity of mitochondrial hydrogen peroxide dropped significantly in the sensitive genotype as a consequence of HeD treatment. The amount of cytoplasmic superoxide was elevated in both cultivars in response to stress. In control Cappelle Desprez the mitochondrial superoxide was four times higher than in Plainsman V, but the quantity declined significantly in response to HeD treatment. The ROS detoxifying enzyme activities per dry weight decreased to a significantly greater extent under stress conditions in the sensitive cultivar. Significantly smaller amount of viable pollen was observed in the anthers of treated plants. The majority of the pollen cells developing in the upper half of treated Cappelle Desprez spikes proved to be unviable after treatment. As a consequence of combined stress, the number of up-regulated genes with more than two-fold increase in expression was ten times higher in the stress-sensitive genotype compared to the stress tolerant one.

Acknowledgments

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How to reduce DON contamination by 90% in wheat by breeding and fungicide use in wheat?

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The Fusarium head blight (FHB) is one of the most dangerous diseases of wheat, in some years the yield losses can be considerable, but the most damages is caused by toxin, mostly deoxynivalenol (DON). The breeding efforts are going back to the 1910s when the devastating epidemics in the US started the research (Atanasoff, 1920). Resistance, more exactly disease severity differences were detected very early, but a systematic breeding work started in Europe in the 70s (Szeged, Hungary) and in the early 90s in US in many different locations following devastating epidemics.

Methodically it was important that Fusarium damaged kernels (FDK) have a much higher significance for breeding than visual symptoms. However, we detected DON overproduction in several lines, the toxin measurements are very important to exclude these genotypes from breeding. The best differentiation of the genotypes was made by the spraying inoculation combined with polyethylene bags.

The long year's screening works proved that most of the commercial cultivars are susceptible or very susceptible, but in every breeding material from different breeder more resistant genotypes were identified. For this reason the first task is to make this work and according to the results the breeding work, so that the crosses can be planned better and step by step the resistance level of the new lines will be higher. This is utilization of the variability existing in the given wheat population. This is very important as all Szeged moderate resistant wheat cultivars were identified by this method. The resistance level can be very high, even if it occurs not very often. Adapted resistance sources can also be applied, we used a number of American, French, German lines in the crossing and breeding program and also highly resistant genotypes were bred. We used also exotic resistance source like Sumai-3 and Nobeoka Bozu, and we transformed this resistance to winter and adapted type, so a crossing partner series was bred that is suitable also for variety development.

In the later phase of the breeding only moderate or higher resistant partners were used, and the locally adapted, adapted and exotic blood containing lines were crossed together to secure a wide base of resistance and bring together QTLs as much as possible. As most of the moderately and higher resistant donors are not often known, QTL screening is not used, phenotypic selection is done, because this way we can utilize unknown QTLs and their unknown interactions.

However, the high FHB resistance is not enough to breed compatible cultivars. In the last ten years, keeping the high level of FHB resistance, yellow rust, leaf rust, Septoria leaf spot and powdery mildew resistance was added and now we have line with multiple resistance to these diseases. We have to consider also quality, e. g. excellent baking quality the Hungarian wheat was known. For this reason quality sources were intensively used, possibly having resistance to one or more diseases and many of the multiresistant materials have 16–18% protein and some of the high baking quality. We have to add yields also, this program is going on, the bet lines achieve the control level. From the moderate resistance, a fungicide use helps to secure food safety and the combination of the resistance and fungicide reduced DON higher than 90%.

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Durum wheat evaluation using Carbon Isotope Discrimination and numerical analysis of image for leaf senescence

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Keywords: durum wheat, Carbon Isotope Discrimination, leaf senescence, Algeria

Drought is a wide-spread problem seriously influencing durum wheat (*Triticum durum* Desf.) production and quality, but development of resistant cultivars is hampered by the lack of effective selection criteria. The overall objective of this study was to investigate if leaf senescence can assist in breeding wheat for drought tolerance under such conditions. Specific objectives included (i) to investigate the relationship leaf senescence and grain yield under contrasting climatic conditions with special reference to drought (ii) to identify lines varying in Δ (Carbon Isotope Discrimination) in wheat breeding programs in Algeria. Senescence was also estimated by following the decrease in chlorophyll concentration, using a SPAD-502 portable chlorophyll meter which measures leaf transmittance at red (650 nm) and near infrared (940 nm) wavelengths. Measurements were done on the same leaf used for Numerical Image Analysis measurements, at five dates (148.4, 188.2, 271.0, 352.8 and 443.8°C from anthesis stage). Some durum wheat genotypes from ICARDA and CIMMYT are studied. Lower Grain Yield and higher senescence rates in season 1 compared to season 2 may be attributed to climatic conditions (rainfall and temperature). Sharp increase in temperature during grain filling stage in season 1 is likely to have accelerated senescence. Lower grain yield is probably the consequence of a lower grain setting caused by freezing at heading and drought and high temperatures around anthesis. Highly significant effects of genotype, season and genotype x season were found on leaf and grain carbon isotope discrimination.

Role of protective compounds in cereals

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Keywords: cereal, polyamine, salicylic acid, stress

Due to the extreme continental climate, there are few years when Hungary does not face severe economic losses related to environmental factors. The predicted climate change may further increase the possibility of unexpected unfavourable environmental conditions. These include long periods of drought, excessive quantities of rainfall in the wrong place at the wrong time, extremely cold winters or hot summers, or the sudden appearance of pests or pathogens in epidemic proportions, all of which are capable of destroying the crop yield of a whole year.

Both plant breeders and crop producers have an interest in finding crops capable of tolerating environmental changes with as little damage as possible. In order to develop such crops,

knowledge of plant defence mechanisms and regulatory processes is essential. Investigations carried out mainly on model plants, such as *Arabidopsis* have revealed the presence of tens of thousands of genes whose expression is influenced environmental stresses. However, it is important to emphasize, that in many cases the results achieved using *Arabidopsis* as model plant can only be generalised to a limited extent, if at all, making them unsuitable for the interpretation of stress adaptation processes in genetically distant plant species such as monocotyledonous cereals.

Investigations on naturally occurring protective compounds capable of reducing the stress sensitivity of plants are of special and great importance. The main aim of our research work is to obtain a better understanding of the responses given to various types of stress by cereal species of outstanding economic importance. It is expected that the results will enable to pinpoint processes that could be manipulated either by conventional plant breeding or using biotechnology to achieve more efficient improvements in plant stress tolerance. The main tasks are elucidation of the mechanisms which enable cereals to adapt to a changing environment, and understanding of principles which make some genotypes tolerant and others sensitive to stress. Changes in salicylic acid – the plant hormone – and the uptake and metabolism of polyamines – another group of protective and signalling compounds – after treatment with exogenous polyamines will be presented in maize, wheat or rice plants grown under various stress conditions.

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Addition of *Aegilops* U and M chromosomes affects protein and dietary fiber content of wholemeal wheat flour

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Keywords: wheat, *Aegilops*, dietary fiber, β -glucan, arabinoxylan, U and M genomes

Aegilops geniculata and *Aegilops biuncialis* are tetraploid species with U and M genomes. They are known to have resistance to biotic and abiotic stresses, but also have unusually high nutritional value (high dietary fiber, Fe and Zn content). The aim of our work was therefore to determine the effects of the addition of *Ae. geniculata* chromosome on the dietary fiber content and composition of Chinese Spring wheat. Prebreeding material with increased level of dietary fiber was therefore also developed by adding *Ae. biuncialis* chromosomes to the Mv9kr1 wheat line. In addition to thousand kernel weight (TKW), Kjeldahl protein content and glutenin composition, the contents of dietary fiber (β -glucan and total (TOT) and the water-extractable arabinoxylan (WE-AX)) were measured by spectrophotometric assays of wholemeal samples. The structures of the β -glucan and AX fractions were also compared by enzyme fingerprinting, based on HPAEC (high performance anion exchange chromatography) analysis of oligosaccharide fragments released by digestion with endoxylanase and lichenase enzymes. The chromosomal positions of putative orthologs of the key genes determining these components were also identified using *Ae. umbellulata* chromosome sequences.

This study showed that the addition of chromosomes 2U^g, 4U^g, 5U^g, 7U^g, 2M^g, 5M^g and 7M^g of *Ae. geniculata* and 3U^b, 2M^b, 3M^b and 7M^b of *Ae. biuncialis* into bread wheat increased the seed protein content. Chromosomes 1U^g and 1M^g increased the proportion of polymeric glutenin proteins, while the addition of chromosomes 1U^b and 6U^b led to its decrease. Both *Aegilops* species had higher proportions of β -glucan compared to arabinoxylan than wheat lines, and elevated β -glucan content was also observed in wheat chromosome addition lines 5U, 7U and 7M. The arabinoxylan content in wheat was increased by the addition of chromosomes 5U^g, 7U^g and 1U^b while water-soluble arabinoxylan was increased by the addition of chromosomes 5U, 5M and 7M, and to a lesser extent by chromosomes 3, 4, 6U^g and 2M^b. Chromosomes 5U^g and 7M^b also affected the structure of wheat arabinoxylan, as shown by the pattern of oligosaccharides released by digestion with endoxylanase. These results will help to map genomic regions responsible for edible fiber content in *Aegilops* and will contribute to the efficient transfer of wild alleles in introgression breeding programs to obtain wheat varieties with improved health benefits.

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Dry matter remobilization and compensatory effects in different plant parts of durum wheat genotypes under water stress

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Keywords: wheat, water stresses, dry matter change, grain filling, compensatory effect

The main aim of the present study was to analyse changes in performance of CIMMYT-derived spring durum wheat cultivars under conditions typical of those prevailing in the irrigated WANA (West Asia, North Africa) areas. This research was performed in Hatay / Turkey (36° 15' N, 36° 13' E; D 80 m) in 2009/2010 and 2010/2011. Six durum wheat cultivars were evaluated under two irrigation regimes: irrigation until physiological maturity and irrigation until anthesis. The cultivars were sown on 27 November and 10 December first and second year. Sowing was performed in eight lines of 6 × 1.2 m, each 0.2 m apart. Seeds were sown at 450 seeds m⁻². Whole phosphorus (60 kg P₂O₅ ha⁻¹) was mixed with the soil, while nitrate was given during planting, tillering and stem elongation (30 + 30 + 20 N ha⁻¹). Transport of dry matter from vegetative organs to grains was significantly greater in water stressed conditions (1374 mg plant⁻¹) than in unstressed conditions (1119 mg plant⁻¹). Significant differences have been identified among the varieties in terms of dry matter translocation. The translocation occurred most at Ceylan-95 (1725 plant⁻¹), at least (1589 mg plant⁻¹). In both conditions the maximum dry matter transport to the grain was attributed to the pre-anthesis period (96,51%). A significant negative correlation ($r^2 = -0.48$ *) was found between the plant height and the DM transport before flowering in case of stress. In the unstressed condition, a positive correlation ($r^2 = 0.50$ **) was found between the transport of the pre-anthesis DM and the transport of the DM after flowering, and vice versa under stress condition (i.e. $r^2 = -0,60$ **). As a result, it was determined that the most important dry matter source for the grain was pre-anthesis reservoir in the water stress of the grain filling period. This contribution could reach to 100% according to the stress intensity and there was significant genetic difference among the varieties. In areas like Mediterranean ecology where water stress is frequently encountered, it has been concluded that these issues are very important and that the breeding strategies to be implemented should be taken into consideration.

Poster presentations

The Effect of salinity (NaCl) on germination and seedling growth of seven ecotypes of sorghum (*Sorghum bicolor* L moench) cultivated in North West of Morocco

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Keywords: *sorghum bicolor*, salt tolerance, physiological parameters, North West of Morocco

In arid and semi-arid areas, Salinity and drought are the main factors responsible for the reduction of productivity and degradation of agricultural lands. The aim of this work was to evaluate salinity tolerance of seven Moroccan local (1P1, 1P3, 2P4, 2P7, 2P11, 3P4 and 3P8) ecotypes of sorghum (*Sorghum bicolor* L., Moench) with respect to the performance of some physiological parameters such as germination, shoot and root development. The shoot growth of the studied sorghum ecotypes were significantly affected by the exposure to 250 mM of NaCl. Root growth was different among ecotypes even when treated with distillate water. The ecotypes 1P3, 2P4 and 3P8 were observed as more salt tolerant and ecotypes (1P1, 2P7, 2P11, 3P4) were more salt sensitive on the basis of the germination-ability and shoot development.

Results of this experiment revealed that ecotypes 1P3, 2P4 and 3P8 can be good candidates for a genetic resource and expanding of sorghum cultivated area in Morocco.

A genome-wide association study for powdery mildew resistance in a Nordic spring barley panel

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Keywords: *Blumeria graminis* f.sp. *hordei*, *mlo*, GWAS, *Hordeum vulgare* L, plant breeding, resistance, QTL

Powdery mildew disease caused by the fungus, *Blumeria graminis* f.sp. *hordei* (Bgh) is a world-wide threat to barley (*Hordeum vulgare* L.) production. To develop resistant cultivars for the future, preferable with broad-spectrum resistance, it is important to identify both currently exploited and new resistance loci and alleles for powdery mildew. For this purpose a spring barley panel of 169 breeding lines and cultivars, chosen to represent the available genetic variation in current elite Nordic barley germplasm, were phenotyped for powdery mildew resistance in three Nordic countries during three years (2012–2014). The panel was genotyped with the Illumina iSelect 9K SNP barley chip and with 48 microsatellite markers spanning over the seven chromosomes¹. The best model to account for population structure, out of total nine general and mixed linear models tested, was chosen for the final GWAS analysis. In total four QTLs for powdery mildew resistance, located on chromosome 4H and 6H, were identified in the GWAS analysis. This study has provided marker candidates that can be exploited for combining resistance loci in marker-assisted selection for powdery mildew resistance in Nordic barley breeding programs as well as important information about the current effective resistance QTLs/genes under Nordic conditions.

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The implementation of yellow rust resistance gene *Yr15* in winter wheat lines based on the use of protein and molecular markers

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Keywords: wheat, yellow rust, Yr15 gene, GluB1 locus, protein and DNA markers

Yellow rust is one of the most harmful winter wheat diseases, which significantly reduces its yield and aggravates the phytosanitary situation in the cereal-growing regions. The area of yellow rust dispersion in Kazakhstan is the mountainous zone of the southern, eastern and south-eastern regions. In Central Asia in 1998–2013, yellow rust spread 5 times to the epiphytotic level in winter wheat and markedly reduced its productivity (Kokhmetova et al. 2010, Ziyaev et al. 2011). Most commercial varieties of winter wheat cultivated in the South and South-East of Kazakhstan were susceptible to yellow rust.

A number of resistance genes to yellow rust, effective in various regions of Central Asia and Transcaucasia including the *Yr15* gene derived from the wild emmer wheat *Triticum dicoccoides* Koern, are known. Its resistance to highly virulent prevailing pathogens of Kazakhstan yellow rust population is shown by Rsaliyev et al. (Rsaliyev et al. 2010). This gene, among others, can provide adequate protection for wheat varieties grown in areas prone to rust (Peng et al. 2016).

The isogenic line of Aroona Yr15 / 5 * was crossed with winter wheat varieties occupying the largest areas in the South and Southeast of Kazakhstan: Steklovodnaya 24 and Krasnovodopadskaya 210. The reason to choose the isogenic line of the Aroona variety was that it has a pair subunits 14 + 15 in the high molecular weight glutenin spectrum, controlled by the locus *Glu B1* (*h* allele), which is not found in bread wheat varieties of domestic breeding. The breeding lines developed with participation of Aroona Yr15 / 5 * could be characterized not only by resistance to yellow rust, but also by diversity of quality traits.

It is known that the *Yr15* gene is located on the short arm of chromosome 1B in its proximal part (McIntosh et al., 1996), while the *Glu B1* locus, also in the proximal region to the centromere, is in the long arm.

Using the electrophoresis of F₄ generations seeds protein, it was found that a significant number of lines with 14 + 15 subunits showed immunity and high resistance to yellow rust pathogens in the seedling stage.

The close arrangement of the *Glu B1* locus and the *Yr15* gene to the centromere in the donor for resistance to yellow rust causes the coupled inheritance of these genes, and therefore the specific subunit pair 14 + 15 can serve as a protein marker for transferring the gene to the desired genetic object. At the same time, 50% of immune and resistant lines in the combination Steklovodnaya 24 x Aroona Yr15/5* had maternal form of high molecular weight glutenin subunits (7* + 8), which indicates the dependence of the recombination frequency

in the “*Yr15* gene-centromere-*GluB1* locus” region from the gene background. The use of SSR markers *Xgwm11* and *Xbarc8* surrounded the location of the *Yr15* gene on chromosome 1B confirmed the presence of it in the resistant to rust samples both with and without the presence of the *h* allele of the *GluB1* locus and allow to identify promising lines valuable for breeding. The cumulative use of protein and DNA markers makes it possible to control the dimensions of the chromosomal region translated into the hybrid lines and to select the forms with the desired combination of resistance and quality genes.

Effects of alleles of dwarfing genes on elements of drought tolerance of winter wheat

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Keywords: winter wheat, *Rht*, drought tolerance

Plants are often exposed to stressful environmental influences that limit their growth and productivity. Drought is a major problem all over the world, which seriously affects the quality and yield of crops.

There are controversy dates about adaptation of dwarfing wheat genotypes to growth in drought conditions. According to one group of authors dwarfing genotypes are more tolerant to drought. Gasperini et al. (2012) assume that selection of genotypes of wheat with the *Rht8c* gene has a high potential in conditions of moisture deficiency, Hu Yin-Gang (2012) concluded that dwarfing genes should be used to improve water use efficiency and increase wheat yield potential, Alghabari et al. (2015) have shown that dwarf *Rht* alleles are better able to enhance tolerance to high temperature and drought stress in wheat. The other authors – Orljuk, Goncharova (2002), Shamanin, Likhenko (1990) have revealed that dwarfing genotypes have worth adaptation to drought condition.

The aim of our work was to analyze the effects of alleles of dwarfing genes on elements of drought tolerance of winter wheat. As material for investigations we have used analogous lines that were developed in the PBGI, (Odessa, Ukraine) by crossing of the parental forms with the donors of dwarfing genes (Odes'ka semidwarf or Krasnodarskiy Karlik 1) and by six back_crossing with recurrent forms. Dwarfing alleles were identified by molecular markers. The tasks of the work were:

- to investigate the length of the primary roots of line-analogous in the phase of seedlings under conditions of osmotic stress;
- to evaluate the effects of alleles of *Rht8*, *Rht-B1*, *Rht-D1* genes on coleoptile length in analogue lines during the germination under conditions of osmotic stress;

- to estimate the effects of the alleles of the dwarfing genes on the water-holding capacity of the wheat leaves;
- to estimate the elements of crop structure of lines-analogous that were grown in rainfed and irrigation conditions.

A negative influence of osmotic stress was shown on the development of primary roots, except cv. Stepnjak, its analogue line Stepnjak 3 and cv. Albatros odes'kyy. The index of drought tolerance (the ratio of the length of primary roots under stress compared with control conditions) for these genotypes were 1.02, 1.04 and 1.2 respectively, while for other genotypes studied it varied from 0.57 to 0.89.

It was found that the length of the coleoptile under conditions of osmotic stress in comparison with the tall analogous lines significantly was affected by the alleles of the genes: *Rht8c* (25.4 and 26.9% (on different background), *Rht-B1b* (30, 3%), *Rht-B1e* (31 and 42.1% (on different background)), *Rht8c + Rht-B1e* (58%), *Rht8c + Rht-B1b* (61.6%) and *Rht8c + Rht-D1b* (25, 1%).

According to our data the *Rht8c* gene does not affect the level of leaves water-holding capacity of wheat plants. At the same time, gibberellin-insensitive dwarfing genes *Rht-B1e* and *Rht-D1b* present in the genotype alone or in combination with the *Rht8c* gene, have a positive effect on the leaves water-holding capacity of bread wheat.

According to preliminary results, Stepnjak, Odes'ka 51, Karlik 1 were characterised by higher WTK on the rainfed condition in comparison with other investigated lines. On the other hand the line-analogous Stepnjak 2K (*Rht8c + Rht-B1e*) has significantly lower WTK in comparing with the tall Stepnjak. The preliminary results does not reveal clear effects of dwarfing genes on yield parameters of bread wheat in rainfed conditions.

Introgressing resilience and resource use efficiency traits from Scots Bere to Elite Barley Lines (REBEL)

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Keywords: barley, landraces, Bere, biotic stress, abiotic stress, phenotyping

With the increasing population it is important to increase the total yield of most crops, importantly the staple cereal crops. Whilst modern elite barley varieties are high yielding and responsive to high levels of agronomic inputs they have plateau in 'on farm yield', with little evidence of an overall increase in abiotic and biotic stress tolerance due to the low priority in breeding for increased stress, low input or marginal environments.

A potential source of viable resilience and resource use efficiency traits are landraces local to areas of marginal land, such as the Scots Bere from the Highlands and Islands of Scotland. The Bere are a deeply historically rooted landrace of barley which has been grown on predominately marginal land for the last half millennia; allowing them to yield well un-

der marginal conditions with impoverished soils. By introgressing them into elite cultivar backgrounds they may contribute biotic and abiotic stress-tolerance genes and nutritional genes that enable them to efficiently and resiliently yield under low input and stress-prone environments.

The overall aim of the proposed project is to assess and genetically characterise these traits thereby improving low-input performance and yield stability in elite barley. Screening for biotic stress resistance to pathogens such as *Rhynchosporium commune* & *Puccinia hordei*, abiotic stress resistance to stresses such as Mn deficiency & salt toxicity, and a combination of one abiotic and one biotic stress to assess the interaction within the genotype.

Influence of leaf trichome density of different winter wheat cultivars on cereal leaf beetle larval leaf feeding

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Keywords: cereal leaf beetle, *Oulema*, leaf trichome density, wheat cultivars

Cereal leaf beetles (CLBs, *Oulema* spp.) are important pests of small grain cereals. Both adults and larvae are feeding on the leaves of the host plants, however, usually only the larval damage affects the yield. Their feeding destroys large parts of the assimilating leaf tissue, which causes severe distress and can lead to yield loss (Philips et al. 2011).

We examined the effects of the leaf trichome density of three different winter wheat cultivars – Alcantra with a high density of short trichomes, Altigo with a low density of long trichomes and Buzogány with a middle density of medium-length trichomes – on the extent of leaf damage caused by the CLB larvae. We placed CLB adults (n = 3) under each isolator tent for a week (16 May 2017 – 23 May 2017). Then, the adults were removed, and the eggs were counted. For the next few days, we monitored the egg hatching and the larval development. We set the number of larvae to 10 different levels in 3 replications, from two larvae/isolator to 20 larvae/isolator in steps of two. We recorded the larval density every second day until pupation. If no larva was found in an isolator within 4 days, we removed the tent, took photos of each infested leaf to measure leaf damage and counted the number of leaves as well as leaf stems. We used Adobe Photoshop to quantify the percentage of leaf surface consumption. For calculating pest pressure we standardized the number of larvae to the number of wheat plants per isolator and to the number of days they were recorded because of larval mortality. Generally, higher pest pressure led to higher leaf damage. However, the highest leaf damage values were observed on plants of Buzogány cultivar suggesting more favourable conditions for the larvae and, therefore, higher risk of severe CLB damage.

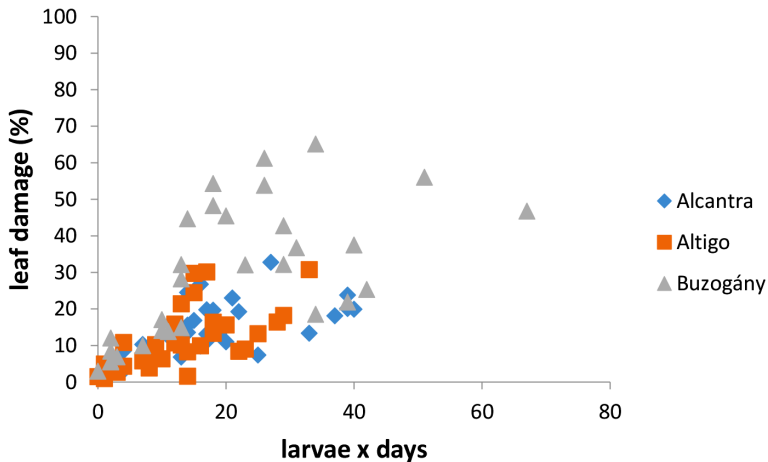


Figure 1: Relationship between the leaf damage and the pest pressure of CLB larvae for three winter wheat cultivars

Reference:

Philips, C. R., Herbert, D. A., Kuhar, T. P., Reisig, D. D., Thomason, W. E., & Malone, S. (2011). Fifty years of cereal leaf beetle in the US: an update on its biology, management, and current research. *Journal of Integrated Pest Management* 2: C1–C5.

Mutations disrupting the formation of the wax layer impact on biochemical and physiological traits in rye under drought

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Keywords: drought, phenolics, photosynthetic pigments, *Secale cereale* L., sugars, chlorophyll *a* fluorescence

Rye (*Secale cereale* L.) is one of the important cereal crops of the world. Its grain is mainly used for animal feed and to make rye whiskey, and, to a lesser extent, is milled to make bread. Rye has a number of positive attributes, such as outstanding cold hardiness, excellent drought tolerance and strong disease resistance. Some of these favorable traits may be due to the intense waxy bloom on the stem and leaf sheath, however such a direct connection has not previously been studied. The aim of the study was to determine the differentiation of near-isogenic lines (NILs) of rye in terms of selected biochemical and physiological parameters as

a result of the impact of the mutation disrupting the formation of the wax layer. Three pairs of rye inbred lines (811w*, 811bw**, L35*, L35bw**, RXL10*, RXL10bw**) were research material. Each pair consisted of the typical line and the one with recessive mutation causing disorder in forming proper wax coating. All tested lines were exposed to a 3-week drought stress, each line was represented by at least 8 plants. Following measurements and analysis were conducted: photosynthetic pigments content (PPC) estimation, spectrophotometric analysis of soluble sugars content (SSC) and total phenolics content (TPC) measurement and determination of chlorophyll concentration in SPAD units. As a result of drought stress each of the lines (with a few exceptions) reported significantly decreased level of all biochemical parameters compared with the control. PPC levels, the concentrations of total chlorophyll *a+b* (TChl) and carotenoids (Car), varied between the lines. TChl content differed significantly within one pair of NILs, 811 and 811bw. The waxless line showed 3 times higher TChl content under drought stress than the waxy one. 3-week drought stress resulted in a significant difference of Car content between lines of two pairs, L35 - L35bw and RXL10 - RXL10bw. The waxless lines reacted in different ways, the level of Car content decreased in line L35bw and increased in line RXL10bw in comparison to waxy lines. The analysis of SSC level under drought conditions showed statistically significant differences between the lines of two pairs of NILs, 811w - 811bw and L35 - L35bw, waxless lines showed respectively an increased and decreased SSC level. Long water deficit induced a significant increase of TPC in a waxless line L35bw. The measurement of chlorophyll content in SPAD units confirmed the tendency of the changes observed in TChl content analysis, but the pair of NILs which showed significant increase of chlorophyll content under drought conditions were lines L35 and L35bw. Conducted experiments are an introduction to further research on mutations that disrupt wax formation in rye as well as their impact on morphological, biochemical and physiological features.

*waxy, **waxless

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Quality of grain varieties of soft spring wheat in adverse weather conditions of harvesting in Northern Kazakhstan

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Keywords: spring soft wheat, quality of grain, the nature of the grain, gluten

The Republic of Kazakhstan is one of the largest grain producers in the world. Due to its high qualitative characteristics Kazakhstan wheat has traditionally been used by importing countries to improve domestic grain to ensure its high baking qualities. The formation of

high quality grain is promoted by the soil and climatic conditions of the grain-bearing regions (Kostanay, Kokshetau, North Kazakhstan, Akmola, and Pavlodar). Almost 85% of the sown area and a gross yield of grain wheat are concentrated in them. The general requirement for the wheat grain, irrespective of its purpose, comes down to keeping the wheat clean, free of weeds and grain impurities, uniform in size and colour, undamaged by pests and unaffected by diseases. The main criteria for the evaluation quality of grain wheat in Kazakhstan are the quantity and quality of gluten. The presence of gluten endows the wheat dough with a good stability of form under fermentation and baking. The research was conducted in 2014–2016 at A.I. Barayev Scientific Production Centre for Grain Farming (Shortandy). In the demonstrative nursery of soft spring wheat of 42 varieties were sown. In order to determine the possible damage and losses from adverse weather conditions, first and second replicates were harvested on time, on September 15, when the grain was fully ripe, third and fourth replicates were kept in the field for a month in conditions of excessive moisture, low temperatures and weak frosts (-3°C). Excessive moisture during harvesting contributed to a slowing of the ripening process and a disruption of normal gluten accumulation. This situation led to a decrease in the level of technological indicators (the nature of the grain, protein content, quantity and quality of gluten) of the investigating varieties of spring soft wheat). Characterizing the grain quality it should be said that before precipitation the grain obtained was typical for the red hard-grained spring wheat in a healthy state, without loss of colour and alien smells; after being kept in the unfavorable conditions the grain lost its natural shine, and discolourisation of 2–3 levels appeared. The nature of the grain varied from 733 to 782 g/l. The best sorts in terms of this indicator were Pyrotrix 28 (782 g/l), Tselinnaya 20 (780g/l). Evaluating the nature of the grain harvested from the third and fourth replicates showed that losses in the actual weight in the grain of the studied varieties were from 23 (Akmola 3) to 57 g/l (Soltustik). When assessing the quality, the content of mass proportion of protein to dry matter in the grain of studied varieties was found in the range of 12,95–16,98%, the highest-protein variety was Ekaterina. As a result of the analysis the grain subjected to prolonged the aqueous and temperature stress, a decrease in protein content of 30 varieties from 0,07 to 1,19% was conducted. The level of mass content of raw gluten varied from 28,0–40,6%, in two varieties of wheat Akmola 3 and Akmola 40 retained their gluten content, in the remaining varieties losses were from 0,6 to 8,6%. By this a high gluten content, it was characterized as a weak, tensile in 88% of the varieties. After the exposure to the negative night temperatures (frosts), the gluten became stronger and less tensile in 59,5% of varieties, which subsequently adversely affected the volumetric yield of bread and its stability of form. The yield of pan loaf volumes from 100 grams of flour showed different results depending on the flour type, such as 640 ml for (Shortandinskaya 2012) and 820 ml for (Uralsiberian). The shape of the bread crust was quite good; golden in colour, crumb was elastic with the uniform porosity. The trial laboratory bakery products are from flour, in varieties harvested after being kept in the field showed variability in the volume yield of bread from 600 to 800 ml. Maximally it became less in terms of varieties: Astana 2 (by 90 ml), Omsk 18 (89 ml), Asyl Sapa (80 ml), Astana (78 ml), while the stability of form, porosity and overall baking evaluation was deteriorated. All varieties had a dome-shaped bread crust, more often with a rough surface, its colour changed; it became light brown, sometimes with a white coating. The porosity was uneven and the inside was slightly elastic and insufficiently resilient when pressed.

Optimization of biolistic genetic transformation of durum wheat with pANIC5E plasmid containing the BAR gene

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Keywords: drought tolerance, biolistic transformation, durum wheat, Basta selection

Since 80s and due to succession of drought events, wheat imports are becoming more and more important in Morocco. Genetic engineering has undoubtedly opened a new avenue to overcome crop losses due to various abiotic stresses. For this purpose, several national programs are undertaken to develop new drought tolerant varieties. One of the biggest obstacles limiting genetic transformation of wheat was the lack of an efficient regeneration system for bombarded tissues. Therefore, it is very important to target cells that are competent for both transformation and regeneration. Our study aims to optimize culture conditions for regenerating bombarded calli from mature embryos of three durum wheat varieties, through comparing the effects of phytohormones and nitrogen sources on callus induction and plants regeneration. The pANIC5E plasmid containing the bar gene as selectable marker for resistance to the herbicide phosphinothricin “Basta”, was used. After biolistic transformation, the induction of embryogenic tissue in the absence of selective agent Basta was successful for all varieties studied. However, the percentage of survival plants reduced drastically as they were subcultured on the selective media. The results of callus induction and plants regeneration depends on the genotypes, media composition and explant types.

Wax composition is important for drought tolerance of rye near isogenic lines

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Keywords: drought, rye, near isogenic lines, wax composition

Drought is considered one of the most important environmental factors that limit crop productivity. This is valid not only in the arid and semi-arid areas, but also applies to central Europe, where the rainfall tend to vary from year to year. Physiological traits affected by drought can be correlated with the CO₂ assimilation rate, PSII photochemical activity, leaf water conservation (stomatal conductance, transpiration rate, relative water content – RWC), but eventually they are all mirrored in the most important trait which is yield. Plants show a plethora of

morphological, physiological and biochemical responses to drought stress and use different adaptive mechanisms including waxes layers. Plant waxes are complex mixtures of hydrocarbons, alcohols, aldehydes, ketones, esters, acids, and diverse combinations of these. The wax layer is deposited outside the epidermal cells. This lipid covering not only protects plant from invasion by foreign organisms but also helps to regulate transpiration.

The aim of the study was to answer the questions how strongly wax coating participates in protecting plants in conditions of optimal and reduced water access, and how it correlates with wax quantity and wax composition.

The experiment was conducted on three pairs of rye near isogenic lines (811w, 811bw*; L35, L35bw*; RXL10, RXL10bw*). The second of the pair (wax-less, marked with *) bears recessive mutation disturbing proper wax layer formation. Plants were exposed to a 3-week drought stress, then wax layer was collected. Wax was weighted and analysed by GC-FID.

Tested lines differ in yield, expressed as thousand grain weight. In optimal conditions wax-less lines were characterized by lower yield. Interestingly after drought stress they reacted less sensitive with yield reduction than mutation free “wild type”. The wax content varied among lines from 3 to 20 mg/g DW. All lines increased wax content under drought conditions. Wax-less lines tend to react less sensitive exhibiting lower wax accumulation than in optimal vegetation conditions. Lines differ in wax composition expressed as hydrocarbon chain length. Wax-less mutation causes lowering of even chain hydrocarbons in favor of odd chain ones. Lines responded differently to drought taking account quality of wax hydrocarbons.

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Gene ontology analysis of miRNA targets involved in soybean chilling stress response

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Keywords: soybean, microRNA target genes, chilling stress response

Stress response in plants is mediated through broad spectrum of cellular mechanisms and one of them is alteration in expression of microRNAs (miRNAs) molecules. As a consequence of its action we can observe differentiation in expression levels of genes targeted by those miRNA. Most plant miRNAs perform their gene expression regulation function through target cleavages. The resulting slicing sites on the target transcripts could be mapped by sequencing of the 3'-cleavage remnants, called degradome sequencing. For selected miRNA previously described as involved in chilling stress response in plants, we performed analysis of potential

target genes in soybean – economically important crop species, which is highly used in feed industry. The analyzes were performed based on data from PMRD: plant microRNA database, structural properties of [RNA: RNA] complex and data from degradome sequencing available from Phytozome database. For most probable target genes we performed also gene ontology analysis to indicate its function in chilling stress response. Studied genes encode, among others, transcription factors and molecule of antioxidant function which are key players in sustaining status quo of cell during stress condition.

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The comparison of impact of water shortage on wheat cultivars in container and field experiments

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Keywords: root system, root depth, water stress, irrigation, ^{13}C discrimination

Root traits play an important role in the screening and selection of genotypes tolerant or adaptive to water shortage. The selection under controlled conditions would enhance screening for target traits. The objective of the study was to compare the effect of water shortage on wheat under semi-controlled and field conditions.

Six lines of winter wheat selected according to different root size measured by electrical capacity (Heřmanská et al. 2005) were grown under optimal and insufficient water supply in container and field experiments. Winter wheat plants were grown in plastic tubes of 60 cm height and 12 cm diameter filled with the mixture of soil and sand in 2015/2016 season. Water shortage (S treatment) was induced from full tillering, irrigated treatment (W) was kept at optimal water content level. Plants were harvested at heading, leaves and ears were analysed for ^{13}C and ^{12}C isotopic content, the ratio of isotopes was calculated ($\delta^{13}\text{C}$), root mass (RM) and total length (RL) were determined.

In the field experiment the same wheats were grown in the season. Water shortage (S) was induced from heading with mobile rain-out shelter, drip irrigation was used for optimal water supply (W) and rain-fed crop served as a control (R) (Raimanová et al. 2016). Various growth and yield traits were determined, including root depth and distribution and $\delta^{13}\text{C}$ of leaves and grains.

Pot experiment: Stress reduced total plant dry mass by 72%–82% of W treatment. WUE (total DM) of wheats in containers ranged from 5.5 to 6.8 g (W) and from 7.0 to 8.8 g.kg⁻¹ of water (S), the effect of genotype was significant ($p < 0.016$) but not the interaction with water. The genotypes significantly differed in RDM and total RL ($p < 0.002$), pronounced differences were observed in the rate S/W treatments (0.65 – 0.96). The effect of water shortage

on the $\delta^{13}\text{C}$ of leaves and ears was significant but the effect of genotypes not. The genotype effect on the value of differences between stressed and irrigated ^{13}C signature of leaves and ears were highly significant ($p < 0.004$). The genotypes with the highest RDM and RL in W treatment showed the highest difference between S and W treatment in RDM a RL ($r = 0.87$) and, oppositely, the lowest difference in $\delta^{13}\text{C}$ between S and W ($r = 0.86$).

Field experiment: The water stress (S) reduced grain yields by 3.7 to 5.2 t.ha⁻¹ against W, and by 2.4 to 4.0 t.ha⁻¹ in comparison with rain-fed control (R). Soil moisture at the start of grain filling suggested differences in water depletion only in deep subsoil layers but with great variability. Observed differences in root depth were also accompanied by great variability of replications, and were not in relation to water depletion. $\delta^{13}\text{C}$ of grain followed expected changes due to different water supply, stress increased $\delta^{13}\text{C}$ on average by 1.4‰ to 2.1‰ in comparison with W, $\delta^{13}\text{C}$ of R was reduced by 0.2 to 1.4‰ in comparison with W, however, there were not relationships with container data.

The results suggest the two experimental environments represent strong interacting factors to obtain consistent results for the examined traits.

Acknowledgments

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Dynamics of wheat stress response in ABA-dependent and ABA-independent pathways induced by drought stress

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Keywords: *Triticum aestivum*, drought tolerance, dehydrine genes

The activation of drought stress reaction on the molecular level is controlled via ABA-dependent and ABA-independent pathways. Interactions between these pathways can be initiated under different stress conditions. The intensity and dynamic of stress response can affect the resulting level of plant tolerance to drought and simultaneously can be influenced by the varied mechanisms of adaptations to stress related to different origins of wheat genotypes. The work was aimed at analysis of the differences in dynamic of activity of several genes, which are involved in both ABA pathways, particularly two genes from *COR/LEA* group (*WDHN13*, *WRAB17*) and four genes for transcript factors (*WAB15*, *WDREB2γ*, *WRKY19* and *TaPIMP1*).

The relative expression of these genes was evaluated in young plants of Czech, Hungarian and Syrian winter wheat varieties, which were exposed to continuous drying. Wheat lines of Czech origin showed higher level of gene expressions (*WDHN13*, *WABI5* and *WDREB2γ*) in control plants, whereas in Hungarian and Syrian lines the activity of those particular genes was higher in stressed plants. Transcription factors *WABI5* and *WDREB2γ* seemed to respond early to stress, while the activation of the transcript factor *TaPIMP1* was postponed. The timing of gene activations was also influenced by a genotype.

Winter and spring wheat FHB resistance breeding

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The main pathogens associated with FHB in Czech Republic are *F. culmorum*, *F. graminearum* (Schwabe) and its teleomorph, *Gibberella zeae* (Schw.) Petch. Other *Fusarium* spp. (*F. poae* (Peck) Wollenw., *F. avenaceum* (Corda ex Fr.) and *Microdochium nivale* (Ces ex Berl. & Vogl.) Sammuels & Hallett and its teleomorph *Monographella nivalis* (Schaffnit) Muller are also present in FHB complex. Recent studies revealed prevailing occurrence of *F. poae* and *F. graminearum* with the Czech Republic.

Fusarium head blight occurs in winter wheat fields every year in the Czech Republic. Its intensity depends on season and local climatic conditions, especially on temperature and air humidity. It was found that condition above 6°C together with higher humidity (fog and dew often due to nearness of ponds and rivers) leads to presence of higher occurrences of FHB and mycotoxins accumulation. Mycotoxins content exceeding hygienic limits is usually connected with other favorable conditions (no tillage, corn precrop).

After all developing varieties with high levels of FHB resistance has become an essential component to the wheat breeding program. For effective selection in our breeding program inoculation techniques are used. The mostly used technique at the breeding programme is inoculation by spraying heads with the conidial suspension (Mesterházy). Because of screening about 1000 lines every year we prefer to inoculate about 10–20 heads/a line in a hill plots (about 40 seeds is sowed for one plot), so the size of nursery can be kept manageable. The inoculations are done once at the stage of flowering (we are carefully looking for flowering heads and label them). After inoculation the screening nursery is under mist irrigation for about one month. Although *F. graminearum* and *F. culmorum* do not have vertical races, isolates differ significantly in their aggressiveness. Therefore, over the entire inoculation period and all locations we use the same inoculum. The first symptoms are observed 7–10 days after inoculation.

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 intro-

gression of FHB resistance QTL into elite high-yielding cultivars often brings a potential risk of worsening of important agronomical traits. Recently were registered winter wheat varieties Carmina and Ibarra which were derived from FHB tolerant variety Sakura. These varieties keep improved FHB tolerance as well as good agronomical traits and have good potential for safety cultivation in fusarium favorable areas of Czech Republic and Central Europe.

Gene effect on partial resistance response to *Puccinia triticina* in perspective Romanian lines

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Leaf rust caused by *Puccinia triticina* Erikss. (*Pt*) is the most prevalent and damaging rust disease of wheat globally. Regular impact on yield is the result of premature leaf senescence and poorly developed kernels. Cultivars that deploy major resistance genes that operate qualitatively on a gene-for-gene pattern are widely used for disease control. Therefore, in practice, their effectiveness proved to be often short under the continuous and dynamic evolution of pathogen virulence. Adult plant *slow-rusting* partial resistance, quantitatively expressed in terms of a slower rate of disease progress and less spore production comparatively to qualitative resistance, has proven to be more durable, while a cultivar possessing it is widely cultivated under pathogen pressure. The cumulative effect of *slow rust* traits result and could be quantified by a smaller area under disease progress curve (AUDPC) and lower values of the final disease severity (FS,%), when resistant and susceptible cultivars are compared. Among the >70 genes catalogued as conferring plant resistance to *Pt*, at least four *slow-rusting* adult plant resistance genes (APR) have been designated to date: *Lr34*, *Lr46*, *Lr67* and *Lr68* while their additive and pleiotropic effects on other rusts and powdery mildew are better documented.

In the wheat breeding program carried out at NARDI Fundulea, the development of new germplasm with more durable and multiple resistance to diseases, even partial, conferred by such genes is of great concern. In this respect were developed six populations for each of the APR genes *Lr34*, *Lr46* and *Lr67*, derived from crosses with two adapted Romanian varieties, *Glosa* and *Miranda*, widely cultivated and appreciated by farmers. The presence of the targeted *slow rusting* resistance genes has been performed by molecular analyses with functional markers: *cssfr5* (*Lr34*), *csLV46/TaqI* (*Lr46*) and *Lr67PLUSHSUTF/R*, *Lr67HSPSUTF/R* (*Lr67*). As a result, the 400 F₆RILs were classified as i) non gene carriers, ii) single gene carriers and iii) carriers of combinations of two genes (*Lr34/Lr46* and *Lr34/Lr67*). In order to evaluate the individual effect of the APR genes on resistance to *Pt*, parents and all recombinant lines were phenotypically evaluated under controlled inoculation in three field environments: Fundulea (44°27'N/ 26°30'E), Albota (44°46'N/ 24°49'E) and

Livada (47°87'N/ 23°13'E). Susceptible spreader rows planted as borders were inoculated with prevalent local *P.t.* isolates. Disease severity (DS) and host response to infection in each line were three times recorded, based on the modified Cobb Scale (Peterson, 1948) and Roelfs et al. (1992), respectively. According to AUDPC and RAUDPC (as % of the AUDPC values in the susceptible spreader), calculated for each line, significant differences between non and carriers of *Lr* genes were found as well as between single gene carriers. The mean lower values for the both criteria were found in lines carrying the pyramided *Lr34/Lr46* genes, derived from cross with Glosa: AUDPC = 427 and RAUDPC = 46%, suggesting their additive effect on *slow rusting*, as compared to this of each gene, separately and with combination of the *Lr34/Lr67* genes. *Lr34* gene proved a higher efficiency on slow rusting traits irrespective of the analysed background, in comparison to *Lr46* and mainly with *Lr67* gene that show a weaker effect on this type of resistance. Further investigations aimed to clarify the interaction environment/*slow rusting* response to *P.t.* in the Romanian perspective lines are necessary.

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Soybean microRNAs expression during chilling stress

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Keywords: soybean, microRNA, chilling stress, digital droplet PCR

Soybean is the major legume crop that owes its importance to high protein (40%) and oil (20%) content, as well as the ability to host the nitrogen fixating bacteria in root nodules. Two major fields of soybean use are food production and animal feed enrichment with latter being the cause of import of more than 20 million tons of soybean meal in Europe. Low temperatures in spring, common for Polish climate, render it not suitable for effective soybean cultivation. In order to remedy this issue and introduce soybean to Polish agriculture efforts have been made to breed cultivars resistant to chilling stress. To better understand the mechanism of abiotic stress tolerance related to low temperatures in plants we employed cultivar Fiskeby V, established in Sweden. Based on literature data survey we selected microRNAs (miRNAs) that potentially play a role in plant low temperature response. Gene ontology analysis indicated that their target genes encode various transcription factors and antioxidants. We determined expression levels of these miRNAs and therefore highlighted their significance in soybean's stress response. Small RNAs were isolated from leaves and seedlings of plants cultivated in stress conditions. Assessment of miRNA expression levels was performed using novel technique called digital droplet PCR, which precisely defines the concentration of target sequence in each probe. We confirmed that all studied miRNAs are involved in chilling stress response in soybean cultivar Fiskeby V.

Dissecting the genetic background of stem rust resistance present in the winter wheat cultivar Mv Zelma

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Keywords: stem rust, resistance breeding, molecular markers, wheat (*T. aestivum* L.)

Stem rust, caused by the fungus *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn., is an impending disease for wheat production worldwide hence breeding for stem rust resistance is a very important challenge of wheat breeders all around the world. Although the disease has been under effective control in Hungary through the use of host resistance, there are needs for new sources of resistance because of the threatening new pathogen races in Africa and Middle East. The Hungarian winter wheat variety 'Mv Zelma' possesses a high level of resistance against the local stem rust races being durable over the past years and in addition, this variety proved to be resistant against the Ug99 race-group, as well.

The main objective of this work was to identify the genetics of resistance in Mv Zelma. We crossed Mv Zelma with two susceptible Mv lines, Mv12-13 and Mv32-13. We started the anther culture from the anthers of the F₂ plants, in order to fix the F₃ segregation in homozygous state by creating doubled haploid lines. We investigated the frequency of the *Sr38* and *Sr15* stem rust resistance genes in both populations and the parents using the gene specific VENTRIUP-LN2 (for *Sr38*) and STS638 (for *Sr15*) molecular markers (Helguera et al. 2003), (Neu et al. 2002). The stem rust resistance of the plants were scored in adult plant stage by the modified Cobb scale, evaluating the reaction type and the severity at the same time. The field experiments were set up in two replications in the artificially inoculated stem rust nursery of the Agricultural Institute, Centre for Agricultural Research, Martonvásár in 2017. Statistical analysis of the data was performed to determine the connection between the two Sr genes and the phenotypic responses of the doubled haploid lines.

Both of the stem rust resistance genes originated from Mv Zelma. Based on the phenotypic data, the *Sr38* resistance gene showed a very strong correlation with the resistance level of the doubled haploid lines. This major gene alone explained 75.6% and 74.9% of the phenotypic variation in stem rust resistance and its presence reduced the Cobb scale values in the Mv12-13/Mv Zelma and Mv32-13/Mv Zelma doubled haploid lines with values of 71.6 and 68.5, respectively. On the other hand, *Sr15* gene alone had no significant effect on the susceptibility of the doubled haploid lines. We identified however a cross specific interaction between *Sr38* and *Sr15*. In the case of the Mv12-13/Mv Zelma lines, *Sr15* seemed to decrease the protecting effect of the *Sr38*, while in the Mv32-13/Mv Zelma combination *Sr15* had no significant effect on *Sr38*.

The results prove that the resistance of Mv Zelma against the Hungarian races of *Puccinia graminis* f.sp. *tritici* is primarily due to the presence of the *Sr38* resistance gene.

Acknowledgments

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Variation in grain filling of winter cereals in Pannonian Plain

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Keywords: grain weight, grain growth, growing season, Pannonian environments

Grain weight and grain number are two most important grain yield components in different winter cereal crops. In the Pannonian environment, grain filling often occurs under undesirable environmental conditions represented by water deficit and increased temperatures at the end of the growing season. Knowledge about variation in grain weight and grain filling parameters across different environments and its association with grain yield is important for further improvement of small grain production. Therefore, the main objective of this paper was to compare grain filling traits in winter cereal crops across different growing seasons. Trials with 12 winter cereal genotypes (barley, wheat and triticale) that are widely grown in Serbia and surrounding countries, were sown in four successive growing seasons in Rimski šančevi Experimental Station of Institute of Field and Vegetable Crops, Novi Sad, a typical location within the Pannonian plain cereal production area in northern Serbia. Results from our study showed that the grain filling parameters were under significant influence of species, cultivar and growing seasons. Although high temperatures occurred at the end of growing seasons, decreased grain filling duration was compensated by increased grain filling rate, resulting in low year-to-year variation in grain weight. The highest grain weight was reported in triticale cultivars, while wheat and six-rowed barley cultivars had the lowest grain weight. The longest duration of grain filling period was noted in triticale cultivars. Furthermore, two-rowed winter barley cultivars had the highest values of grain filling rate, and the lowest grain filling rate was recorded in wheat. The presence of genotypic variation indicates that breeding for higher grain filling rate and duration in different winter cereals should enable the development of new cultivars characterized by higher grain weight and yield.

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Identification of QTLs associated with salt tolerance in bread wheat cult. Roushan in cross with Falat, Sabalan, and Superhead II using DArT markers

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Keywords: bread wheat, salt stress, recombinant inbred line, QTL, grain yield, linkage

For identification of QTLs controlling salt tolerance and associated with grain yield, physiological and agronomical traits in three populations of winter bread wheat derived from cross between Roushan×Falat, Roushan×Sabalan, and Roushan×Superhead-II, 186 recombinant inbred lines (RILs) from cross Roushan×Superhead-II, 272 RILs from cross Roushan×Sabalan, and 319 RILs from cross Roushan×Falat, were evaluated for salt tolerance under seedling and reproductive growth stages. The linkage maps for these populations were developed using SSR and DArT markers. We found 127 QTLs in the RILs from cross Roushan×Superhead-II under normal and salt stress conditions. 25 QTLs on 13 linkage groups (10 chromosomes) including 1A₁, 2A, 4A, 5A, 7A, 2B₁, 2B₂, 2B₃, 3B, 6B₂, 6B₁, 1D, and 2D₁ were identified, which 10 and 15 QTLs were related to normal and saline conditions, respectively. The major QTLs for grain yield were on chromosomes 1A₁, and 3B sandwiched between wPT-668205 and wPT-731282, and wPT-666738 and wmc505.2 which described 11.02 and 10.30 of the total grain yield variations, respectively. Chromosomes 3B and 2D₁ showed the highest numbers of QTLs under normal and saline conditions. It seems that several clusters of QTLs for salt stress are located on these chromosomes which probably play an important role in salt tolerance of wheat. Hence, these chromosomes can be considered for further investigations in wheat breeding programs. 164 QTLs were detected in RILs population derived from cross Roushan×Falat by using composite interval mapping method. From these QTLs 110 and 54 QTLs were associated with reproductive and seedling stages, respectively. Five QTLs for grain yield (GY) were detected on chromosomes 2B₁, 4D₂, 5B₂, 7B and 7D₁ which two of

them were under saline conditions. The major QTL for GY was detected on chromosome 7B sandwich between wPt-6484 and wPt-6657 which describes %14.99 of total variation in GY. We used MCMC method to identify QTLs with additive and epistatic effects as well as their interactions with environment. Finally, 44 QTLs were detected, which 28 of them associated with reproductive stage. Out of 44 QTLs, 29 QTLs showed only additive effects and 15 QTLs had both additive and environmental effects. We also detected 24 QTLs with epistatic and environmental effects. Results showed that QTLs associated with physiological traits such as shoot and root sodium content at seedling stage, in comparison with morphological traits relatively better shows the environmental (treatment) effects on plant growth and development. Biochemical pathways for accumulation of Na⁺ and K⁺ were different. In RILs population of Roshan×Sabalan, by using QTL Cartographer software 142 QTLs were detected at seedling and reproductive stages under normal and saline conditions. The major QTL for GY were detected on chromosome 2B sandwiched between wPt-3561 and wPt-0408 markers which describes %10.2 of total variation. Chromosomes 2B and 1A showed the highest numbers of QTLs at reproductive stage under normal and saline conditions. Major QTLs for some other measured traits were also detected on these chromosomes. Probably, those loci on these two chromosomes have important role in salinity tolerance of wheat which can be considered in further studies. 29 QTLs were detected for sodium concentration on 13 chromosomes where 16 and 13 QTLs were associated with sodium concentration in shoot and roots, respectively. Several QTLs for shoot and root sodium concentration were detected on chromosomes 1A, 6A, and 2B which described high percentage of phenotypic variations. For other measured traits, 17 and 5 QTL clusters were detected on different chromosomes under seedling and reproductive stages, respectively. SSR markers qwm88, gwm55, gwm526, gwm626, and gwm540 were significantly correlated with the different identified QTLs. These markers can be used in further studies for MAS.

Creation of salt-tolerant breeding material of clover (*Melilotus Mill.*)

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Keywords: sweet clover, salt tolerance, soil, variety, complex hybrid population

In Kazakhstan, natural forage lands occupy an area of 187 million hectares. A considerable part of them is found on different levels of saline soils and their complexes with the normal soils by the area 10–50% of solonetz. Taking into account the need for the production in stress-resistant crops and varieties, the goal of the research is to create a salt-tolerant breeding material of sweet clover, with the increased productivity of fodder mass and seeds, and high quality of feed for the conditions of Northern Kazakhstan.

The study for the salt tolerant forms of 57 varieties used and the promising complex hybrid population (CHP) of Volgian sweet clover (*Melilotus wolgicus* Poir.), yellow sweet clover (*Melilotus officinalis* (L.) Pall.), white sweet clover (*Melilotus albus* Medik.), and dentated sweet clover (*Melilotus dentatus* (Waldst.et Kit) Pers.).

A biological salt-tolerance under the seeds germination was in a 1,05% aqueous solution of chemically pure sodium salt (NaCl), the laboratory germination of the sweet clover varied from 10,0 to 86,0% and was lower than the control sample (distilled water). Five classes of salt tolerance were identified with an interval of 20%: the 1 class is very weakly resistant, less than 20%, the 2 class is weakly resistant, 21–40%, the 3 class is the medium-resistant, 41–60%, the 4 class is resistant, 41–80%, the 5 class is a highly stable, more than 81%. Most samples of sweet yellow clover were in the 1–2 classes of stability, Volgian sweet clover was in the 3–4 classes of stability, the sweet white clover was in the 2nd class of resistance. When comparing 4 species of sweet clover among themselves, it was revealed that the most salt tolerant species is Volgian variety. The high biological tolerance to salinity (62,0–87,5%) characterized the following varieties (CHP) yellow sweet clover KD-591, 1728, the sweet Volgian clover was Akbas variety, (CHP) KD-1410, 1690, 1731, 1828, 1829, 1830, 1898, 1689.

For the level determination of the varieties and (CHP) sweet clover of two field experiments were conducted on poorly saline soil (a salt content 0,2–0,3%) and in the southern carbonate black soil (control). In the first year, the survival rate of plants before leaving for the winter was 76,6–96,2% in the control, 70,6–93,9% in the saline soil. The best survival (77,5–93,9%) on the saline soils possess the varieties and CHP of Volgian sweet clover of Akbas and Bars, (CHP) KD-1829, 1689, 1793, 1831, 1732, 1690. In the second year on the saline soil the more yielded by green mass and dry matter were (CHP) Volgian sweet clover KD-1828, 1829, 1823, 1689, 1830, 1832, 1690, the excess over the standard grade was 16,3–51,1%, by the standard yield 91.5 c/ha and 24,7 c/ha respectively. The high agronomic resistance to salinity showed (CHP) Volgian sweet clover KD-1689, 1828, 1829, 1830, 1690, thus the decrease of the green mass yield in the saline soil was 3,8–9,0%, comparing the yield on the control samples. Thus, the combination of biological and agronomic salt tolerance is indicative for the (CHP) Volgian sweet clover KD-1828, 1829, 1689, 1830, and 1690. The best (HCP) of yellow sweet clover is KD-1829 that undergo preliminary multiplication and subsequently will be transferred to the state strain testing.

Comparative Transcriptome Analysis of ‘Koshihikari’ rice seeds before and after treatment of hot water disinfection method

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Keywords: heat stress, hot water disinfection, rice seed, RNA-seq, transcriptome

Seed disinfection is an indispensable step during rice cultivation process to prevent the emergence of seed-borne diseases in the rice plants. Hot water disinfection is an environmentally friendly disinfection method without expensive agricultural chemicals by only soaking the seeds to 60°C water for 10 minutes. To kill the pathogens effectively, higher temperature

or longer treatment is necessary but may suppress the germination of several rice cultivars especially Indica, which is widely cultivated in South East Asia. To spread the application of hot water disinfection method across the countries, understanding the heat-stress tolerance mechanism under hot water treatment in the seeds of Japonica rice is important. ‘Koshihikari’ is a Japonica rice cultivar that is able to maintain its high germination rates even after treatment at 70°C for 10 minutes (64.7%) compared to an Indica cultivar ‘Habataki’ (10%). Hot water treatments upon de-hulled seeds and seed embryos of ‘Koshihikari’ indicated that the tolerance feature was located inside the embryo. Comparative transcriptome analysis between the embryos of ‘Koshihikari’ before and after treatment of hot water at 53°C for 10 minutes using RNA-seq detected 153 and 26 up and down-regulated differentially expressed genes (DEGs) respectively. Gene Ontology enrichment analysis reveals 20 genes involved in DNA-binding (GO:0003677) which contained genes mostly related to heat-stress, germination and ethylene signaling pathway. Real-time RT-PCR analysis towards three selected genes included in the DNA-binding family showed that the expression of Hsf- C1b was up-regulated significantly after hot water treatment both in the seeds with or without hull compared to ‘Habataki’. Thus, genes expressing DNA-binding proteins for early germination and defense against heat-stress, one of which is OsHsfC1b, may play important roles to maintain ‘Koshihikari’ high seed germinability under severe condition of hot water disinfection.

The effect of drought on the activity of GS genes and protein content in barley grain

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Keywords: barley, drought stress, protein content, glutamine synthetase

Barley (*Hordeum vulgare* L.) is widely used as food or feed for animals. For malting and brewing purposes specific quality parameters protein content is very important (Gous et al. 2015), which is determined by the agronomic practise and by the enviroment. Excessive rates of nitrogen fertilization can increase protein levels. Hot and dry weather can cause higher protein content. Barley suitable for malting should have moderately low grain protein content (10–11.5%). High protein content will not only reduce malt extract, but also deteriorate final beer quality (Wu et al. 2015).

Tobin and Yamaya (2001) described the dependence between protein content and glutamine synthetase (GS) gene activity, which is a key enzyme for the synthesis and transport of nitrogenous substances in the plant. *HvGS2* gene and three isoforms of the *HvGS1* enzyme, which differ in localization and function within plant tissues, have been described in cereals. The expression of genes *HvGS1_1*, *HvGS1_2*, *HvGS1_3* can be indirectly influenced by abiotic stress associated with accelerated leaf senescence (Goodall et al., 2013).

Aim of this work is evaluation of GS gene expression and the resulting protein content in grains in relation with two different water conditions and two levels of fertilization. The

five genotypes of barley with different grain quality and drought tolerance are cultivated in a pot experiment in partially regulated conditions to assess genotype dependence on these processes. First variety Tadmor from Syrian is characterized by very good adaptation to drought, higher osmotic potential, high efficiency of water use and high protein content in grain (Tardy et al. 1998, Teulat et al. 1997). Other varieties Jersey (NL) and Sebastian (CZ) are varieties with a high malting quality. Two lines (1/3, 1/40) were derived from reciprocal crosses between Tadmor and Jersey variety.

Acknowledgments

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The effect of crop rotation on cellulolytic activity of soil under climate change conditions

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Keywords: southern carbonate chernozem, crop rotations, cellulolytic activity

At the present stage, one of the directions for improving cultivation technologies of agricultural crops is the transition to a biologized farming system that contributes to the protection of the environment and the production of environmentally friendly products. An important role in this process is played by fore crops, since they contribute to increasing the yield of cultivated crops and improving the balance of nutrients in the soil. In this regard, special attention is paid to such agro-practices as crop rotation. In the context of agricultural technology and chemistry, the advantage of crop rotations is well known, but it will be incomplete

without considering the microbiological processes occurring in soil, including cellulolytic activity. Therefore, the diversification in a crop rotation can potentially affect the group and numerical composition of soil microorganisms, which subsequently leads to a change in the level of biological activity of the soil. The study of cellulolytic activity was carried out at the field fixed-site laboratories of the crop rotation of LLP “SPCGF named after A.I. Barayev” in the steppe zone of Northern Kazakhstan in the period of in 2015–2016. The soils are southern carbonate chernozem. The four-course crop rotations with different crops alternation were selected for investigation: 1) pea-wheat-flax-barley; 2) rape-wheat-pea-wheat; 3) mustard-wheat-pea-wheat; 4) flax-wheat-pea-wheat. Cellulolytic activity was determined by the application method, where cotton goods were instilled into the soil on a depth of 0–30 cm in triplicate. The exposure lasted for 90 days. Then, the linen was removed from the soil, cleaned of the ground, dried and weighed. The intensity of the cellulose-decomposing activity of the soil was judged by the difference in weights of the control cotton linen unexposed to the soil and the decomposed cotton linen extracted from the soil, where the activity was expressed as a percentage (Kazeev, 2003). The conducted studies showed that the cellulolytic activity of the soil changed over the years depending on the crop rotation culture. In 2015, the intensity of cellulose decomposition under crop sowings in all studied crop rotations ranged from 7.6 to 24.8%, in 2016 – from 47.6 to 63.6%. Such differences can be explained by the weather-climatic conditions of the year. A more active decomposition of cellulose was preceded in the moistened conditions of 2016. Analyzing the data obtained, it should be noted that under the conditions of 2015, the active decay of cellulose was carried out in pea sowings after barley and amounted to 24.8% (crop rotation 1) and in pea sowings after wheat –13.3% (crop rotation 3). A similar phenomenon was traced in 2016 on the same crop rotations, but already with other fore crops. It should be noted that the cellulolytic activity changed along the layers of the soil profile. Depending on the crop rotation and culture, cellulolytic activity has increased with the depth, reaching a maximum in the 20–30 cm layer in crop rotations 1 and 2, and averaged 53.4% and 41.0% for two years, respectively. The cellulosic complex was represented by various physiological groups of microorganisms (fungi, bacteria and dominating actinomycetes). The most intensive process of cellulose decay was in 2015 in pea sowings after barley (24.8%) and in wheat crops after peas 63.6% in 2016.

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Creation of initial forms of spring soft wheat due to the climate change

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Keywords: spring wheat, gene pool, hybrids, climate

The importance of developing the issue of the consequences of climate change in agriculture is confirmed by the figures of economic damage caused by adverse weather conditions, which even in countries with a relatively stable and favorable climate accounts for more than half of the losses in all other combined industries [Martina, 2011; Menzhulin, 2009].

Agricultural production has always been and remains dependent on climate change occurring in nature. The grain belt of Northern Kazakhstan, basically, is located in the zone of so-called risky farming, characterized primarily by a short vegetation period and pronounced continental climate.

The creation of the initial forms of spring wheat took place in the zone of Northern Kazakhstan, the soil and climatic potential of which ensures the formation of high-quality grain, but does not guarantee the obtainment of a stable yield by years. Environmental conditions are very rarely favorable for the plants development in critical periods, both in terms of temperature and moisture. Data from the meteorological station at the SPCGF named after A.I. Baraev, over the past 20 years, confirm a gradual decrease in the amount of precipitation during the vegetation period and their extremely unequal distribution by months.

The world-gene pool of spring wheat with a known spectrum of variability of characteristics, varieties-donors on earliness, productivity, responsible for the reaction to photoperiod etc. is involved in the selection process to enrich the new initial forms with hereditary information.

As a consequence of the undertaken studies, the peculiarities of inheritance of the main quantitative characteristics in hybrids of different generations and the main regularities in the manifestation of the correlation dependence between the traits have been revealed, a high degree of positive transgressive variability in the cereal productivity of the main spike and the number of grains in the spike.

A different contribution of the factors “genotype”, “years”, “genotype x years” to the total variability in the main elements of the crop structure was obtained. Genetic formulas of parental forms and hybrids are composed representing the selection value. The presence of specific alleles of Gli-A1g, Gli-B1e, Gli-D1i and Gli-D1f in the created hybrids allows making a conclusion about their resistance to drought and diseases in the conditions of Northern Kazakhstan.

The new spring wheat hybrids that have undergone laboratory analyses and field studies are used to create new varieties adapted to the conditions of Northern Kazakhstan relative to the climate change.

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What we even want to know about CBF transcription factors in cereals...

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Keywords: CBF, transcription factor, cereals, RNA Sequencing, trichome

It was proved in earlier experiments, that two CBF transcription factors (namely CBF14 and CBF15) have outstanding role in the development of frost tolerance in wheat (Vágújfalvi et al. 2005, Knox et al. 2008). The genes encoding these transcription factors were isolated from winter wheat (*Triticum aestivum* L. ssp. *aestivum* cv. ‘Cheyenne’) then over-expressed in spring barley (*Hordeum vulgare* L. cv. ‘Golden Promise’) (Soltész et al. 2013) and spring wheat (cv. ‘Cadenza’) (not published yet). The experiments showed that transgene increased the frost tolerance; some known genes (*HvCOR14b*, *HvDHN5*, and *HvDHN8*) were up-regulated by TaCBF14 and TaCBF15, and the transgenic lines exhibited moderate retarded development, slower growth, and minor late flowering compared with the wild type, with enhanced transcript level of the gibberellin catabolic *HvGA2ox5* gene.

The TaCBF14 and TaCBF15 transgenic barley germplasm were selected after repeated frost tests. These lines are considered as valuable materials for further molecular biological analysis and functional genomics studies. The aim of our recent work is to understand the mechanism of this improved freezing tolerance at the molecular level, by RNA Sequencing method. The purpose of the research is to investigate which genes are under the control of the

given two CBF transcription factors, which genes belong to the CBF regulon. Furthermore, this analysis also highlights the functional differences in the CBF14 and CBF15 regulon. Monitoring of the transcriptome profiles might lead to the discovery of new CBF-dependent pathways.

The RNA Sequencing method determines the target genes and their expression level in the CBF-overexpressing transgenic barley lines comparing with the spring cultivar ‘Golden Promise’ (wild type). The wild type and the most frost tolerant TaCBF14 and TaCBF15 transgenic barley lines were grown in plant growth chamber in the Phytotron of MTA ATK Agricultural Institute, Martonvásár. After control conditions, plants were treated by short-term cold-treatment. Samples were collected under control conditions and after the short-term cold-treatment too. RNA samples with adequate quality were sent for RNA Sequencing analysis. Bioinformatics analysis of the results of RNA Sequencing will identify the genes belonging to the CBF14 and CBF15 regulon. The interesting target genes will be selected for further studies by Real-Time RT-PCR in barley and wheat lines too. Monitoring of the transcriptome profiles may lead to the discovery of new CBF-dependent pathways.

As the leaves of transgenic barley lines show hairy phenotype comparing to the hair-free wild type, the structure of the trichome were analysed by microscopic methods. In addition, the reason for the development of trichome is investigated *in silico* by bioinformatics methods and at gene expression level by the quantitative Real-Time RT-PCR.

Acknowledgments

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Relationship between *Fusarium* head blight and pigments in wheat grain

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Keywords: wheat, grain colour, anthocyanins, *Fusarium*, mycotoxin, deoxynivalenol

Cereals are of major importance in human and animal nutrition, and *Fusarium* head blight (FHB) caused by *Fusarium* species is a devastating fungal disease of wheat (*Triticum aestivum*) worldwide that causes great health and economic concern for the food sector and consumers. FHB may lead to significant grain yield losses and end-use quality reduction with contamination by mycotoxin deoxynivalenol (DON). Disease control and prediction is difficult because of the many *Fusarium* species associated with FHB. The development of resistant cultivars is the most effective way for managing this disease. Important target in breeding programmes is searching new sources for FHB resistance, which may be represented by the bioactive components in the grain. Cereal grains contain many phytochemicals, some of which significantly influence the colouration of grain. Different pigment accumulation in the grain is represented in the studied collection of wheat genotypes, which contains carotenoids and anthocyanins in blue aleuron, purple pericarp, white or red grain.

The aim of the research was to examine a relationship between FHB resistance and colour of wheat grain and compare differences in deoxynivalenol (DON) and symptomatic evaluation (SE) in total of 42 genotypes of bread wheat; 26 samples with coloured grain and 16 varieties with conventional grain colour. The wheat varieties in three replications were screened for FHB resistance using artificial inoculation technique by spore suspension of *Fusarium culmorum* under field conditions. SE for all genotypes was determined. DON levels in harvested grain samples were used to evaluate the FHB resistance of wheat cultivars and breeding lines. The occurrence of DON in 26 wheat samples with colored grain were compared with 16 wheat varieties with conventional grain colour of varieties using immunochemical determination by ELISA. Deoxynivalenol was detected in all samples at concentrations ranging from 5.5 mg/kg to 95.3 mg/kg and mean DON content was 24.0 mg/kg in 26 wheat samples with coloured grain and from 11.5 mg/kg to 50.3 mg/kg with mean DON content of 27.3 mg/kg in 16 wheat varieties with conventional grain colour.

The study determined that genotypes Tschermaks blaukörniger Sommerweizen (DON 8.4 mg/kg; SE 7.7) with blue aleuron and genotype ANK-28A (DON 5.5 mg/kg; SE 7.4) with purple pericarp showed the best resistance to *Fusarium sp.* among the studied collection of coloured wheat, compared to varieties with conventional grain colour, where the best genotypes achieved values of DON 13.7 mg/kg; SE 7.8 (Quintus) and DON 13.0 mg/kg; SE 7.4 (KWS Chamsin).

Although the results are very interesting in our study, the wheat genotypes with content of anthocyanins and flavonoids should be further studied and evaluated. Lower levels of *Fu-*

sarium mycotoxin contamination of cereals with coloured grain compared with conventional grain have been reported, but the causes of these differences are not well understood.

However, these genotypes could be promising candidates for breeding programs intended to obtain *Fusarium* head blight resistance.

Acknowledgments

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miRNAs involved in stress responses and senescence in glyphosate-treated maize are altered in herbicide-treated maize

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Glyphosate is an active compound of a systemic, nonselective and most widely used herbicide in the world – RoundUp. It causes broader range of physiological alterations than previously assumed and some plants gain higher level of resistance without the need to use genetic engineering methods. The holistic understanding of RoundUp mechanism of action is of great importance since it has been shown that glyphosate affects the growth of plants not only by inhibiting EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) but also through altering several crucial plant physiological processes (e.g., photosynthesis, carbon metabolism, mineral nutrition, oxidative events). However to understand the mechanisms of height-ened resistance to RoundUp we must first know the processes mediating the plants' death in response to glyphosate treatment.

To achieve precise and timely regulation of stress-responsive gene networks organisms have employed gene expression regulation by small RNA molecules (sRNAs). We performed miRNA expression profiling (Next Generation Sequencing) in maize leaves 7 days after RoundUp administration (late response to herbicide). We show that 12 miRNAs, belonging to miR167, miR396, miR159, miR156, miR169, miR444 and miR827 families, are significantly upregulated, and one, miR166, downregulated following glyphosate treatment. These miRNAs have been previously shown to be involved in abiotic stress responses and implicated in senescence. Moreover, our results suggest that prominent role in mechanism of glyphosate toxicity could be associated with the disruption of phosphate transport pathways (enabling glyphosate uptake into cells by using the transporters specific for Pi and thus diminishing Pi uptake) and regulated through the action of miR827-3p and miR444.

Heat-priming induced thermo-tolerance in wheat (*T. aestivum*) genotypes

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Keywords: chlorophyll fluorescence imaging, heat priming, heat tolerance, photosynthesis, wheat

The optimum growth temperature is around 20 °C for wheat. Pre-exposure of plants to mild heat stress may activate a “heat stress memory” in plants, which can facilitate a fast protective response to a subsequent high temperature stress event. Photosynthesis is one of the most heat sensitive processes in which both the carbon assimilation and the photosynthetic electron transport are affected. Therefore, the heat tolerance of genotypes is often characterized by the thermo-tolerance of the photosynthetic apparatus. In heat-susceptible genotypes, the heat stress inhibits the photosynthesis and promotes the senescence, while the heat tolerant varieties are able to maintain the photosynthesis and chlorophyll contents. Under severe heat stress, both the CO₂ assimilation capacity (due to the metabolic limitation of the carboxylation process) and the efficiency of the photosynthetic electron transport are reduced. The inhibition of PSII activity manifests in the changes of chlorophyll fluorescence signal, which is considered to be a sensitive and reliable method for the detection and quantification of the temperature-induced changes of the photosynthetic apparatus.

In the present study, the seedlings of winter wheat (*Triticum aestivum* L.) genotypes were pre-treated at a moderate (30 °C) temperature (heat priming, HP) then were subjected to a more severe high temperature stress (above 40°C) and the thermo-tolerance of the photosynthetic apparatus was studied in relation to the non-primed plants (NP). The plants experienced priming (HP) was associated with the elevated stomatal conductance (which indicates more opened stomata) and transpiration, while the CO₂ assimilation capacity changed less intensively. In spite of the fact that there was no significant difference in the effective quantum yield of PS II between the HP and NP plants under growth conditions, temperature-dependent decline in the photochemical utilisation of absorbed light energy was shifted to higher temperature in HP plants as compared to the NP plants. In addition, the high temperature stress resulted in a lower effective quantum yield of PS II in NP plants than those in HP. These later plants survived better the extreme high temperature stress. These results indicated that the heat priming effectively could improve the thermo-tolerance of photosynthetic apparatus in wheat seedlings subjected to a later high temperature stress. Genotype variations of these effects were also found and they will be presented.

Acknowledgments

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Diagnostic value of markers linked to the eyespot resistance genes in breeding lines of hexaploid wheat (*Triticum aestivum* L.)

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The aim of this study was to search the breeding collection of hexaploid wheat in terms of eyespot resistance identification. Eyespot is one of the common fungal stem-base disease of wheat (*Triticum aestivum* L.) and other cereals in temperate regions. The causal agents of eyespot are *Oculimacula acuformis* (syn. *Tapesia acuformis*; anamorph: *Helgardia acuformis* syn. *Pseudocercospora herpotrichoides* var. *acuformis*) and *O. yallundae* (syn. *T. yallundae*; anamorph: *H. yallundae* syn. *P. h.* var. *herpotrichoides*). Eye-shaped, elliptic lesions on the lower portion are the symptoms of this disease, which can weaken stem bases and cause them to bend or break. Crops from infected plants are slighter with reduced quality and yield of even up to 50%. There are three eyespot resistance genes (*Pch1*, *Pch2* and QTL on chromosome 5A), which have been characterized in hybrid forms of wheat. *Pch1* located on chromosome 7DL is the most important and most effective resistance gene and was introduced into wheat from the wild grass *Aegilops ventricosa*. However, *Pch1* locus is associated with lower yield in the absence of the disease, which is a limiting factor considering elite varieties breeding. On the other side, both *Pch2* and *Pch-QTL-5A* originate from the French wheat cultivar Cappelle Desprez, but confer only a moderate level of resistance and are unlikely to prevent yield loss under high disease pressure. The main aims of this study were (1) to screen the collection of 150 winter wheat breeding lines in order to identify genotypes with *Pch1*, *Pch2* and *Q.Pch.jic-5A* loci, (2) to analyze the resistance expression in those lines using inoculation tests at BBCH10-12 (seedling) and BBCH31-32 growth stage and (3) to compare the yield components with the results of markers identification and inoculation tests in order to evaluate the influence of the genotype on technological values of breeding lines. We have identified 7 genotypes carrying cumulated two or three *Pch* genes. The results of inoculation tests confirm the resistance expression in those genotypes.

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Multivariate analysis of yield, yield component traits of Iranian wheat genotypes grown under full and less irrigated conditions at two locations

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Keywords: wheat, breeding programs, drought stress, factor analysis

Field experiment was conducted at Shahed and NIGEB sites, Iran in cropping seasons of 2014 and 2015 to study the impact of less and full irrigated conditions on agro-morphological traits in 30 bread wheat genotypes. Plots were arranged on basis of randomized complete block design with three replications. Combined analysis of variance revealed a significant difference among genotypes and different locations for all the traits studies. Disregarding spike density and spikelet number per spike, rest of traits were noticeably influenced by water stress. A diverse correlation responses pattern was depicted as for different environments. Factor analysis based on principal component analysis method and varimax rotation at Shahed field under irrigation regimes indicated 4 important factors accounting for around 78.20 and 77.68 percent of the total variation among characters for full and less irrigated conditions, respectively. The cumulative variation at NIGEB site was 62.65% for 4 factors in normal irrigation and 84.79% for 5 factors in less irrigated condition. This study believes that characters such as, plant height, peduncle length, spike density, 1000-grain weight, harvest index and biological yield can be considered as a selected suitable criterion in wheat breeding programs and production of prolific varieties.

Optimization of *Brachypodium distachyon* cultivation *in vitro*

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Keywords: model object, callus, optimization

Wild cereal *Brachypodium distachyon* L., as model object for breeding purposes is used in Kazakhstan for the first time in order to increase wheat resistance to *Puccinia recondita*. Standard line Bd21 seeds were obtained from the RIKEN BioResource Center (BRC; Japan). The objective of this work was choice of the explant and optimization of the culture medium

for cultivation *in vitro*. Three types of extracts were used: 1) the inflorescences and unripe germs allocated from green ears of a vegetating plant; 2) the isolated germs from mature seeds of the PSB 00001 0049 line; 3) nodal segments of sprouts of the young plants received from the initial seeds grown in the conditions of the greenhouse. The mode of sterilization of plant material was developed depending on the explant nature. Allocation of germs was carried out using a bipolar magnifying glass. The isolated germs were placed on a surface of a nutrient medium with scutum down. For introduction into culture side seedlings of plants 5 cm long were cut off with 3-4 interstices. As the main nutrient medium for cultivation two mediums were tested: Linsmeier and Skoog (LS), Murashige and Skoog (MS) with introduction of various concentrations of phytohormones: 2,4-dichlorophenoxyacetic acid (2,4-D), β -indolyl-acetic acid (IAA), 6-benzylaminopurine (BAP), kinetin. In order to initiate callusogenesis LS1 medium with addition of 2,5 mg/L of 2,4 D is picked up. Use of LS and MS nutrient mediums at use of various phytohormones led to regeneration of shoots in callus: 1. LS2 with addition of 0,5 mg/L of BAP; 2. LS3 and IAA hormone 0,5 mg/L + 0,3 BAP mg/L; 3. LS3 and phytohormones – 0,5 mg/L of IAA + 0,3 BAP mg/L. When cultivating unripe inflorescences callus formation is noted in LS1 with introduction of 2,4 D in concentration of 2 mg/L. Callus has light yellow coloring and friable structure. At further cultivation small accumulation of biomass without visually visible structural changes was noted. Since maturing of wheat grain within one plant happens unequally, it was difficult to isolate the germs which are at the one stage of development. In this regard 0.3-0.4 mm long unripe germs allocated from green cones, more mature germs (≥ 0.5 mm) were isolated from moist wheat grain. Callus formation in the scutum area of the isolated germ is noted on day 20–25 day of cultivation on LS inducing environment with 2 mg/L of 2,4 D. Callus yield from unripe germs does not exceed 37.5%. Callus is not dense and not friable. After 30 days of cultivation on LS1, obtained callus was passed on LS2 with 0.5 mg/L of BAP. Obtained callus is more dense, nodular structure of yellowish coloring, but, regeneration of shoots in obtained callus is not noted.

Acknowledgments

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Antioxidative system activity as the marker for freezing and drought tolerance in winter barley (*Hordeum vulgare* L.)

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Keywords: abiotic stress tolerance, doubled haploids, antioxidative enzymes, low molecular weight antioxidants

Any disturbance in physiological homeostasis of a plant can lead to the overproduction of reactive oxygen species (ROS), often resulting in a cascade of uncontrolled oxidation. For that reason plants evolved a sophisticated defence system, in which the most important role is played by antioxidative enzymes – superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) – working in concert with low molecular weight (LMW), non-enzymatic antioxidants to protect plant cells from oxidative damage. In the presented study, the role of the antioxidative system in plant acclimation to low temperature and drought was studied with the use of doubled haploid (DH) lines of winter barley (*Hordeum vulgare* L.), produced by anther culture method from F1 generation of Polish breeding materials. Ten DH lines selected for the study showed significant variation in respect of freezing and drought tolerance level (Gołębiewska-Pikania *et al.* 2017a, b).

A generally uniform mechanism of plant defence against oxidative stress induced by low temperature and drought was revealed. Analysing the effects induced by freezing tolerance acquisition (20-day cold hardening of seedlings at 4/2°C (day/night)) and drought treatment (14 days at 35% soil water content at the generative phase of development) in selected DH lines of barley, it could be supposed that higher tolerance to both studied stress factors was associated with stable (drought) or increased (freezing) SOD activity. Moreover, although both stress factors significantly decreased CAT activity in all studied DH lines of barley, the amplitude of changes was significantly smaller in the case of more tolerant barley genotypes. In contrast, a greater increase in the activity of POX in cold/drought treated plants was characteristic for less tolerant DH lines of barley, whereas the activity of LMW antioxidants was genotype-dependent without clear relation to stress tolerance level.

The results obtained indicate a potential for using the analysed parameters as physiological markers of freezing/drought tolerance level in winter barley.

Acknowledgments

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T8: FOOD SAFETY AND QUALITY

Oral presentations

Development and characterisation of wheat with low-viscosity grain extracts using novel alleles of IRX9 orthologue TaGT43_2

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Viscosity of extracts from wheat grain is a detrimental trait for non-food uses, leading to sticky deposits resulting in down-time for whisky distilleries and necessitating enzyme treatment in animal feed. In wheat (unlike barley) extract viscosity is almost entirely due to the soluble fraction of the cell wall polysaccharide arabinoxylan (AX). We have shown that suppression of genes responsible for synthesis of AX can decrease extract viscosity by 6-fold using RNAi driven by an endosperm-specific promoter [Freeman et al., 2016, *Plant Biotech. J.* 14: 109-116]. In order to develop a non-GM variety with the low viscosity trait, we decided to look for non-functional alleles of these genes using TILLING. However, AX is a vital component of every cell wall in wheat so we needed a gene target where constitutive knock-out would only affect the grain tissues that produce the water-extractable AX (WE-AX) responsible for viscosity. From expression data, we selected TaGT43_2 as a suitable target; TaGT43_2 is one of several orthologues in wheat to IRX9, a glycosyl transferase 43 family gene required for xylan extension in Arabidopsis. Using high resolution melt analysis approaches [Botticella et al., 2011 *BMC Plant Biol.* 11, 156], we identified alleles with premature stop codons in all three homeologues of TaGT43_2 in the mutagenised Cadenza population. We crossed lines carrying these alleles and selfed them to produce lines with homozygous stacks of aabbDD, AAAbdd, aaBBdd and aabbdd mutants. We found that the amounts of WE-AX and extract viscosities from the grain of these lines were significantly decreased in one of the double stacks and substantially decreased in the triple stack. We are currently studying these triple stack plants in more detail to characterise transcriptome and cell wall effects. Limagrain have introduced these novel alleles into their elite breeding streams and are backcrossing to minimise background mutations. We will be yield testing the MAS converted lines for any negative pleiotropic effects. We are on course to deliver one of the first new wheat varieties developed using reverse genetics.

Kebari, Gluten-free barley: from lab to market

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Gluten proteins in cereals are a serious health challenge for people with coeliac disease and the less well understood non-coeliac gluten sensitivity. Many more choose to avoid gluten. Coeliac disease occurs in about 1% of the population, and requires lifelong exclusion from the diet of the various gluten proteins found in wheat (gliadin and glutenins), barley (hordeins), rye (secalins), and for some individuals also oats (avenins). Untreated coeliacs suffer from painful intestinal malignancy greatly damaged mucosal villi, and resulting in poor absorption of nutrients. They frequently suffer from low bone density, and their alternative diets are often low in fibre and high in fat and sugar. To address these issues we have combined null mutations of each of three classes of hordein. The triple-null barley lines, called *Kebari*, have more than 10,000 fold reduction in gluten compared to control barley¹. The initial hulled *Kebari* lines had smaller grains, making processing and malting less efficient. With further breeding the seed weight has been largely recovered, and the agronomic performance is approaching commercial malting barley. We have also developed a hull-less version of *Kebari* suitable for use in the food industry, in order to make healthy wholegrain cereal options for those who must avoid gluten. The hordein levels in these *Kebari* lines, even as pure grain, are well below the World Health Organisations recommended level of 20 ppm for classification as gluten free¹. *Kebari* is already being used in Germany to make a gluten free barley beer, and it is anticipated a growing number of food and beverage options will soon become available for the estimated 5% of people in the world who suffer from coeliac disease or non-coeliac gluten sensitivity.

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Dough and bread qualities of waxy and high-amylose wheats and the nutritional and functional improvements of these wheats during germination

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Keywords: waxy wheat, high-amylose wheat, breadmaking, functional properties, germination

Starch is major component of wheat, therefore in order to improve eating quality or food processing of wheat, the characteristics of wheat starch must be investigated. Recently, waxy (amylose-free, 0~3%) (Yasui et al. 1997; Morita et al. 2002) and high-amylose (Yamamori et al. 2000; Pham et al. 2015) wheats which had unique starch functional properties were produced through the development of new physicochemical and biological techniques. Waxy wheat flour (WWF) and high-amylose wheat flour (HAF) were characterized and used as a substitute for normal wheat flour in breadmaking; formation of resistant starch (RS) in bread during storage and retardation of bread staling were determined. Substitution using HAF decreased peak and final viscosities, breakdown, and setback of the dough. Dough with HAF substitutions were weaker and less elastic, and absorbed more water than those of the normal wheat flour. Against our expectations, bread made from WWF and HAF had significantly lower specific volume and large gas-cell distribution as compared to that from commercial wheat flour (CWF). However, the WWF bread was significantly softer than that of the CWF bread during storage. After baking, RS contents in breads with 10%, 30%, and 50% HAF substitutions were 1.6%, 2.6%, and 3.0% (db), respectively, higher than that of the CWF (0.9%, db). In addition, whole WWF had lower protein and lipid contents, but higher dietary fiber content. Bread made from the WWF had significantly lower specific volume and was significantly softer during storage. The addition of pentosanase improved loaf volume of bread and increased the firmness of breadcrumbs.

Germination was considered to improve the nutritive value, antioxidant capacity, and functional properties of the grains because germination is the start of life activity. Therefore, changes in the chemical composition, nutritive value and antioxidant capacity of WWF and HAF during germination were examined. Results showed both germinated WWF and HAF had a better nutritional composition, such as higher dietary fibre, free amino acid, and total phenolics, than ungerminated wheat flours.

The amounts of soluble dietary fiber as well as essential and functional amino acids (including isoleucine, leucine, phenylalanine, valine and gamma-amino butyric acid) in the 48 h-germinated wheat increased by 3–10 times. Total phenolic contents of both free and bound phenolics and their antioxidant capacities significantly increased after 24 h of germination and the free lipids in germinated HAF increased with increased germination times.

From the results described above, germinated WWF and HAF should be used to improve the nutritional and functional qualities, including antioxidants, of cereal-based products.

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Poster presentations

Reduction of yield and mycotoxins accumulation in oats genotypes after *Gibberella intricans* Wollenw. (*Fusarium equiseti*) inoculation

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Keywords: oats, inoculation, mycotoxins, *Gibberella intricans*

Gibberella intricans obtained from kernels of oats (Kosiak et al. 2005, Mielniczuk et al. 2015) may contaminate it by the trichothecenes from groups A and B (Mielniczuk 2015). Due to the limited information on the harmfulness of this pathogen for oats, the field experiment on Fusarium panicle blight of oat caused by *G. intricans* was conducted. A strictly controlled experiment with panicle inoculation was conducted in 2015–2016 in experimental fields near the city of Zamość. The study included 8 genotypes of oats prepared for the practice by breeding specialists: DC 06011-8, DC 07116-11/2, DC 14-8, POB 4109/10, POB 6020/10, POB 961-1344/13, STH 2.388, STH 2.102 and *G. intricans* strain No. 20. The harmfulness of *G. intricans* to panicles of these genotypes was determined on the basis of a field experiment with inoculation of panicles with *G. intricans* No. 20 during flowering. The method of inoculation was the same as in the case of oats (Mielniczuk et al. 2015). After the harvest the yield, number of kernels per panicle and the 1000 kernels weight were measured and compared with controls and the chemical analysis of the mycotoxins in grain of 8 oat genotypes was made. All yield traits as well as the metabolite concentrations are presented in Table 1.

Table 1. Number of kernels per panicle (KN), yield (Y), 1000 kernels weight (TKW), concentrations of trichothecenes from groups A and B, and repeatabilities (mean and range) for 8 oats genotypes inoculated with *G. intricans* in years 2015–2016

Trait	2015		2016	
	Mean	Range	Mean	Range
KN	72.61	57.1-87.9	38.58	19.80-55.8
Y (g)	21.20	16,51-27,49	11,94	7,03-13,65
TKW (g)	28,81	23,91-32,62	31,82	24,07-36,56
T-2 toxin ($\mu \cdot \text{kg}^{-1}$)	not detected	not detected	0,343	0,00-2,74
HT-2 toxin ($\mu \cdot \text{kg}^{-1}$)	51,00	7,00-37,00	8,84	0,40-30,67
DAS ($\mu \cdot \text{kg}^{-1}$)	0,375	0,00-1,00	2,230	0,42-6,55
STO ($\mu \cdot \text{kg}^{-1}$)	80,375	75,00-86,00	5,234	1,35-12,41
T-2 triol ($\mu \cdot \text{kg}^{-1}$)	1.125	0.00-3.00	0,264	0,00-1,54
T-2 tetraol ($\mu \cdot \text{kg}^{-1}$)	6.00	2.00-17.00	6,285	0,17-22,52
DON ($\mu \cdot \text{kg}^{-1}$)	20.00	17.00-31.00	16,193	0,00-34,52

Trait	2015		2016	
	Mean	Range	Mean	Range
3-Ac DON ($\mu\cdot\text{kg}^{-1}$)	22,30	22.00-24.00	not detected	not detected
15-Ac DON ($\mu\cdot\text{kg}^{-1}$)	16.00	15,00-17.00	3,334	0,00-13,40
NIV ($\mu\cdot\text{kg}^{-1}$)	74,13	39.00-119.00	111,76	0,00-519,10
FUS X ($\mu\cdot\text{kg}^{-1}$)	24.40	24.00-27.00	4,903	0,21-36,19

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Hungarian small grain cereals to serve craft beer brewing.

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Keywords: barley, einkorn, triticale, brewing

Main aim of our project was to set up a complex innovation system which gives a chance to produce high value special organic beer made of einkorn. Therefore all the stakeholders of the production chain from breeding till the end user brewery were involved in the innovation. This kind of research resulted in a marketable high value organic beer product. It belongs to the recently favourable beverage fashion of healthy foods in a premium category market niche. The field technology for successful einkorn production and growing seed production was developed thanks to this project. As no long cultivation knowledge was in the background, several technological steps were still open. To be able to set up economically seed production technology, half-farm and farm scale experiments were carried out to optimize several important factors, such as plant density, fertilizer needs, optimal harvest time, etc. The technology improvement was run together with the development of seed production system under diverse ecological conditions.

Our new R & D & I work in Elixbeer project is aimed at creating an organic beer brand based on Hungarian Cereal varieties, aiming at the development of special organic beers and malted drinks and organic malt.

Several type of organic cultivation of malting barley, triticale, wheat and rye will be tested on farm level at Körös Maros Biofarm Ltd. under supervision of GK Ltd. and MTA ATK and a new organic cultivation technology will be developed.

The preceding Alkobeer project focused on organic bred einkorn wheat and on the organic einkorn beer made from it. The compliance of organic ingredients and the brewing process is certified over a batch certificate. The batch certification process covers the whole value chain starting from the breeding of modern einkorn varieties, across the recipe and technology development, the production and purchasing of row materials, the brewing and the trading process. The einkorn beer is certified organic, from the field to the bottle.

Researchers of MTA ATK focused their activity mainly on organic einkorn breeding in ALKOBEEER project. MTA ATK produced several new crossing combinations using einkorn lines, to produce new einkorn varieties for brewing. The results of their activity are two new einkorn genotypes. One of them, Mv Menket was registered in 2011. Mv Menket is a semi-dwarf einkorn variety with outstanding lodging resistance, recommended for intensive organic farming. The new line, MvA6-13, is a traditional type of einkorn. It was applied for variety registration in 2013. MvA6-13 is a high yielding quality with low protein content. Methods of maintenance breeding and organic seed production of einkorn were developed by MTA-ATK.

Cereal Research Ltd. has also strong crop plant breeding programmes, producing a wide range of varieties, two row, six row, feed, malting barley, triticale and wheat which are successfully marketed across Eastern Europe. Our spring type malting barley varieties GK Habzó and GK Toma are potentially promising raw materials for craft beer brewing.

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Assessment of Near Infrared hyperspectral imaging for the detection of fraudulent adulteration of durum wheat kernels

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Keywords: durum wheat, fraud detection, morphology, NIR hyperspectral imaging, protein, vitreousness

Several major pasta production countries, as Italy, France or Greece have decided that only pasta produced from Durum wheat – DW (*Triticum durum*) is permitted and that the use of common wheat – CW (*Triticum aestivum*), should be considered as fraud. Mixtures of DW and CW can happen in some cases of supply problem or depending of the fluctuation of prices. According to current Italian regulations¹, only a maximum of 3% of CW is allowed

to account for cross-contamination that may happen during post-harvest steps. Efficient analytical methods for the detection of accidental or intentional contamination of CW to DW products within the limits defined by the regulation are therefore required. Until date, all the studies dealing with the detection of CW in DW use macroscopy, microscopy or molecular biology based methods. In the present work, NIR (Near Infrared) hyperspectral imaging has been studied for the discrimination at the kernel level between both species of wheat.

For this study, 77 samples of DW and 180 samples of CW collected respectively in Italy and Belgium in 2015 and 2016 were analysed using NIR hyperspectral imaging. NIR images at kernel level (16 kernels) and at bulk level (200 g subsample) were acquired for each sample.

To discriminate DW from CW, 4 approaches were studied based on morphological criteria, NIR spectral criteria, protein content (< 12% or > 12 %) and ratio vitreous/not vitreous kernels. Partial Least Squares Discriminant Analysis (PLS-DA) was used as classification method for the construction of the discrimination models. Models were developed on samples collected in 2015 and validated on samples collected in 2016. Once the models were built, they were applied either on the morphological criteria or to all the spectra at pixel level of the images. The results are presented at the kernel level (4112 kernels) and at the sample level (257 samples of +/- 4000 kernels) based on the individual approaches or by combining the approaches.

This study has shown the potential of NIR hyperspectral imaging combined with chemometrics to propose solutions for sorting kernels at the entrance of the production chain according to the species (morphological and spectral criteria), the protein content and the vitreousness.

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