

BOOK OF ABSTRACTS



7th Conference on Cereal Biotechnology and Breeding
7-9 November 2023

18th EWAC
The European Cereals Genetics Co-operative Conference
6 November 2023

Wernigerode, Germany

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CONTENTS

EWAC18 – Oral	1
EWAC18 – Poster	17
CBB7 – Invited	23
CBB7 – Oral	32
T1: Genetic Resources for Crop Improvement	32
T2: Environmental Adaptation	40
T3: Biotic Stress Response	51
T4: Plant-Microbe Interactions	52
T5: Yield and Quality Improvement	54
T6: Bioinformatics, Genomics and Genome Editing	57
T7: Phenotyping Technologies (Generally)	63
T8: Phenotyping Technologies – Wheat Initiative	67
T9: Phenotyping Technologies (CePPG)	75
CBB7 – Poster	81
T1: Genetic Resources for Crop Improvement	81
T2: Environmental Adaptation	89
T3: Biotic Stress Response	100
T4: Plant-Microbe Interactions	108
T5: Yield and Quality Improvement	112
T6: Bioinformatics, Genomics and Genome Editing	119
T7: Phenotyping Technologies (Generally)	125
T8: Phenotyping Technologies – Wheat Initiative	127
T9: Phenotyping Technologies (CePPG)	128

EWAC18 – Oral

Effect of plant age, light-spectra, and winter/spring vernalization alleles on the cold acclimation of barley photosynthetic apparatus

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Keywords: blue light, far-red light, cold acclimation, vernalisation, barley

Cold temperature has a Janus-face effect on winter-hardy cereals: cold acclimation increasing their frost tolerance, while it also induces vernalization, resulting in a vegetative-generative transition. The degree of cold hardiness is determined by the CBF-regulon while the vernalization requirement is determined by the VRN1 gene. VRN1 has an epistatic effect on the CBF genes, as plants lose their freezing tolerance when the vernalization requirement is fulfilled. However, sudden frost spells could occur both during the vegetative-generative transition phase as well as when the generative transition has completed. By the approaching of the cold season in temperate climate, red:far-red (R:FR) photon ratio decreases, while blue:red (B:R) photon ratio increases in the sunlight spectrum. In contrast to this, from spring to summer, changes with the opposite direction could be observed. Many publications discuss the influence of light spectra on the development of frost tolerance in cereals. Recently, we reported that when frost-tolerant winter barley genotype ‘Nure’ illuminated by White light (W) with FR enrichment (WFR) increased its frost tolerance. The process was more successful, when WFR light was further enriched with blue light. These effects have been observed even at 15°C, where the cold-induced acclimation processes are mostly inactive. However, this effect is known to be negligible in spring varieties. Diminished freezing tolerance can also result from less efficient cold acclimation of the photosynthetic apparatus, however, the spectral dependence of the process is less investigated. Chlorophyll fluorescence parameters could serve as a physiological marker of photosystem II acclimation to cold as well as an indicator of freezing damages. Therefore, we aimed to investigate how winter/spring VRN1 alleles (*vrn-H1/Vrn-H1*) influence the light-induced cold acclimation of the photosynthetic apparatus of barley plants under different spectral illumination (W, WFR, WFRB) at 15°C for 10 days after a pre-growing phase in W. The pre- and early post-freezing chlorophyll fluorescence parameters of detached leaves were compared in vegetative-generative transition- and early generative developmental phases between two reciprocal Near Isogenic Lines (Cod.43s: Nure background carrying the Tremois Fr-H1 and Nure Fr-H2 alleles; Cod.30s: Tremois background carrying Nure Fr-H1 allele and Tremois Fr-H2 alleles) and the two parents. Interestingly the Nure *vrn-H1* allele improved the quantum yield of photosystem II in the Tremois background after freezing, which was further enhanced by WFR treatment.

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Candidate genes for seed longevity of barley stored in the ambient storage

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Keywords: longevity, ambient storage, barley, candidate genes

Investigation of seed longevity under ambient storage conditions is time consuming as seeds age slowly and lose 50% seed germination after about 9.2 years. However, we unique gene-bank material multiplied in 2014 and stored at 20°C and 15% relative humidity to identify candidate genes that affect longevity of barley seeds under ambient storage conditions.

The so-called “Ecoseed panel” (184 spring barley accessions from 23 countries) was genotyped by Illumina 9K. For phenotyping, seed vigour including percentage of total seed germination (%TG) and normal seedlings (%NS), the the area under the germination curve (AUC), the time to reach 50% (T50) and 10% (T10) of maximum germination were recorded according to ISTA rules in 2014, 2018, 2022 and using the curve-fitting module of the Germinator software (Joosen et al., 2010). In total, 67 significant ($-\log(p) > 5$) marker trait associations (MTAs) were revealed based on the FarmCPU model and candidate genes were identified.

We observed changes of allelic effect of the MTAs. For example, the marker 11_10357 co-localized with the candidate gene *Horvu.MOREX.1H01G499100* that encode thioredoxin has no significant allelic effect on %TG in 2014, but in 2018 the allelic effect was the highest -8.54 [$-\log(P_M)=10.87$] and then declined to -6.30 [$-\log(P_M)=5.90$] in 2022. Thus seed ageing impacts of wide range of genes at different stages during storage. Overall, we identified new candidate genes as key regulators for longevity of seeds stored under ambient conditions in barley that can be functionally validated in the further investigations.

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Current status of wheat drought tolerance investigations in Ukraine

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Keywords: wheat, draw tolerance, *TaSnRK2.8*, *Dreb-1*

In the changing climate conditions drought tolerance is one of the most valuable traits for wheat yields in Ukraine. From the beginning of the 21st century desertification occurred in the South Steppe region of Ukraine and growing zones changed for 100 km inside the country. On the other hand, rare rains became more severe and soil can't swallow the whole water amount after the rain. Historically old Ukrainian varieties were well adapted to the high temperature and drought conditions, but was high and logged. After introduction of dwarfing genes with the help of breeding, the average plant height decrease, but there was no molecular breeding program directed to the increase of drought tolerance. The presence of drought tolerance/susceptibility genes still remains unknown in the genetic pool of Ukrainian wheat varieties, because of the lack of investigations on molecular-genetic level attended to the fields and laboratory experiments.

The aim of our work is to apply known molecular markers for the drought tolerant genes to the Ukrainian varieties genepool and to identify effects in artificial and natural environmental conditions of Ukrainian South Steppe zone.

Wheat varieties adapted for Kherson Steppe region created in the Institute of Irrigated Agriculture of NAAS have grown in the drought (rainfed) and under the irrigated conditions during the 2016, 2017, 2018 years. Drought tolerance index was assigned as ratio between the average values of yield in the stress and in the irrigated (control) conditions. Wheat varieties that did not decrease the yield significantly in the drought conditions in comparison with the irrigated conditions were classified as drought tolerant. The average yield of the studied varieties of bread winter wheat on irrigated land was 2.4 times higher than on rainfed land over three years of observation.

Genotype x Environment interactions observed, whereas significant effects detected in one year of investigation were not confirmed in the other years. For example, wheat varieties with the *G* allele at position 5917 of the *TaSnRK2.8-A* gene had higher yields grown under drought conditions in 2018, but no significant differences were found under drought conditions in 2016 and 2017. Genotypes *aa* (amplification fragment of 1113 bp with P21F/P21R) for the *Dreb1-A* gene were more productive under irrigation in 2016 and 2017, then plants with *nn* (null) alleles, but in 2018 no significant yield differences were detected. In the rainfed environment *Dreb1-A aa* and *nn* genotypes did not differ in yield.

During artificial drought (12% PEG 6000) in laboratory conditions significant differences between the varieties with different alleles of *TaSnRK2.8-A* and *Dreb1-A* were not found for coleoptile, roots and shoots length. According to our data *Dreb1-A* molecular markers did not permit to distinguish drought tolerant/susceptible genotypes in the investigated genetic pool of varieties, whereas *TaSnRK2.8* can be used.

Our work shows the possibilities of approbation of modern molecular markers but require application of genome-wide association study for unique determinants investigations.

Allelic-polymorphism of *Gli-A1*, *Gli-B1* and *Gli-D1* loci in Ukrainian wheat varieties revealed by molecular markers and their correspondence to the allelic variants of gliadins

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Keywords: bread wheat; *Gli-A1*, *Gli-B1*, *Gli-D1* loci; molecular markers.

Polymorphism of *Gli-A1*, *Gli-B1* and *Gli-D1* loci was analyzed using allele-specific primers developed by Zhang et al. (2003), and primers to the *Taglgap* microsatellite on the world collection of bread wheat cultivars, which reflects the maximum diversity of allelic variants of gliadins encoded by the *Gli-B1* locus (provided for research by Dr. E. Metakovskiy (Universidad Politécnica de Madrid)) and on the modern Ukrainian collection of cultivars and lines of bread wheat from different Ukrainian breeding centers.

According to the PCR with allele-specific primers to the *Gli-B1* locus results, a polymorphism of the amplification fragments of the *Gli-B1.1* and *Gli-B1.2* alleles, caused by a microsatellite within the amplified sequence (Devos et al., 1995), was revealed. Seven alleles were sequenced, the obtained nucleotide sequences confirmed the presence of a polymorphic microsatellite with a CAA motif with the number of repeats from seven (Gabo cultivar) to 31 (Chinese-spring cultivar). Additionally, a *Taglgap* microsatellite primer pair was used, and 12 alleles in the worldwide and Ukrainian collections of bread wheat cultivars were detected. The correspondence between “SNP allele of the *Gli-B1* locus – allele of the *Taglgap* microsatellite – allelic variant of gliadins” was revealed.

To analyze the genetic polymorphism of the *Gli-A1* locus, two pairs of allele-specific primers developed by Zhang et al. (2003) were applied. In contrast to similar primers for the *Gli-B1* locus, the amplification fragments of *Gli-A1.1* and *Gli-A1.2* alleles were of the same length. Based on the results obtained for the *Gli-A1* locus, correspondence between the *Gli-A1.1/Gli-A1.2* alleles and allelic variants of gliadins encoded by the *Gli-A1* locus was established. As a result of bioinformatic analysis, a microsatellite, which is part of the coding sequence of the *Gamma gliadin-A1* gene was found, and a pair of primers MsA1 was developed. Eight different alleles that correspond to allelic variants of gliadins encoded by the *Gli-A1* locus were identified.

According to the results of PCR with allele-specific primers for the *Gli-D1* locus, in comparison with the results for the *Gli-A1* and *Gli-B1* loci, a significant number of heterogeneous cultivars were found. Correspondence between alleles determined in PCR with allele-specific primers to the *Gli-D1* locus has not been established.

Obtained results demonstrate application of molecular markers for detecting of allelic variants of gliadins encoded at *Gli-A1*, *Gli-B1* loci using PCR.

Genetic diversity in wheat pre-breeding program at NARDI Fundulea

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Keywords: synthetic amphiploids, *Aegilops tauschii*, MAS, pre-breeding

The wheat pre-breeding program at NARDI Fundulea is focus on two directions: (1) use of wild species to introduce genetic variation into modern wheat cultivars/lines; (2) molecular marker assisted selection to pyramid/cumulate the traits of interest such as resistance to biotic and abiotic stress, quality and yield in wheat genotypes.

In case of the first objective, synthetic amphiploids have been developed by hybridizing tetraploid durum wheat with *Aegilops tauschii*, followed by hybridization with *Triticum aestivum* L. cultivars.

Molecular markers assay revealed that some synthetic amphiploids carry the resistance gene to *Zymoseptoria tritici*, *Stb16q* (E1A, E16A, E18A and E28A amphiploids); a QTL for heat resistance (Heat_ chr6D_6276646) in E2A, E3A, E5A, E7A, E20A, E21A, E26A, E29A and E30A) and possible new alleles variants in case of two QTLs, previously reported in literature, associated with chlorophyll content, located on 2D (SSR markers CFD53 and BARC228) and 5D (SSR markers WMC215) for E1A, E16A, E17A, E18A and E19A.

Also, this year, under artificial inoculation field with teliospores, eight synthetic amphiploids (E5A, E15A, E22A, E26A, E30A, E32A, E34A and E35A) showed resistance to *Tilletia spp.* The percentage of infected heads, for these samples, ranged from 0% to 5%.

Regarding the markers assisted selection for disease resistance genes to rusts, common bunt, septoria tritici blotch, resistance genes/QTLs to heat and drought (osmoregulation *or*, *TaSnRK2.3-1B*, *TaSnRK2.8* and *TaSnRK2.9*), genes/QTLs for protein (*Gpc-B1*) and yield (*TaGASR7-A1* and *TaSBEIII-A*) highlighted wheat elites with four, five, seven and eight cumulative favorable alleles (Table).

Another approach in the pre-breeding program was the epicuticular wax layer, a trait for plant protection. Our study showed significant role of the *Iw2* locus, clearly separating the wax and non-wax genotypes from the winter wheat (*Triticum aestivum* L.) except pre-breeding lines obtained by crossing with wild species related to wheat, such as *Aegilops ventricosa* and *Agropyron junceum*. These results further require more complex study regarding the composition of the epicuticular wax layer in the adaptation of wheat to unfavorable conditions.

These studies prove the importance of wheat pre-breeding program regarding the genetic diversity and value of MAS breeding strategy, for new wheat cultivars development, in an uncertain climate.

Table Favorable alleles present in wheat elites

Name of wheat elite	Genealogy	Disease resistance alleles	Favorable alleles for abiotic stress, quality and yield component	No. of favorable alleles
17061G2-3	11248G2-2/13248G4	<i>Lr34/Yr18//Sr57/Pm38/Ltn1/Bdv1 + Lr37/Yr17/Sr38 + Lr46/Yr29//Sr58/Ltn2 + Stb16q</i>	NA	4
17061G2-5		<i>Lr34/Yr18//Sr57/Pm38/Ltn1/Bdv1 + Lr37/Yr17/Sr38 + Lr46/Yr29//Sr58/Ltn2 + Lr68/Ltn4</i>	NA	4
17061G2-6		<i>Lr34/Yr18//Sr57/Pm38/Ltn1/Bdv1 + Lr37/Yr17/Sr38 + Lr46/Yr29//Sr58/Ltn2 + Lr68/Ltn4 + Stb16q</i>	NA	5
18022G-4	11248G3-1/13248G4	<i>Lr34/Yr18//Sr57/Pm38/Ltn1/Bdv1 + Lr37/Yr17/Sr38 + Lr46/Yr29//Sr58/Ltn2 + Lr68/Ltn4 + Yr36</i>	<i>or+Gpc-B1</i>	7
18022G-11		<i>Lr46/Yr29//Sr58/Ltn2 + Lr68/Ltn4 + Yr36</i>	<i>or+Gpc-B1</i>	5
18148G1-3	DURES-118-1/BOGDANA	<i>Lr37/Yr17/Sr38 + Lr46/Yr29//Sr58/Ltn2</i>	<i>TaSnRK2 (2.3-1B, 2.8-5A și 2.9-5A) TaGASR7-A1 (H1c) TaSBEIII-A (T)</i>	7
18148G1-4		<i>Lr34/Yr18//Sr57/Pm38/Ltn1/Bdv1 + Lr37/Yr17/Sr38 + Lr46/Yr29//Sr58/Ltn2</i>	<i>TaSnRK2 (2.3-1B, 2.8-5A și 2.9-5A) TaGASR7-A1 (H1c) TaSBEIII-A (T)</i>	8

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Effect of spring (*Vrn-A1*) and winter (*vrn-A1*) vernalization alleles on light spectrum and temperature induced frost tolerance of wheat

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Keywords: cereals, cold acclimation, freezing tolerance, light regulation, vernalization, wheat

Adaptation to cold temperatures in winter cereals is a two-fold process: on the one hand, it is a cold acclimatization process, during which the plants' resistance to frost increases, and on the other hand, it induces vernalization, which results in a vegetative generative transition. The degree of cold hardiness is determined by the CBF-regulon while the vernalization requirement is determined by the *VRN1* genes, which belong to the same linkage group as the CBF genes on homeolog chromosome group 5. *VRN1* gene has an epistatic effect on CBF-regulon, as plants lose their tolerance to frost when the vernalization requirement is satisfied (shoot apex double ridge formation). It is known that the length of day and the circadian clock affect both the degree of frost resistance and the vernalization. There have also been many publications on the influence of the spectrum of illuminating light on the development of frost resistance. For example, frost-tolerant winter wheat illuminated by White light (W) with low Red:Far-red (R:FR) ratio increases its frost resistance. However, this effect is negligible in spring cereals. Therefore, we aimed to investigate how winter/spring *VRN1* alleles (*vrn-A1/Vrn-A1*), inserted in the same genetic background, affect the frost resistance of wheat plants under different spectral illumination at 5°C and 15°C. Reciprocal near-isogenic lines (NILs) produced from the crossing of the non-hardy spring-habit (*Vrn-A1*) cultivar 'Manitou' and the very cold-hardy winter-habit (*vrn-A1*) cultivar 'Norstar' were used. The survival rate of different lines and the related gene expression pattern will be presented.

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Genome-wide association mapping of plant height, thousand kernel weight, grain protein content, and normalized difference vegetation index in Bulgarian bread wheat

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Keywords: grain protein, GWAS, NDVI, plant height, TKW, *Triticum aestivum* L.

Association mapping in a crop enables identification of genomic loci pertinent to breeding. Here, we conducted a genome-wide association study (GWAS) in a set of 179 Bulgarian winter wheat varieties, comprising modern semi-dwarf and traditional tall accessions to detect SNP markers associated with four agronomic traits: final plant height (PH), thousand kernel weight (TKW), grain protein content (GPC), and normalized difference vegetation index (NDVI). The association panel was genotyped with an optimized 25K Infinium iSelect array. The population structure (Q-values), kinship (K), and linkage disequilibrium (LD) across each chromosome were determined with 19019 polymorphic SNPs. For GWAS, the mixed linear model (MLM, Q+K model), and Bonferonni correction to control the false positive rate were used to analyze phenotypic data from three crop seasons, the means across the seasons, and the calculated Best Linear Unbiased Estimator Values. Many significant ($-\log_{10}(p) > 5.53$) environment-specific SNPs associated with the studied traits were identified on almost all chromosomes. Adjacent significant SNPs were combined within a LD block as a quantitative trait locus (QTL). Thus, we defined in total 70 QTLs linked with PH (9), TKW (35) and GPC (26). Stable associated markers, detected in at least two environments, were identified for PH (98 on 16 chromosomes) and TKW (14 on 5 chromosomes) (Figure). Two hot spot genomic regions for PH and GPC were found on chromosomes 3A and 6A. The GWAS results could be used for searching putative candidate genes within the detected genomic regions and better understanding of the genetic relationships between studied traits, and for gaining useful information for modern marker-aided breeding.

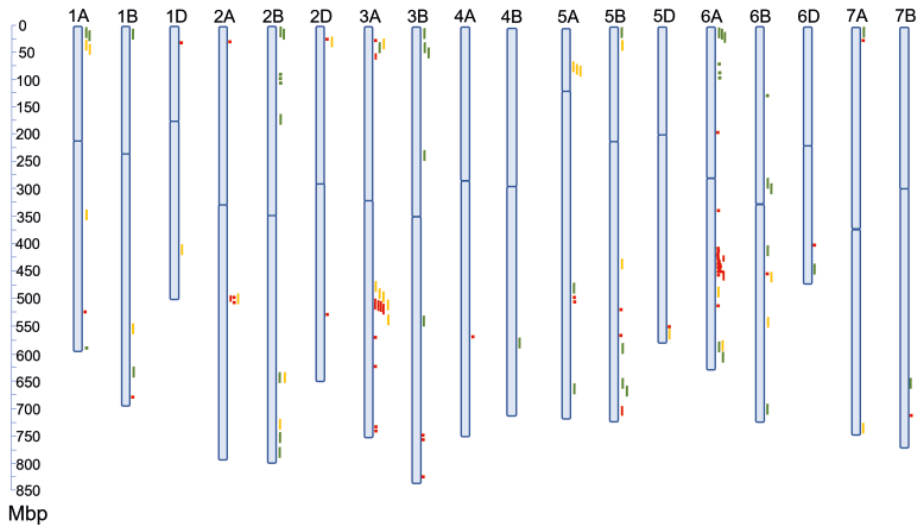


Figure. Summary of stable significant SNPs (dots) and QTLs (lines) for plant height (red), thousand kernel weight (green) and grain protein content (yellow).

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The influence of chromosomal localization of DArT markers on the genetic variation and population structure assessment in hexaploid triticale (*X Triticosecale* Wittmack)

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Keywords: DArT markers, genetic variation, population structure, triticale

Hexaploid triticale is an artificially created species of cereal with many desirable characteristics. Triticale enabled the combination of rye's resistance to diseases and pests as well as tolerance to abiotic stresses with wheat productivity and quality attributes. However, due to its origin, it is predicted that triticale has narrow genetic variation, which is a limitation for the breeding of new cultivars. Diversity Array Technology (DArT) is a high-throughput marker technique now used very widely in plant genetic research. The aim of the study was determination whether codominant and dominant markers assigned to individual triticale chromosomes work better than using the whole marker pool to group the analyzed materials. The second objective of the presented research was to determine which type of marker (silicoDArTs or DArTseq) would work better for this kind of analysis.

The plant material of the study consisted of 466 homogeneous breeding lines of winter hexaploid triticale obtained from Polish plant breeding companies. DNA isolated from examined genotypes was the template for marker analyses. Two marker types were generated, namely, SNP (codominant) and silicoDArT (dominant). Both types of markers were filtered with quality control parameters. Molecular marker chromosomal assignment and position were evaluated based on wheat and rye sequences in reference genomes that were homologous to the marker sequence. For calculation of the genetic indices including expected heterozygosity (*He*) and the polymorphism information content (PIC) of the total SNP and silicoDArT markers, as well as analysis of molecular variance (AMOVA) the GenAlEx software was applied.

Obtained results revealed that the average expected heterozygosity (*He*) values obtained for markers assigned to the single chromosomes were different. A similar result was observed in the case of the polymorphism information content (PIC). The genetic distance (GD) matrices evaluated on markers assigned to triticale chromosomes were positive and significantly correlated, but the GDs varied considerably. The results of correlation analysis using different types of markers were similar for some pairs of chromosomes, but in some cases, differences were noted between them. The marker type seems to differ in their ability to group plant materials. The choice of DNA marker system should be based on *He* and PIC values; however, using both on molecular data from different chromosomes may prove to be the best solution.

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Evaluation of the effectiveness of powdery mildew resistance sources identified in *A. sterilis*

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Keywords: *A. sterilis*, resistance, powdery mildew, oat

Powdery mildew caused by *Blumeria graminis* DC. f. sp. *avenae* Em. Marchal. is nowadays one of the most serious foliar disease of common oats. This pathogen is distributed widely throughout the world and causes significant grain losses. To date, 12 resistance genes to powdery mildew were identified and characterized in oats. However, only a few of these genes are still effective against existing pathogen races. A large genetic variability and the ability to generate new forms through mutations and DNA recombination causes the fungus adapt very quickly to new conditions. Therefore, there is a necessity to identify and characterize new and effective sources of resistance. So far, several potential resistance sources to fungal diseases have been identified in oats, both in cultivated forms *A. sativa* and wild species *A. sterilis*, *A. strigosa*, *A. occidentalis*, *A. pilosa*, *A. macrostachya*, and *A. barbata*. Among these species, *A. sterilis* is known as the most potential source of powdery mildew resistance genes. However, identification of effective sources of resistance requires more extensive research using a much larger group of differentiated isolates of *B. graminis* f. sp. *avenae*.

The aim of the presented study was the characterize the effectiveness of resistance identified in *A. sterilis* genotypes identified in our previous study as resistant to powdery mildew.

The subjects of this research were *A. sterilis* genotypes, from the collection of the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben (Germany) which were characterized by the highest level of resistance in screening host-pathogen tests.

The effectiveness of resistance was evaluated in host-pathogen tests based on a set of 55 different *B. graminis* f.sp. *avenae* isolates collected in different regions of Europe and characterized by different infection patterns to describe powdery mildew resistance genes.

Our previous screening analysis showed that among 88 tested *A. sterilis* genotypes 28 were resistant to two *B. graminis* f.sp. *avenae* isolates used in tests. In the first step of our investigation, we use 5 different *B. graminis* f.sp. *avenae* isolates. As a result, we selected only seven resistant genotypes. Additional tests based on 50 different pathogen isolates showed that among these genotypes only one was characterized by very high effectiveness.

The studies reveal that effective sources of resistance require testing with a range of pathogen isolates. Tests based on only 2-3 isolates are suitable only for preliminary screening.

Identification and chromosomal localisation of SNP markers for the oat crown rust resistance gene *Pc59*

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Keywords: disease resistance, DArTseq, marker assisted selection, *Puccinia coronata* f. sp. *avenae*

Crown rust is one of the most destructive fungal diseases of oats worldwide, caused by *Puccinia coronata* f. sp. *avenae*. Breeding disease-resistant oat cultivars is the preferred method of preventing the spread of rust and potential epidemics. The subject of the study was *Pc59*, a race-specific seedling crown rust resistance gene, highly effective at all growth stages, derived from *A. sterilis* originated from Israel. The surveys carried out for more than 10 years in Poland has shown that virulence against this gene is extremely rare and most often the symptoms of infection are accompanied by many signs of the immune response of the infected plant in the form of chlorosis or necrosis and small-sized uredinia. Crown rust response tests were performed and the proportions of phenotypes in segregating populations derived from a cross *Pc59* differential line with two crown rust susceptible Polish oat cultivars, Kasztan × *Pc59* and Bingo × *Pc59*, confirmed the monogenic inheritance of the gene.

Effective gene introgression depends on reliable gene identification at early stages of plant development, so the aim of the study was to develop molecular markers closely linked to *Pc59*. Segregating populations of Kasztan × *Pc59* and Bingo × *Pc59* were genotyped using DArTseq™ technology based on Illumina next-generation short-read sequencing. Markers associated with *Pc59* were located on chromosome 7D of the current version of the oat reference genomes, *Avena sativa* OT3098 v2 [1] in the region between 306,171 and 306,689 bp and *Avena sativa* cv. Sang [2] in the region between 738,878 and 739,392 bp. Based on the SNPs discovered in this region, allele-specific markers were subsequently developed using KASP and TaqMan technologies. Using KASP technology, the best marker was found to be incompatible in 5.3% of the 244 plants tested. In contrast, the best marker adapted to TaqMan technology misdiagnosed the phenotype in only 3.33% of the 268 plants tested. The newly developed co-dominant markers will be a valuable tool for marker-assisted selection in breeding programmes and may be useful for oat improvement.

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Generation of haploidy inducers in barley by site-directed mutagenesis

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Keywords: cas endonuclease, CRISPR, domain of unknown function, doubled haploids, phospholipase, DUF 679 membrane protein, genome editing, uniparental genome elimination

The use of doubled haploid (DH) lines is one of the most effective biotechnological measures in modern plant breeding. Individual DH lines are genetically unique results of meiotic recombination, while they are possessing the important characteristic of being entirely true-breeding. This means that once a useful DH line is selected, it can be identically reproduced through selfing. In barley, DH lines can be efficiently obtained via microspore-derived plant regeneration. However, this principle is genotype-dependent to some extent. An alternative means to produce DH lines is to employ haploidy-inducing lines as paternal parents (Satpathy *et al.* 2021). Among the progeny resulting from such crosses, maternal haploids can be found that have lost the paternal genome during early embryogenesis. The phenomenon of uniparental genome elimination was reported to occur in mutants including those for *Centromeric histone 3 (CenH3)*, *Phospholipase (PLA1, PLD3)* as well as *DUF 679 membrane protein (DMP)* genes of various species (Liu *et al.* 2017). Primary barley mutants carrying Cas9-triggered mutations in *PLA1* produced about 6% haploid progeny upon pollination of wild-type plants. The haploidy-inducing capacity of homozygous *plal* M₂ mutants was then validated by pollination of various barley accessions, which resulted in haploid formation from 6% to 16%. In a further approach, we are employing *cas9/gRNA*-transgenic *plal* mutant barley to deliver these transgenes and their respective products from sperm cells to zygotes via fertilization. Any maternal parents of choice may thus be subjected to genome editing, while the transgene-carrying paternal genome is expected to getting lost in some cases during embryo formation (Budhagtapalli *et al.* 2020). This concept holds great promise for barley genome editing with considerably reduced genotype dependency.

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EWAC18 – Poster

Using Artificial Intelligence for estimation of Population Structure and Genetic Diversity of groups of varieties

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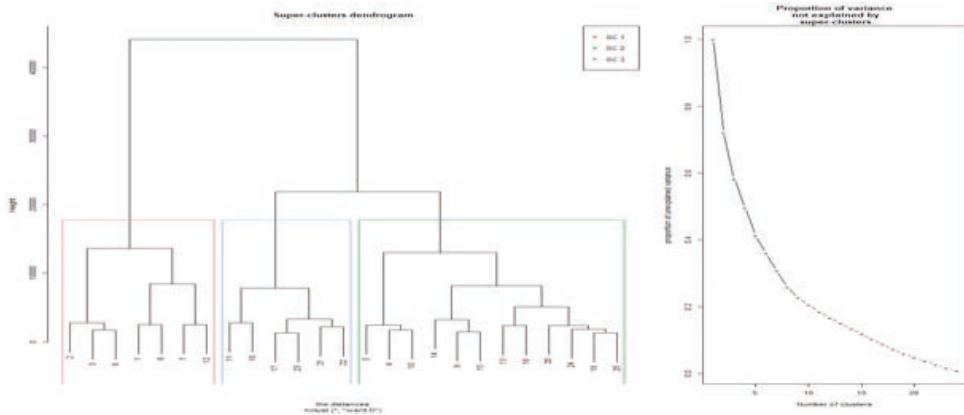
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Keywords: population structure, genetic diversity, Self-Organized Map, Independent Component Analysis, Artificial Intelligence, wheat

Knowing a given crop's genetic diversity and population structure is essential for selecting species with desirable agronomic traits. In this study we use a combination of two mathematical models to investigate genetic diversity and population structure in a panel of 179 bread wheat cultivars from Bulgaria, using an optimized wheat 25K Infinium iSelect array. In a previous paper, we have already shown this panel's genetic diversity and population structure using traditional bioinformatics methods. In this paper, we use Independent Component Analysis and Artificial Intelligence algorithm (Self-Organized Map – SOM) to determine the population structure and genetic diversity of 179 bread wheat varieties. First, we used only SOM to estimate the population structure of wheat varieties. The results are highly correlated with the results obtained by the k-means clustering algorithm and the STRUCTURE software. With k-mean clustering and STRUCTURE, we found 3 subpopulations SP1(structure)=125; SP1(clustering)=109, SP2(structure)=41; SP2(clustering)=49, SP3(structure)=13; SP3(clustering)=16 and 5 unmixed, respectively. With SOM algorithm SP1=102, SP2= 45, SP3=32. The combination of Independent Component Analysis (ICA) and SOM improves the distribution of cultivars by subpopulations. One of the advantages of SOM over other methods for determining the population structure of a group of cultivars is that once the network is trained, it can assign each new cultivar to the given subpopulation. This allocation happens quickly without requiring a new calculation of the population structure. On the other hand, the results obtained from the Independent Component Analysis applied to the population provide information about its genetic diversity.

Figures



Superclusters dendrogram shows the relationships between the groups of accessions, which strongly correlate with the relationships between the accessions obtained from the k mean clustering algorithm

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Towards the localisation of minor genes that restore male fertility in rye with the C sterility-inducing cytoplasm

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Keywords: genetic structure, hybrid cultivars, *Secale cereale* L.

First hybrid cultivars of rye were registered almost 40 years ago in Germany. Since then, this type of cultivars have become more and more popular every year, mainly due to higher yielding performance of hybrids vs traditional population varieties. Cytoplasmic male sterility (CMS) systems are necessary for efficient production of hybrid rye seeds. Currently, there are known only two genetically different CMS systems available for rye breeding: cytoplasm Pampa discovered by Geiger and Schnell (1970) and CMS-Vavilovii represented by various sources reported by different scientists and breeders (Kobyljanskij 1969; Łapiński 1972; Madej 1975; Adolf and Winkel 1985 and others). Majority of registered hybrid cultivars of rye are based on the CMS-Pampa system. The C cytoplasm used in our research was discovered by Łapiński (1972) and belongs to the group named CMS-Vavilovii. Restoration of male fertility of rye hybrids with the C cytoplasm stays under control of the *Rfc1* major gene located on 4RL chromosome and undefined number of minor genes (Stojalowski et al. 2004).

The mapping population used for our research was developed from a hybrid of male sterile line 544C and effective restorer Ot0-20 (the same combination cross was used for localization of the *Rfc1* gene by Stojalowski et al. 2004). A set of molecular markers located on 4RL chromosome was used for selection of male fertile genotypes that did not contain *Rfc1* gene. Male fertility of these genotypes was considered as an effect of minor restorer genes activity. Segregating generations of following progenies were used to identify PCR-based COS markers and DARtseq markers, revealing correlation with male fertility/sterility plants. Mapping DNA sequences of DARtseq markers to the rye reference genome (Rabanus-Walles et al. 2021) allowed us to identify two chromosomal regions abundant in sequences significantly associated with male fertility in hybrid genotypes. The first group of sequences was located on 2RL chromosome and the second group of markers was assigned to 5R. Our results allow to formulate a hypothesis, that two unknown minor restorer genes interacting with the CMS-C are located on 2RL and 5R chromosomes of rye.

Acknowledgments

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Varietal purity assessment using molecular techniques in wheat seed quality control

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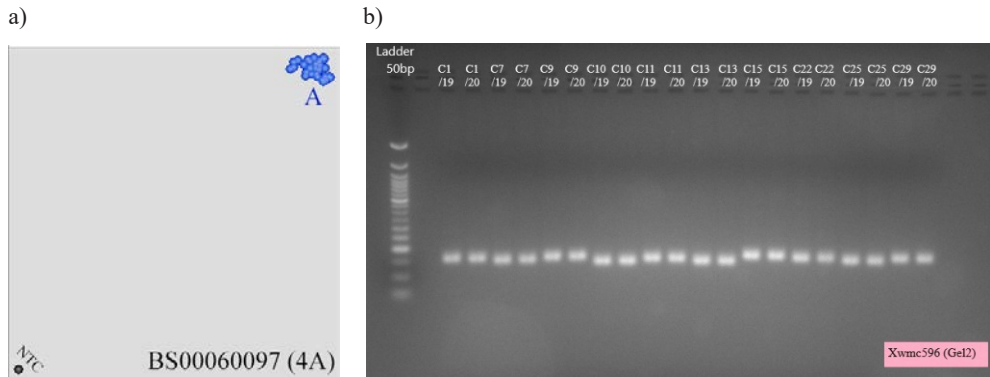
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Keywords: SSR markers, KASP markers, varietal purity, wheat

Assessing genetic purity and varietal identification are important topics in wheat seed quality control.

Several approaches that can be used to exploit new methods for genetic purity assessment and varietal identification of wheat are currently available in various international laboratories. Techniques based on morphological identification involve intense effort, making it sometimes difficult to verify crops varieties. Thus, using molecular techniques in order to determine the purity and variety identification of different seed types seems to be a better approach.

The aim of this study was to assess the varietal purity and genetic diversity among different wheat cultivars grown in Romania using 22 SSR markers (DuPw167, DuPw217, DuPw004, DuPw115, DuPw205, Xgwm155, Xgwm413, Xgwm003, Xgwm372, Xbarc184, Xbarc347, Xbarc074, Xgwm052, Xgwm095, Xwmc603, Xwmc596, Xwmc418, Xbarc170, Xgwm469, Xwmc474, Xwmc533 and Xgwm71) and KASP markers BS00023119_6A, BS00060097 and 1-Feh-w3. In order to establish that varietal purity is maintained 14 certified wheat cultivars were analysed from two harvesting campaigns (year 2019 and 2020) and seeds of four of them were also compared with the author's seed. Seeds belonging to the same certified wheat variety, multiplied in seven different locations were also compared to the author's seed. No contamination of the varieties was observed in these harvesting campaigns since all 22 SSR markers tested showed high similarity for the certified wheat cultivars harvest analysis. The use of KASP markers: showed that the results obtained with the selected SSR markers are reproducible, since no differences were observed (Fig. a, b). The results showed that in the present study the good practices guidelines in seed production are maintained.

Figure

a) KASP clustering results for marker BS00060097 for the certified wheat variety, multiplied in different locations, b) Amplification products obtained with Xwmc596 marker for certified wheat cultivars analysed from year 2019 and 2020

CBB7 – Invited

Needs and opportunities of field-like environment simulation for indoor plant phenotyping and performance assessment

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In open fields, plants are exposed to ever-changing dynamic environmental conditions. In order to sustain high and stable yields, crop plants need to constantly adjust to these changes and optimize the use of available resources. However, research devoted to gene function discovery was hitherto mainly carried out using constant growth conditions. To identify genetic factors influencing plant performance under changing environmental conditions, vegetative growth was assessed using high-throughput phenotyping at high temporal resolution. Genome-wide association studies were performed in a collection of 382 *Arabidopsis thaliana* accessions cultivated under constant or fluctuating light intensities, respectively. Quantitative Trait Loci (QTL) detected for growth-, coloration-, and photosynthesis-related traits were predominantly light regime (condition) specific and displayed distinct temporal activity patterns. Candidate genes expected to be of fundamental importance were identified at QTL regions shared between both light regimes. On the other hand, genes in fluctuating light-specific QTL regions encode factors involved in phytochrome signaling and several photosynthesis-related physiological processes, which are prime candidates for mediators of *Arabidopsis*' adjustment to fluctuating light. These observations emphasize the need for detailed experimental analyses under well-defined field-like environmental conditions to effectively unravel the complexity of gene x environment interactions that determine the expression of important plant traits.

To enable reproducible assessment of plant performance in simulated field-like environments, the IPK PhenoSphere, a large indoor plant cultivation and phenotyping facility was recently constructed and thoroughly tested. The growth and development of 11 diverse maize inbred lines was assessed in the PhenoSphere in an emulated single season and in a simulation of an averaged season (across several years). Results were compared to those in a standard glasshouse and in four years of field trials. The observed close match of growth and development progression in the simulated weather regime of the single season in the PhenoSphere with that expressed in the corresponding field season confirms the viability of the concept of this unique facility: It enables detailed analyses of performance-related trait expression and elucidation of causal biological mechanisms by assessing plant populations exposed to weather conditions of current and anticipated future climate scenarios.

Wheat for 9 billion people

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Keywords: wheat, yield, yield stability, quality, nitrogen

Wheat (*Triticum aestivum* L.) acreage has varied between 200–220 million hectares since 1960 when FAO started recording global wheat statistics. Average global grain yield has reached 3,5 t/ha, resulting in a global annual production of c. 770 Mt. Over the last 5 decades, global wheat production and average yield has linearly increased with an average increase of 400 kg/ha per decade. However, since the early 2000, yield increases occurred among the top ten producers mainly in the Global South (S-and E-Asia) and Russia, whereas in the high income countries and in particular in the European Union average yield stagnated, and a tendency for increased year to year yield variation is observed.

Wheat is the most important protein and 2nd most important calorie source for humans, therefore global food security demands a production increase of around 25–30% by 2050 to feed a population of 9,7 billion. Since area expansion is not an option, the increased production must come from higher grain yield.

Challenges to achieve this are significant. Increasing temperatures and in particular high night temperatures can reduce yield significantly at around 6–8% for every C⁰ increase. Models show that increasing CO₂ levels and temperature may result in increased wheat yields of up to 17% in the Northern hemisphere, but for the Global South wheat yield reductions of 15% are predicted. Weather extremes become more regular as part of GCC.

Wheat breeding with new traits is a promising climate change adaptation option, and the International Wheat Yield Partnership (IWYP), an international partnership of research funders and research organisations is exploiting such options. The phenomics capacity to characterize wheat accessions has increased tremendously during the last decade and this coupled with the progress in the molecular research area, bioinformatics to analyse huge data sets and development of better models to predict yield and other important traits suggest that wheat scientists will be able to develop the varieties that can mitigate the effects of future GCC. A particular challenge will be N management, since many traits could be limited under rainfed conditions where water and N stress limit benefits of traits for heat tolerance, early vigor, and delayed flowering adaptations. An increased N availability will often be required for new traits to express a higher yield potential. Since nitrogen application has reached non-sustainable levels in many regions of the world but in particular in Asia, promising options to improve NUE are shown.

Considering the importance wheat has for global food security, the consequences of GCC on wheat quality, protein quality and nutritional values are also discussed. The presentation focuses on challenges for global wheat improvement and options to produce sufficient nutritious wheat for 9 billion people in 2030.

Molecular diversity in the resistance interactions of wheat and its fungal pathogens

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Keywords: wheat, resistance breeding, leaf rust, powdery mildew, resistance genes, avirulence genes

We recently contributed to the establishment of high-quality reference genomes of wheat and its fungal pathogen powdery mildew. These resources provide the basis for the identification of genes involved in host-pathogen interactions and the establishment of a wheat resistance gene/pathogen avirulence gene atlas that includes the proteins specifically recognized from the pathogens. Novel and diverse genes were identified in wheat using genomics. There is a surprising diversity in the proteins determining race-specific resistance in wheat against powdery mildew and leaf rust pathogens. One of the identified proteins (Lr14a) is proposed to act as an executor protein specifically induced by avirulent pathogen isolates. Given the identification of many avirulence genes in the wheat powdery mildew pathogen, we propose that identification and monitoring of avirulence gene diversity in pathogen populations becomes an integral part of introgression breeding to ensure effective and durable resistance in wheat. Thus, the knowledge of the wheat-fungal pathogen “interactome” promises to support breeding strategies for increased durability of resistance. The molecular basis of non-NLR based race-specific resistance remains to be explored and provides a rich field of research expected to identify novel types of mechanisms involved in plant immunity.

Contribution of wheat landraces to wheat breeding and their current status in Turkiye

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Wheat is a staple crop in Turkiye with around 7.5 million ha acreage and over annual 20 million tons production. Turkiye has a unique example for the wheat production; though there are many modern wheat varieties in the market, there are still some wheat landraces (WLR) grown in mainly remote areas. The WLR in the farmers' hand are inherited from their ancestors and grown for decades. The WLR's do not only provide income for the farmers but also, they have rich diversity that enable breeders to use in their breeding programs. The WLR usage in breeding programs in Turkiye can be studied in 3 different eras: Direct selection of the pure lines from the WLR populations; it is in the early days of breeding program, in 1930's, the populations have been collected, pure lines selected, tested and suitable ones are registered, seeds multiplied and given to the farmers for productions. The varieties developed through this way were in production till mid 1960's. Second step of the WLR usage was using them as parents in crosses that started in late 1930's. There are many varieties of such grown till the beginning of 1990's. Especially, after 1990's the contribution of the WLR's are more of behind the scene, they are within the pedigree of one of the parents used in the cross that is secondary contributor; this era could be considered as third cycle of the WLR contribution to modern wheat breeding, though some of them were used as source of the trait of interest to be transferred to the modern germplasm such as quality, disease resistance, nutritional value, etc. International Winter Wheat Improvement Program (IWWIP) is a joint program among Turkey, CIMMYT and ICARDA operating in Turkey since 1986, developing Winter Wheat germplasm and distributing it globally. IWWIP collected more than 1700 WLR's from all over Turkey during 2009–2014. They are characterized and provided to Genebank in Turkiye. IWWIP also made comparisons of the selected WLR's from Turkiye, Iran and Afghanistan. While Turkiye WLR's were much more different than Afghanistan WLR's, they were overlapping with Iranian WLR's. In this study, the contribution of the WLR's to wheat breeding in Turkiye, comparisons of them with other countries WLR's will be presented.

Pangenomics in crop plants: the example of barley

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Pangenomes are collections of annotated genome sequences of multiple individuals of a species or higher taxonomic unit. These catalogues of genetic diversity are useful resources for evolutionary studies, functional genomics and plant breeding. Cost reductions in high-throughput sequencing and advances in assembly algorithms have made it possible to sequence many more genomes than was conceivable a mere five years ago. In this talk, I will summarize our work in barley pangenomics: the selection of representative core sets by genebank genomics, the assembly of chromosome-scale reference sequences, and the discovery of structural variants associated with agronomic traits. A future challenge will be the development of pangenome interfaces for easy access by breeders and geneticists.

Impact of wheat evolutionary history on interactions with microorganisms in the rhizosphere

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Keywords: PGPR, rhizosphere, symbiosis, QTL, wheat history

Wheat roots interact with a diversified microbial community in soil, and wheat genotypes can differ from one another in their ability to select soil bacteria. These differences are likely to relate to the evolutionary history of wheat, which has undergone genomic hybridizations and several domestication events. To assess the impact of wheat evolutionary history on interactions with soil microorganisms, we used different species of wheat to characterize microbial selection patterns and responses of plant-beneficial bacteria. Bread wheat and durum wheat, as well as current representatives of their ancestors, were chosen to measure their interactions with four microbial functional groups, i.e. free nitrogen fixers, 1-aminocyclopropane-1-carboxylate deaminase producers (modulating ethylene metabolism in plants), indole-3-acetic acid (IAA) producers via the phenylpyruvate decarboxylase pathway and 2,4-diacetylphloroglucinol producers (stimulating root branching), as well as with the Plant Growth-Promoting Rhizobacterium (PGPR) *Pseudomonas ogarae* F113. Data show that the abundance (measured by quantitative PCR), diversity (measured by metabarcoding) and activity (enzymatic assays and reporter gene monitoring) of beneficial microorganisms in the rhizosphere fluctuated according to wheat genome content and ploidy status. Rather often, similar results were obtained for wheats possessing the D genome (inherited from the ancestor *Aegilops tauschii*), suggesting that this genome may significantly influence wheat interactions with beneficial microorganisms. Moreover, domestication and breeding had an impact on wheat colonization ability of these microorganisms, but this impact varied according to the microbial functional group. Overall, our results show that considering wheat evolutionary history can be useful to understand the interaction potential of current wheat genotypes with microorganisms involved in plant-growth promotion.

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Validation of cross progeny variance genomic prediction using simulations and experimental data in winter elite bread wheat

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Keywords: cross value, progeny variance, usefulness criteria, genomic prediction, bread wheat

Genomic prediction is used in many crop breeding programs to select parental lines to ensure high performance of their progeny. Taking into account progeny variance estimates on top of parental mean is expected to increase the probability to get outstanding progenies. Several Cross Selection Criteria have been proposed and the Usefulness Criterion (UC) that accounts for Parental Mean (PM) and progeny Standard Deviation (SD) has been shown as a good compromise to secure genetic gain as well as genetic diversity in the next generation using simulation studies.

In this study, we predicted the three cross value components (PM, SD and UC) of 73 winter bread wheat crosses whom progenies have been evaluated in the field. The Training Population (TP) used to estimate marker effects was composed of 2,146 French varieties registered between 2000 and 2021 and INRAE-AO breeding lines.

We first evaluated different factors influencing the prediction ability of the cross value components based on simulations, starting from the same crosses as the experimental design, simulating phenotypes with increasing heritability, number of QTLs and progeny size. As expected, increasing the number of QTL decreased the prediction ability for all cross value components, and increasing heritability or increasing progeny size improved prediction abilities. The prediction of SD was the most impacted. We used as a reference a TRUE scenario, i.e. an optimal situation where TP is optimal and where marker effects are perfectly estimated. Once again, SD was strongly impacted by the quality of marker effect estimates. For polygenic traits (10 QTL), Bayesian models showed higher prediction ability. For quantitative traits (more than 300 QTL) with low heritability, using a progeny variance estimate that takes into account the error of marker effect estimates improved significantly SD prediction ability.

We validated our findings using experimental data for four traits evaluated on the same crosses: yield, grain protein content, plant height and heading date. Prediction abilities were assessed for each cross value component, and overall, predictions aligned well with experimental values. PM and UC were reasonably predicted for most traits, while SD was more challenging, especially for yield. To our knowledge, this study is the first to experimentally validate the genomic prediction of progeny cross variance and showed that prediction abilities strongly depend on trait architecture. This study also revealed that it is essential to generate a very large number of progenies per cross to obtain reasonable prediction abilities of SD.

Acknowledgments

We thank the FSOV for financing the project called “Estimation of the genomic prediction ability of the value of a cross in bread wheat”. The authors acknowledge all the PrediCopt FSOV project partners: Laurent Falchetto, Sandrine Berges and Kevin Bargoin (INRAE UE PHACC), Patrice Walczak (INRAE UE FERLUS), Paul Bataillon (INRAE UE GC) for experimental evaluation. They also thank Marie-Hélène Bernicot and Solène Barraïs (GEVES) for providing the training population datasets.

CBB7 – Oral

T1: Genetic Resources for Crop Improvement

Optimized SNP arrays for genotyping cereals

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Keywords: Single Nucleotide Polymorphism, genotyping, wheat, barley, rye

Single Nucleotide Polymorphism (SNP) genotyping is nowadays the method of choice for molecular marker analysis in the cereals. In the last decade a large wealth of SNP markers has been identified and characterized. SGS IF TG has been involved in the development of several public cereal SNP genotyping arrays and has served the breeding and scientific community for wheat, barley, and rye using both the Illumina Infinium and Thermo Fisher Axiom technology. During the course of these services with first generation SNP genotyping arrays, it was noted that these arrays contain a large number of non-functional markers as well as markers that are always in perfect linkage disequilibrium (LD). Furthermore, the high costs of these arrays prevented their use in routine breeding where often many thousands of samples need to be investigated.

Because of that SGS IF TG has developed a number of novel optimized genotyping arrays that contain functional markers, provide less redundant data, where the markers are well-distributed on the recombinational landscape, contain published markers for specific traits and can be analyzed at low costs especially for large sample numbers as needed for genomic selection or other breeding applications. For wheat, we describe the development of a 135K Axiom array for large scale SNP genotyping and two smaller arrays with 25K and 7K markers for routine analysis. For barley, we have developed a 15K and a 4K genotyping array mostly based on markers from the 50K barley array. A new and optimized SNP array for rye contains more than 5,000 markers (5K). For Triticale, we have developed a 30K array that combines the 25K wheat markers and 5K rye markers. We describe the specific steps involved in the development and improvement of these arrays and provide examples for the use of these arrays in genetic research and plant breeding in which by now a total of hundred thousand samples have been analyzed with these arrays.

Genetic analysis of flag leaf size diversity in a multi-parent population of barley

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Keywords: QTL, fine mapping, barley

The flag leaf is an important source of assimilates for developing grains in cereals. The aim of our study was to dissect the natural variation and identify the genetic loci controlling flag leaf size in barley using a multi-parent population developed from a genetic cross between 23 diverse barley inbreds in a double round-robin design (HvDRR). We observed a significant genotype effect for flag leaf length and width with a broad sense heritability of 0.8 and 0.6, respectively. We identified 70 quantitative trait loci (QTLs) linked to flag leaf length and 45 QTLs associated with flag leaf width. The explained variance by the QTLs ranged from 5 to 46% for flag leaf length while it was 4 to 44% for flag leaf width. Further, we validated two major QTL for flag leaf size on chromosome 2H (*qFL_Hv14_2H_1*) and 4H (*qFW_Hv33_4H_1*) using recombinant inbred lines (RILs) polymorphic for the respective QTLs but close to isogenic for the rest of the genome. *qFL_Hv14_2H_1* affected flag leaf length by regulating epidermal cell proliferation rate during leaf expansion while the control of flag leaf width by *qFW_Hv33_4H_1* might be attributed to difference in epidermal cell size. The fine-mapping of the *qFL_Hv14_2H_1* and *qFW_Hv33_4H_1* is ongoing in heterogenous inbred families developed from the RILs used for QTL validation. The results of our study indicate that flag leaf size in barley is a complex trait controlled by the number and size of the epidermal cells of the leaf blade. We can use the major effect QTLs detected in this study for gene isolation and marker assisted breeding to optimize the flag leaf size.

Acknowledgments

We thank Deutsche Forschungsgemeinschaft (DFG) for funding the project, CRC trr341 for the organisation and providing training opportunities. We also thank all the members of trr341, HHU, iGRAD and QGGP for their help and support. Also, we would like to thank gardeners for taking care of plants.

Genomics-assisted gene introgression from tertiary gene pool species into wheat

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Keywords: introgression breeding, *Aegilops*, *Secale*, *Thinopyrum*, segregating genetic map

Wild *Secale*, *Agropyron* and *Aegilops* species have been used for transfer of useful genes into modern wheat cultivars in the frame of Martonvásár introgression-breeding program. The perennial rye cultivar 'Kriszta', a hybrid of *S. cereale* and *S. montanum*, is resistant to leaf rust, stem rust, stripe rust and powdery mildew. The stripe rust resistant bread wheat (Mv9kr1) x Kriszta BC₂F₉ line '179' was selected under field condition and the cytogenetic analysis confirmed presence of a disomic 1RS·1BL centric fusion, which has different allele composition from the well-known Petkus derived 1RS arm. *A. glael*, a hybrid between *Th. intermedium* and *Th. ponticum*, showed resistance to leaf rust under field conditions. Molecular cytogenetic screening with probes for J-, S^t-, and D-genomic DNA (McGISH), DNA repeats pSc119.2, Afa family, pTa71, and (GAA)₇ identified a genotype WT153397 carrying a 6DS·6J^{vs} centric fusion. Field trials over three growing seasons revealed that the introgressed 6J^{vs} chromosome arm significantly increased the number of productive tillers, which manifested as higher grain yield relative to the parental wheat cultivars. A wider use of gene introgression from wild relatives into wheat has been hampered by the lack of genome reference sequence and scarcity of molecular tools. In case of tetraploid wild goatgrass, *Ae. biuncialis* (U^bU^bM^bM^b), we demonstrated that high throughput marker system DArTseq, which doesn't require preliminary sequence information, is suitable for production of a high resolution genetic map. The MvGB642 x MvGB382 F₂ map highlighted the wheat-*Aegilops* synteny and facilitated gene introgression into wheat. The DArTseq genotyping of wheat (Mv9kr1) x *Ae. biuncialis* (MvGB642/382) BC₃F₁₋₃ populations in combination with GISH and FISH confirmed the presence of full 1MS, 3M, 4M, 5M and 6M chromosomes, while majority of U-genome chromatin was lost and only 1U-, 2U-, 4U- and 7U chromosomal fragments were present in some lines. This approach allowed identification of new lines with deletions, translocations, chromosome additions and substitutions. The new introgression and addition lines together with the chromosome-specific markers will facilitate development of new wheat cultivars adapted to changing environmental conditions and offers an ideal base for agronomically important genes cloning.

Acknowledgments

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Improvement of the fatty acid profile in a camelina cultivar facilitated by novel methods of plant regeneration, *Agrobacterium*-mediated transformation and genome editing

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Keywords: *Camelina sativa*, adventitious shoots, targeted mutagenesis

Camelina (*Camelina sativa* (L.) Crantz) is an oilseed plant that tolerates various adverse conditions, such as low temperatures, drought, and nutrient-poor soils. In addition, the unique fatty acid profile of its seeds renders camelina particularly suitable for the production of food and health products. While genetic engineering may greatly contribute to improving yield and seed composition, it requires efficient plant regeneration from cells that are accessible to DNA transfer. In camelina, the current limitations of plant regeneration from such cells constitute a bottleneck for the development and application of conventional as well as site-directed genetic engineering in elite germplasm. In the present study, a method of adventitious shoot formation from immature zygotic embryos was established and used for *Agrobacterium*-mediated transformation. To optimize the system, we compared the performance of the *Agrobacterium* strains LBA4404 and AGL1 carrying a vector for the delivery of hygromycin phosphotransferase and green fluorescent protein genes. In addition, the duration of explant pre-cultivation, wounding of explants, *Agrobacterium* cell density at inoculation, acetosyringone concentration, duration of co-culture, and hygromycin concentration for selection was varied using the spring cultivars Ligena and Calena. Transformation frequencies of 10% and 13% were achieved in Ligena and Calena, respectively. Based upon these results, this novel method was applied for Cas9-mediated targeted mutagenesis of the FATTY ACID ELONGASE 1 (FAE1) gene. In this approach, the aim is to increase the formation of the particularly valuable long-chain fatty acids at the expense of the harmful very long-chain fatty acids gondoic and erucic acid. After having simultaneously targeted all FAE1 alleles present in the three subgenomes of camelina, triple-homozygous *fae1* knockout mutants were identified in Ligena upon selfing of the primary transgenic plants. A fatty acid profiling analysis revealed that the very long-chain C20 to C24 fatty acids were indeed eliminated in these plants, while the long-chain fatty acids were slightly increased.

Green Revolution *Rht* genes affected anther extrusion and floral traits related to cross pollination efficiency in wheat

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Keywords: *Triticum aestivum* L., green revolution, dwarfing genes, grain yield, hybrid seed, reduced height, yield potential, elite pollinators

Hybrid wheat breeding is a promising strategy to increase grain yield. Due to the prevalence and usefulness of the *Rht* dwarfing alleles, it is important to gain a better understanding of their effect on traits related to hybrid seed production. Field experiments were performed at the IPK Gatersleben (Germany) and the National University of La Plata (Argentina) during four growing seasons. Traits associated with cross-pollination efficiency were studied using four sets of Near Isogenic Lines (NILs) carrying the alleles *Rht1* (semi-dwarf), *Rht2* (semi-dwarf), *Rht1+2* (dwarf), *Rht3* (extreme dwarf), *Rht2+3* (extreme dwarf) and *rht* (tall). Results showed that the extreme dwarfing alleles *Rht2+3*, *Rht3* and *Rht1+2* presented the greatest effects in all the traits analyzed ($P < 0.001$). Plant height showed reductions from 21–23% (*Rht1* and *Rht2*), 49% (*Rht1+2*), 56% (*Rht3*), and 64% (*Rht2+3*) compared to *rht* (tall). Spike length was increased up to 9.4% (*Rht1+2*), whereas spikelets/spike were increased up to 5.2% (*Rht2+3*). Floral organs were negatively influenced by *Rht* dwarfing alleles compared to *rht*. Decreases up to 20.2% (*Rht2+3*) in anther length and -33% in anther filament length were observed. Anthers extrusion decreased from 40% (*rht*) to 20% (*Rht1* and *Rht2*), 11% (*Rht3*), 8.3% (*Rht1+2*) and 6.5% (*Rht2+3*). Positive correlations were detected between plant height and anther extrusion, anther and anther filament lengths, suggesting the negative effect of dwarfing alleles in traits with importance for hybrid seed production. The magnitude of these detrimental effects was closely related to the level of dwarfing of the alleles studied: *Rht2+3/Rht3* (severe dwarfing) > *Rht1+2* (dwarf) > *Rht2/Rht1* ('green revolution' semi-dwarfs) > *rht* (tall). Our results indicate that *Rht* alleles are involved in multiple traits of interest for hybrid wheat production and the need of selecting alternative sources for reduced height/lodging resistance for hybrid breeding programs.

Figures

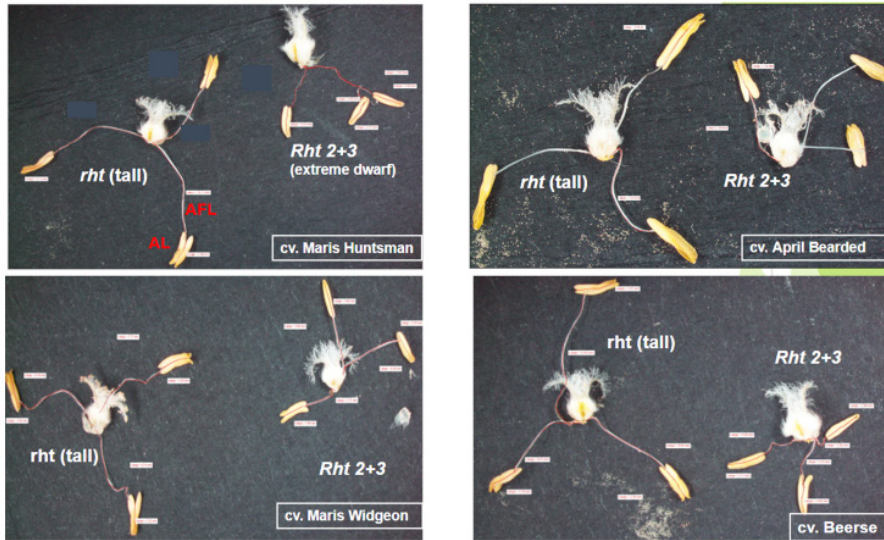


Figure 1. Comparison between *rht* (tall) and *Rht2+3* (extreme dwarf) for Anther (AL) and anther filament (AFL) lengths in the four genotypes evaluated. Images were taken using a stereo dissecting microscope equipped with a digital camera (NIKON SMZ25 stereomicroscope)

Acknowledgments

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Can we perpetuate and transfer to valuable lines of rye the trait of androgenesis ability?

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

The research presented here indicates that the ability to androgenesis in rye can be transmitted by crossbreeding. There is growing interest among scientists and breeders in homozygous lines. They can be used in basic research as "pure" material and in experimental plant breeding to obtain lines whose progeny do not segregate. At the same time, doubled haploid lines are a source of variability, since each microspore has a unique set of alleles. For this reason, cereal breeding in recent years has relied on doubled haploids. Despite developing methods using molecular markers and automated phenotyping, still *in vitro* induced androgenesis is the most efficient biotechnological method used in practical breeding. In this presentation, we summarize our experiments on androgenic ability in rye, and demonstrate the transferability of androgenesis ability from lines with high androgenesis efficiency to lines with high breeding value.

Seeds from a selected F1 line, obtained from a cross between a control androgenic line and a line with low androgenesis efficiency, were germinated. Seedlings from the resulting F2 population, were vernalized. They were then grown under conditions that induced intensive tillering. These singles from the F2 population were brought to earing. Spikes at the appropriate developmental stage were subjected to cold stress, and then served to collect anthers and establish their *in vitro* culture. During the establishment of anther cultures, material was collected for RNA isolation and further transcriptomic analyses. After collecting results on the efficiency of androgenesis, we conclude that the trait of androgenesis ability can be transmitted through crossbreeding, although not to all genotypes, but more than 70% of the tested lines showed androgenesis capacity. 16% of the tested lines showed androgenesis ability with higher efficiency than the positive control. Control lines confirmed their negative (Line 12S) or positive (Line 8J) predisposition to induce androgenesis.

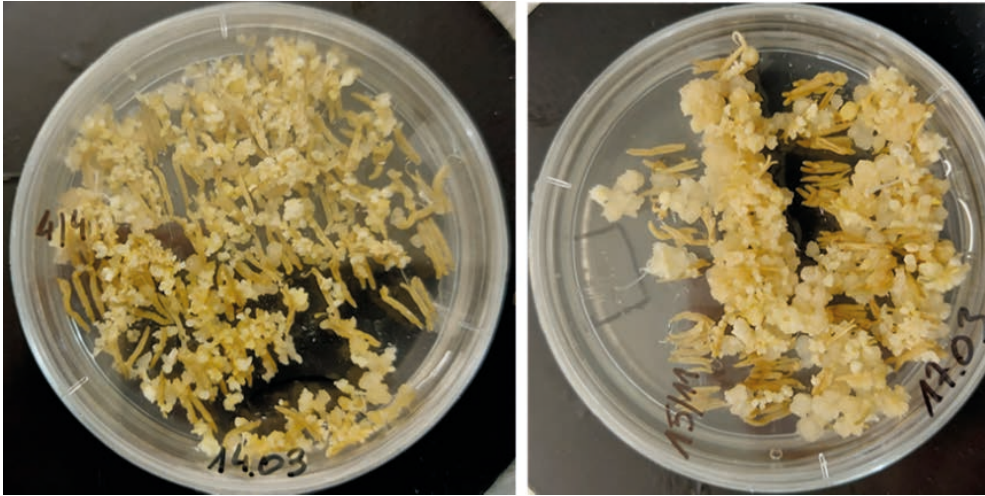


Figure 1. An example of androgenic response of anthers from crosses between an androgenic line and a line with low androgenesis efficiency

Acknowledgments:

We would like to thank Jola Kubiak and Ola Zimny for their contribution in performing the experiments

T2: Environmental Adaptation

Stem structural biomass and water-soluble carbohydrate's role in wheat grain filling under water deficit and high temperature

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Keywords: water soluble carbohydrates, grain filling, stem traits, wheat breeding

Under terminal stress when wheat canopy senesces, assimilate supply for grain filling depends on stem water-soluble carbohydrates (WSC) (Blum 1998). Following previous hints on congruence between stem traits, WSC content, and grain yield (GY) under drought (Saint Pierre et al., 2010) here we investigate in bread wheat stem structural biomass investment and its association with grain filling under drought and heat stress based on two experimental setups using spring wheat: (1) Set of 25 genotypes was evaluated under four environments: drought, heat (late sowing), and optimum conditions (Obregon, Mexico) and Mediterranean-rain-fed conditions (Israel). (2) wheatMAX associactin panel of 300 genotypes was evaluated in two environments: well-watered (WW 700mm) and water-limited (WL 350mm) in a rainout shelter facility (Rehovot-Israel). The phenotypic evaluation concentrated on stem morphology traits such as internode diameter (ID), stem solidness (%), peduncle length (PL) and stem water-soluble carbohydrates (SWSC) (via NIR analysis), biomass (BM), thousand-grain weight (TGW) and GY. Genome-wide association study (GWAS) was applied to the wheatMAX data collected in the second experiment to detect genomic regions associated with stem traits. First, based on the genotypic set in environments except heat, stem-WSC accumulation reached the peak 25 days after heading (DAH) with the highest values recorded under drought and Mediterranean environments. Under heat and drought increased investment in stem ID resulted in a higher accumulation of SWSC at 25 DAH. Drought and heat stress negatively affected not only crop productivity [from mean GY of 626 g/m² (irrigated) to 311, 287, and 213 g/m² for Mediterranean, drought and heat respectively] but also structural stem biomass as expressed in lower ID and PL. However, PL, SWSC content, and remobilization were positively associated with and significantly enhanced grain filling (TGW and GY) under drought and heat stress. Interestingly across environments, there was no trade-off between increase crop investment in both ID and PL and GY [$r=0.42$, ($<.0001$) and $r=0.44$, ($<.0001$) respectively]. The wheatMAX GWAS identified major QTLs for stem solidness on chromosomes (3B,3D), ID (3A), and PL (6A) under both well-watered and water-limited

conditions. In summary, our results indicate the reward of selecting for spring wheat genotype with enhanced ID, PL SWSC content and remobilization suggesting a useful strategy to offset TGW reduction under water deficit and high-temperature conditions.

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Evaluating the wavelength specific role of blue light in light-induced cold acclimation of barley

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Keywords: blue light, far-red light, cold acclimation, frost tolerance, barley

During late autumn, when the temperature become colder, plants undergo an intensive transcriptional and physiological remodelling to tolerate the upcoming freezing temperatures which is called cold acclimation (typically 2–7 weeks). However, before the full development of the process, plants could be susceptible to sudden frosty days, which may occur in this season. As the temperature, the spectrum of sunlight changes dynamically throughout the year. As autumn approaches, red:far-red (R:FR) photon ratio start to decrease, while blue:red (B:R) photon ratio start to increase in the temperate climate zone. Low R:FR ratio light could induce cold acclimation in plants at temperate climate, as well as in winter cereals, even at warmer temperatures to enhance their readiness to sudden drops in temperature. Recently, it was also demonstrated, that the addition of extra B light into the incident FR enriched white light (W) further improve the frost tolerance of barley. Thus, the number of evidence is growing, that cereals could enhance their readiness to frost by sensing the seasonal alterations of sunlight spectrum, even at higher temperatures, when cold acclimation process is inactive. However, the exact role of B light in the process is barely known. Fortunately, in plant growth chambers equipped with controllable LED light ceilings, the contribution of many spectral components to the process can be studied. Accordingly, the effects of monochromic B light with different emission peak LEDs, 410 nm (B_{410}) and 450 nm (B_{450}) on freezing tolerance was investigated. Moreover, the incident W light as well as FR enriched W light was supplemented either B_{410} or B_{450} . All light treatments were carried out at different temperatures (15 °C or 5 °C). To estimate the success of the light-induced cold acclimation, the freezing tolerance of barley plants was evaluated by measuring electrolyte leakage of detached leaves after freezing. The expression of cold acclimation and light signalling related genes was also investigated. The wavelength specific effect of blue light (monochromatic or in mixed light) on subsequent freezing injury rates and the related gene expression pattern in barley leaves will be presented.

Acknowledgments

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Changes in Vernalisation and Photoperiod Response in Australian Wheat Over 130 years

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Keywords: vernalisation, photoperiod, flowering time

Australia's wheat breeding history has been marked by germplasm introductions that have influenced vernalisation and photoperiod responses, which are key drivers of flowering time. The first of these was germplasm improvement immediately after European settlement, which improved adaptation of English cultivars to the local climate. Crossbreeding for traits suited to drier regions led to the release of the fast developing cultivar Federation in 1901. The introduction of semi-dwarf CIMMYT lines in the late 1960s led to an influx of genotypes with further accelerated flowering. To examine the effect of these introductions on vernalisation and photoperiod sensitivity, we measured thermal time to anthesis (TTA) in 260 genotypes from the OzWheat diversity panel, which has been curated to represent Australia's wheat breeding pedigree. TTA was recorded across four controlled environments; vernalised long days, non-vernalised long days, vernalised short days and non-vernalised short days. Principal component analysis of TTA in each environment as well as year of release identified four distinct clusters of cultivars. While winter wheat clearly separated from spring wheat, spring genotypes were split into pre-1930 releases, and two groups of post-1930 releases with either high or low photoperiod sensitivity. On average, genotypes flower 3.4 °C degree days faster for each passing year from 1880 to 2012. While faster flowering was initially desirable for wheat crops to escape drought stress and disease pressure, increasing the duration of the spike growth stage (critical period) through enhanced photoperiod sensitivity could increase yield under warming climates. Re-evaluation of photoperiod sensitivity in breeding programs may be an important strategy for future yield increases.

Involvement of the different *Rht* dwarfing alleles in the light-regulated cold acclimation of wheat and barley

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Keywords: cereals, cold acclimation, freezing tolerance, light regulation, *Rht* genes

The cold acclimation process of winter-hardy plants is highly influenced, apart from cold ambient temperatures by other light-dependent environmental factors like the intensity and spectrum of the incident light, day length and circadian clock. It was reported by studying winter varieties of wheat and barley that the freezing tolerance of plants in the early pre-hardening phase increased both at non-acclimating temperatures (+15 °C) and even in higher rate applying cold treatment (+ 5°C), respectively, when they were illuminated by white light (W) with low Red:Far-red (R:FR) ratio. More recently, the involvement of blue light in this light regulated cold hardening process also was reported. This light spectrum induced cold acclimation is depending on a phytochrome regulated signaling pathway affecting through the CBF-regulon. By investigating the altered incident light spectrum induced changes in lipidome, hormone homeostasis, and metabolome of barley and wheat plants we could elucidate the physiological background of increased freezing tolerance. CBF-regulon interact with both the gibberellin (GA) biosynthesis and DELLA (*Rht*) genes. Consequently, our interest is to work both with wheat GA insensitive /sensitive *Rht* mutants to elucidate how these mutations alter the light spectrum induced freezing tolerance and GA content. The alteration in the modified light induced hormone homeostasis and freezing tolerance caused by the presence of different *Rht* alleles at ‘Maris Huntsman’ wheat genetic background will be presented.

Acknowledgments

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Agroecological genetics of allometry and allocation plasticity of wheat to light limitations

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Keywords: biomass, partitioning, shade, growth, genotype-by-environment interactions

How plants distribute biomass among organs influences resource acquisition, reproduction, and plant-plant interactions and is essential in understanding plant ecology, evolution, and yield production in agriculture. However, the genetic mechanisms regulating allocation responses to the environment are largely unknown.

We studied recombinant lines of wheat (*Triticum* spp.) grown as single plants in mixtures under sunlight and simulated canopy shade to investigate genotype-by-environment interactions in biomass allocation to the leaves, stems, spikes, and grains. Size-corrected mass fractions and allometric slopes were employed to dissect allocation responses to light and plant size.

Modeling QTL-by-environment interactions of size-corrected allocation loci revealed light-responsive alleles associated with adaptation to the crop environment. Combined with an allometric approach, we demonstrated that polymorphism in the DELLA protein is associated with the response to both shade and size. While the gibberellin-sensitive allele amplified allocation to the stem when shaded, size-dependent effects of this allele drive allocation to reproduction, suggesting that consequences of shade responses are conditioned by the ontogenetic trajectory of the plant.

Our novel approach provides a basis for exploring the genetic determinants underlying investment strategies in the face of different resource constraints, and will be useful in predicting social behaviors of individuals in a crop community.

Accelerated induction of reproductive development in winter wheat to shorten the generation time in breeding programs

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Keywords: vernalization, reproductive development, winter wheat, generation time

The reduction of generation time is of great advantage for cereal breeding. While the duration of generative development can already be reduced considerably under so-called Speed Breeding conditions, minimizing the period required to vernalize winter cereals poses a further challenge. The competence of shoot apical meristems of winter-type temperate cereals for ear formation is typically acquired under short-day conditions in combination with a temperature just above freezing for a period of about two months. The vernalization requirement of winter wheat cultivar Certo was investigated by varying the durations of germination and vernalization treatments. The experiments revealed a reduced requirement for vernalization without alteration of plant performance upon short germination prior to vernalization treatment. Recent studies of others demonstrated the potential for a further accelerated phase change to generative development by germination of the grains at the soil surface and exposure of seedlings to extreme long-day conditions at a temperature of around 10 °C (termed speed vernalization). When applying these conditions in our facilities, the appropriate provision with water turned out to be a major challenge under such stress conditions. Therefore, various soil substrates and measures of coverage were compared regarding their impact on germination and plant development. The accelerating effect of extreme long day and 10 °C was confirmed, but further investigations are required to establish conditions suitable for genotypes with particularly high vernalization requirement.

Acknowledgments

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Effect of *PPD-D1*, photoperiod sensitivity gene on yield related traits under stress-free conditions in wheat

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Keywords: wheat, developmental patterns, adaptation, *PPD-D1*, minor developmental loci, field experiment

The dynamics of plant development not only has an impact on ecological adaptation but also contributes to the realization of genetically determined yield potentials in various environments. Dissecting the genetic determinants of plant development becomes urgent due to the global climate change, which can seriously affect and even disrupt the locally adapted developmental patterns. In order to determine the role plant developmental loci played in local adaptation and yield formation, a panel of 188 winter and facultative wheat cultivars from diverse geographic locations were characterized with the 15K Illumina Single Nucleotide Polymorphism (SNP) chip and functional markers of several plant developmental genes, and included into a multi-season field experiment. Genome-wide association analyses were conducted on five consecutive developmental phases spanning from the first node appearance to full heading together with various grain yield-related parameters. The panel was balanced for the *PPD-D1* photoperiod response gene, which facilitated the analyses in the two subsets of photoperiod-insensitive and -sensitive genotypes in addition to the complete panel. *PPD-D1* was the single highest source, explaining 12.1%–19.0% of the phenotypic variation in the successive developmental phases. In addition, 21 minor developmental loci were identified, each one explaining only small portions of the variance, but, together, their effects amounted to 16.6%–50.6% of phenotypic variance. Eight loci were independent of *PPD-D1*. Seven loci were only detectable in the *PPD-D1* insensitive genetic background, and six loci were only detectable in the sensitive background. The combination of *PPD-D1* insensitivity and sensitivity with the extremities of early or late alleles in the corresponding minor developmental loci resulted in significantly altered and distinct plant developmental patterns with detectable outcomes on some yield-related traits.

In conclusions, the contrasting combination of the early-late alleles of the minor loci with *PPD-D1* insensitivity–sensitivity alleles may lead to a series of developmental range that may be utilized to ensure a greater ecological plasticity of plant development. *PPD-D1* together with the minor loci also contribute to several morphological traits, but their effects on yields and yield-related traits depend more on the environment. The indirect effects of *PPD-D1* on yield-related traits may appear mostly as causal consequences of altered plant developmental patterns under unfavourable growing conditions that may occur randomly from the start of the intensive stem elongation for any of the two *PPD-D1* allele phases.

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T3: Biotic Stress Response

An Assessment of the Relationship between Seed Nutritional Components and Resistance of Maize to *Sitophilus zeamais*

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Keywords: betacarotene, seed size, infestation, storage insects, susceptibility

Maize is the mainstay of food and animal feed globally. Maize weevil, *Sitophilus zeamais*, accounts for a significant global loss of stored maize annually. Twenty-one maize genotypes were evaluated for their reaction to attack by maize weevil and the influence of nutritional contents on their response to the weevil attack. Fifty grams of whole grains of each genotype were infested in three replications with 20 unsexed weevils in a 250 ml test tube and kept under ambient conditions for 36 days. The nutritional attributes of the maize genotypes were determined. Accession TZB-SR-A had the thickest (7.32 mm) grains, while TZEE-WSTR had the thinnest grains (3.46 mm). Crude protein ranged from 8.43 to 12.78% for NG/SA/07/029 and TZB-SR-B, respectively. Beta carotene ranged from 38.91 to 51.22% for Ig-bogbo Local and EVDT-Y2000-STR, respectively. Accessions TZM-212, TZM-1296, TZB-SR-B, and TZM-1311 showed no appreciable loss in grain weight, while EVDT-Y2000-STR, which had the highest beta-carotene content, showed the highest weight loss of 2 g. These indicate sufficient differences among the maize accessions for improvement for weevil resistance. Furthermore, the findings of the present study suggest a potential role of beta-carotene in influencing the feeding preference of *S. zeamais* on maize grains. Accessions TB87/97/15, TZB-SR-A, TZB-SR-B, TZM-1311, and TZM-144 should be targeted for future improvement of the trait.

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T4: Plant-Microbe Interactions

Bacterial priming may facilitate enhanced resistance of barley to leaf rust and net blotch

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Keywords: priming, barley, leaf rust, net-blotch, GWAS, QTL, field resistance

Leaf rust (*Puccinia hordei*) and net blotch (*Pyrenophora teres f. teres*) are among the most harmful barley (*Hordeum vulgare* L.) diseases, leading to yield losses up to 60% and 70% respectively. The most effective means of their control are the use of resistant cultivars and fungicide application. For leaf rust, resistance genes Rph1 to Rph28 are known, however most of them are already overcome. Upon priming, plants respond stronger and faster to a pathogen attack, often securing higher yield. We studied if bacterial priming facilitates enhanced resistance of barley to leaf rust and net blotch in the greenhouse and the field.

Recent reports suggest that bacterial quorum sensing molecules, such as N-acyl homoserine lactone (AHL), might induce priming in plants. AHL is produced by numerous Gram-negative bacteria, e.g. *Ensifer meliloti*. We previously investigated *E. meliloti* priming efficiency in a diverse set of 198 spring barley accessions (Matros et al., 2023). Repaired *E. meliloti* natural mutant strain expR+ch producing a substantial amount of AHL and a transformed *E. meliloti* strain carrying the lactonase gene attM from *Agrobacterium tumefaciens*, which inhibits AHL production, were used. Greenhouse grown plants were treated three times with priming inducer prior to the infection with *P. hordei*. Twelve days post-inoculation the diseased leaf area and the infection type were scored, and the corresponding relative infection and priming efficiency were calculated. The same protocol was applied to another priming challenger, the net blotch-causing fungus *P. teres f. teres*. (unpublished). Significant effects ($p < 0.001$) of the bacterial treatment were observed, indicating a positive effect of priming on resistance to both *P. hordei* and *P. teres f. teres*. For both systems genome-wide association studies (GWAS) based on the observed phenotypic differences from three independent experiments and 493,846 filtered SNPs were performed. Eleven quantitative trait loci (QTL) associated to improved resistance to *P. hordei* after AHL-priming, were identified with a peak on the short arm of the barley chromosome 6H. GWAS revealed 18 significant (LOD > 4) associated markers in 12 QTL, for priming efficiency regarding net blotch infection. Among specific QTLs for each priming challenger, three common QTL regions were observed. In the current phase, we will develop and validate KASP markers, which can be used to identify primable accessions. A first field experiment with 10 selected barley genotypes was performed at JKI Quedlinburg, in order to confirm the priming effect of AHL producing bacteria

on naturally occurring fungal diseases. Seeds were coated with *B. pumilus* and *B. velezensis* (kindly provided by ABiTEP GmbH) prior to sowing and diseases were recorded during the growth period of the plants. In addition, parameters relevant to growth and yield formation were monitored. The presence of applied *Bacillus* strains in the rhizosphere was confirmed 10 and 13 weeks after sowing. We observed differences in growth and yield parameters related to the priming treatment and dependent on the genotype investigated, which are currently statistically evaluated.

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T5: Yield and Quality Improvement

Development and characterization of durum wheat *lpa* mutants by modulating the accumulation of phytic acid

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Keywords: durum wheat, biofortification, phytic acid, microelements, TILLING

The lack of micronutrients such as iron (Fe), zinc (Zn), iodine (I) and vitamins is the main cause of malnutrition defined as hidden hunger. In recent decades, the international community has faced challenges to reduce malnutrition by implementing different strategies such as diet supplementation, fortification and diversification. To date the biofortification of staple food crops remains the most valid strategy to address the problem of hidden hunger.

Phytic acid (PA) represents the major phosphorus storage sink within the kernel. In seeds PA is considered an anti-nutritional compound since it limits the bioavailability of essential mineral cations such as iron (Fe), zinc (Zn), potassium (K), calcium (Ca) and magnesium (Mg) by chelating them in the form of phytate salts poorly digested by monogastric animals, including humans, due to the lack of phytases in the digestive tract.

The research here presented is focused on the development and characterization of durum wheat genotypes biofortified in essential minerals by silencing the genes encoding a Multidrug-Resistance associated Protein 3 (MRP3), transporter involved in the accumulation of PA inside the vacuole.

The TdMRP3 mutant lines showed a significant reduction in the content of PA and were able to accumulate a higher amount of essential micronutrients (Fe, Zn, Mn) compared to the control. Agronomic analyses performed on the selected mutants revealed a reduction in number of spikelets and seeds per spike, compared to the control, but the negative effect was in part balanced by the increased grain weight. The TdMRP3 mutant lines showed morphological differences in the root apparatus such as a significant decrease in the number of root tips, root length, volume and surface area and an increase in root average diameter compared to the control plants.

These materials represent a promising basis in breeding programs focused on the obtaining new commercial durum wheats with higher nutritional value any legal restriction associated with genetically modified organisms.

Impact of epigenetic factors on the induction of bread wheat microspore embryogenesis and plant regeneration

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Keywords: microspore embryogenesis, wheat, demethylation, acetylation, doubled haploid, plant regeneration

Microspore embryogenesis (ME) occurs under the influence of stress and growth hormones. Male microspores change the path of development to the sporophyte and form embryos that regenerate into haploids or spontaneously doubled haploids (DH). The induction of this process has a complicated, multi-stage course, and its molecular basis is still not fully understood. In recent years, more and more evidence has been collected that epigenetic processes regulating gene transcription and affect the adaptive responses of cells in developmental changes, and thus also ME. Epigenetic factors play an important role in the response to abiotic stress that induces ME, as well as in plant regeneration (PR), thereby affecting the reproduction of DH. DNA demethylation and histone acetylation are two epigenetic modifications that result in over transcription. They significantly affect ME and can be successfully used in an effective strategy to increase the yield of DH in rapeseed, barley and triticale. In addition, it has been proven that trichostatin A as an inhibitor of deacetylation in wheat ME effectively influences the developmental reprogramming of bread wheat microspores towards the embryogenic pathway.

The aim of the study was to analyze the effect of DNA demethylation and histone acetylation factors on the induction of ME, PR and ploidy level (PL) of selected bread wheat genotypes.

Plant material varied in pedigree. Four winter genotypes were tested: two F1 hybrids obtained in Polish breeding companies (K393 and K20290); DH line (DH1/2) developed in anther cultures in Department of Genetics and Plant Breeding Poznan University of Life Sciences; PO19 line supplied by The Franciszek Górski Institute of Plant Physiology Polish Academy of Sciences in Krakow. In addition, two spring varieties were analyzed: DC356/08-4-5/09 (DC) supplied by National Small Grain Collection, United States Department of Agriculture, Agricultural Research Service Aberdeen-Idaho (USA) and Ac Abbey (AC) shared by Agriculture and Agri-Food Canada (AAFC), Semiarid Prairie Agriculture Research Centre, Canada).

Four compounds modifying the level of methylation at two concentrations (2.5µM; 5µM) and two exposure times (2 and 7 days) were studied: 5-azacytidine, 5-Aza-2'deoxyctidine,

zebularine and trichostatine A. Controls without treatment were used for all combinations. Each treatment was repeated 3 times.

Studied epigenetic factors have influenced the induction of ME, as well as PR of bread wheat. Differences in the efficiency of these parameters were observed between the analyzed treatments. Embryos were obtained from all tested genotypes, but not in all experimental combinations. Lower concentration of compound in induction media with a longer exposure time appeared to be the best treatment for green PR.

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T6: Bioinformatics, Genomics and Genome Editing

A new 7K SNP array for oats (*Avena sativa*) provides an efficient and informative genotyping tool for research and breeding

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Keywords: *Avena sativa*, spring oats, SNP, diversity, gene bank, genetic resources, genotyping

The development of genomic tools for oats has long lagged behind that of other cereals such as barley and wheat. However, with the recent release of several different oat genome sequences (Kamal *et al.* 2022, Peng *et al.* 2022), and an ongoing pan-genome project in oats, this situation is rapidly improving. Nevertheless, there is a need for a simple and easily accessible genotyping platform for hexaploid oats that can be used by researchers and breeders alike. Previously, a 6K Illumina™ SNP array was developed based on North American germplasm by Tinker *et al.* 2014, but this array is no longer maintained. Here, we describe a novel 7K Illumina™ SNP array for oats, developed from European germplasm. The array has been tested on >10,000 breeding lines, gene bank material, and commercial varieties of diverse origins. Compared to the previous 6K array, the 7K array produces nearly 2 times as many useful polymorphic SNP in the investigated material. We also report on the pattern and distribution of genetic diversity in a sample of 900 spring oat gene bank accessions of Nordic origin, as well as 600 advanced breeding lines using the new 7K array.

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Generation of new allelic diversity for durable rust resistance of wheat and barley by editing *SUGAR TRANSPORT PROTEIN 13*

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Keywords: genome editing, Stp13

Fungal diseases such as leaf rust (*Puccinia triticina*), stripe rust (*Puccinia striiformis*), stem rust (*Puccinia graminis*) and powdery mildew (*Blumeria graminis*) pose a constant threat to global cereal production. Changing climatic conditions in temperate regions are likely to increasingly facilitate the spread of rust fungi, leading to the accelerated emergence of new pathotypes and associated epidemics. The most effective and environmentally friendly way to control these diseases is the use of resistant varieties. Therefore, establishing broad-spectrum and durable rust resistance has tremendous potential to reduce fungicide use in cereal crops. The *Lr67* resistance gene was first described in wheat and encodes the hexose/proton symporter STP13. *Lr67*-mediated resistance is causally based on a base exchange in the second exon of the sugar transporter gene *TaSTP13-D*, which causes amino acid exchange G144R (Moore et al. 2015). The *Lr67res* allele of wheat confers universal resistance to leaf rust, stripe rust, stem rust and powdery mildew. However, this gene has not yet been exploited for breeding of modern, short-straw varieties due to genetic linkage with the *Reduced height 1* wild-type allele. A wide range of new *Stp13* allelic variants was generated in wheat and barley by targeted mutagenesis (Figure 1 A-B), which in principle may confer resistance in the same way as *Lr67res*. In preliminary tests, some of the generated barley mutants were found indeed to be less susceptible to the leaf rust fungus (Figure 1 C-D). The barley and wheat mutants generated so far provide a promising basis for research on the *Lr67* resistance mechanism and the development of varieties with broad and durable resistance to fungal diseases.

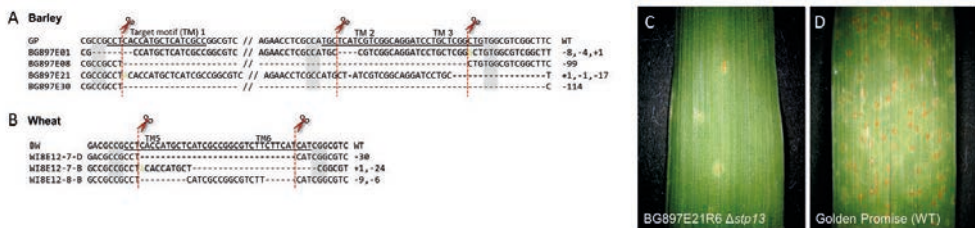


Figure 1: Most frequent mutations obtained in STP13 of barley (A) and wheat (B). Reduced leaf rust susceptibility of barley *stp13* mutants (C) in comparison with the wild-type (D). GP, BW: wild-type sequences of cvs. Golden Promise and Bobwhite

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Targeted mutagenesis for virus resistance in barley

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Keywords: biotechnology, CRISPR, genome editing, plant pathology

The Potyviridae are the largest family of plant-pathogenic viruses. Members of this family are the soil-borne bymoviruses *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) which, upon infection of young winter barley seedlings in autumn, can cause yield losses as high as 50%. Resistance breeding plays a major role in coping with these pathogens. The *EUKARYOTIC TRANSLATION INITIATION FACTOR 4E* (*EIF4E*) is a well-known susceptibility gene for potyvirus infections in many plant species. Its resistance-conferring alleles are widely used in winter barley breeding, but new virus strains have overcome these resistances. Thus, there is a need for novel sources of resistance. In ancient landraces and wild relatives of cultivated barley, alleles of the susceptibility factor *PROTEIN DISULFIDE-ISOMERASE-LIKE 5-1* (*PDIL5-1*) were identified to confer resistance to all known strains of BaYMV and BaMMV in Europe. Here, we present the employment of different approaches of Cas9-mediated targeted mutagenesis, including base editing, of *HvEIF4E* and *HvPDIL5-1* in winter and spring barley to generate new alleles for bymovirus resistance, circumventing the tedious and time-consuming introgression by crossing to current elite lines. We induced knockouts as well as new functional alleles with base substitutions for *HvEIF4E* and *HvPDIL5-1* similar to those that were described to render certain landraces resistant. Primary homozygous mutants were produced in winter barley, and transgene-free homozygous M₂ mutants were produced in spring barley. A variety of mutants was mechanically inoculated with BaMMV, by which a number of the new alleles were demonstrated to confer resistance to the used virus strain.

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BRIDGEcereal: a webapp streamlining unsupervised learning to survey and graph indels from pangenomes

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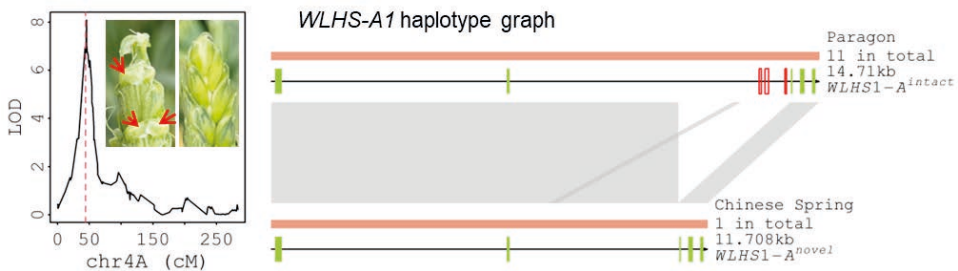
Keywords: pangenome; insertion and deletion; QTL; GWAS

Large insertion and deletion (indel) polymorphisms contribute significantly to phenotypic variations through altering gene structure or expression. However, surveying and graphing large indels across assemblies for specific genes are challenging and painstaking tasks.

To overcome the challenge, we devised two unsupervised learning algorithms, CHOICE (Clustering HSPs for Ortholog Identification via Coordinates and Equivalence) and CLIPS (Clustering via Large-Indel Permuted Slopes). CHOICE automatically retrieves the segments harboring the ortholog from each assembly for the desired All-vs-All comparison while CLIPS groups accessions sharing same indels into haplotypes for concisely haplotype graphing.

We then constructed an interactive webapp BRIDGEcereal (<https://bridgecereal.scinet.usda.gov/>) to expedite this process. Over hundred assemblies from 5 major cereal crops (Wheat, Barley, maize, rice, and sorghum), were compiled. The only required input is a gene model ID or a transcript sequence. Two adjustable parameters, up- and down-stream search boundaries, enable to survey the unknown sizes and locations for indels outside of the gene body. We demonstrated that mining pan-genome through BRIDGEcereal could accelerate gene discovery and characterization with multiple wheat genes underlying QTL/GWAS intervals, such as *WLHS-A1* is a promising gene for the classic *Hooded* QTL. The versatile design enables to seamlessly incorporate newly released assemblies.

Figures



The haplotype graph generated by BRIDGEcereal suggests *WLHS-A1*, a MADS-box gene segregating a 3-kb indel containing 3 exons (open red boxes), is a promising candidate for the classic *Hooded* QTL.

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T7: Phenotyping Technologies (Generally)

Integration of *in vitro* androgenesis in wheat breeding

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Keywords: androgenesis, common wheat, spelt wheat, breeding

About haploid plants, the first scientific publication was reported (Blakeslee et al. 1922) more than 100 years ago in Science. Routine production of *in vitro* haploid plants started in the 1980-90s and since then plant breeders have been attracted to its potential in acceleration and modernization of traditional breeding programmes. By *in vitro* androgenesis, this genetic-based technology and its many different aspects become a new field of plant breeding. Lots of laboratories started intensive research projects to use and integrate *in vitro* androgenesis into the breeding methods.

For breeding use, the most important is to have (a) a well-working anther culture system from the induction of androgenesis for green plant production, (b) acceptance of doubled haploid (DH) lines in a traditional, but modified breeding process, (c) integration of DH breeding gains for a business orientated variety policy. The lecture looks through these details of androgenesis-based bred and spelt wheat breeding (Lantos et al. 2013, 2019).

(a) Since 1973, many different induction media were published, but for today the Chinese published and modified induction medium (W14mf) looks the best medium to produce embryoids in large scale. For plantlet induction, the copper included medium (190-2Cu) is the best, where we regenerate the plantlets for transplantation in soil and later we transfer them to the nursery. In winter wheat, the different abiotic stresses have a positive effect on the spontaneous rediploidization of haploid wheat plants.

(b) When we induce the DH lines from segregated breeding material, the produced lines have perfect homogeneity (pure lines), but these aren't selected pure lines, these are random induced lines. We can say that the homogenous line (DH) is ready, but the breeder has to place the new lines in the breeding program. At this point, the breeder(s) have an important challenge to make the decision via selection using the traditional methods with a combination of the modern (MAS, phenotyping etc.) selection methods.

(c) After a relatively quick process, we can release a DH new variety (or line for hybrid wheat breeding program) which is better than the controls and it's ready for marketing. The integration of a new genetics- and plant physiology-based phenomenon in a plant breeding program gives a new challenge to the breeders and businessmen, too. The time-saving in the breeding process is important, but a good market policy is also very important to generate a higher profit. For a genetically homogenous variety strict variety maintenance (pedigree method) is also very important to avoid out-cross and mechanical mixing.

Through the integration of androgenesis in our common and spelt wheat breeding program, we generated more than 5- thousand DH lines per year. New varieties of common wheat were released in the last 30 years and related to spelt wheat, the method was used for a dietetic-goal (FODMAP) breeding program.

Acknowledgments

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Predicting the unpredictable: A novel approach to screening for inner leaf tipburn

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Cultivated lettuce (*Lactuca sativa*) often show inner leaf tipburn, a condition characterized by necrotic lesion development on younger leaf tips associated with insufficient calcium (Ca) supply. This problem is more pronounced in lettuce grown during high-growth seasons, like summer and spring. Tipburn can be particularly detrimental to heading lettuce varieties such as 'icebergs' since symptoms appear just before harvest, leading to substantial economic losses for growers and packing companies. The existing tipburn screening methods involve time-consuming and costly field trials that extend until lettuce reaches maturity. In this study, we introduce a novel hydroponics-based screening method to induce early-stage tipburn using a systemic low Ca approach. We employ the high-throughput phenotyping system Phenovator-II at NPEC for quantitative tipburn assessment in addition to traditional ordinal scoring. In our tipburn scoring method, we show the potential to use maximal photosynthetic efficiency (dark Fv/Fm) as a proxy for tissue damage along with classical RGB images. We apply this approach to the LK200 lettuce diversity panel to identify sensitive and tolerant lettuce varieties. Furthermore, we conduct a correlation analysis of one timepoint with a field trial of the same population, cross-validating tipburn severity observed in the hydroponics system. Our results demonstrate the reliable induction of early-stage tipburn under specific conditions. If successfully implemented, this screening approach has the potential to significantly reduce the screening duration from months to weeks. This would offer a swift and predictable solution for phenotyping the tipburn trait, thereby expediting breeding and the identification of quantitative trait loci (QTL) associated with the complex genetic architecture of tipburn.

Wheat biomass and leaf area prediction by machine learning

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Keywords: High-Throughput Phenotyping (HTP), phenomics, biomass, shoot area, machine learning, i-Traits, wheat

Phenomics has emerged as important tool to bridge the genotype-phenotype gap. To dissect complex traits such as highly dynamic plant growth, and quantification of its component traits over a different growth phase of plant will immensely help dissect genetic basis of biomass production. Based on RGB images, models have been developed to predict biomass recently. However, it is very challenging to find a model performing stable across experiments. In this study, we recorded RGB and NIR images of wheat germplasm and Recombinant Inbred Lines (RILs) of Raj3765xHD2329, and examined the use of multimodal images from RGB, NIR sensors and machine learning models to predict biomass and leaf area non-invasively. The image-based traits (i-Traits) containing geometric features, RGB based indices, RGB colour classes and NIR features were categorised into architectural traits and physiological traits. Total 77 i-Traits were selected for prediction of biomass and leaf area consisting of 35 architectural and 42 physiological traits. We have shown that different biomass related traits such as fresh weight, dry weight and shoot area can be predicted accurately from RGB and NIR images using 16 machine learning models. We applied the models on two consecutive years of experiments and found that measurement accuracies were similar suggesting the generalised nature of models. Results showed that all biomass-related traits could be estimated with about 90% accuracy but the performance of model BLASSO was relatively stable and high in all the traits and experiments. The R^2 of BLASSO for fresh weight prediction was 0.96 (both year experiments), for dry weight prediction was 0.90 (Experiment 1) and 0.93 (Experiment 2) and for shoot area prediction 0.96 (Experiment 1) and 0.93 (Experiment 2). Also, the RMSRE of BLASSO for fresh weight prediction was 0.53 (Experiment 1) and 0.24 (Experiment 2), for dry weight prediction was 0.85 (Experiment 1) and 0.25 (Experiment 2) and for shoot area prediction 0.59 (Experiment 1) and 0.53 (Experiment 2). Based on the quantification power analysis of i-Traits, the determinants of biomass accumulation were found which contains both architectural and physiological traits. The best predictor i-Trait for fresh weight and dry weight prediction was Area SV and for shoot area prediction was projected shoot area. These results will be helpful for identification and genetic basis dissection of major determinants of biomass accumulation and also non-invasive high throughput estimation of plant growth during different phenological stages can identify hitherto uncovered genes for biomass production and its deployment in crop improvement for breaking the yield plateau.

T8: Phenotyping Technologies – Wheat Initiative

Using high-throughput functional phenotyping to increase understanding of plasticity of drought response behavior in barley

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The production of cereals like barley faces increasing challenges due to climatic change entailing, among others, more frequent and intense drought events. Breeding drought resilient barley cultivars requires in-depth knowledge on their physiological drought response mechanisms. We used a high-throughput functional phenotyping platform to autonomously, continuously and simultaneously measure water flux in the soil-plant-atmosphere-continuum of four European spring barley cultivars. The aim was to examine their response behavior to a standardized drought treatment implemented around heading through gradual deficit irrigation mimicking the gradual drought development in the field. Non-conserving, productivity maximizing water use as observed in cv. *Chanell*, is characterized by maximum transpiration rates under well-watered conditions, yet rapid transpiration reduction under drought. In our tested drought scenario this resulted in poor drought recovery and large yield penalties. Water-conserving, survivability-enhancing behavior, as observed in cv. *Baronesse* and cv. *Formula*, with low pre-drought transpiration, yet a more gradual transpiration reduction under drought coupled with good recovery (resilience), was found to prevent large yield losses.

cv. *RGT Planet*, which demonstrated a plastic water use behavior, i.e. non-conserving water use under ample water supply and moderate transpiration decrease under drought like a conserver, showed high resilience and produced the highest and most stable yields. Such a dynamic water use combined with high drought resilience and favorable root traits could potentially make up an ideotype resilient to intermediate drought. Prospective studies will further examine these results through field experiments and crop growth simulations.

Remote sensing for plant breeding and variety characterization

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INNOVAR is a H2020 European project where the principal aim is to update and augment valuable information in bread and durum wheat Distinctness, Uniformity and Stability (DUS) and Value for Cultivation and Use (VCU) variety registration procedure using latest advancements in genomics, precision agriculture/remote sensing and machine learning.

Two panels of ca. 270 each bread and durum European varieties were assembled for field trials across 13 locations for 3 years (2020-2023). Data are being collected using actual DUS CPVO varietal registration protocol (30 traits) as well as additional phenotypes related to wheat disease response and vegetation indexes through UAV. The two panels were genotyped with a common Illumina iSelect SNP 90K Chip array, broadly used for wheat genotyping, and UAV high throughput phenotyping technology.

UAV technologies used in 9 key wheat developmental stages played an important role by acquiring more than 20 vegetation indexes (VI). In particular, vegetation indexes were used to estimate dynamic parameters associated to: a) ground coverage, b) biomass accumulation, c) nitrogen uptake and chlorophyll content, d) senescence rate. Results showed clear differences regarding stay green/maturity associated to varieties coming from different breeding programs.

As regards to molecular technologies, the panels were surveyed based on haplotype SNP analysis for improved genetic relationships and GWAS.

GWAS analysis identified QTLs in common between manual assessment measurements and vegetation indexes for some traits, for example culm glaucosity. This strengthens the importance of considering high-throughput phenotyping technologies for a more accurate traits discrimination in varietal registration protocols.

Finally, these results will provide a framework for CPVO and breeders to set new and improved methods and practices for updating and improve in efficiency the plant varietal registration procedure, including the assessment of candidate variety against panels of already registered varieties.

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Combining models, standardized experimental setup and specialized protocols to achieve physiological phenotyping in wheat

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With the increase capacity in phenotyping, experiments using large number of genotypes (from 50 to 1000) become more common in the current crop research. Experiments using standardized experimental setup offers consistency, comparability, cost-effectiveness through optimization and interoperability of data that enable widespread collaboration and enhance statistical power. In this work, we first used the strength of large-scale field experiments with standardized protocols to quantify the sensitivities of yield components (thousand kernel weight, kernel per spike and spike number) to five environmental variables in 220 winter wheat cultivars during 81 developmental sub-phases, ranging from double-ridge to seed desiccation. Triple-interactions between short-term environmental fluctuations, phenology and genotype-specific sensitivities determine the formation of yield components. Furthermore, we showed significant additive effects of different environmental variables. In contrast to standardized phenotyping protocols, tailored techniques for measurements or specific experimental designs are required for addressing specific research questions. Here we combined three platform experiments with specialized designs to understand the phenotypic differences between plants grown under controlled or field environment. Our results showed that inter- and intra-genotypic competitions within a platform experiment were triggered by the phenotypic plasticity in response to the platform-specific environment and explain to a large extend the differences in phenotype between controlled- and field environment.

Magnetic Resonance Imaging of early wheat seedlings and possible relevance for root water uptake

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Seed germination and seedling establishment are the first important steps in a plant's growing cycle. We optimized a measurement sequence to quantify the early stages of root development in young seedlings in natural soil. We used magnetic resonance imaging (MRI), providing us with 3D information about root structures non-invasively. By growing up to 18 seedlings per pot ($\varnothing=12.5\text{cm}$, 10cm height) and by focusing on early growth (up to 4 days after start of germination), a much higher plant throughput compared to traditional 3D root measurement protocols in soil was achieved. Due to high temporal resolution of the acquired data (4 images per day), dynamic traits such as shoot and root emergence time were obtained accurately. We used this 'deep phenotyping' approach, with several temporal and spatial layers of data, to investigate phenotypic differences within the 8 parent lines of the NIAB MAGIC population. Clear phenotypic differences in structural (e.g. root angle, root lengths, and number) and temporal (e.g. time of root emergence, shoot emergence) were quantified. The initial root angle may be important for rooting depth at later stages which can potentially influence root water uptake (RWU) depth profiles. We show preliminary data using our home-build Soil Water Profiler (SWaP) on localized RWU for wheat plants in relatively wet soil and compare these with results we found for other species. This new MRI automation approach offers a promising tool for high throughput root seedling screening in natural soil environments, along with an opportunity to link the results with physiological measurements on root performance.

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Global wheat full semantic segmentation dataset

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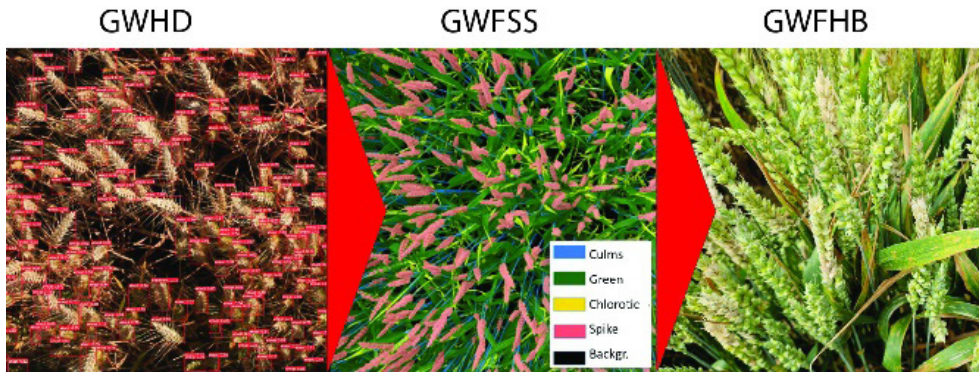
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Keywords: global wheat, deep learning, semantic segmentation, wheat canopy, field phenotyping

Deep learning methods for image processing are rapidly advancing and imaging techniques have become a standard for classification and quantification in agriculture. Several studies already tackled organ segmentation of wheat under field conditions (Anderegg et al., 2023; Dandrifosse et al., 2022; Serouart et al., 2022). However, these studies rely on small datasets from single experiments. Global wheat (<http://www.global-wheat.com/>) aims to combine such efforts to train robust algorithms which can segment almost anything in a wheat canopy. To do so, we assembled a large dataset of around ~40000 images collected from field phenotyping platforms around the globe. These red-green-blue (RGB) images were taken with a spatial resolution below 0.5 mm at different developmental stages under different viewing angles. The image information will include geographic location, developmental stage, genotype, or agricultural treatment. Based on manually allocated image tags and available meta-information, we will select a diverse subset of ~4000 images to label all relevant features observable in a wheat canopy. The dataset will serve as public benchmark for training and validation of deep learning algorithms. We will present the state-of-the-art of organ segmentation of wheat and highlight how to use the technology for physiological breeding and disease quantification.

Figures



The full semantic segmentation dataset builds up on the earlier Global Wheat Head Detection dataset and will further be used for within organ segmentation, e.g. for Fusarium head blight (FHB) quantification. Respective training image will be provided within the framework of the EU-Project PHENET.

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Genome-wide scan and haplotype analysis identified candidate loci for nitrogen use efficiency under drought conditions in winter wheat

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Keywords: Drought, nitrogen deprivation, NUE-related traits, GWAS, photosynthesis, allele effect, transcript abundance.

Climate change causes extreme conditions like prolonged drought, which results in yield reductions due to its effects on nutrient balances such as nitrogen uptake and utilization by plants. Nitrogen (N) is a crucial nutrient element for plant growth and productivity. The understanding of the mechanistic basis of nitrogen use efficiency (NUE) under drought conditions, is essential to improve wheat yield. Here, we evaluated the genetic variation of NUE-related traits and photosynthesis response in a diversity panel of 200 wheat genotypes under drought and nitrogen stress conditions to uncover the inherent genetic variation and identify quantitative trait loci (QTL) underlying these traits. The results revealed significant genetic variations among the genotypes in response to drought stress and nitrogen deprivation. Drought impacted the plant performance more than N deprivation due to its effect on water and nutrient uptake. GWAS identified a total of 27 QTL with a significant main effect on the drought-related traits, while 10 QTL were strongly associated with the NUE traits. Haplotype analysis revealed two different haplotype blocks within the associated region on chromosomes 1B and 5A. The two haplotypes showed contrasting effects on N uptake and use efficiency traits. The *in silico* and transcript analysis implicated candidate gene coding for cold shock protein. This gene was the most highly expressed gene under several stress conditions, including drought stress. Upon validation, these QTL on 1B and 5A could be used as a diagnostic marker for NUE and drought tolerance screening in wheat.

Barley response to drought: current achievements based on high-throughput image analyses

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The domestication followed by genetic selection had major impact on genetic erosion in most crop species resulting in narrow gene pools and threatening future breeding activities. Wild progenitors are one promising solution to enrich breeding pools with exotic desired alleles. Within the BRACE (Barley Response and Adaptation Changing Environments) consortium a set of 400 Halle Exotic Barley lines (termed HEB-400) was selected which have been deeply characterized under field conditions in multiple environments and controlled atmosphere phenotyping platforms with the aim to identify wild barley alleles which improve resilience to drought. For this, all 400 HEB lines were grown under control and stress conditions in repeated experiments in the medium sizes plant phenotyping platform of IPK. Drought stress was applied individually for each plant to avoid effect of growth stage on drought escape mechanisms (BBCH31 cereal growth stage). The platform is equipped with an automated watering system and cameras imaging at different wavelengths, enabled us to extract extensive list of traits from non-destructive plant images over time. In this regard, the image-derived parameter biovolume was proved as potential proxy for biomass and other agronomic traits as it was able to clearly differentiate phenotypic profiles of plants between drought and control treatments. In addition, the time-lapse image-derived traits like biovolume were proven to be highly valuable to understand growth dynamics and crop performance. For this case, we applied various growth models to predict biomass accumulation and extract relevant parameters that can interpret the biological nature of plant growth and stress tolerance. Exome capture data are currently available for HEB-400 collection, such image-based traits and model-derived parameters are now used for subsequent genetic mapping to uncover the genetic basis of complex agronomic traits. Therefore, image-derived traits such as biovolume provide a basis for functional mapping and detection of dynamic QTLs underlying complex dynamic process of plant growth. Taking all together, the current research and the related analyses provide further evidence of potential suitability of image-derived traits such as biovolume to fill the gap between genomics and phenomics for efficient detection of genes controlling complex traits and stress tolerance.

T9: Phenotyping Technologies (CePPG)

Phenomics of clock and growth plasticity in barley: a tango of two genomes

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In plants, the role of chloroplasts and mitochondria (plasmotype) in controlling circadian clock plasticity and overall plant robustness has not been elucidated. It is not also clear what is the relationship between plasticity of circadian clock rhythms and fitness of the plants under changing environments, a timely topic in the scenarios of current and future climatic change. We utilize the SensyPAM high content phenomics platform for photosynthesis rhythmicity measurements, combined with field trials, to explore consequences of domestication and genetic diversity on clock output and its thermal plasticity, using barley as a model crop. Our studies identified drivers of the clock (DOC) loci that control the loss of clock plasticity between wild and cultivated populations; in these loci we identify significant signature of selection in the barley Pangenome². In the wild populations, we identified and experimentally validated key DOCs, including the chloroplast genome in which alleles of the *rpoC1*, a member of the RNA polymerase complex, are modulating the clock plasticity in barley³. Furthermore, population analysis shows a non-random association of alleles for chloroplastic *rpoC1* and nuclear DOC loci, thereby indicating the signature of selection for clock plasticity before domestication. The pleiotropic effects of the DOC on plant fitness in the field, including that of the plasmotype, prompt us to develop advanced mapping populations for pre-breeding and additional phenomics modelling in different environments. I will also present the first built cytonuclear multiparent population (CMPP) in crops, which captures the segregation of ten wild organelles and nuclear genomes in the background of an elite cultivar. I will also show preliminary data that highlight its potential to study hitherto un-explored cytonuclear by environment interactions for growth and grain phenotypes.

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Tracking dynamic root responses to nitrogen in barley with an automated rhizotron platform

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Increased nitrogen (N) use efficiency is key to stabilize crop yields under the challenging conditions imposed by climate change and more restrictive fertilization policies. Although the ability of crops to acquire N is highly dependent on their root systems, root traits, especially those that are responsive (i.e., plastic) to temporal and spatial changes in N availability, are still rarely considered in breeding programs. To investigate the potential of such traits in increasing N uptake efficiency, we used the automated rhizotron platform installed inside IPK's PhenoSphere, a facility with fully controlled environmental conditions. This platform can image roots throughout most of the vegetative phase of barley plants, thus allowing us to track dynamic responses of complex root systems. In our experiments, we manipulated the spatial distribution of N in the rhizotrons and followed growth trajectories and developmental outputs as roots grew through low-N patches or as they encountered placed N fertilizer. To investigate which root architectural changes are associated with increased N uptake, elemental tracers were distributed in specific patches within rhizotrons and their concentrations assessed in shoots. The daily imaging revealed how barley roots mount a strong localized response when encountering a N fertilizer by increasing lateral root development in sections of axial seminal and nodal roots that have direct contact with the fertilizer and decreasing root branching elsewhere. We are now using this approach to screen root system architecture and N accumulation in a panel of up to 200 barley accessions from IPK's Gene Bank. Large phenotypic variation for several root traits have already been identified and, for some dynamic traits, such as seminal root elongation, a positive correlation with N acquisition detected. The obtained results will help to map genetic factors underlying the phenotypic diversity of barley root systems and their responses to N.

Can selection on phenotypic traits replace selection for yield in arid environments?

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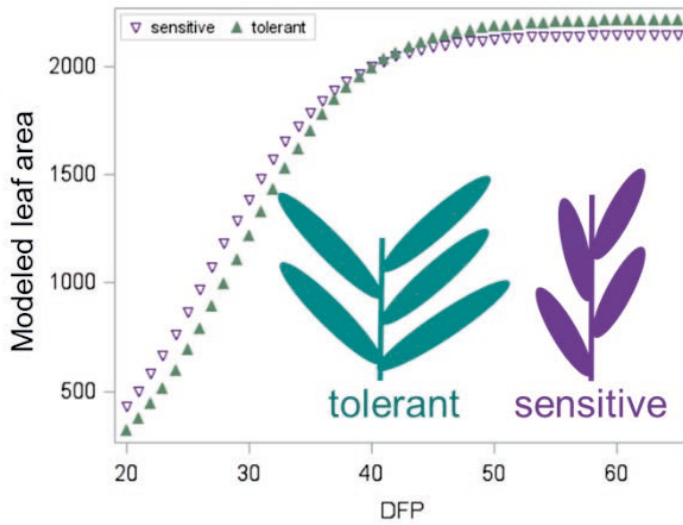
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Keywords: drought tolerance, abiotic stress, laser scanner, non-linear regression, decision trees

As climate changes, maintenance of yield stability requires efficient selection for drought tolerance. Yield-based selection in arid environments increases yield stability, but takes decades. Our previously published metabolite/transcript based selection model (DOI:10.1111/pbi.12840) efficiently selected tolerant, but failed to detect sensitive genotypes in a validation trial (DOI: 10.3389/fpls.2020.01071). Therefore, we studied whether a second selection layer based on phenotypic markers derived from automatic phenotyping systems can improve selection. Our test population comprised 63 *Solanum tuberosum* ssp. *tuberosum* genotypes selected from a population segregating for drought tolerance. The validation population contained 13 genotypes from the test population plus 7 unrelated cultivars. We determined drought tolerance indices for these genotypes based on starch yield in three test systems representing Central European drought stress scenarios. An automobile laser scanner system monitored shoot growth continuously to estimate features like plant height, leaf area and leaf movement. We developed a data evaluation pipeline to estimate descriptive parameters for the growth of control (c) and drought-stressed (s) plants. For shoot height and leaf area, the parameters initial slope of growth (k), inflection point of the growth curve (T_m) and maximum (Max) were estimated by logistic regression. For leaf angle, means were estimated for six intervals of the diurnal cycle and three different age ranges (vegetative, tuber initiation, tuber filling). Analysis by general linear model revealed significant genotypic variation for all features. Leaf movement and growth curve parameters for plant height were significantly affected by the treatment and varied between years. The relatively small environment effect on leaf area made the trait a good candidate for tolerance selection. Decision tree analysis selected T_m (leaf area)(s), plant height(c) at the end of the vegetative growth and the leaf angle(c) before noon as the most important features for tolerance class prediction. Multiple regression analysis on data from the genetically more diverse validation population confirmed leaf area and leaf position parameters as predictive tolerance traits.

Figures



Modelled growth of leaf area in drought-tolerant and -sensitive ideotypes

Acknowledgments

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Digital assessment of leaf rust resistance and water use in wheat (*Triticum aestivum* L.) at the seedling stage

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The production of bread wheat, *Triticum aestivum* L., is an essential component of global food security. Given the need to increase wheat production to meet the growing demand for food, combating various biotic and abiotic stresses that significantly affect wheat production is becoming a key priority.

Two relevant challenges among these stress factors are leaf rust, initiated by the fungus *Puccinia triticina*, and drought stress, a consequence of water scarcity. Both cause considerable economic losses and pose a serious threat to wheat production. Leaf rust leads to substantial yield losses and contributes to the impairment of grain quality. Simultaneously, drought stress, further exacerbated by climate change-induced weather extremes, exerts a decisive influence on wheat production by inhibiting growth, reducing yields, and affecting wheat crop quality.

Against this background, we tested two phenotyping approaches at the early juvenile stage to analyze leaf rust resistance and drought stress tolerance in a medium-size collection of wheat genotypes. On the one hand, the Macrobot is used as an image-based procedure to quantify seedling resistance; on the other hand, the Plantarray (DiTech) is deployed to quantify the transpiration patterns of the plants in extremely high temporal resolution. In addition to external traits such as symptoms, we also investigated physiological parameters such as biomass production.

Here we show preliminary data and provide a first insight into most recent phenotyping methods that allow to assess traits that are not obtainable under field conditions. The integration of these analyses enabled a comprehensive assessment which support pre-breeding approaches of wheat and contribute to a sustainable crop production.

Integrated phenotyping of root and shoot growth dynamics in maize

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Most automated plant phenotyping platforms are either only able to phenotype shoots or roots of plants but not both organ systems simultaneously. In order to offer the opportunity to assess the growth and development dynamics of both shoot and root systems jointly, a root phenotyping installation was integrated into an existing automated non-invasive high-throughput shoot phenotyping platform. The amended platform is now capable of conducting high-throughput phenotyping at the whole-plant level, and it was used to evaluate the vegetative root and shoot growth dynamics of five maize inbred lines and four hybrids thereof, as well as the responses of five inbred lines to progressive drought stress. The results showed that hybrid vigour (heterosis) occurred simultaneously in roots and shoots. Under water deficit, the root growth dynamics responded faster than shoot growth. While total root volume (TRV) was significantly reduced 10 days after the onset of the water deficit treatment, the estimated shoot biovolume was significantly reduced about 6 days later.

Multiple automated high-throughput phenotyping facilities were applied to investigate the physiological and morphological mechanisms involved in maize resilience to drought and high temperature stress under controlled environmental condition within the DROMAMED project. A selected maize panel from the Mediterranean area was used for non-stress (control) and stress (drought and heat) experiments. Root phenotyping of a subset of these lines was conducted in the PhenoSphere_Rhizotron system. A subset of the lines was selected based on results of these studies and further deep phenotyping was performed under simulated field-like conditions in the PhenoSphere_PhenoCrane system.

CBB7 – Poster

T1: Genetic Resources for Crop Improvement

Exploring the diversity of European spelt genebank collection: high-throughput SNP genotyping of the spelt gene pool compared with bread wheat

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Keywords: spelt, wheat, SNP, genebank genomics

Spelt wheat (*Triticum aestivum* ssp. *spelta* L. em. Thell, $2n = 6x = 42$, AABBDD) represents one of the most ancient food grains in Europe. Its natural adaptation to a wide range of environmental conditions leads to good performance in low-input agriculture, and its outstanding stress resistance and specific quality parameters are of great interest for the diversification of cultivated bread wheat. For instance, today's wheat production is facing new challenges from biotic and abiotic stresses imposed by climate change. The eroded genetic diversity of cultivated wheat due to the continuous selection for high yielding ability cannot surmount such difficulties. A large set of the spelt wheat and bread wheat accessions were collected and preserved in the European Gene Banks representing an important legacy and providing a hitherto unexploited source of genetic variation, which can be utilised for wheat improvement.

The aim of the present work was to provide detailed genotypical and phylogenetic characterisation of the complete spelt wheat and bread wheat collection, encompassing 188 spelt lines in parallel to 80 modern bread wheat cultivars. Genotyping was carried out using a high-density 25K Illumina SNP genotyping array. Maximum likelihood tree and principal coordinate analysis showed considerable differences within the different spelt accessions and between spelt wheat and bread wheat lines indicating that wheat and spelt form two distinct gene pools. We identified 406 chromosome-specific markers applicable for the reliable identification of spelt chromosomes in the wheat background.

Our results provides a solid basis for future crop improvement by offering new breeding strategies where new wheat varieties can be developed by selecting suitable genetically distant parents from the genebanks and transfer hitherto untapped alleles into modern cultivars.

Acknowledgments

The authors extend their sincere appreciation for AGENT Project (H2020-SFS-2019-2) from the Research and Innovation Action of the European Union (Grant agreement ID: 862613) and TKP2021-NKTA-06 provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-NKTA funding scheme. AC was supported by János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00416/23/4).

Automated micro- and macro-phenotyping of Powdery mildew infection in a diverse collection of *Aegilops biuncialis* to support resistance gene discovery and transfer into wheat

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Keywords: *Aegilops*, powdery mildew, automated microscopy, microphenomics, interspecific hybridization, BluVision

Powdery mildew (Pm) is one of the major diseases affecting wheat. Pm causes substantial reductions in yield and quality of wheat. The most environmentally responsible approach to safeguarding crops seems to involve harnessing natural genetic resources and engaging in selective breeding to enhance resistance against diseases.

Goatgrasses (*Aegilops*) are close relatives of *Triticum*, which makes them promising gene sources for increasing wheat genetic diversity through interspecific hybridization. The annual goatgrass, *Aegilops biuncialis* is a rich source of genes responsible for drought and salt tolerance, as well as resistance to pests and diseases, including Pm¹⁻³.

An automated phenotyping pipeline for plant-pathogen interactions was developed at IPK Gatersleben. This state-of-the-art system, known as the BluVision Micro- and Macrophenomics Framework, allows precise, high-throughput quantification of disease-related phenotypes, focusing on powdery mildews and rusts.

In the present work, a unique collection of 168 accessions of *Ae. biuncialis* were investigated for resistance to Pm using the BluVision system. Detached leaves were infected with wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*), and phenotyped at micro- and macroscopic levels. The macrophenotyping analysis did not reveal visible infections in the *Ae. biuncialis* population; it appeared that the pathogen did not infect the plants. However, the BluVision Micro system allowed much closer insights on a cellular and subcellular level into the resistance mechanisms. The number of fungal microcolonies, an indicator of resistance in the early stages of interactions (pathogen penetration and colony establishment), revealed a wide range of responses. Whereas 57 of the tested genotypes remained completely resistant, allowing no colony formation, the rest were moderate to fully susceptible, in some cases even exceeding the number of colonies in the susceptible wheat control (cv. Kanzler).

This wide phenotypic variability indicates a valuable range of genetic diversity of *Ae. biuncialis* making it excellent for mapping Pm-resistance-associated loci and a suitable gene source for improving Pm tolerance in wheat through interspecific hybridization. The robust and detailed phenotypic data on the powdery mildew–*Aegilops* interaction will be used for a genome-wide association study (GWAS) to explore new resistance QTLs, suitable targets for chromosome-mediated gene transfer from the identified *Ae. biuncialis* genotypes.

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Comparison of RAPD and SSR molecular marker methods for classification of khorasan wheat genebank accessions

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Keywords: gene bank collection, tetraploid wheat, genetic diversity, RAPD, SSR, molecular marker, dendrogram

Khorasan wheat (*Triticum turanicum* Jacubz.) is a tetraploid wheat species, originates from the fertile crescent, present day Iran. Regarding its taxonomic classification, someone describes it as a subtaxon of *T. turgidum* subsp. *turanicum* (Jakubz.) A. Love & D. Love, while others describe it as an independent species. In addition, many synonyms can be found in the databases. An important task in the management of germplasm collection is the systematic classification of the items, which can be done by both traditional and molecular taxonomy methods. PCR technique based molecular marker methods make possible to identify the genotypes and establish their relationship. In this study, we used 19 Khorasan wheat gene bank items and as controls, 3 additional tetraploid wheats and one bread wheat. Our goal was to determine whether Random Amplified Polymorphic DNA (RAPD) or Simple Sequence Repeat (SSR) is more suitable for identifying gene bank items. A fundamental difference between the two methods is that a single primer is used during the PCR reaction in RAPD while a pair of primers is used to amplify the target sequence in SSR. We worked with 8 decamer RAPD primers and with 15 SSR primer pairs which detected 50 and 79 bands, respectively. In both cases, we determined the degree of polymorphism. Based on the binary matrix of the RAPD, the smallest distance with a Jaccard coefficient of 1 was found between items 3 and 13 and items 8 and 11. The highest genetic distance with a Jaccard value of 0.479 was obtained between the Khorasan genotypes 15 and 22. Concerning SSR, with a Jaccard value of 0.054, the largest genetic distance occurred between items 2 and 17, while items 8 and 10 showed the smallest distance (0.947) indicating a very close relationship. On the dendrogram obtained by both methods together, the *T. turanicum* Jakubz. genotypes intended for identification were well separated from the four control species. Our results reveal that SSR method gave a more accurate picture about genetic relationships between the tetraploid wheats in this experiment.

Acknowledgments

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Assessment of the possibility of using wild species of the genus *Triticum* spp. as effective sources of resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*) in wheat and triticale

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Keywords: wild relatives, powdery mildew, resistance, *Triticum* spp.

Wild plant species related to cultivated forms are a valuable source of many genes associated with increased tolerance to abiotic stresses and conferring resistance to various types of biotic stresses. The introduction of disease-resistant varieties into cultivation is one of the current trends in reducing the occurrence of crop pathogens. Powdery mildew is the main fungal pathogen of cereals, which significantly reduces the quality and quantity of yield. The study aimed to check which of the diploid species belonging to the genus *Triticum* spp. can be a valuable source of resistance to powdery mildew for wheat and triticale cultivars.

The subject of the study were genotypes belonging to the species *Triticum timopheevii* (Zhuk.) Zhuk. subsp. *armeniacum*, *Triticum urartu* and *Triticum monococcum* subsp. *monococcum*, *Triticum monococcum* subsp. *aegilopoides*. Analyses were performed at the seedling stage on leaf fragments of 10-day-old plants using host-pathogen physiological tests. The tests were based on 3 isolates of *Blumeria graminis* f. sp. *tritici*. Infection was assessed 12 days after inoculation on a 5-point scale, where 0 means no infection and 4 means infection, in which the pathogen covers more than 50% of the leaf surface.

The conducted research showed that the most promising source of resistance to powdery mildew is *T. timopheevii*. 16 out of 19 tested genotypes of this species were completely resistant to the pathogen isolates used in the tests. *T. monococcum* genotypes were also characterized by a high level of resistance. Most susceptible genotypes were identified among *T. urartu*.

Preliminary screening studies have shown that different diploid species belonging to the genus *Triticum* spp. have different potential and the possibility of using them in breeding programs as a source of resistance to powdery mildew. The greatest potential for searching for resistance genes was identified in *T. timopheevii*.

Molecular characterization of cis- and trans-variation and its impact on hybrid vigour in maize

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Keywords: hybrid vigour, allele specific expression, transcription factor, ZmBZR1, cis-and trans-regulatory variation

One of the most remarkable achievements in maize breeding is the development of maize hybrids. The superior performance of hybrids compared to their parents is attributed to hybrid vigour, which exhibits polygenic inheritance in maize¹. This project aims to elucidate the differential expression of genes in hybrids compared to parents at a molecular level, particularly regarding their allele-specific expression (ASE). Differential expression of two alleles may result from variations in various cis and trans-regulatory elements².

The primary objective of the initial study is to identify and validate causative sequence variations responsible for the differential expression of target genes, focusing on upstream-acting transcription factors (TFs). It has been revealed through hybrid allele-specific chip-seq analysis that variations in brassinosteroid-responsive transcription factor (ZmBZR1) binding are linked to maize traits³. DAP-seq analysis for ZmBZR1 identified candidate genes regulated by this TF.

Furthermore, we intend to focus on identifying TFs contributing to ASE through trans-regulatory variation. Proteoforms of drought stress related TFs displaying coding sequence variations, will be selected. DAP-seq will be used to profile the DNA binding landscape of the selected TFs proteoforms. In the final phase of this project, we will endeavour to identify and characterise enhancer sequences involved in gene regulatory networks in maize.

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Evaluation of *Avena sativa* landraces resistance to *Puccinia coronata* f. sp. *avenae*

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Keywords: crown rust, oat, disease resistance

The fungal diseases of oats cause large yield losses in this crop. One of the most economically important diseases is crown rust, caused by the fungus *Puccinia coronata* f. sp. *avenae*. Breeding cultivars with effective resistance genes is the most efficient and environmentally beneficial method of controlling crown rust. To date, about 100 genes conferring resistance to crown rust have been described and characterised, but their use in breeding programmes is very low, especially in Europe. The main concern is the reduction of cultivar value by introducing undesirable traits through crosses with *Pc* gene donors. Therefore, it is necessary to search for new, effective sources of resistance to this pathogen within landraces, which are better accepted by breeders as components for crosses.

The aim of the present study was to search for potential sources of resistance to crown rust among *A. sativa* landraces. The study was carried out on 190 accessions. The oat seeds were provided by the Gene Bank of the Crop Research Institute, Ruzyně (Czech Republic) and the National Centre for Plant Genetic Resources (Radzików, Poland). Most of the landraces tested originated from Poland and the former Czechoslovakia, Yugoslavia and the Soviet Union. The host-pathogen tests were used to assess the resistance of individual genotypes to five crown rust isolates with different virulence profiles. Response to infection was scored on a 6° scale. Disease symptoms were assessed 10 days after inoculation using the qualitative infection type (IT) scale, where HS = highly susceptible - large pustules with little or no chlorosis; S = susceptible - moderately large pustules with little or no chlorosis; MS = moderately susceptible - moderately large pustules surrounded by extensive chlorosis; MR = moderately resistant - small pustule surrounded by chlorosis or necrosis; R = resistant - chlorotic or necrotic mottling; and HR = highly resistant - no visible reaction.

The studies showed that the oat landraces analysed were mainly classified as highly susceptible. It is known that local varieties are heterogeneous as they have not undergone rigorous selection, and therefore individual plants resistant to individual isolates were identified within the genotypes analysed. This variation within the accession should be taken into account when selecting forms for crosses. Only two landraces from Morocco (52300 and 52309) from the Polish genebank and the landrace from Turkey (03C0702060) from the Czech genebank showed a high level of resistance. The resistance of these accessions was uniform but limited to three or two of the *P. coronata* isolates used in the host-pathogen test. The identified genotypes can be used as a potential source of resistance to this pathogen after verification of the mode of inheritance.

Molecular characterization of local Serbian and Bulgarian wheat accessions for their contribution to sustainable agriculture

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Keywords: genetic resources, molecular markers, wheat breeding, low-input farming

The increasing pressure of climate change, reduction of genetic diversity on farms and constant decrease in arable land present the most challenging aspects for today's crop production. Reintroduction of diverse adapted local varieties and underutilised landraces of wheat, potentially tolerant to different biotic and abiotic stresses, could ensure stable yield under low-input agriculture. The uncharacterized local varieties and landraces of bread and durum wheat from Serbia and Bulgaria, were collected from small-scale farms and gene banks within a project funded by the Benefit-Sharing Fund of the International Treaty on Plant Genetic Resources for Food and Agriculture, aiming to evaluate their genetic diversity. As a part of this comprehensive project, the selected material was evaluated using different molecular marker techniques. The level of genetic diversity and relatedness of a set of 90 Serbian and Bulgarian local varieties and landraces of bread and durum wheat was assessed using microsatellite molecular markers and 25K wheat Infinium Array. The total of 141 alleles was detected at 20 microsatellite loci with an average number of 7.05 alleles per locus. The PIC value varied from 0.344 to 0.837. Markers of A genome were the most informative. The structure analysis revealed presence of three subpopulations, while principal coordinate analysis showed almost clear separation of durum wheat, Bulgarian and Serbian bread wheat accessions. For each group private alleles were detected. A total of 17,930 high-quality SNPs were retained after filtering and the highest number of SNPs was recorded for the B genome. The UPGMA analysis showed clear separation between Serbian and Bulgarian accessions. The obtained results indicate genetic richness of the collected material and contribute to better characterization and completion of the passport data. These results, together with the results of phenotypic evaluation, will build up to the knowledge required for utilization of local wheat genetic resources in breeding climate-resilient varieties suitable for sustainable farming.

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T2: Environmental Adaptation

Investigation of heat stress tolerance in winter barley (*Hordeum vulgare* L.) varieties based on yield response types

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Keywords: winter barley, heat stress, yield components

Multiple abiotic environmental factors can affect the development and crop production of plants. The overall grain yield and yield stability specific to a variety are determined by the combination of yield components. The biodiversity of varieties is also relevant in the case of tolerance because the genotypes of different geographic origins may have different environmental adaptability. From a population of 184 winter barley varieties of different geographic origins, we selected 28 genotypes based on a high-throughput SNP marker genotyping (GWAS). In addition, we considered spike type and attempted to preserve genetic diversity, thus selecting 14 two-row and 14 six-row varieties for the experiment. To investigate acclimatization to extreme weather conditions, we initiated a controlled climate chamber experiment in Phytotron. Based on the changes in yield data, we analyzed the types of reactions that adaptation capacity, variety characteristics, or tolerance levels could result in regarding high-temperature stresses. The selected barley genotypes were treated with heat at different developmental stages: a single treatment at the stage of grain filling (ZD49), and a combined treatment at the stage of stem elongation (ZD31) and then at booting (ZD49). Barley plants at the ZD31 stage were subjected to a high-temperature treatment for 5 days, while at the grain filling stage they were exposed to a 10 days high-temperature treatment. For heat stress treatment, we applied 30°C at the ZD31 stage and 35°C at ZD49. The plants received continuous water supply during both the control and heat stress treatments.

Overall, we measured 18 different morphological traits and yield component parameters. We found that the varieties can be divided into four groups based on the responses of stress treatments. Significant differences were observed between the groups for both the single and combined treatments. In both treatment types, we identified a group consisting of varieties with lower parameter values and below-average performance in terms of treated average, indicating sensitivity to heat stress. The groups that responded better to the treatments were separated based on response types, but overall, these groups either reached or exceeded the average of the 28 genotypes subjected to stress. In both heat stress treatment, one group showed clearly worse main spike results and better side spike yield, while the other group had higher main spike values but showed a decreasing tendency in overall side spike yield. Based on the analyses, certain varieties grouped together in terms of adaptability and resistance for both treatment types.

Taking everything into consideration, we will further investigate which varieties belonging to different response types can be more effectively utilized in a breeding program aimed at enhancing stress tolerance and upgrading the gene pool.

Acknowledgments

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Proteome profiling revealed the adaptive reprogramming of barley flag leaf to drought and heat stress

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Keywords: abiotic stress, combined stress, flag leaf, proteomics, regulatory components, stress-induced proteins

Plants, as sessile organisms, permanently struggle for survival under various environmental stressors and in field conditions they are usually exposed to several hazards rather than just one; for example, drought is often accompanied by an elevated temperature. The response to environmental stress is manifested at the whole-plant level, and the plant reaction to combined stress cannot be easily deduced from the effect of each stress alone. Recent advances in omics approaches have facilitated the exploration of plant genomes; however, the molecular mechanisms underlying the responses of barley and other cereals to multiple abiotic stresses remain largely unclear. Hence, the extensive investigation of molecular responses to combined stresses is of great importance. The enrichment of transcriptomics by illustrating proteome remodeling appears to be a milestone in deciphering the mechanisms and pathways of plant responses to stress. Moreover, research conducted on barley is of particular value since it is now being used as a model system for genetic/molecular studies in cereals. Significant progress has been made in unraveling the genome of barley, which has accelerated our understanding of the molecular background of its behavior in a changing environment. Unfortunately, there are only limited studies on molecular characterization of the barley's flag leaf, whose vitality under stress is fundamental for grain yield formation.

In the present study, we employed liquid chromatography coupled with high-resolution mass spectrometry to identify stress-responsive proteins on the genome-wide scale of barley flag leaves exposed to drought, heat, or both. Profound alterations in the proteome of genotypes with different flag leaf sizes were found, including proteins involved in the photosynthetic apparatus. Proteins induced by specific stress treatment were identified as well as candidates underlying the universal stress response were proposed, including dehydrins. Moreover, the putative functions of several unknown proteins that can mediate responses to stress stimuli were explored using Pfam annotation, including calmodulin-like proteins. Finally, the confrontation of protein and mRNA abundances was performed. A correlation network between transcripts and proteins performance revealed several components of the stress-adaptive pathways in barley flag leaf. Taking the findings together, promising candidates for improving the tolerance of barley and other cereals to multivariate stresses were uncovered. The presented proteomic landscape and its relationship to transcriptomic remodeling provide novel insights for understanding the molecular responses of plants to environmental cues.

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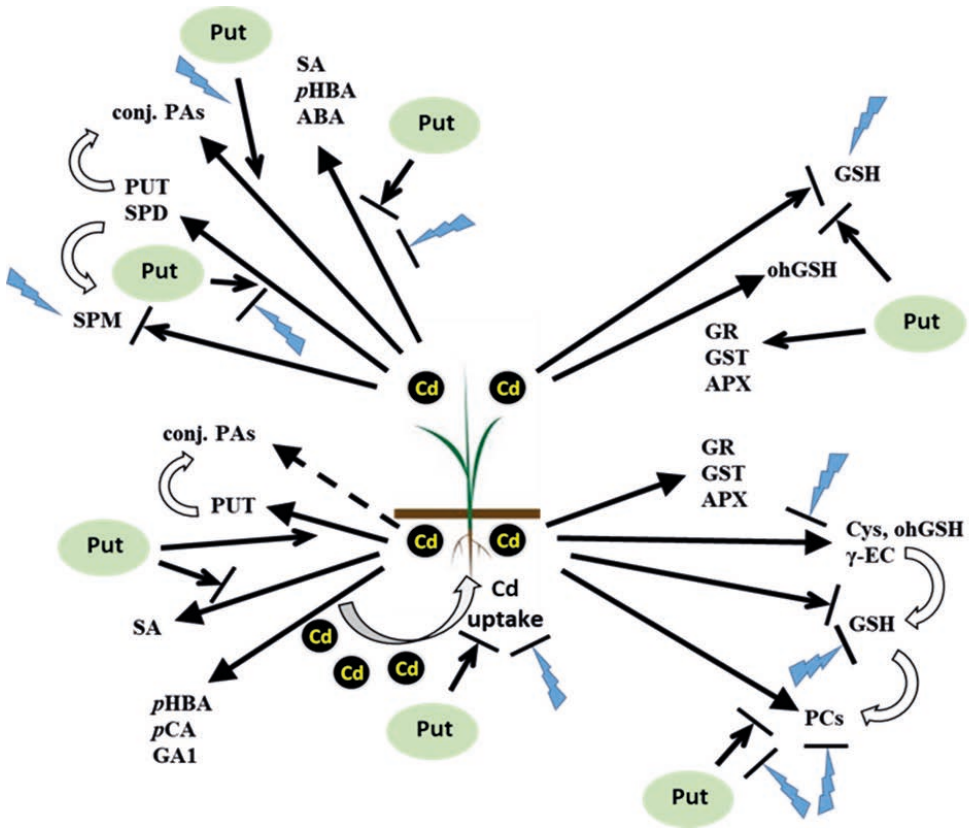
Different responses of wheat to cadmium stress under white and blue light, modulated by pre-treatment with putrescine

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Keywords: oxidative stress, polyamines, phytochelatins, plant hormones, thiol compounds

Numerous aspects of plant biology, including morphology, photosynthesis, and metabolism, are significantly influenced by blue light. Although some studies have indicated that blue light may have a beneficial effect on plants experiencing stress, the precise mechanisms involved remain largely unexplored. Concurrently, new research highlights the significance of polyamines in enhancing plant stress tolerance. In this study, we hypothesized that blue light induces distinct adaptive responses in wheat plants under Cd stress compared to white light and that blue light may also modify the potential protective effects of exogenous putrescine. Our findings reveal that exposure to blue light mitigated the adverse effects of Cd stress, in contrast to white light conditions. At both the metabolic and gene expression levels, blue light exerted its influence, resulting in decreased Cd absorption, increased levels of conjugated polyamines, and decreased phytochelatin production. Pre-treatment with putrescine exhibited protective effects under both light conditions, with a more pronounced benefit observed under white light. In addition, putrescine pre-treatment revealed disparities between blue and white light conditions under Cd stress, specifically in the synthesis of phytochelatins, polyamine metabolism, and accumulation of phenolic compounds and plant hormones. These findings highlight the function of blue light in regulating Cd tolerance in wheat and its ability to alter defense strategies in the presence of excess putrescine.



Summary figure about the most interesting and pronounced changes induced by cadmium stress (Cd), putrescine pre-treatment (Put), or blue light treatment (blue lightning sign) in the investigated metabolite contents and enzyme activities

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Evaluation of the influence of microbial consortium on the root system of wheat subjected to drought

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Keywords: water deficit, root, electrical capacitance, *Triticum aestivum*

The tolerance of crops to environmental stresses (both biotic and abiotic) determines the economic efficiency of farm production. Among the abiotic stresses, periodic water shortages are the main factor limiting plant production and causing economic losses. On the other hand, it is believed that the size of the root system influences the amount of water and minerals taken up by plants, improving drought tolerance. In the present experiment, we studied the wheat root system growth performance under water deficit and modification of its response to the applied microbial consortium.

Materials for studies consisted of eleven winter wheat cultivars: Arkadia, Artist, Asory, Euforia, Grana, Impresja, Informer, Jutta, Opoka, Yukanand Wilejka. First, seed germination and eight weeks of vernalization were made in the phytotron. After that, the seedlings were planted into stonewares dug into the ground. The experiment was performed in three variants: control (watered), drought, drought + microbial consortium. The microbial consortium was composed of 4 isolates: DPGb6, *Bacillus* sp. (isolate with 4% NaCl); DAB 1, *Arthrobacter globiformis*; K50XA, *Enterobacter* sp., all three producing auxins; and TES10B3 isolate (isolate with 10% NaCl) mobilizing phosphorus from sparingly soluble phosphorus compounds, having chitinolytic activity and producing siderophores.

The electrical capacity of the roots was measured with a capacitance meter at a voltage of 1 V between the ground electrode, inserted into the soil, and the electrode covering the plant shoots, according to the method described by Cseresnyés et al. (2018). It is a non-invasive technique based on the electrical properties of the root system. However, it has some limitations resulting from the sensitivity of the measurement to external factors, such as soil water content, texture, and ion composition, and the position of the soil electrode relative to the plant electrode. The advantages of this method are simplicity and low price on the one hand and, on the other hand, the potential resulting from the properties of the electric current (flowing almost exclusively through the absorbing and not suberized parts of the roots) to assess the functional size (activity) of the root system, including the hairs. Unlike other commonly used techniques, the capacitive method measures the activity of the roots.

Compared to the control, the application of beneficial microorganisms increased the growth characteristics of the roots, as well as the above-ground parts of wheat plants in most of the tested cultivars, growing under the conditions of a half-reduced water dose.

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Functional analysis of HvHY5, a key transcription factor of photoreceptor mediated signalling in barley

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Keywords: light spectrum, light signalling, HY5, barley

Increasing frequency of weather extremities represents a constantly growing risk to decrease crop yield, thus, understanding the relevant defensive gene regulatory systems is crucial. The spectral composition of sunlight, the ratio of the different photon components changes throughout the year. For example, by the approaching of the cold season in temperate climate, red:far-red (R:FR) photon ratio decreases, and blue:red (B:R) photon ratio increases, while from spring to summer, changes with the opposite direction could be observed. Altering the spectra of the illuminating light could be beneficial to plants under several types of abiotic stresses, for example freezing (as a light pre-treatment), which is one of the most researched area of this field. Winter hardy cereals achieve their frost tolerance through a 2-7 weeks cold acclimation process. However, sudden frost spells could occur before the completion of this process during autumn. It is well known that FR enrichment of the incident white light (W) would enhance or induce cold acclimation, but the effect of B light on the process has become the most recent target of scientific papers. Photoreceptors absorb light signals and transmit them to specific transcription factors (TFs), which orchestrates the development of the light response. In *Arabidopsis*, HY5 (ELONGATED HYPOCOTIL 5) is one of the most important TFs integrating light and temperature signals, since HY5 could receive light signals from a wide range of sunlight spectrum. Despite its fundamental role in developmental and stress physiological processes in *Arabidopsis*, it is currently uncharacterised in barley. Since light quality can markedly influence cold acclimation in *Arabidopsis* via the HY5 protein, it could be presumed that this TF is also an important element of this process, and light signalling in barley as well. Thus, our aims are to confirm the physiological function of the putative HvHY5 protein by expressing it in *Arabidopsis* *hy5* mutant (SALK) to complement the *hy5* phenotype. According to our results, putative HvHY5 was able to complement *Arabidopsis* *hy5* mutant phenotype under different light environments. HY5 related gene expression of the complemented lines, and the yeast II hybrid analysis results of HvHY5 will also be presented.

Acknowledgments

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Impacts of the water shortage and waterlogging in combination with the elevated atmospheric CO₂ concentration on the rooting habits of an early ripening winter wheat (*Triticum aestivum* L.) variety

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Keywords: climate change, drought stress, cereal production, belowground biomass, root structure

Cereal production faces challenges in the Carpathian Basin nowadays and the negative impacts of climate change are expected to get more intense in the future. The temperature is increasing in the Central European region while the yearly precipitation does not change significantly. However, the frequency of precipitation-related extreme events such as heavy rainfalls and dry periods are increasing. The spatial heterogeneity of the climate, hydrological and soil conditions in the central Carpathian Basin can cause frequent waterlogging in the early phase of the plant development and water shortage in the late phenophases could occur in the same region. Therefore the present study aimed to examine the effects of the waterlogging simulated in the BBCH 21 phenophase and the water shortage induced at the beginning of heading in the BBCH 51 stage on the root length and the root biomass. Besides the well-watered control (C), three treatments were involved in the study: waterlogging (WL), water withdrawal (DS) and the combination of the two stress treatments (WL+DS). The rise in the atmospheric CO₂ concentration could stimulate plant development and production, therefore, the impacts of the stress treatments on the root system were determined both under atmospheric and increased (750 ppm) CO₂ levels. The experiments were carried out in two chambers of the greenhouse of the HUN-REN, Centre for Agricultural Research, Martonvásár, Hungary between February and July 2021. The examined winter wheat variety was Mv Ikva, the earliest genotype in the variety assessment of the institute. Two germinated and vernalized plants were planted in PVC tubes (diameter of 110 mm and length of 750 mm) containing polyethene bags filled with graded sand (particle size between 0,1 and 0.7 mm). The plant number was reduced to only one vigorous plant per tube on the 10th day of the experiment. Nutrients were supplied with a half-strength Hoagland solution; plants were watered every two days. At full maturity, the plants were harvested and the phenological and yield properties were determined. The polyethene bags were taken out of the PVC tubes and removed. Roots were gently washed out of the sand with running tap water. The total root length per plant and the average diameter of the roots were measured with the WinRHIZO Pro system (Regent Instruments Ltd. Canada). The root length and dry biomass decreased by 15.7 and 17.4%, respectively as a result of the CO₂ enrichment by optimum watering. The waterlogging led to a significant increase in root length and root dry biomass by 95.46% and 80.81, respectively, compared to the control, but this trend remains detectable only at ambient CO₂ concentration. At 750 ppm CO₂ level, only the root dry biomass decreased by 26.23% under WL compared to the C but the root length did not enhance. The water shortage at the BBCH 51 stage resulted in a decrease in root length and biomass under both CO₂ concentrations. Under the ambient CO₂ level, the root length decreased by 38.28% but the change in root dry

biomass was not significant. As a consequence of the combination of waterlogging with the drought stress (WL+DE) the root lengths of the plants were significantly shorter than in the control (13.8% and 41.4% at 400 ppm and 750 ppm, respectively).

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T3: Biotic Stress Response

Role of WAK1, wall-associated kinase in barley biotic stress tolerance against *Pyrenophora teres f. teres*

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Keywords: *Hordeum vulgare* L., *Pyrenophora teres f. teres*, biotic stress tolerance

Pyrenophora teres f. teres (PTT), the causal agent of the net form of net blotch disease of barley. This is one of the most important fungal pathogens of barley that can cause significant yield loss. Deployment of adaptable, resistant cultivars is one of the main tasks of plant breeders. This method reduces the use of fungicides, environmentally sustainable and is the most economical way of plant protection.

Several genes play a role in developing resistance to the net form of net blotch, the effectiveness of these depends on the developmental stage of barley and the fungal pathotypes present in the local population of the pathogen. Relatively few net blotch resistance sources have been mapped and the majority of the resistance sources were identified in spring genotypes, but winter cultivars also have a great economic impact. Therefore, our aims were to characterise the resistance of the barley collection against the pathogen in seedling and adult plants and the identification of chromosome regions conferring the net form of net blotch resistance.

We determined the seedling and adult resistance of 260 barley cultivars to the net form of net blotch in greenhouse. The investigation of seedling and adult resistance were characterized based on the infection with four and three monoconidial isolates, respectively. Resistance of the cultivars were determined based on the symptoms (Tekauz's scale) caused by the infection.

Twenty-one percent of them were found to be resistant, 34.7% moderately resistant, 18% moderately resistant-moderately susceptible, and 7-7% moderately susceptible and susceptible. There was no significant correlation between seedling and adult resistance to different PTT isolates, suggesting an independent regulation behind these resistant barley genotypes in this study. Examining the genetic background of resistance to net blotch, we identified 285 significant marker-trait associations (MTAs) using genome-wide association analysis. The strongest association was observed on chromosomes 3H, 6H, and 7H. The 178 of MTA connected with seedling and 107 with adult resistance. The A/T difference between susceptible/resistant cultivars at the JHI_Hv50k-2016-183207 marker locus was closely correlated

($1.75E^{-11}$) with cultivar resistance (resistant cultivars carried thymine) on chromosome 3H. This mutation hotspot is located in the transcription terminator of the WAK1 (wall-associated kinase receptor) gene. Terminators influence the strength of gene expression. In the case of weak terminator, a short polyA tail is added to the mRNA and the mRNA is rapidly degraded or overrun can occur and the next gene is also transcribed. The next gene on the chromosome is PR5, this and WAK1 may also play a role in fungal resistance. Pathogenesis Related 5, also known as thaumatin proteins are classic, well-known antifungal defense proteins. The strong termination of WAK1 was paired with adenine. The PTT infection resulted the increase of WAK1 and PR5 gene expression in the resistant genotypes. Based on our results, we can conclude that the expression of the WAK1 and PR5 genes affect the PTT resistance of barley, but further experiments are underway to clarify their role.

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Comparison of various inoculation methods to study seedling resistance of barley varieties to *Pyrenophora teres*

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Keywords: *Hordeum vulgare*, *Pyrenophora teres* f. *teres*, *Pyrenophora teres* f. *maculata*, resistance, artificial inoculation

Barley (*Hordeum vulgare* L.) is among the four most substantial cereals in the world and the third in Hungary. It has a significant role in the world's various healthy foods, a source of fermentable material for beer and livestock feed and it is a model plant for research. *Pyrenophora teres*, the causal agent of net blotch disease, is known as one of the main fungal pathogens of barley worldwide. Deployment of resistant cultivars is the most economic and eco-friendly method to control plant diseases.

The objective of this study was to evaluate the resistance of young plants of various barley genotypes after artificial inoculation of both intact seedlings and detached leaves with *Pyrenophora teres* f. *teres* (PTT) and *P. teres* f. *maculata* (PTM), by applying different inoculation methods.

Intact seedlings were treated under greenhouse conditions whereas detached-leaf assays were carried out in the laboratory. Altogether the response of 11 barley genotypes to 2 PTT and 1 PTM monoconidial isolates were studied. Fungal isolates were originated from Hungary and grown on V8PDA and V8 agar media. The applied inoculation methods are as follows: intact seedlings inoculated by hand sprayer or a brush with and without washing the leaves with water before inoculation, detached-leaves inoculated with the droplets. The area under the disease progress curve (AUDPC) was calculated from the Tekauz lesion types at various times.

Multiple analysis of variance on data from the intact seedling and detached-leaf assays revealed that there were no significant differences among the different inoculation methods, however, the interaction A (washing before inoculation) x B (spray/brush or droplet inoculation), BxC (genotype) and AxBxC was significant. So, the response of some varieties was significantly influenced by the applied inoculation method(s). Based on the AUDPC values from the detached-leaf assay, there were no significant differences in susceptibility between the first or second leaves. With detached-leaves, resistance of a large number of barley genotypes to several pathogens or different isolates of pathogens can be tested at the same and in a relatively short time, regardless of the environmental conditions. In our studies, we showed a moderately strong correlation between the resistance of intact seedlings evaluated in the

greenhouse and the resistance determined by detached-leaf technique. Based on our results, this latter technique may be suitable for routine testing of the resistance of barley breeding materials to net blotch.

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Identification of stable reference genes for RT-qPCR studies of oat (*Avena sativa* L.) response to powdery mildew (*Blumeria graminis* f. sp. *avenae*) infection on transcriptome level

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Keywords: reference genes, powdery mildew, oat, RT-qPCR, genes expression

The reverse transcription quantitative real-time PCR (RT-qPCR) technique is widely applied in the analysis of gene expression due to its high sensitivity and specificity. However, for reliable analysis of gene expression, a normalization step is necessary. The most common strategy is based on reference genes (RGs), which are internal controls with stable expression levels in the tested material under the experimental conditions. Therefore, the selection of appropriate RGs is one of the most crucial points in the RT-qPCR experiment. Numerous genes that are necessary for proper cellular metabolism are widely used as RGs in many studies. Nevertheless, many reports indicate that the expression profiles of these genes can be unstable in certain experimental conditions, species, or tissues. In this study, we conducted to select the most suitable RGs for the determination of transcriptomic changes in oat plants during powdery mildew infection.

The research material consisted of F₂ oat hybrids obtained by crossing the Av1860 genotype containing the *Pm4* resistance gene with the Fuchs cultivar susceptible to powdery mildew infection, together with both parental forms. Fourteen-day-old seedlings of analyzed genotypes were inoculated with powdery mildew spores in the phytotron. Plant leaves for RNA isolation were collected 1 h, 3 h, and 6 h after plant inoculation. As a control forms pre-infection plant tissue samples were used.

Obtained genomic RNA was reverse transcribed to cDNA and used as a template for qPCR with SYBR Green as a reporter dye. In the presented study 10 putative RGs were analyzed. Seven of these were reference genes used most often in gene expression analyses for oat (ACT, GAPDH, TUB α , EF1 α , EIF4A, TBP, ADPR). The other three genes encoding: ferredoxin-NADP reductase (FNR), phosphoribulokinase (PRK), and lipid transfer protein 1-like (LTP) were orthologs of genes with the highest stability of expression in powdery mildew-infected wheat seedlings. These genes were selected *in silico* using the RefGenes tool from the Genevestigator platform [<https://www.genevestigator.com>]. The sequences of all tested gene transcripts were obtained from the GrainGenes database. Primers for qPCR were designed using the Primer-BLAST and PrimerQuest tools and verified *in silico* through the OligoAnalyzer tool. The PCR amplification efficiency was determined for each primer pair by the analysis of the slope obtained from a standard curve generated from a serial dilution of pooled cDNA. Based on the obtained results, the amplification efficiency (E) and correlation coefficient (R²) of the primers were calculated. The stability of expression of analyzed genes

in examined oat tissue was determined by four different algorithms: geNorm, NormFinder, BestKeeper, and RefFinder.

The amplification efficiency value for analyzed RGs ranged from 82.905% for the *AsLTP* gene to 109.162% for the *AsPRK* gene. Of the 10 potential reference genes tested, the *AsTBP2* gene encoding TATA-binding protein II subunit showed the most stable expression in the tested material. The *AsTUBa* gene encoding α -tubulin also showed high stability of expression in analyzed conditions. The results obtained were the same for most of the algorithms tested, except for BestKeeper.

Based on the results, it was concluded that the combination of *AsTBP2* and *AsTUBa* genes appears to be optimal for normalization in analyses of gene expression changes in oat plants at the seedling stage in response to plant infection by powdery mildew.

The differentiations in stress reactions of barley plants with contrasting wax and trichome structure compositions

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Keywords: abiotic and biotic stresses, cereal plants, drought, *Fusarium*, phenology, stress combination responses, chlorophyll fluorescence parameters

Global warming has led to the concurrence of several abiotic and biotic stresses in plants. Moreover, the developed models predict a rapid increase in the frequency of this type of environmental events. Plants activate a specific and unique stress response when subjected to a combination of multiple stresses. Superficial tissues (epidermis) and structures (cuticle and trichomes) of plant organs, as the first barrier in plant–environment interaction, play a crucial protective role against multiple abiotic and biotic stresses. Recent studies have confirmed that trichomes can support fungal infection by enabling attachment of the fungal spore/hyphae to the surface of the trichomes, which allows for further plant colonization. Although the aerial epidermis of plants plays a crucial role in environmental interactions, the defence structure shaping of this plant part in terms of plant development is still not fully understood.

In this study, the lines derived from the crosses combination between Bowman *eceriferum* (*cer*) mutants related to glaucous or nonglaucous phenotypes and early/late heading barleys were employed to reveal the role of the epidermis structure in stress response of barley plant with different phenology. Chlorophyll fluorescence dynamic changes, as a convenient and sensitive indicator of plant stress responses, were investigated in our experiments to evaluate the plant reactions on both drought and fungal infection (*Fusarium* Head Blight). In addition, to explore the differences in plant reaction to specific level of water scarcity, two type of drought treatments (mild and severe drought) were applied. In order to compare the fungal infection symptoms severity between different genotypes, the SEM observations were performed. In this study, artificial modification modulators were also used to highlight the plant phenology effects (Gibberellin - GA and Trinexapac-ethyl - TR).

Based on the conducted analysis, it can be concluded that there was a significant impact of abiotic and biotic stresses on the efficiency of the photosynthetic apparatus, and such studied plants' response was also dependent on the genotype and development points (DPs), in which the measurements were performed. It should be noted that the mild drought, regardless of the combination with the biotic factor, caused smaller changes in the course of the OJIP curves. In the last term (DP) of measurements for almost all analysed plant material, the most negative impact was observed in severe drought, *Fusarium* and GA treatment. During the recovery process, all plants returned to their proper functioning to varying degrees.

Our results provide a new insight into the underlying mechanisms contributing to tolerance to combination of multiple stresses in cereal crops and highlight the effects of heading time to plant stress reactions.

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T4: Plant-Microbe Interactions

Progression of *Fusarium* infection in alternative hosts

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Keywords: wheat disease, *Fusarium culmorum*, mycology, weeds, alternative hosts, phytopathogenic fungi

Weeds can play an important role in facilitating the infection of wheat fields with the fungal phytopathogen *Fusarium* by acting as an alternative host. It has already been shown that infected perennial weeds on the edges of agricultural fields can significantly increase the presence of *Fusarium* in nearby wheat ears by acting as overwintering vessels for the phytopathogenic fungi. Some empirical studies have also reported that gramineous weeds host more overwintering *Fusarium* than non-gramineous weeds, but it is unclear whether or not these observations are due to innate susceptibility differences in the weeds. The main objective of this study was to identify differences in the reaction of different weed species to infection by *Fusarium culmorum*. In order to do this, we grew gramineous and non-gramineous weeds in a climate chamber and infected them with a pathogenic *Fusarium culmorum* strain through the roots. The plants were analysed after 3 and 9 weeks after infection in order to observe the development of the infection in the rhizo-, endo- and phyllosphere of the weeds over time.

We determined that while gramineous weeds were more infected in the roots than non-gramineous weeds, they were less infected in the shoots. This implies that our hypothesis that gramineous weeds are just generally more susceptible to *Fusarium* infection cannot be confirmed in this experiment. We also found that the roots were already infected after three weeks and that there was a large increase in the infection load of all weeds between three and nine weeks post initial infection. Despite high infection loads in infected plants, there were no clear visual differences between them and the control plants. The large differences in the mean fungal load detected in the different weed species was also very high. Further research should focus on whether the trends mentioned above are observed when different *Fusarium* species or strains and other infection methods are used to assess the susceptibility of the different weed species. Alternatively, further research could also focus on exploring how the large differences in the susceptibility of common weed species to fungal pathogens such as *Fusarium culmorum* could be used for practical ecological applications and phytopathogen infection mitigation.

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Evaluation of 1,3,4-thiadiazole derivatives as stimulants for cereal plant resistance to fungal diseases

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Keywords: 1,3,4-thiadiazole, fungal pathogens, cereals, resistance

The rapidly adapting population of plant pathogens necessitates effective solutions to protect crops from fungal attacks. Introducing genetic sources of resistance to cultivated forms is an effective, but long-term process. An alternative solution is the use of chemicals that can replace existing therapeutic strategies in combating plant diseases. In recent years, the development of heterocyclic agrochemicals has become a major trend due to their flexible structure and low mammalian toxicity. Numerous literature reports indicate that 1,3,4-thiadiazole derivatives exhibit a wide spectrum of biological activity, such as insecticidal, fungicidal, or herbicidal activity. These compounds are plant activators with the potential to induce disease resistance in plants.

This study aimed to determine whether 1,3,4-thiadiazole derivatives can inhibit the growth of fungal pathogens that cause cereal diseases.

The subject of the study was 33 compounds being derivatives of 1,3,4-thiadiazoles obtained at the Department of Chemistry of the University of Life Sciences in Lublin. The biological activity of the compounds was tested *in vitro* for selected oat and wheat fungal pathogens: *B. graminis* f.sp. *avenae*, *B. graminis* f.sp. *tritici*, *Puccinia coronata* and *Puccinia recondita* f. sp. *tritici*. The analyzed compounds were added to the agar medium (6g/l) at a 10 µg/ml concentration. Leaves of oat (Fuchs) and wheat (Błyskawica) cultivars susceptible to fungal diseases were placed on such prepared plates and inoculated with pathogen spores. 10 days after inoculation, the reaction of the plants was evaluated. The presence of pathogen colonies, their number, and the presence of chlorosis as a reaction of the plant to the applied chemical compounds were taken into account.

Of the tested compounds, 21 showed inhibitory activity on the growth of *Blumeria* fungi, and 14 inhibited the growth of *Puccinia* fungi. In the case of three compounds, no chlorosis was observed, and the leaves of the analyzed cultivars remained green with no signs of fungal diseases.

The conducted research is an initial stage for further work aimed at determining the mode of action of these compounds on the plant-pathogen system and demonstrating that 1,3,4-thiadiazole derivatives can be used in plant protection strategies against fungal pathogens.

Bacterial Priming to Enhance Resistance of *Triticum aestivum* Against the Fungal Pathogen *Puccinia triticina*

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Keywords: wheat, leaf rust, priming, induced resistance, GWAS, QTL

Leaf rust (*Puccinia triticina*) is one of the most destructive diseases that threatens global wheat production. The development and utilization of resistant cultivars and fungicide application are considered the most effective and sustainable methods to control fungal diseases in crops. Using beneficial microorganisms is an emerging alternative to protect plants against abiotic and biotic stresses. The positive effect of plant interaction with their associated microbiome on plant defense against diseases has been reported. For instance, N-acyl-homoserine lactones (AHLs) produced by *Ensifer meliloti* are known for inducing systemic resistance in different plants.

In the first phase of our study, a priming system using *Ensifer meliloti* (two bacteria strains: *E. meliloti* strain *expR₊ch* (producing AHL) or transformed *E. meliloti* carrying the lactonase gene *attM* (non-AHL producing, control) as the priming trigger was established in wheat and QTLs regarding leaf rust resistance were identified. A Genome wide association study (GWAS) by using phenotypic and genotypic data of 175 diverse wheat genotypes, identified four, eleven and nine significantly ($p < 0.001$) associated markers for relative infection under control and primed condition, as well as for priming efficiency, respectively. Identified markers were assigned into 15 QTLs on chromosomes 1A, 1B, 2A, 3A, 3B, 3D, 6A and 6B.

In total, 21 wheat genotypes showed a significant difference ($p < 0.05$) between treatments, which might be a valuable genetic resource for increasing resistance to pathogens through AHL in wheat. Ten out of 21 identified genotypes were considered to respond to AHL-priming by bacteria against fungal diseases in field conditions, too. On the other hand, ten selected genotypes and two control genotypes (Borenos as susceptible and Tabasco as resistant) and 18 genotypes, which were provided by our project partners (RAGT Saaten Deutschland GmbH and Limagrain), were tested in three different locations. The seeds were coated with *Piriformospora indica* or *Bacillus velezensis IT45* (provided by ABiTEP GmbH) and phenotypic performance was tested in three replications under field condition at three locations (Quedlinburg, Silstedt, and Peine). The seeds of uncoated genotypes were used as control in all mentioned locations. Several traits such as early growth, soil coverage, plant height, number of ears per 1m², thousand grain weight, and number of grain per ear were measured.

Furthermore, three different diseases (powdery mildew, stripe rust and brown rust) were recorded. We observed differences in growth and yield parameters related to the priming treatment and dependent on the genotype investigated, which are currently statistically evaluated.

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T5: Yield and Quality Improvement

Specificity of expression of *TaIPT* family genes in different organs of developing wheat plants and their correlation with cytokinin content during early grain development

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Keywords: cytokinin, isopentenyl transferase, *TaIPT*, wheat

The early phase of kernel development is one of the most relevant growth stages in determining wheat yield. At that phase, cell division predominates and two weeks after anthesis grain length and number of cells in the endosperm are established. These traits contribute to the grain filling capacity and, therefore, to the final grain mass. Cytokinins, a class of plant hormones that stimulate cell division and promote accumulation of assimilates, are one of the key regulators that impact early grain growth.

Cytokinin homeostasis is maintained by multigene families encoding enzymes that regulate cytokinin metabolic processes. Gene family members tend to have tissue- and developmental-specific expression patterns. In our research, we focused on a gene family encoding isopentenyl transferase (IPT), an enzyme that catalyses the first, rate-limiting step of cytokinin biosynthesis. To determine which of the members of the *IPT* gene family (GFM) may have the most significant effect on cytokinin content during the early kernel development in two common wheat cultivars (*Triticum aestivum* L.), we performed expression analysis (RT-qPCR) of each of nine *TaIPT* GFMs in four spike stages: 0, 4, 7 and 14 days after pollination (DAP). Additionally, we performed qualitative and quantitative analysis of cytokinins in these spikes using LC-MS/MS technology.

We found that there are five *TaIPT* genes with higher expression levels in tested spikes (*TaIPT1*, 2, 5, 9, 10) and the expression levels of three of them (*TaIPT1*, 2, 5) differed significantly between spikes in both cultivars. The *TaIPT1* and *TaIPT2* genes exhibit opposite expression patterns in 0, 4 and 7 DAP spikes. These two genes appear to be the most important for early grain growth considering that both had very low expression in 14 DAP spikes and their transcripts were not detected in additional qRT-PCR analysis that we performed with different organs such as seedling roots and leaves.

Most of the identified cytokinins were *trans*- and *cis*-zeatin-type, and most of them were accumulated until the 4 DAP spike. Their concentration generally decreased with time, but *cis*-zeatin-type cytokinins lasted longer.

The conducted work is part of a wider research project. The next step will be to perform an analysis of plants with disturbed hormonal homeostasis obtained using the CRISPR-Cas9 system.

Acknowledgments

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Photoperiod and vernalisation requirements in Argentinian oat genotypes

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Keywords: oat adaptability, flowering time, photoperiod × vernalisation interaction, earliness *per se*

Flowering time (FT) is a critical trait determining grain yield in oats. The study of the environmental factors that regulate the FT is essential to predict the adaptability and agronomic performance of the genotypes. Although, the influence of cold hours [vernalisation] and long days [photoperiod] has been well documented in wheat and barley, the limited evidence in oats makes it necessary to conduct new research to understand the response to these factors, and their interaction, which could be useful for adaptation to the new challenges posed by climate change and improve crop management globally. For this, a trial was carried out in the Argentinian Pampas, where nine oat genotypes (Ge) were sown on six sowing dates [from June to December, +/-10 hours (h) to +/- 15 h] and three vernalisation treatments [40 days (V40), 20 days (V20) and 0 days (V0) at 4 °C]. The Ge included lines and commercial cultivars widely grown in Argentina. Days from emergence to flowering were evaluated, and then converted to growing degree days. The adjustment of duration from emergence to flowering (DEF) using an average photoperiod was performed using bi-linear regressions to determine the photoperiod sensitivity (Ps) and threshold (Pt), and Earliness *per se* (Eps). Our findings confirmed differences in vernalisation requirements among the Argentinian oat genotypes. Some genotypes appeared to be insensitive to vernalisation, others had minimal requirements (< 480 cold hours at 4 °C) while materials with high requirements were not found, indicating a reasonably constrained range of variability. Different photoperiod responses were found between the genotypes, which were explained by differences in Ps and Pt. In this sense, most oat genotypes showed high Ps [slopes from -310 to -158 °Cday.h⁻¹]. Furthermore, our results suggest a link between Ps and crop cycle length. Likewise, the *photoperiod × vernalisation* interaction confirms that the vernalisation is a pre-requisite for long-day response, even in those genotypes with low requirements. We reported that Argentinian oat genotypes showed a wide range of variability in the Earliness *per se*, with values ranging from 759 to 1023 °Cday. This study is the first report of vernalisation and photoperiod requirements in Argentinian oat genotypes, and it complements recent studies from our research group reporting the critical period for yield generation in this crop under Argentinian conditions. The results generate valuable information to optimise crop adaptability and tackle challenges arising from climate change.

Table 1: Characterization of Argentinian oat genotypes based on their response to vernalisation, photoperiod and Earliness *per se*.

Genotype	Cycle-length	Vernalisation response	Photoperiod response	Ps (°Cday)	Pt (h)	Earliness per se response	Eps (°Cday)
Line 1-FA	Intermediate	Insensitive	Low	-180 [e]	13.94 [a]	High	1017 [a]
La Plata FA	Long	Insensitive	High	-306 [a]	13.02 [ef]	High	1023 [a]
Los Hornos FA	Long	Low (<480 cold hours)	High	-310 [a]	13.56 [c]	Medium	949 [c]
Line 6-FA	Intermediate	Low (<480 cold hours)	Medium	-259 [b]	13.23 [d]	Medium	920 [d]
Calén	Intermediate	Low (<480 cold hours)	Medium	-230 [c]	12.91 [gh]	Medium	899 [e]
Carlota	Intermediate	Low (<480 cold hours)	Medium	-272 [b]	12.95 [fg]	Medium	899 [e]
Graciela	Intermediate	Low (<480 cold hours)	Medium	-275 [b]	12.81 [h]	Medium	964 [b]
Maja	Short	Low (<480 cold hours)	Low	-158 [f]	13.83 [b]	Low	810 [f]
Mana	Short	Low (<480 cold hours)	Low	-201 [d]	13.08 [e]	Low	758 [g]

Different letters show significant differences between genotypes for the variables ($p \leq 0.05$)

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Image-based quantification of pollen shedding in a hybrid component system in barley

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Keywords: barley, hybrid seed production, pollen shed, male floral traits

Hybrid breeding offers potential for boosting grain yield, stability and disease resistance in many important crops [1,2,3]. In barley (*Hordeum vulgare* L.), the superior performance of hybrids compared to their inbred parental components, i.e. heterosis, has been documented in the literature on several occasions [4,5,6]. In addition to significant levels of heterosis, the success of hybrids relies on efficient cross-pollination of potential parental lines. Hybrid seed production systems will be more cost-efficient if fewer male plants are required to produce a sufficient pollen quantity [7]. For this reason, ways of measuring and understanding the genetic bases of male floral traits such as pollen shedding are crucial. To date, several studies have found significant genetic variance and moderate to high heritability for traits associated with pollen quantity and dynamics in wheat [8,9]. Some studies have even identified genes associated with pollen quantity in some species [10,11,12]. However, many of these studies tend to focus on the total pollen mass produced per male line rather than the amount of pollen shed.

As well as for the remaining male floral traits, pollen shedding is largely unstudied in barley. With the presented pilot experiment we aim 1) to develop a protocol for simple, precise and scalable phenotyping of pollen shedding in six-rowed winter barley male lines and 2) to determine whether pollen shedding is a heritable trait in barley and thus suitable for improvement through genomic tools. Quantitative measurements of pollen shedding were performed on nine different restorer (male) lines and one maintainer (female) line grown in field locations in Denmark and Germany during a 14-day period in 2023. The trapped pollen was enumerated using image-based quantification based on 3619 micrographs. The protocol allowed us to capture pollen and quantify shedding of lines. We observed significant differences in the amount of pollen shed per line. With some adjustments, the protocol can be applied to 150-200 lines next year and eventually provide data for genome-wide association studies and genomic selection.

Acknowledgements

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Genetic homogeneity and heterozygosity of three new Polish hybrid varieties of rye

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Keywords: genetic structure, hybrid cultivars, *Secale cereale* L.

Rye (*Secale cereale* L.) is a small-grain cereal cultivated mainly in Nord-eastern part of Europe. Due to its open-pollinated character, the effect of heterosis is crucial for yield potential. Hybrid cultivars outperform the traditional population forms and are in recent years the choice of many farmers. Predominance of hybrid cultivars compared to population ones is determined by their genetic structure. Both types of cultivars are in some way populations consisted of genetically different individuals, but hybrids are considered as more uniform and more abundant of heterozygotes. We applied the DArTseq markers for assessment of genetic structure of three newly developed hybrid cultivars from Polish breeding companies: DC2424 (last year registered as Gulden), RPD1273 and SMH604. For comparison we included into experiment two registered cultivars: Horyzo (population) and Dolaro (hybrid). Genotyping was performed on 93-94 individuals of each variety. Genetic similarity indexes and the dendrograms illustrating relationships between individual plants of each variety were calculated using NTSys 2.2 software. As it was expected the most variable was population cultivar Horyzo (average similarity index 0,74). Three new Polish varieties revealed average similarity between 0,81 and 0,84. Dolaro cultivar consisted of the most uniform genotypes (mean value of Similarity Index=0,86). Result of heterozygosity estimation was slightly different than expectations. The average frequency of heterozygous loci in population cultivar Horyzo was 15.06. The same parameter calculated for three new and one registered hybrid cultivars was only slightly higher. Values were between 16.17 for Dolaro to 17.33 for RPD1273. These preliminary results indicate that the heterozygosity of hybrid cultivars may be not significantly higher than that present within traditional population varieties.

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T6: Bioinformatics, Genomics and Genome Editing

Re-engineering amino acid metabolism of wheat grain using CRISPR/Cas9

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Keywords: genome editing, wheat, CRISPR/Cas9, lysine, DHDPS

Unlike plants and microorganisms, humans and other animals cannot synthesise lysine, and so must acquire it in their diet. Wheat (*Triticum aestivum*) and other cereal grains are low in lysine and people who live a plant-based lifestyle or do not have access to meat can suffer from lysine deficiency and malnutrition. Soybeans and other legumes do contain higher concentrations of lysine, but if we are to reduce our dependency on meat, which is one of the recommendations for achieving net zero CO₂ emissions, a broader and more sustainable source of plant-based lysine will be required.

Lysine is synthesised by the DAP (diaminopimelate) pathway, of which the rate-limiting step is the conversion of L-aspartate semialdehyde and pyruvate into (4S)-4-hydroxy-2,3,4,5-tetrahydro-(2S)-dipicolinic acid, catalysed by the enzyme DHDPS (dihydrodipicolinate synthase). High levels of lysine are prevented from accumulating by feedback inhibition of DHDPS by lysine.

We will produce wheat with a lysine-insensitive DHDPS using gene editing via CRISPR/Cas9 and homology directed repair, with the aim of increasing free lysine levels in the grain. DHDPS inhibitors have been synthesised and will be used as selective agents to identify successfully edited plants.

Dissection of the role of strigolactones in barley during drought stress by taking a targeted mutagenesis approach

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Keywords: barley, strigolactones, drought

The INSIGHT (Investigation of the role of strigolactones in barley in response to drought) project, funded by the DFG and the Polish National Science Centre (NCN), aims to uncover the function of strigolactones (SLs) in the response to water stress. SLs are carotenoid-derived phytohormones that regulate many processes, both *in planta* (tillering, photomorphogenesis, root development, internode elongation, leaf development, senescence etc.) and in the plants' micro-environmental context (communication with soil microbes, defence against biotic and abiotic stresses). The involvement of these compounds in drought stress responses has been confirmed in several species, such as *Arabidopsis*, rice and tomato; mutants impaired in the SL biosynthesis and signalling pathways were found to be hypersensitive to water stress, while the ones with reduced repression of SL signal transduction exhibited enhanced drought tolerance.

Consistent with these results, our Polish cooperation partner observed that the barley TILLING mutant *hvd14.d*, defective in SL signalling, is hypersensitive to drought stress (1, 2). To confirm and further investigate the function of strigolactones in barley, the availability of mutant lines is crucial. Hence, a collection of plants carrying mutations in SL biosynthetic and signalling genes is being developed by taking a targeted mutagenesis approach based on CRISPR-associated endonuclease technology. The generated mutants, together with their respective wild-type counterparts, are then subjected to control and drought stress conditions. Subsequent physiological, morphological and molecular analyses are carried out to evaluate how the manipulation of SL biosynthesis/signalling affects plant responses to water stress, which may facilitate the development of strategies towards crop improvement.

Acknowledgments

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Identification of polyamine oxidase genes in wheat (*Triticum aestivum* L.) and their expression patterns under cold stress

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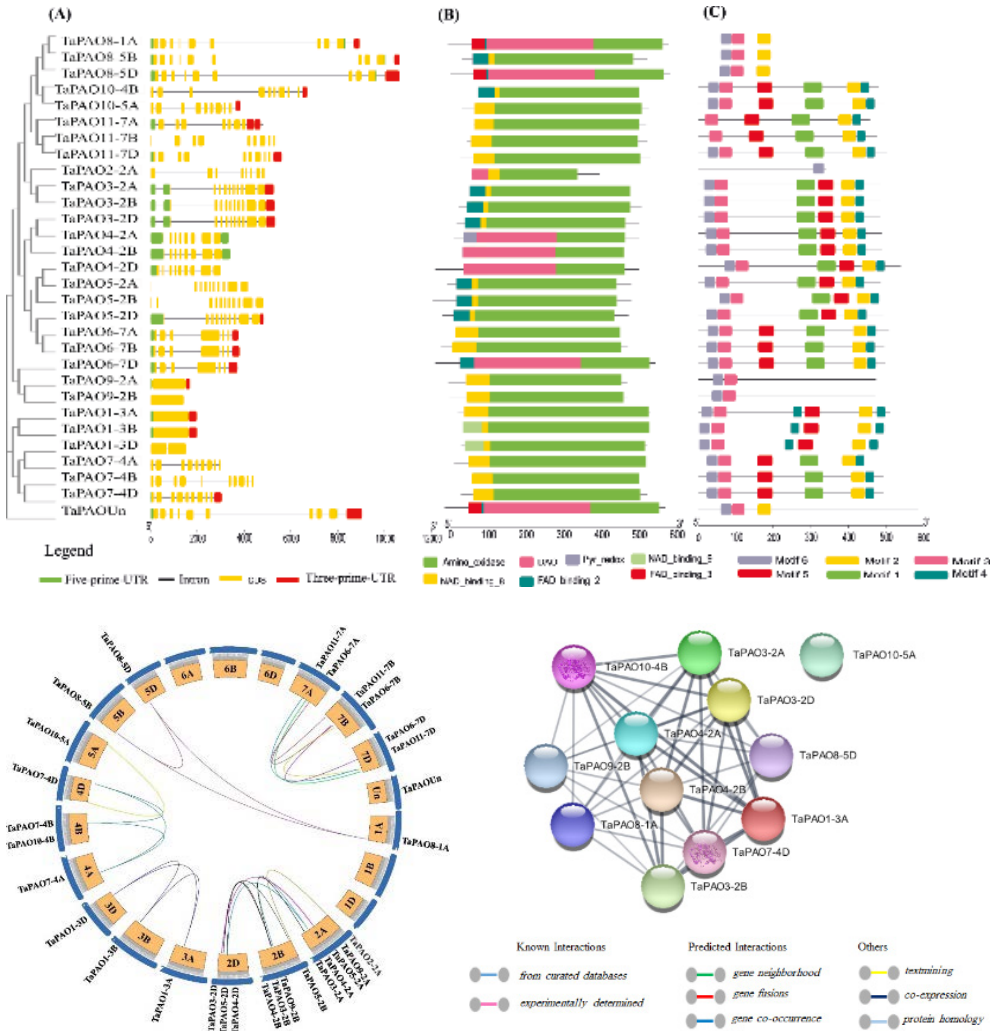
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Keywords: wheat, Polyamine oxidase (PAO), cold stress, gene expression

Polyamines (PAs), are low-molecular weight compounds found in all living organisms. They are involved in many physiological phenomena in plants relating to growth and development and stress responses. Low temperature is one of the most common abiotic stresses experienced by plants and negatively affects plant growth, development, and survival. In the present study, we identified and characterized 30 *PAO* genes in wheat, and they were grouped into 6 clusters based on the phylogenetic relationship. The 30 *TaPAOs* were distributed on 16 of 21 chromosomes, plus the unassembled (Un) part of the genome. The *TaPAO* genes showed uneven distribution across the A, B, and D subgenomes. Based on the wheat genome annotation, most of *TaPAO* genes have introns in their structure and the number of exons varied from 1 to 11. Their gene structures, genome organization, chromosomal locations, and evolutionary relationships were analyzed. Seedlings were foliar sprayed with 10 mg L⁻¹ spermidine (Spd), 10 mg L⁻¹ putrescine (Put), and distilled water as control. Then, the plants were exposed to low temperature stress at 4 °C for seven days. The expression profiles of *TaPAO* in wheat were confirmed through qRT-PCR technique. Expression of PA-treated wheat varieties Mihan and Rakhshan were differentially responsive to low temperature. Cold stress increased the expression levels of *TaADC*, *TaSAMD*, *TaSPDS*, *TaPAO11-7B*, and *TaPAO11-7D* genes compared to control in both cultivars. However, foliar application of exogenous Put and Spd significantly increased gene expression levels, especially in the tolerant variety Mihan. Our results provided a reference for further functional investigation of *TaPAO* proteins.



Application of SSR-markers in local Ukrainian winter wheat breeding program

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Keywords: SSR-markers, alleles, polymorphic information content, clusters, *Triticum aestivum* L.

The simple sequence repeats (SSR) markers are widely used for genetic analysis in plant breeding enabling investigation of the genetic divergence and similarity, identification of the unique alleles and determination of the genetic diversity level. Selection based on markers allows selecting a genotype with the necessary combination of genes, significantly increasing the efficiency of breeding programs. Ukraine has a rich history of wheat breeding spanning over 100 years, which has led to the development of specific allele combinations in Ukrainian cultivars.

The aim of this study is to assess the genetic diversity among the genotypes presented in a local Ukrainian breeding program and to differentiate closely related material using highly informative SSR markers compare with Ukrainian and foreign winter wheat varieties.

The first stage of the our study has been considered assessment of 42 wheat genotypes from the local breeding program of Poltava State Agrarian University by 11 SSR markers (Xgwm 11, Xgwm 44, Xgwm 46, Xgwm 135, Xgwm 174, Xgwm 186, Xgwm 194, Xgwm 219, Xgwm 312, Xgwm 372, Xgwm 389). A total of 80 alleles were identified for the studied 11 loci. Among them, a total of 25 unique alleles were found, which were each allele only occurring in one genotype. The polymorphism information criterion (PIC) values ranged from 0.48 to 0.84. Five major clusters were identified with varying numbers of genotypes within each. The newly developed lines and recently released cultivars did not have any unique alleles according to the investigated markers.

The next stage of the study has been carried out on 82 samples of different geographic origin. A total of 15 SSR markers (Xgwm 003, Xgwm 005, Xgwm 011, Xgwm 044, Xgwm 046, Xgwm 120, Xgwm 160, Xgwm 174, Xgwm 194, Xgwm 219, Xgwm 234, Xgwm 251, Xgwm 325, Xgwm 427, Xgwm 539) distributed throughout the wheat genome were used. A total of 104 alleles have been detected with an average of 6.93 alleles per locus. The PIC values ranged from from 0.16 to 0.79.

The obtained results reveal the genetic similarity among the studied genotypes and can be used as a significant factor in the final breeding decision. The allelic profiles can be used not only to compare with profiles of lines at advanced breeding stages, but also to choose parental pairs for the future crosses, and to identify unique genotypes valuable for breeding.

Hybridization and selection across generations within a given pool with the constant involvement of new sources create a valuable set of alleles for adaptation under certain environmental conditions. The long-term involvement in the hybridization of an extensive ge-

netic material of various origin allows the concentration of valuable alleles that can provide a high level of adaptivity to environmental conditions.

This study has been carried out due to a collaboration of Poltava State Agrarian University with High School of Province Hainaut CONDORCET and Centre for Agricultural research CARAH during 2005-2020 years.

T7: Phenotyping Technologies (Generally)

Drone - based - high throughput - phenotyping system in mapping of cereal diseases

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Keywords: UAV, high-throughput phenotyping, heatmaps, diseases

Unmanned Aerial Vehicle (UAV) or Drone technology has already proven its usefulness in several areas of agriculture, its use can save time and material, as a result minimizing production costs and, not least, environmental impact. In the future, drones can also help plant breeders, as their use can simplify, speed up and make the selection process more objective.

A large amount of data can be easily and quickly recorded from the plots with various cameras, thereby providing an objective picture of the properties of tens of thousands of breeding lines. Multispectral imaging sensor systems allow access to information that is not visible to the naked eye. By analyzing the maps made of the area flown in (orthomosaic and reflectance maps), it is possible to determine the spectral reflectance indices calculated from the light absorbing and reflecting capacity of the leaf tissue at visible ($\lambda=400-750$ nm) and near-infrared ($\lambda=750-1400$ nm) wavelengths. With these vegetation indices, it is possible to show differences between the breeding lines in terms of disease resistance, insect infestation, drought tolerance, nutrient utilization capacity, plant tissue aging and photosynthetic activity.

Drone flights have been carried out at the Agricultural Institute (Martonvásár) since 2019, in addition in the autumn of 2021 and 2022, an expanded reference variety trial was established, made of 11 winter wheat, barley, durum wheat and triticale genotypes which are sensitive and tolerant to disease, drought and heat stress. The trial was inoculated with leaf rust and *Pyrenophora teres* f. *maculata* (only on barley), and 4 out of 8 randomly arranged replications were treated with fungicide. From the spring of the growing season, the drone flew weekly over the plots at a height of 100 meters. The drone forwarded the recorded data to the AGRONMaps platform (AGRON Analytics Ltd.), where they were processed, and the vegetation indices were calculated from this database. Altogether, 17 phenotypic parameters were recorded, 47 spectral data were measured by the drone on 12 occasions, and all plots were harvested in the summer.

The plants showed a special multispectral profile, and clear differences between the species could be detected. It was also possible to differentiate between varieties, which can greatly help plant breeding processes. When plotting the principal component analysis data, it is clear that the ripening process can be followed, and early and late ripening varieties can

be separated. In fungicide experiments, the pattern of the multispectral values of the infected varieties could easily be compared with the resistant varieties. These heatmaps can also be used to speed up selection.

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T8: Phenotyping Technologies – Wheat Initiative

Deciphering the effect of wild emmer (*T. dicoccoides*) QTL introduced into elite wheat varieties on root growth by precision phenotyping

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

Plant genetic resources are a source for crop improvement under difficult climatic conditions. Wild emmer (*T. dicoccoides*) is a wild relative of wheat (*T. aestivum*) and can therefore be used for the introduction of beneficial alleles. A QTL shown to be beneficial under drought stress introduced from wild emmer into the cultivar BarNir has been investigated to improve drought resistance in terms of estimated biovolume and thousand grain weight among other traits (Merchuk-Ovnat et al. 2016a, 2016b, 2017; Lauterberg et al. 2021). In the study presented here, the cultivars BarNir and NIL were tested in rhizoboxes and in the rhizotron system at IPK PhenoSphere to investigate the effects of QTL spatiotemporally on roots. Furthermore, the results were correlated with those of a high-throughput phenotyping (HTP) experiment (Lauterberg et al. 2022). It was observed that for the NIL in question, there was a larger root surface area and a lower root to shoot ratio. Daily phenotyping can shed light on the effect of QTLs on the underground plant organs and thus contribute to the breeding of promising varieties.

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T9: Phenotyping Technologies (CePPG)

From controlled environment to field: confounding factors in container trials

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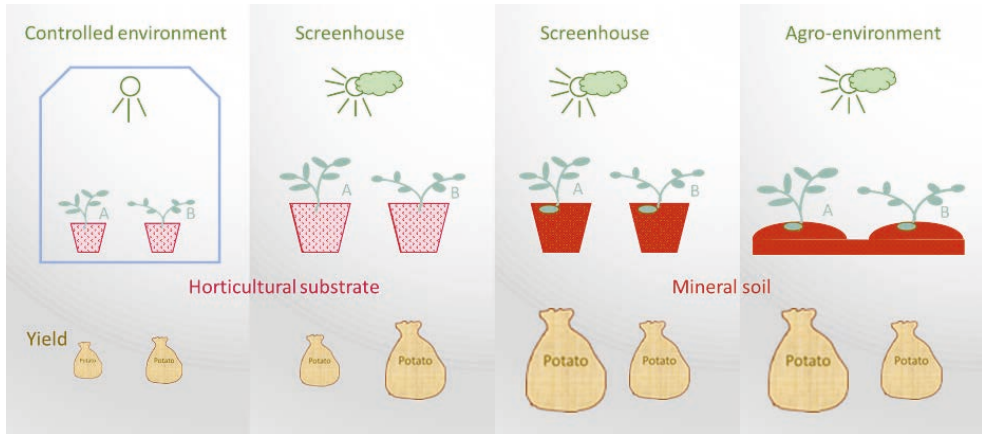
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Keywords: drought tolerance, abiotic stress

Global climate change models predict an increase of extreme weather events, among them drought and heat. Maintenance of agricultural yield requires breeding of resilient crops. The bottleneck in drought tolerance breeding is phenotyping in managed field environments. Fundamental research on drought tolerance uses container-based test systems in controlled environments as a proxy. However, breeders debate the portability of results from these systems to performance under field conditions. Thus, we analysed the effects of climate conditions, container size, starting material, and substrate on yield and drought tolerance assessment of potato genotypes in pot trials compared to field trials. The tolerance ranking in the field was obtained from seven multisite-multiyear trials. The tolerance ranking in controlled environments was highly reproducible, but weakly correlated with field performance. Changing to variable climate conditions, increasing container size and substituting cuttings by seed tubers did not improve the correlation. Substituting horticultural substrate by sandy soil resulted in yield and tuber size distributions similar to those under field conditions. However, as the effect of the treatment \times genotype \times substrate interaction on yield was low, drought tolerance indices that depend on relative yields can be assessed on horticultural substrate too. Realistic estimates of tuber yield and tuber size distribution, however, require the use of soil-based substrates.

Figures**Acknowledgments**

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