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EWAC19 – Oral

Supplementing white light with violet-blue or far-red light restored freezing tolerance in *Rht12* wheat leaves by modulating hormone and metabolite accumulation

Péter Borbély[1*], *Zsolt Gulyás*[1], *Tamás Pálmai*[1], *Kitti Kulman*[1,2], *Zahra Tahmasebi*[1,3], *Zsuzsanna Farkas*[1], *Danuše Tarkowská*[4], *Petre Dobrev*[5], *Radomíra Vanková*[5], *Romina Beleggia*[6], *Valentina Giovanniello*[6], *Pasquale De Vita*[6], *Nicola Pecchioni*[6], *Andreas Börner*[7], *Gábor Galiba*[1], *Mohamed Ahres*[1]

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Keywords: light-regulation, *Rht*, gibberellin, metabolism, freezing tolerance

The incorporation of *Reduced height* (*Rht*) genes into wheat breeding has significantly influenced wheat production; however, these alleles may reduce freezing tolerance. At present, only a limited number of *Rht* alleles are utilized in commercial wheat varieties, indicating the need to characterise alternative alleles. With milder winters, temperature fluctuations and extreme cold events becoming more common, non- or poorly-acclimated or de-acclimated winter cereals are likely to experience severe frost damage. Consequently, brief cold (non-freezing) exposures (pre-hardening) and other environmental factors, such as light quality, will determine the freezing tolerance of these plants. During pre-hardening, gibberellin (GA) levels change dynamically, but many mutant *Rht* alleles exert a dominant effect on GA signalling or availability. While far-red enriched white light (W+FR) induces pre-hardening at 15°C in winter wheat or barley, similar effects of violet-blue light supplementation (W+VB_{410}) is unexplored. Thus, the impact of these light conditions has been investigated after 10 days exposure of young winter wheat 'Maris Huntsman' at 15°C, using a tall genotype, harbouring the wild-type *rht12* allele and a dwarf, GA-deficient near-isogenic line with the mutant *Rht12* allele. Leaf samples were collected to examine hormonal and metabolic changes at the end of the treatments, prior to the freezing tests using excised leaf segments. Both light treatments triggered pre-hardening and rescued the freezing-sensitive phenotype of *Rht12* leaves likely due to hormonal and metabolic remodelling. Additionally, W+FR treatment partially restored the GA_3 and GA_1 content in the leaves of *Rht12* plants, without affecting their height. These findings highlight the role of light signalling in GA homeostasis, and suggest that modifying light conditions or signalling could improve the freezing tolerance of wheat varieties with limited GA synthesis.

Acknowledgments

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References

1. Gulyás et al. (2025). *Physiol*. *Plant*. 117(2), e70112.

Examples of successful collaboration (2024–2025)

A. Börner[1*], S. Okon[2], M. Nowak[2], K. Kowalczyk[2], T. Kartseva[3], S. Misheva[3], Z. Gulyás[4], G. Galiba[4], N. Shvachko[5], E. Khlestkina[5], L. V. Shchukina[6], T. Pshenichnikova[6], M. Schierenbeck[1,7], M.R. Simon[7], R. Šućur[8], D. Trkulja[8]

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Keywords: freezing tolerance, powdery mildew, GWAS, grain quality, cross-pollination efficiency, spike productivity

In this review, we will list examples of fruitful co-operation within EWAC partners, published during the last two years, since the EWAC conference in Wernigerode in 2023. Results on successful collaborative projects span a wide range of activities.

The following publications were considered:

Gulyás Z, Ahres M, Pálmai T, Kulman K, Tahmasebi Z, Singh K, Jobbágy K, Tarkowská D, Dobrev P, Vanková R, Borbély P, Börner A, Galiba G: Blue or far-red light supplementation induced pre-hardening in the leaves of the *Rht12* wheat dwarfing line: hormonal changes and freezing tolerance. Physiol. Plant. 177 (2025) e70112.

Okoń S, Nucia A, Ociepa T, Börner A, Kowalczyk K: When the source of resistance can be considered effective - resistance to *Blumeria graminis* f. sp. *avenae* found in *Avena sterilis* L. Eur. J. Plant Pathol. 172 (2025) 819-827.

Kartseva T, Aleksandrov V, Alqudah A M, Rehman Arif M A, Kocheva K, Doneva D, Prokopova K, Börner A, Misheva S: GWAS in a collection of Bulgarian old and modern bread wheat accessions uncovers novel genomic loci for grain protein content and thousand kernel weight. Plants 13 (2024) 1084.

Kartseva T, Aleksandrov V, Alqudah A M, Schierenbeck M, Tasheva K, Börner A, Misheva S: Exploring novel genomic loci and candidate genes associated with plant height in Bulgarian bread wheat via multi-model GWAS. Plants 13 (2024) 2775.

Pshenichnikova T A, Shchukina L V, Börner A: The phenotypic characterization of Rivet wheat accessions (*Triticum turgidum* L. ssp. *turgidum*) for grain quality and other agronomic properties. Genet. Resour. Crop Evol. 71 (2024) 4919-4928.

Schierenbeck M, Alqudah A M, Lantos E, Avogadro E G, Simón M R, Börner A: Green Revolution dwarfing *Rht* genes negatively affected wheat floral traits related to cross-pollination efficiency. Plant J. 118 (2024) 1071-1085.

Shchukina L V, Klykov A G, Murugova G A, Shamanin V P, Pozherukova V E, Lepekhov S B, Chebatareva M V, Petin V A, Börner A, Pshenichnikova T A: Variability of protein and gluten content in bread wheat lines with introgressions into chromosome 5B from related species. Euphytica 220 (2024) 171.

Shvachko N, Solovyeva M, Rozanova I, Kibkalo I, Kolesova M, Brykova A, Andreeva A, Zuev E, Börner A, Khlestkina E: Mining of QTLs for spring bread wheat spike productivity by comparing spring wheat cultivars released in different decades of the last century. Plants 13 (2024) 1081.

Šućur R, Mladenov V, Banjac B, Trkulja D, Mikić S, Šumaruna M, Börner A: Phenotypic marker study of worldwide wheat germplasm. Ital. J. Agron. 19 (2024) 100002.

Dual-phase cold acclimation in barley under field conditions: expression dynamics of ICE-CBF-COR and vernalization pathways in Nure and Tremois

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Keywords: barley, cold acclimation, vernalization, freezing tolerance, gene expression, *CBF* genes, *VRN* genes

Barley (*Hordeum vulgare* L.) thrives across diverse environments but yield in temperate regions like Italy's Po Valley depends on adapting to seasonal cold. Survival relies on cold acclimation for freezing tolerance and vernalization to align flowering (Feng et al., 2025). While studies under controlled conditions helped to understand the base of ICE-CBF-COR and vernalization pathways, field-based work is needed to capture transcriptional dynamics under natural temperature and photoperiod fluctuations(Vítámvás et al., 2019).

Cold acclimation in barley is driven by the ICE–CBF–COR pathway: *ICE1* activates *HvCBFs* at *FR-H2*, which induce COR effectors genes that stabilize membranes and mitigate oxidative/dehydration stress (Caccialupi et al., 2023). In parallel, vernalization (*VRN*) and photoperiod regulators (*PPD*) genes integrate seasonal cues to coordinate stress tolerance with reproductive timing (Deng et al., 2015).

To investigate the dynamics of these pathways under agronomic conditions, a time-series gene expression experiment was performed in the Po Valley, comparing the winter cultivar Nure with the spring cultivar Tremois. Plants were sampled weekly at sunset throughout the whole vegetative phase, and expression was quantified for *ICE1*, multiple *HvCBF* genes at *FR-H2*, the effector genes *HvCOR14b* and *HvDHN5*, and selected genes of the vernalization pathway (*VRN-H1*, *2*, *3* and *PPD-H1* and *2*).

Our results confirmed that *HvCBF4* is a putative candidate to enhance freezing tolerance, that showed consistently stronger and more sustained induction in Nure than Tremois. Expression of effector genes was higher in Nure at the beginning of the experiment, followed by secondary peaks during later stages, reflecting a two-phase acclimation response. In contrast, no constitutively high expression of effector genes was observed in Tremois showing transient induction events that coincided with minimum field temperatures falling below 0 °C.

Taken together, these findings support a dual-phase model of acclimation in barley: an early phase, where ICE-CBF-COR signaling primes stress-protective responses, and a later phase, where sustained cold maintains or reactivates effector genes expression to reinforce freezing tolerance. These results provide novel field-based evidence of how barley integrates stress signaling with seasonal adaptation, offering insights for breeding strategies aimed at improving winter hardiness under climate variability.

References

1. Caccialupi, G., Milc, J., Caradonia, F., Nasar, M. F., & Francia, E. (2023). *The Triticeae CBF Gene Cluster—To Frost Resistance and Beyond*.
2. Deng, W., Casao, M. C., Wang, P., Sato, K., Hayes, P. M., Finnegan, E. J., & Trevaskis, B. (2015). Direct links between the vernalization response and other key traits of cereal crops. *Nature Communications*, *6*(1), 5882. https://doi.org/10.1038/ncomms6882
3. Feng, Y., Li, Z., Kong, X., Khan, A., Ullah, N., & Zhang, X. (2025). Plant Coping with Cold Stress: Molecular and Physiological Adaptive Mechanisms with Future Perspectives. *Cells*, *14*(2), 110. https://doi.org/10.3390/cells14020110
4. Vítámvás, P., Kosová, K., Musilová, J., Holková, L., Mařík, P., Smutná, P., Klíma, M., & Prášil, I. T. (2019). Relationship Between Dehydrin Accumulation and Winter Survival in Winter Wheat and Barley Grown in the Field. *Frontiers in Plant Science*, *10*. https://www.frontiersin.org/articles/10.3389/fpls.2019.00007

Genetic control of leaf angle in barley

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Keywords: *Hordeum vulgare*, leaf erectness, TILLING, CRISPR-Cas9 gene editing

Leaf angle is a key agronomic trait for optimizing planting density and grain yield in cereal crops; in particular, **erect leaf angle** reduces competition among neighbouring plants and allows for better distribution of photosynthetic light. Maize and rice varieties with upright leaves have been shown to produce increased grain and biomass yields, especially under high-density planting. In contrast, little progress has been made in improving this trait in barley, a global cereal crop with multiple end-uses and a well-established genetic model for the Triticeae tribe.

Identification of genes and alleles for leaf erectness is a promising approach to increase barley productivity. In this work, the genetic control of the leaf angle in barley has been exploited through both forward genetic, by screening and analysing TILLING mutant populations, and reverse genetic, targeting by gene editing a brassinosteroid-related gene known to be involved in the control of plant architecture in maize and rice.

The TILLING mutant HorTILLUS 5061 (HT-5061) was selected for its erect architecture and crossed with Barke cv to generate an F2 population used to map the candidate gene. Mapping-by-sequencing led to the identification of *HvCLAVATA1* (*HvCLV1*) as the most promising candidate gene, with the mutant carrying a G to A transition, introducing a premature STOP codon. Co-segregation between the mutant phenotype and the *HvCLV1* associated mutation was confirmed by analysis of 160 F2 plants. To further explore the involvement of *HvCLV1* in leaf angle formation, gene expression was investigated in meristematic tissues, the ligular region, and the adjacent basal leaf blade tissue, using qPCR and a reporter line. *HvCLV1* was expressed in inflorescence and vegetative meristems, but notably high transcript levels were detected in the ligular region and leaf blade; confocal microscopy analyses also showed a signal in mesophyll cells of this tissue.

Five additional mutant alleles for *HvCLV1* available from the NordGen collection were selected to further study the role of *HvCLV1* in the control of leaf angle. Under field conditions, both HT-5061 and the allelic mutants exhibited significantly more erect leaves than their wild-type backgrounds, corroborating the role of the *HvCLV1* gene in the regulation of leaf angle in barley.

In addition, another TILLING population, TillMore, was screened in experimental field to identify additional erect leaf mutants. Five promising lines have already been identified, and their genomes have been targeted for WGS, in a first attempt to list possible candidate genes and variants, considering also the existing knowledge in the literature. These lines have been crossed with Morex to generate segregating populations for mapping-by-sequencing analysis.

To obtain additional alleles involved in barley leaf erectness, CRISPR-Cas9 gene editing approach was employed to target a gene involved in the biosynthesis of brassinosteroids, which are well known to control plant architecture and to improve the grain yield under high-density sowing conditions. Among 59 screened mutant plants, five independent M1 edited, and Cas9-free lines have been identified and selected for phenotypic characterization.

Acknowledgments

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Drought tolerance of winter wheat (*Triticum aestivum* L.) varieties under laboratory and field conditions in Southern Ukraine

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Keywords: *Triticum aestivum* L., drought tolerance, osmotic stress, molecular markers

Arid conditions are increasingly common in southern Ukraine, with soil moisture deficits observed in both autumn and spring–summer periods. Breeding institutions such as PBGI-NCSCI (Odesa) and ICSA (Odesa, formerly the Institute of Irrigated Farming NAAS (IIF), Kherson) work continuously to develop winter wheat varieties adapted to the Southern Steppe. For decades, breeding has relied on genotypes carrying the photoperiod-insensitive allele Ppd-D1a and semi-dwarfing alleles (Rht8c, Rht-B1b, Rht-D1b), which increase yield potential in stress-prone environments. Most varieties developed in this region carry Ppd-D1a, which induces earlier flowering and enables wheat to avoid terminal heat and drought stress in May. Selection for such alleles is straightforward due to visible phenotypic effects, but drought tolerance itself is more complex, as it is polygenic. Thus, modern breeding requires integrating DNA markers and advanced phenotyping to improve efficiency.

This study compared drought tolerance of winter bread wheat using two approaches: (i) osmotic stress induced by 12% PEG 6000 at the seedling stage, and (ii) field evaluations. Results were associated with allelic variants of the TaSnRK2.8 gene.

Twelve winter bread wheat varieties from IIF were examined. All carried Ppd-D1a and were adapted to the Steppe zone with high yield potential. Seeds (25 per variety) were germinated on filter paper moistened with either 12% PEG 6000 (–0.2 MPa) or distilled water (control). Experiments were conducted at 21±2°C. Drought tolerance was evaluated using a Tolerance Index (TI).

Highly tolerant genotypes included Rosynka (TI = 0.96), Soborna (0.88), and Ovidiy (0.84). Low tolerance was observed in Anatolia (0.31) and Khersonska Bezosta (0.45). Other varieties (Mariya, Burgunka, Konka, Ledya, Koshova, Kokhana, Blaho) showed moderate tolerance without significant differences.

Comparison of PEG-test and field data revealed consistency for Rosynka, which showed high tolerance in both conditions. Soborna displayed high tolerance under PEG stress (TI = 0.88) but only moderate tolerance in the field (40%), indicating that genetic potential can be influenced by agronomic or environmental factors. Anatolia and Khersonska Bezosta were consistently drought-sensitive.

Allelic analysis of TaSnRK2.8-A indicated that the presence of allele A does not always guarantee high tolerance, while varieties with allele G may rely on additional adaptive mechanisms.

Combining PEG osmotic stress tests with field evaluations is effective for identifying drought-tolerant wheat genotypes. Rosynka and Soborna emerged as promising donor varieties for breeding programs aimed at improving resilience to drought conditions in the Southern Steppe of Ukraine. Reliable identification of drought tolerance requires an integrative strategy involving molecular markers, laboratory assays, and multi-environment field trials.

Past and present of pre-breeding program for wheat at N.A.R.D.I. Fundulea – Romania

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Keywords: monosomic, pre-breeding, wild species, synthetic amphiploid

Wheat is a significant crop in Romania and the first among cereals used in human consumption. The wheat pre-breeding program plays a crucial role in the wheat breeding initiative by introducing new options and variability. The National Agricultural Research and Development Institute Fundulea is focused on developing wheat cultivars with advantageous characteristics, including disease resistance, drought and heat tolerance, quality, yield, and low input requirements.

The pre-breeding effort commenced over 50 years ago with cytogenetic and aneuploid studies. Later, as part of an EWAC-cooperative initiative, a monosomic series was created in the variety Bezostaya-1. This whole monosomics set of Bezostaia-1, with two to three sublines per chromosome, was completed in 1981 (Bc.10). Another monosomics full set with two to three sub-lines for each chromosome was created using the Romanian cultivar Favorit, which was completed in 1983.

Monosomics originating from the Romanian cultivar Favorit have been utilized in genetic analyses to elucidate the genetic regulation of certain agronomically important traits, identify novel genes, and evaluate their functionality within a genetic framework comprising cultivars and advanced breeding lines developed at NARDI. Another approach in the wheat pre-breeding program focuses on employing related wild species for gene transfer into the wheat gene pool, encompassing synthetic amphiploids, translocation and introgression lines.

Currently, at NARDI, the wheat pre-breeding program focuses on the production of novel synthetic amphiploids (*Triticum durum* x *Aegilops tauschii*) and the populations between synthetic amphiploids and advanced wheat lines.

The contribution of the EWAC network to the establishment of the molecular basis of frost resistance research from the beginning until now in Martonvásár (Hungary)

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Keywords: EWAC, frost tolerance, history, Martonvasar, molecular methods, wheat

At the very beginning, the EWAC was established as "European Wheat Aneuploid Cooperative" during the 1970s. A group of researchers from various European institutions recognized the immense value of standardized genetic stocks (especially aneuploid series) in advancing wheat genetics. One of the important centers of this activity was the Plant Breeding Institute (PBI) at Cambridge (GB). My mentor, Prof. József Sutka spent half year there with a scholarship and actively participated in the creation of different monosomic and substitution lines. His close professional relationship with Dr. John Snape led to long-term collaboration and several joint publications, including studies using monosomic and single chromosome recombinant lines to investigate the genetic basis of frost tolerance. For example, using single chromosome recombinant lines between T. spelta and (wheat) Hobbit 5A homologues, they located a gene for frost tolerance on chromosome 5A. The RLFP molecular mapping technique was well established in the second half of the 1980s at PBI. In 1993, I was invited to the Cambridge Laboratory (John Innes Centre, Norwich) by Steve A. Quarrie and John Snape to learn this technique and to map the *VRN1* (*VERNALIZATION 1*) and *FR1* (*FROST RESISTANCE 1*) genes using single chromosome recombinant lines developed in collaboration between John Snape and József Sutka. This marked the beginning of the application of molecular technologies in Martonvásár. The other important partner, originated from the EWAC network, was Dr. Andreas Börner and Dr. Nils Stein from IPK Gatersleben. My colleague, Gábor Kocsy revealed how the 5A wheat chromosome affects the transcriptome during cold acclimation and learned some genomic methods at Nils Stein's laboratory in 2005. Other two colleagues from Martonvásár completed part of their PhD studies at the laboratory of Andreas Börner. Focusing on frost tolerance research, Andreas Börner provided us with *Rht* (*REDUCED HEIGHT*) wheat genetic stocks to examine how different *Rht* alleles influence frost tolerance. Furthermore, we are currently investigating the interaction between modulated light-induced frost tolerance and the presence of various *Rht* mutations.

Acknowledgments

Our ongoing research program ('Effects of different *Rht* gene mutations on the vigour- and light-quality-dependent regulation of seedling stress tolerance in cereals') also involves collaboration with EWAC members. It is supported by the National Research, Development and Innovation Office (NKFIH, grants K 147019 and TKP2021-NKTA-06

Current status of the disease resistance genes in common wheat (*Tritium aestivum* L.) in Poland

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Keywords: wheat, resistance genes, rust, powdery mildew

Common wheat (*Triticum aestivum* L.) is a major cereal crop in Poland, but its production is significantly threatened by diseases such as stripe rust, leaf rust, stem rust, powdery mildew, and Fusarium head blight. To combat these threats, Polish breeding programs have increasingly focused on incorporating disease resistance genes (R-genes) into new wheat cultivars. Genetic control is the most effective, economically safe and environmentally friendly approach because it eliminates the need for fungicides and reduces production costs. Resistance genes such as *Lr* (leaf rust) and *Pm* (powdery mildew) are widely studied and used. However, single-gene resistance can be overcome by pathogens through evolution, variability, and selective pressure. This reinforces the need to develop consistent resistance and provides a basis for plant breeders and geneticists to develop durable, fungal disease-resistant wheat varieties using marker-assisted breeding or gene pyramiding. Despite progress, challenges remain, including the limited availability of novel resistance sources and the continuous adaptation of pathogens. The aim of the study was to identify genes for resistance to leaf rust and powdery mildew in common wheat. Forty-five Polish common wheat varieties, both winter and spring, were used for the analysis. The studies were conducted using available molecular markers. The presence of genes for the fungal diseases studied varied depending on the variety. The most frequently identified genes in Polish wheat varieties were *Lr11*, *Lr13*, *Lr16* and *Lr34*, as well as *Pm2*, *Pm4b*, *Pm6* and *Pm8*. Thus strengthening collaboration between breeders, pathologists, and geneticists, along with the use of genomic tools, are crucial for developing cultivars with durable resistance and ensuring sustainable wheat production in Poland.

Twenty years with EWAC: a personal and professional reflection

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Keywords: bread wheat, career advancement, collaborative achievements, genetics

Over the past two decades, engagement with EWAC has played a significant role in influencing both the trajectory and substance of my scientific career. Since attending my first conference in Prague, Czech Republic, in 2005, the organization has provided a consistent platform for scholarly exchange, cooperative research, and professional development. Within the evolving framework of EWAC, from its original focus on wheat aneuploidy to its broader scope as the European Cereals Genetics Co-operative, my research in wheat genetics, cytogenetics, molecular genetics, and physiology has found fertile ground for advancement. These collaborations have contributed to the development of integrative scientific approaches and have supported the dissemination of research through joint publications and funded initiatives. Productive partnerships with colleagues from Germany, Hungary, Russia, Poland, Ukraine, the United Kingdom, Serbia, and Romania have played a central role in this process. The continuity of involvement within EWAC has enabled sustained scientific progress and cultivated a network of trusted academic relationships. This presentation shares a personal perspective on the professional growth and collaborative achievements shaped through twenty years of active participation in the EWAC community, an experience that has been both intellectually rewarding and deeply meaningful beyond the boundaries of science.

"Prof. Jozsef Sutka, the cytogeneticist and his legacy"

Márta Molnár-Láng

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Keywords: monosomic series, frost resistance, intergeneric hybridization, molecular cytogenetics

József Sutka was born in 1936. After working ten years at Agricultural University in Gödöllő, and some months in the newly established Biology Centre in Szeged, he started work in the Agricultural Research Institute of the Hungarian Academy of Sciences in Martonvásár in 1972. From 1982 till 2003 he had been Head of Department of Genetics in Martonvásár. He started work on wheat cytogenetics, and specialised in the development of monosomic series. He successfully developed complete monosomic series for two varieties. Later he developed numerous other basic genetic materials (substitutions, recombinant lines).

He was one of the founding members of EWAC. He spent half year with a scholarship in Cambridge at PBI, Plant Breeding Institute, where he made good cooperation with Dr. John Snape and Dr. Tony Worland. With the help of monosomic and substitution lines the *Fr1* gene on chromosome 5A was mapped. He had many cooperations in the frame of EWAC with colleagues from various part of the world. He organized an EWAC meeting in Martonvásár in 1987.

His first book, entitled Cytogenetics, was published in 1980, and his second book, entitled Plant Cytogenetics, in 2004. The number of his scientific publications were 176, and the number of citations are 1861. He received several awards to recognize his work. He died in 2010.

After his retirement Gabor Galiba continued the research on frost resistance and abiotic stress tolerance and Marta Molnar-Lang worked on wheat cytogenetics. She developed interspecific and intergeneric hybrids and derivatives and analysed those using molecular cytogenetic methods. In the frame of a EWAC cooperation she learned molecular cytogenetic technique in Norwich at the John Innes Institute at the Department Headed by Dr. John Snape and with the help of Dr.Terry Miller and Steve Reader in 1994. From 2003 she was the Head of Cell Biology Department and the leader of the Molecular Cytogenetics group. From 2007 till 2015 she was the Head of Department of Genetic Resources. She retired in 2016.

Figure 1. EWAC meeting in Martonvásár in 1987. József Sutka is the 6th in the first row (from left to right)

Phenomic characterization of drought stress response of the common wheat (*Tritium aestivum* L.) intervarietal substitution lines

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Keywords: common wheat, drought, substitution lines, phenomics, plant imaging

Drought is one of the most critical abiotic stresses affecting plant growth and productivity worldwide. As climate change intensifies, periods of water scarcity are becoming more frequent and severe, posing a significant threat to agricultural sustainability and ecosystem stability. Understanding the genetic background of drought tolerance and adaptive strategies is essential for developing drought-resistant crops and ensuring food security in the face of global environmental challenges.

The research aimed to analyze the response of wheat plants to drought stress at the phenomic level. The research material consisted of a set of 'Cappelle Desprez' ('Bezostaya 1') intervarietal chromosome substitution lines. During the experiment, 3-week-old wheat plants were analyzed using fluorescence, RGB, visible and near-infrared (VNIR) imaging, as well as short-wave infrared (SWIR) imaging, to detect symptoms of stress in the plants. The use of a high-throughput plant imaging system allowed for rapid, non-invasive, and precise monitoring of plant growth and stress responses.

Analysis of the obtained results showed that plants from the control group which were not subjected to drought stress were characterized by a significantly higher degree of tillering than plants subjected to stress. The leaves of plants subjected to drought stress had a smaller surface area and visible changes. Fluorescence analysis showed that the F_v/F_m parameter values for plants from the test group (indicating the efficiency of the plants' photosynthetic apparatus) were significantly lower than those for plants from the control group. Similarly, the NDVI coefficient values for the test plants showed a downward trend compared to the control group. In the case of the WATER1 index (representing the water content in plant tissues), an increase in values and maintenance of increased values can be seen for the group subjected to drought stress from the 6th day after the onset of drought stress. A higher value of this index correlates with a lower water content in the plant.

A more detailed analysis and characterization of the response of individual substitution lines to water stress may contribute to the identification of regions of the common wheat genome associated with increased drought tolerance.

Breeding for drought-resilient hybrids for tropical maize improvement by precision phenotyping

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Keywords: AMMI stability, GGE biplot, drought resilience, genotype-by-environment interaction

The major portion maize cultivation in India is in the ecosystem. Due to climate change, frequency and duration of drought spells are increasing and affecting yields significantly. For developing drought-resilient maize hybrids, identifying inbred lines tolerating moisture stress with appreciable yield per se plays a crucial role. Therefore, we evaluated 94 inbred lines for two years in winter 2021-22 and 2022-23 in field at Dharwad in moisture stress and non-stress conditions. It was interesting to make a note in our study that severe moisture-stress was induced at 652 growing degree days (GDD), and irrigation was not resumed up to harvest of the crop with an objective to address drought tolerance at flowering as well as grain filling stages and to identify reliable inbreds. Based on mean performance in both conditions, we selected 18 inbreds and evaluated in field at four locations viz., Dharwad, Bailhongal, Sankeshwar and Arabhavi in Karnataka in winter 2023-24 and 2024-25 in moisture stress and non-stress conditions in a total of 14 environments to understand genotype-by-environment interaction and stability of these lines. Severe moisture-stress was induced at the same time at all four locations at 652 growing degree days (GDD), and irrigation was not resumed up to harvest of the crop. Furthermore, all 18 lines were screened in PVC pipes in winter 2023-24 under moisture-stress and non-stress conditions to study root traits and biochemical traits related drought tolerance. Fourteen root and shoot traits were analysed using WinRhizoTM 2021 root scanner. The pooled data two years (7 seven environments each in moisture stress and non-stress) indicated that the check inbred CML 580 (35.08 q/ha) recorded highest grain yield followed by GI 755 (33.74 q/ha), GI 722 (32.91 q/ha), GI 701 (26.78 q/ha), DIM 204 (25.08 q/ha) and GI 743 (23.63 q/ha) in normal irrigation condition. Whereas, in the moisture stress condition, inbred line GI 722 (5.76 q/ha), GI 755 (5.68 q/ha), and GI 743 (4.88 q/ha) manifested highest grain yield over the check line CML 580 (4.29 q/ha). AMMI ANOVA revealed 38.90%, 28.62% and 32.46% contribution from environment, genotypes, and genotype-by-environment to total variation for grain yield in non-stress, while genotypes (23.71%) contribution was lower; environment (48.71%) contribution was higher and GEI (27.66%) was comparable in moisture-stress condition. The PC1 (42.72%), PC2 (24.81%) and PC3 (17.35%) together explained 84.89% variation in non-stress compared to 85.95% in moisture-stress (PC1-56.94%, PC2-19.18%, PC3-9.82%). GGE biplot revealed drought tolerant GI 743 was most stable genotype in non-stress along with check CML 580. Along with higher grain yield, inbreds GI755, GI722, GI 743, CML 580, VL 183150 and VL184811 shown lower anthesis-silking interval, leaf rolling, leaf senescence, and yield reduction compared to non-stress condition. Furthermore, these inbreds displayed

higher root surface area, average diameter, root tips, crossings, proline and wax content in stress condition. Six moisture stress tolerant and 2 susceptible lines among 18 lines were mated in an 8×8 diallel fashion and resulting 56 hybrids were screened for drought tolerance. The hybrid GH 24616 (25.30 quintal/hectare) showed higher grain yield in moisture stress compared to drought tolerant check GH 150125 (23.5 quintal/hectare). Hybrids GH 24617 (120.32 & 21.4 quintal/hectare) followed by GH 24635 (113.62 & 18.7 quintal/hectare), and GH 24608 (115.92 & 18.4 quintal/hectare), documented higher grain yield in optimum irrigation and relatively better yield with minimum yield reduction in moisture stress condition, respectively over next best drought tolerant checks LG 36511 (114.63 & 17.0 quintal/hectare) and DKC 9133 (116.47 & 15.8 quintal/hectare) suggesting direct filed application of these hybrids in rain fed ecosystem.

EWAC19 – Poster

Detection of HTP-7H alleles in barley via KASP marker-assisted selection

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Keywords: barley, KASP, vitamin E, HPT-7H, SNP

Barley (Hordeum vulgare L.) serves as a principal feed and is the most prolific grain source of functional ingredients, being the predominant species among functional food crops, particularly in dietary fibre and antioxidant compounds such as polyphenols and vitamin E. Vitamin E comprises a collection of chemicals, specifically tocopherols and tocotrienols. Recent researches identified two genes: *homogentisate phytyltransferase* (*HPT*-7H) and *homogentisate geranylgeranyl transferase* (*HGGT*), which are involved in the production of tocopherols in leaves and tocotrienols in grains, respectively. The HPT-7H gene exhibits two haplotypes: "low tocopherol content" and "high tocopherol content," differentiated by the marker SCRI_RS_225155 at the SNP (C/T). The allele with SNP-C correlated with elevated tocopherol levels in barley leaves, while SNP-T correlated with diminished tocopherol levels.

This study aimed to identify the favourable allele of the HPT-7H gene linked to high tocopherol levels. The identification of favourable alleles of the HPT-7H gene was accomplished via the KASP method (Competitive Allele-Specific PCR). This study investigated 70 barley genotypes, identifying the beneficial haplotype in 37 lines (53%). This outcome presents opportunities for enhancing beneficial components in barley grains through breeding. Also, this study demonstrates the efficacy of Marker Assisted Selection (MAS) as a breeding approach for enhancing barley quality.

Acknowledgments
The research work was carried out with the support of the National Research Authority from the core project „Improvement of wheat and barley crop adaptability to climatic changes from Romania", grant 43N/2023 and the National Agricultural Research and Development Institute Fundulea.

Development of colored wheat lines through synthetic amphiploids

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Keywords: synthetic amphiploid, colored wheat, blue aleurone, purple pericarp

The registered wheat cultivars with colored grains (blue, purple and black) are scarce and the existing varieties possess minimal agronomic value. In colored wheat, the type and content of anthocyanins vary according to their distribution in grains and the cultivation conditions. Due to their antioxidant activity, anthocyanins contribute to the prevention of cardiovascular diseases, diabetes, inflammation, cancer, obesity and aging. Therefore, these biochemical compounds and their health effects have heightened interest in the development of colored wheat varieties.

Although red and white are the predominant hues of wheat grains, blue and purple wheat can be produced by intergeneric and interspecific hybridization methods. However, yield is the primary challenge in the development of wheat varieties with colored grains. The objective of this study was to produce synthetic amphiploids distinguished by colored grains and to transfer these traits to wheat lines. As a result, synthetic amphiploids with blue aleurone and purple pericarp traits were developed.

The blue aleurone trait was achieved by crossing *Triticum durum* variety Condur with *Triticum monococcum ssp. boeoticum*, whereas purple pericarp trait was derived from *Elymus sp*. This purple pericarp trait was introduced into cultivated wheat, resulting in one line exhibiting a reasonably high yield–84% compared to the control cultivar (FDL Columna) in 2024-2025 season.

Analyses of the chemical composition of these lines are underway.

Acknowledgments
This work was funded by the MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT - Romania (projects ADER321/2019-2022 and ADER311/2023-2026)

Relationship between wheat cultivars earliness and yield, test weight and bread-making quality

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Keywords: wheat earliness, grain yield, baking quality, test weight

We analyzed twenty-one winter wheat cultivars tested during two seasons (2022–2023 and 2023-2024) at NARDI Fundulea, ROMANIA under four crop managements differing by N fertilizer rates, disease control and sowing dates. Heading date of these cultivars, averaged over the two years, varied from 122.5 to 130 days after January 1st. Late heading had a negative effect on grain yield under all crop managements, but correlation was only significant under intensive crop management (higher N fertilizer rates and disease control) (r =-0,59*) and when planted two weeks later than optimum (r=-0,57*). Averaged over these two crop managements, released cultivars *FDL Columna, Biharia, FDL Consecvent* and *FDL Abund,* had large deviations (over 400 kg/ha) from the regression of yield on heading date.

On two years average, heading date was significantly correlated with test weight, under all crop management variants (r from -0,39* to -0,54*), reflecting the effect of increasing temperatures on grain filling in later heading cultivars. Heading date showed significant positive correlation with grain protein concentration in three of the four crop managements (except under intensive management). This reflects the negative correlation between yield and protein concentration and the fact that the higher temperatures encountered by later cultivars during grain filling inhibited starch accumulation more than the protein one.

The bread-making quality indices analyzed using the Reomixer (bread volume, peak height, breakdown and end width) were not significantly correlated with heading date.

The present results justify the emphasis on earliness in the wheat breeding program at NARDI Fundulea.

Genetic resistance of oat to powdery mildew: evaluation of ***Pm*** genes effectiveness and pathogen adaptability

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Keywords: *Avena, Blumeria graminis*, resistant genes

Oat (*Avena sativa* L.) is an important cereal crop valued for its nutritional qualities and versatile applications, yet its productivity is increasingly threatened by fungal pathogens. Among them, powdery mildew, caused by *Blumeria graminis* f. sp. *avenae*, represents one of the most widespread and economically significant diseases of oat in Poland, leading to reduced photosynthetic efficiency and considerable yield losses. The most sustainable strategy for controlling this disease is the use of genetic resistance, which combines effectiveness with economic and environmental benefits by reducing reliance on fungicides. To date, 13 major resistance genes (*Pm* genes) have been identified, but their long-term effectiveness is undermined by the rapid adaptive potential of the pathogen. Newly emerging virulent races can overcome previously effective resistance, a process known as resistance erosion, particularly in regions where a narrow genetic base of resistance is deployed. Regular monitoring of the effectiveness of resistance genes and pathogen populations is therefore essential. In Poland, studies on oat cultivars and lines carrying known *Pm* genes revealed variable levels of resistance effectiveness, ranging from weak to strong, depending on the gene and pathogen pathotype. These results confirm that, although *Pm* genes remain a key component in the management of powdery mildew, their utility is not permanent and must be supported by continuous evaluation. Long-term and sustainable control will require integrating resistant varieties with complementary agronomic practices, as well as broadening the genetic diversity of resistance sources in breeding programs..

Exploiting the tetraploid wheat pangenome: a functional resource for the whole wheat community

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Keywords: tetraploid wheat pangenome, genomics, PacBio sequencing

Durum wheat evolved from wild emmer through domesticated forms to modern cultivars. As donor of the A and B genomes of bread wheat, tetraploid wheats are key source of genetic diversity for modern breeding. Herein, we present preliminary results from the chromosome-level assemblies of 40 tetraploid wheat genomes, selected from the Global Durum wheat Genomic Resource (GDGR, passport and genotypic data deposited in GrainGenes, https://graingenes.org/GG3/global_durum_genomic_resources) to represent the full genetic spectrum of sequence variation across wild emmer, domesticated emmer landraces and cultivars for supporting evolutionary studies and pre-breeding efforts. As case studies, two loci related to disease resistance and spike development will be presented, providing insights into the effects of structural variation and allelic diversity and demonstrating the value of the pangenome in crop breeding. Short- and long-read RNA-sequencing data from multiple tissues of ten core accessions enabled pan-transcriptome analysis, classifying transcripts as core, shell or cloud. Early findings show genotype-specific expression in genes linked to metabolism, hormone signalling, and transcription. Ongoing co-expression network analysis aims to identify expression profiles tied to kernel development, with further investigation into links between transcript abundance, gene copy number and structural variation.

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Spike and seed morphometric traits phenotyping of some winter barley DH lines

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Keywords: winter barley, phenotyping, spike and seed traits, DH lines

Barley developmental stages and yield component traits data (e.g., spike and seed traits) are valuable for robust phenotyping and reliable genotyping. Accurate phenotyping is essential for genome-wide association (GWAS) studies in breeding programmes aimed at developing new barley varieties. This study describes the phenotyping process for spike and seed traits in winter barley lines obtained through the biotechnological bulbosum method (Hordeum vulgare x Hordeum bulbosum) from F1 hybrids of hulled two-row and six-row genotypes (Kasha and Kao, 1970; Adamski, 1979), which included 11 doubled barley haploid (DH) lines (winter type). Various related traits, such as spike length (SL), awns length (AL), rachis weight (RW), spike weight (SW), number of grains per spike (NGS), grains weight (GW), one thousand grain weight (TGW), and individual seed characteristics like seed length (SL), seed width (SW), seed circularity (SC), seed area (SA), and length/width seed ratio (L/W), were measured. Images of over 1000 individual seeds acquired using the MARVIN instrument were utilised to characterise the analysed traits. The obtained values exhibited a low coefficient of variation, indicating good stability of the analysed traits under the climatic conditions of 2024. These phenotyping data can support marker-assisted breeding and be linked to genomic information to validate candidate genes that regulate spike and seed architecture.

Acknowledgments

The research work was carried out with the support of the National Research Authority from the core project PN 23.18.02.01 „Improvement of wheat and barley crop adaptability to climatic changes from Romania", grant 43N/2023 and the National Agricultural Research and Development Institute Fundulea.

References

1. Kasha K.J. and Kao K.N., 1970. High frequency haploid production in barley. Nature, 225, 874–876.
2. Adamski T., 1979. The obtaining of autoploid barley lines using haploids from the cross Hordeum vulgare L. x Hordeum bulbosum L. Genet. Pol., 20, 31–42.

CBB8 – Opening

Some historical moments in 52-year history of Cereal Research Communications - CRC

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Keywords: scientific journal, manuscript, publication

The scientific journal, Cereal Research Communications (CRC) was established in 1973, when Zoltan Barabás – the talented researcher, plant breeder and manager – arrived back to Szeged, Hungary from University of Missouri - Columbia, USA. Zoltan spent a longer term in laboratory of George P. Rédei, who is the father of Arabidopsis research on the word. He was very deeply touched by the wind of scientific research, the importance of high level publication. It was a very impulsive stress of Zoltán. Arriving home, he inspired his director and important persons in Ministry and in 1973 the first issue of CRC was published by the Cereal Research Institute, Szeged. The main idea was to make scientific chance for publication of **cereal** scientist of the world. In the first editorial board was involved famous researchers, namely N. Borlaug, R.A. McIntosh, G. Kimber, J.F. Mackey, P.P. Lukyanenko, J. Bojanowski, Á. Kiss and others.

Cereal Research Communications (Figure 1.) from the first issue publishes original papers, at times invited scientific reviews and short book reviews in the field of cereal genetics, physiology, pathology, quality and utilization, breeding, and agronomy of primarily wheat, barley, rye, triticale, rice, oat, maize and other cereals. High-level scientific content is recommended in manuscripts, where the new scientific results are well discussed along with the international results.

In first period of the CRC's history the owner of the journal was Cereal Research Institute, Szeged and the journal was printed by the printing company of Hungarian Academy of Sciences. Close to the 100-year anniversary of the journal, Academy bought the ownership of CRC. Today the CRC member of the Springer family. CRC's impact factor is 1.9 (2024), it's very close to the best plant breeding- and plant science journals.

The Chief Editor is Andreas Börner from Gatersleben, Germany and the editorial board includes more than 40 members from the different part of the word. They try to keep and increase the scientific quality of the Cereal Research Communications. The lecture will flash some important moments from the 52-year old history of CRC. Welcome among the authors of CRC in future.

Cereal Research
Communications
A QUARTERLY OF THE CEREAL RESEARCH NON-PROFIT COMPANY

Figure 1. Well- known logo of the CRC from the front page of the Journal

CBB8 – Invited

Genome editing in rice: major achievements and future prospects

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Rice, as a staple food crop for more than half of the global population, faces increasing pressure from climate change, evolving pathogens, and the urgent demand to feed 10 billion people by 2050. Conventional breeding and transgenic approaches, while important, are increasingly limited in their capacity to deliver timely improvements in yield, resilience, and nutritional quality. Genome editing is a transformative platform, offering targeted, efficient, and often transgene-free modifications that mirror the processes of natural evolution and domestication. CRISPR/Cas systems, together with refinements such as base editing, prime editing, and multiplexed editing, are redefining the scope and market potential of rice breeding. Coupled with advances in delivery technologies and computational design, these tools provide flexibility to address long-standing and emerging challenges in rice production.

Our group is using genome editing to dissect and modulate traits of agronomic importance, with a particular focus on starch biosynthesis and pathogen resistance. By targeting enzymes central to starch metabolism, we have generated rice lines with altered amylose–amylopectin ratios, opening opportunities for both improved grain quality and tailored nutritional profiles. Additional efforts have demonstrated how genome editing can enhance resistance to devastating diseases such as rice blast, through the knockout of susceptibility genes. These case studies exemplify the versatility of genome editing to accelerate functional genomics while simultaneously creating improved germplasm.

The deployment of genome-edited rice in European production systems will require solutions to major bottlenecks, including the development of robust editing tools for diverse cultivars, scalable delivery methods, and harmonized regulatory frameworks. Equally critical will be the adaptation of rice to cultivation under reduced water input, drought, and salinity, major constraints in Mediterranean European rice-growing regions. Addressing these challenges will be central to transitioning laboratory innovations into resilient and sustainable rice varieties for future breeding programs.

IT and AI in the digital transformation of agriculture

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Rapid advances in information technology (IT) and artificial intelligence (AI) are revolutionizing agriculture, improving efficiency, sustainability, and productivity. AI-powered data analytics, remote sensing, and precision farming techniques are enabling farmers to optimize resource use, predict crop yields, and mitigate risks associated with climate change. Smart sensors and IoT devices collect real-time data on soil conditions, weather patterns, and crop health, enabling data-driven decision-making. Automated machinery, such as drones and autonomous tractors, further streamline farming operations, reducing labor costs and increasing production. Machine learning algorithms improve pest and disease detection, minimizing the need for chemical treatments and promoting environmentally friendly practices. Despite these advances, challenges such as data security, high implementation costs, and digital literacy gaps must be addressed. This presentation will explore how IT and AI are reshaping modern agriculture, highlighting key benefits, challenges, and future trends.

Acknowledgments

Modelling predicts that soybean is poised to dominate crop production across Africa
https://doi.org/10.1111/pce.13466
Statistical modelling of crop yield in Central Europe using climate data and remote sensing vegetation indices
https://doi.org/10.1016/j.agrformet.2018.06.009
Cereal yield gaps across Europe
https://doi.org/10.1016/j.eja.2018.09.003

Harnessing wild diversity to identify a novel resistance gene for cereal eyespot disease

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Eyespot, caused by the fungal pathogens *Oculimacula yallundae* and *Oculimacula acuformis,* is a historically important disease of wheat and other cereals. To date, limited resistance loci have been deployed in wheat, with breeding largely focusing upon the usage of a single R gene, *Pch1*. However, whilst *Pch1* has shown an enduring efficacy against both eyespot causative pathogens, dependency upon a single or handful of resistance sources is a strategy that has proven persistently unwise. With numerous examples of R gene breakdown often associated with single gene deployment. Perhaps worryingly, efforts to identify further sources of resistance amongst wheat landraces and cultivars has failed to yield any new major effect loci. Indeed, whilst *Pch2* (and a separate QTL, QPch.jic-5A) were identified in French wheat cultivar Capelle deprez, both *Pch1* and *Pch3* were derived or detected in wheat wild relatives *Aegilops ventricosa* and *Dasypyrum villosum* respectively. Here we have focused on identifying the causative sources of resistance to *O. yallundae* infection that has been reported in *Ae. tauschii*. Utilising historical and newly generated data combined with *k*-mer based association genetics we have uncovered an NLR gene on chromosome 1D that provides protection against *O. yallundae*. We provisionally designated it as *Pch4* and show that resistance segregates in an additive dominant manner, conveying a similar resistance phenotype when transferred into wheat. *Pch4* provides an equivalent resistance in wheat as *Pch1* and will therefore be useful for introducing into breeding programs. Furthermore we show judiciously selecting *Ae. tauschii* germplasm will enable the targeted introgression of *Pch4* alongside other agronomically relevant *R* genes.

Use of genomic selection in plant breeding: after GBLUP

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Keywords: genomic selection, multi-trait, rapid-cycle breeding, functional prediction, phenomics, complex traits, gene bank

Genomic selection (GS) is the method of choice to improve selection for complex traits. GS is now about 20 years old and has been taken up in many plant breeding programs using the standard “work horse” GBLUP. GBLUP is good to predict breeding values of close relatives, making it very suited for use within breeding cohorts to improve efficiency of selection in field trials. This can be expected to give modest improvements in genetic gain, but breeders will also use the improved efficiency to reduce costs. Current use of GS, however, does not shorten breeding cycle time, which can potentially make much larger improvements in genetic gain.

Looking forward, replacement of GBLUP by improved prediction tools will be needed. Although GBLUP is good to predict close relatives, it is the poorest method to predict distant relatives. Thus, GBLUP cannot well exploit rapid-cycle breeding schemes, use of external (public) data, or combining of breeding populations. The approach to improve distant relatives’ prediction is use of a weighted-SNP approach, where SNP weights can be derived from many sources: genomic annotations, functional genomic information, gene (-tissue) expression atlases, public or private gene mapping information, LLM predictions of variant effects, and the output of Bayesian prediction models. SNP weights can be implemented in weighted GBLUP, making routine use relatively straightforward, but relationship matrices will become trait-specific and multi-trait expansions are still challenging.

A significant next step in the improvement of plant breeding programs with GS tools will be implementation of rapid-cycling breeding schemes where selection is purely based on genomic information without phenotyping. This can shorten breeding cycle time to 3 months for spring cereals – a potentially 20-30x speed-up compared to current 5-8 years cycle time. Long-term rapid-cycling schemes could be designed based on parallel field-phenotyping and greenhouse-based rapid breeding. Many questions remain and need to be investigated, among others if phenotyping and breeding should run on inbred or outbred plants, how large the parent-pool should be to avoid significant genetic drift, optimizing the distance between training data and test candidates with the performance of prediction tools, and use of multi-trait prediction to avoid undesired correlated responses.

Using rapid-cycle breeding schemes, novel opportunities to use complex trait variation from gene bank material can become available. Rapid-cycle breeding could improve yield of older landrace material to an acceptable level to consider use as crossing parents for (re) introducing lost complex trait variation for yield or yield components. For disease resistance, where current use of gene bank material is limited to single-gene resistances, rapid-cycle breeding can open a new field of using quantitative disease resistance.

Use of phenomic tools and phenomic prediction should be carefully considered. Phenomic tools applied on field trials, for instance predicting yield based on field imaging, can have limited value because true yield is available at the end of the season, and there are no opportunities to speed up breeding, but phenomic tools applied in rapid-cycling greenhouse generations can be interesting if they are well predictive of field performance. The utility of phenomic prediction tools for breeding should be properly evaluated for predicting the genetics of candidate lines, and not just based on phenotype prediction, which can be misleading.

Combining high productivity, good baking quality and nutrients along wheat supply chains

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Wheat is a very important stable crop dealt along worldwide supply chains. Breeders address requests of the different stakeholders of the wheat supply chain to get advantages in selling their varieties. Consequently, many traits from agronomy but also techno-functional quality have to be worked and are successfully improved since decades in parallel by breeders. Owing to climate change, political restrictions, etc., farming practices as well as availabilities of agrochemicals changes leading to the need of adapting breeding targets. For instance, tremendous investments have already be realized for more resistance breeding owing to expected reduction strategies for pesticides. Similarly, nitrogen- and water-use efficiency are further traits getting more relevant the last decade.

In that presentation I show the breeding success for wheat in Germany the last decades for classical and new traits. Genome-wide association analyses showed intermediate major QTL for protein yield, grain yield as well as new traits like minerals and proteins. Moreover, the implementation of minerals in breeding wheat can be realized especially into the high quality wheat class, as minerals seem to correlate positive with protein content and baking quality. Nevertheless, this requires investments, which need to be financed by higher prices for wheat batches with increased nutrient profile. I expect that this is only realizable when the consumer shows higher interest in whole grain products and when the supply chain is willing and able to separate wheat with high nutritional quality along the supply chain.

Acknowledgments

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References

1. El Hassouni, K., M. Afzal, P.H.G. Boeven, J. Dörnte, M. Koch, N. Pfeiffer, F. Pfleger, M. Rapp, J. Schacht, M. Spiller, M. Sielaff, S. Tenzer, P. Thorwarth, and C.F.H. Longin. 2025. Wheat breeding to better feed a growing world: historic insights and future potential elaborated using a diverse cultivars collection and extended phenotyping. Scientific reports, https://doi.org/10.1038/s41598-025-13678-w
2. Longin, C.F.H., M. Afzal, K. El Hassouni. 2023. From farm to fork: future supply chains need to measure and trade nutrient content, Trends in Plant Science, 28: 1237–1244, doi.org/10.1016/j.tplants.2023.05.011

From roots to shoots: multi-scale phenotyping approaches at IPK

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Keywords: high-throughput phenotyping, controlled conditions, rhizotron

Climate change is placing unprecedented pressure on global agriculture, with rising temperatures, prolonged droughts, and emerging plant diseases threatening crop productivity. At the same time, a growing world population demands higher yields, produced sustainably and with fewer resources. Meeting these challenges requires a deeper understanding of how plants respond to environmental stress. Plant phenomics plays a key role in this effort by enabling high-resolution, quantitative analysis of plant traits under diverse and controlled conditions—accelerating the development of resilient, high-performing crop varieties. While field research remains vital, its reproducibility is limited by environmental variability and the unpredictability of extreme weather events. To investigate plant responses to such stressors systematically, we must be able to simulate them in a controlled and repeatable way. To facilitate controlled, reproducible studies under dynamic environmental scenarios, the IPK Gatersleben has established the PhenoSphere—a globally unique phenotyping facility designed to simulate future agricultural conditions. Within this facility, key environmental parameters including light intensity and spectral composition, air and soil temperature, relative humidity, wind, and CO_2 concentration can be precisely regulated, systematically varied, and reliably reproduced. The PhenoSphere is organized into two highly specialized areas: the Rhizotron system and the Container/PhenoCrane system. The Rhizotron allows for non-invasive monitoring and analysis of root development over time, while the Container/PhenoCrane system enables fully automated imaging of plant stands using a rail-mounted camera crane that traverses the entire experimental area. Together, these systems offer an integrated, high-resolution view of plant development, capturing both below- and above-ground traits under controlled yet field-relevant conditions. The rhizotron system enables automated imaging of up to 360 plants using two imaging towers equipped with one monochrome camera and two high-resolution RGB cameras, allowing for parallel monitoring of both root and shoot development. In the container-based PhenoSphere system, plant populations can be cultivated in up to 108 individual containers, each with a volume of 1 m^3, simulating small-scale field plots across the full growth cycle. Above-ground phenotyping is conducted using the PhenoCrane system, which integrates multiple imaging modalities, including RGB cameras, a FluorCam for chlorophyll fluorescence, a hyperspectral camera operating in the VNIR range, and a 3D laser scanner for structural analysis. Both of the systems allow for automated watering. Artificial intelligence (AI) and machine learning (ML) techniques are employed for the analysis of the large and complex datasets generated by the phenotyping systems. In particular, self-learning algorithms are applied to image-based data to extract phenotypic traits and identify patterns, enabling scalable, high-throughput analysis with minimal human intervention. In the proof-of concept study in maize it could be demonstrated that in the PhenoSphere a field-like environment can be simulated in terms of the evoked plant growth rates and the progression of developmental stages, resulting in field-typical plant growth performance. A comprehensive diversity atlas of root system architecture (RSA) has been developed through daily imaging of seventeen crop species. It reveals species-specific patterns of RSA development over time and uncovered species-specific correlations between root and shoot growth and the key traits contributing to both intra- and interspecific RSA diversity. By combining controlled environmental simulation with advanced phenotyping and AI-driven analysis, the PhenoSphere enables a new level of precision in plant phenotyping—bringing us closer to developing climate-resilient crops for a more sustainable agriculture.

Diversity and breeding potential of wild wheat relatives in Türkiye: a focus on *Triticum araraticum*

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Keywords: wild wheat, Türkiye, Triticum, Aegilops

Wild genetic resources are pivotal for advancing crop evolution and breeding by expanding the genetic diversity of cultivated cereals. Turkey, a global hotspot for wild cereal relatives, harbors a rich array of species that exhibit valuable traits, such as tolerance to abiotic stresses like drought and salinity, resistance to biotic stresses including pests and diseases, and adaptability to diverse agroecological environments. Among these, *T. araraticum*, a wild tetraploid wheat, stands out for its unique genetic variation, offering significant potential to enhance modern wheat varieties for improved yield and resilience. This presentation will explore the genetic diversity of Turkey's cereal germplasm, with a particular focus on *T. araraticum*, and elucidate its applications in cereal breeding. By integrating these wild relatives into breeding programs, we can address challenges in crop improvement and support global food security through advancements in crop evolution studies.

The power of arbuscular mycorrhizal fungi in plant-microbe interactions: past, present, future

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Keywords: arbuscular mycorrhizal fungi, drought and heat stress, P-efficiency, sustainable agriculture

The symbiotic association between plant roots and arbuscular mycorrhizal fungi (AMF) has persisted for over 400 million years and played a pivotal role in the early colonization of terrestrial ecosystems by plants. Initially regarded primarily as facilitators of water and nutrient acquisition, AMF are now recognized as integral components of the plant–microbe–soil continuum, influencing a wide range of ecological and physiological processes.

This presentation examines the ecological, physiological, and agricultural significance of AMF, with particular emphasis on their roles in crop production systems, including cereal crops. Drawing on both our recent original research and findings from the broader scientific community, it highlights how AMF contribute to nutrient cycling, enhance drought tolerance, increase resistance to abiotic stresses, and promote overall soil health.

Advancements in systems biology and biotechnology have significantly expanded the potential for AMF application in modern agriculture, offering promising strategies to enhance crop productivity and promote environmental sustainability. The presentation also explores the role of AMF in addressing global challenges such as climate change, soil degradation, and food security, and discusses future directions for their integration into sustainable agricultural practices. These insights could be particularly relevant for plant breeding programs aiming to develop crop varieties with improved nutrient efficiency, stress resilience, and compatibility with sustainable agricultural.

Navigating the cereal QTLome to enhance environmental adaptation to climate change

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Keywords: QTLome, QTL cloning, climate change, pangenomes

Food security is increasingly threatened by climate change and requires the adoption of targeted actions to select cultivars better able to mitigate the impact of global warming and reduce the environmental footprint of agriculture. An informed breeding-by-design approach is crucial to identify the key haplotypes that enhance yield, quality and environmental plasticity. Notwithstanding the key role played by the QTLome in the genotype-to-phenotype path of any trait, in cereals only a limited number of QTLs have been cloned and shown a notable impact on grain yield, except for rice. Examples of QTL cloning in maize for root architecture (Tassinari et al. 2025) and fine mapping of two major QTLs in durum wheat for SBCMV resistance (Bruschi et al. 2024) and root angle (Sciara et al. 2025), will be presented. Additionally, novel opportunities offered by a comparative pangenome analysis of species with different phylogenetic/ploidy levels in order to identify orthoQTLs (Sow et al. 2025) will be addressed, particularly between durum and bread wheat.

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References

1. Bruschi M., Bozzoli M., Ratti C. et al. (2024). Dissecting the genetic basis of resistance to *Soil-borne cereal mosaic virus* (SBCMV) in durum wheat by bi-parental mapping and GWAS. Theor. Appl. Genet. 137, 213. doi.org/10.1007/s00122-024-04709-7
2. Sciara G., Bozzoli M., Fiorani F. *et al.* (2025). Genetic dissection of the root system architecture and shoot QTLome in tetraploid durum wheat. The Plant Genome. In press.
3. Sow M.D., Forestan C., Pont C. *et al.* (2025). Striking convergent selection history of wheat and barley and its potential for breeding. Molecular Plant. In press.
4. Tassinari A., Salvi S., Urbany C. *et al.* (2025). Cloning of *qRoot-Yield-1.06,* a major QTL for root architecture in maize (submitted).

CBB8 – Oral

T1: Genetic Resources for Crop Improvement

Development of a new bread wheat (*Triticum aestivum* L.) cultivar adapted to the Rainfed Areas of Northern Iraq – Kurdistan Region

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Keywords: wheat, genotype, breeding, Rainfed condition

To improve wheat productivity under rainfed conditions in the Kurdistan Region of Northern Iraq, a long-term field evaluation was conducted to identify superior bread wheat (*Triticum aestivum* L.) genotypes adapted to local agroecological environments. A total of 28 genetic lines introduced from the International Maize and Wheat Improvement Center (CIMMYT), Mexico, were cultivated and assessed over eight consecutive growing seasons in the Shahrizor Plain, a representative rainfed area. The primary objective was to evaluate these genotypes' productivity and adaptability and select the most promising ones based on yield potential and environmental suitability. In the final three seasons, nine superior genotypes were selected and evaluated alongside three widely grown local cultivars using a randomized complete block design (RCBD) with three replications. The analysis was based on data from two consecutive agricultural seasons (2022/2023 and 2023/2024). The results revealed that Genotype No. 22 consistently outperformed all others in terms of grain yield, yield components, and stability under rainfed conditions. Its superior agronomic performance and genetic stability prompted its nomination for official registration. The selected genotype (22), later named the variety "Wafaa Halabja", showed superiority in the traits of number of spikes per square meter, 1000-grain weight, and grain yield per unit area, as well as stability in these important yield traits over the past three seasons, according to the results and statistical analysis, despite the variation in rainfall amounts during these seasons. Following further evaluation by a special committee at the Iraqi Ministry of Agriculture in Baghdad, Genotype No. 22 was officially approved and released as a new cultivar under the name ‘Wafaa-Halabja’, recognized for its high productivity and suitability for rainfed farming systems in Northern Iraq. This achievement marks a significant step toward sustainable wheat production and food security in semi-arid regions.

Acknowledgments

I have more than 33 years of experience in crop production, especially wheat crops. Since I was employed at the Biological and Agricultural Research Center I've been working in the Iraqi Atomic Energy Commission (Ministry of Sciences and Technology now) and exclusively in the field of special research of mutation breeding of Wheat. Where I was directly responsible for the experiments of maintenance and multiplying the new registration and dependable varieties, as the field of breeder seed production of the Wheat crop, Then we conducted comparing experiments to protect them from vanishing. as I prepared special reports related to research, moreover I carried out the statistical analysis over the annually implemented experiments

References

1. Abate GT, Bernard T, Brauw AD, Minot N (2018) The impact of the use of new technologies on farmers' wheat yield in Ethiopia: evidence from a randomized control trial. Agric Econ. 49: 409–421.
2. Abraha MT, Hussein S, Laing M, Assefa K (2017) Genetic variation and trait association of tef [Eragrostis tef (Zucc.) Trotter] evaluated under optimal and moisture stressed environments. Aust J Crop Sci. 11:241–247.
3. Ali MA, Nobe N, Nawab, Abbas A, Zulkiffal M, Sajjad M (2009a) Evaluation of selection criteria in Cicer arietinum L. using correlation coefficients and path analysis. Aust J Crop Sci. 3: 65–70.

RNA roadmaps of seed aging during long-term genebank storage

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Keywords: genebank, seed, aging, RNA

Seeds *in ex* situ collections inevitably lose viability over time, with stochastic failure often occurring after reaching a threshold in otherwise homogeneous lots. This process is accompanied by oxidative stress, macromolecular damage, and membrane deterioration, and a decrease in RNA integrity is correlated with the loss of germination. However, ribosomal RNA–based metrics (RINs) only partially capture the integrity of the mRNA fraction in long-stored dry seeds. Consequently, transcriptome-level studies of dry-stored seeds with declining viability remain scarce despite methodological advances in RNA-Seq that enable the profiling of coding and noncoding RNAs in degraded materials. Noncoding RNAs play essential roles in seed developmental checkpoints and stress responses. In plants, microRNAs (miRNAs) and small interfering RNAs (siRNAs) modulate gene expression through cleavage or translational inhibition. They also interact with hormone signaling and chromatin pathways. Dynamic small RNA programs accompany dormancy release and early germination across species. In this study, we examined barley (*Hordeum vulgare*) seeds from a single historical lot that were divided based on age-induced viability and a field-regenerated control. We integrated small RNA sequencing, degradome analysis, and RNA-Seq libraries that were optimized for low RIN inputs. In dry seeds, conserved miRNA families were dominant and stable across viability classes. A single novel miRNA (hvu-new41) was elevated in seeds with the lowest viability, suggesting that it could be a deterioration indicator. During germination, specific miRNAs vary with the imbibition stage and seed quality. Degradome-supported targets were enriched for nucleic acid binding, nuclear organization, and primary metabolism. Parallel siRNA profiling revealed temporally structured 21–22 nt cohorts (including ta-siRNAs), whose peak timing shifted with viability. This finding implicates processes ranging from cytochrome complex function to carbohydrate metabolism. Notably, the siRNA pools remained relatively stable despite RNA degradation.

Our findings suggest actionable small RNA markers for genebank quality control (e.g., RT–qPCR panels integrating hvu-new41 with conserved families). Our results also advocate for RIN-aware, yet fragmentation-retaining, library preparation for aged seeds and motivate cross-collection validation and functional assays to establish causality. Routine integration of small RNA, degradome, and mRNA profiles could enable longitudinal surveillance of seed longevity trajectories across species.

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INNOVAR project combines genomics and morphological studies to characterize the breeding value of durum wheat varieties

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Keywords: genomics, GWAS, DUS, VCU, varietal registration

The EU H2020 FP7 INNOVAR project aims at revising wheat CPVO Distinctness-Uniformity-Stability (DUS) and Value for Cultivation and Use (VCU) protocols by proposing a combination of traditional phenotyping and innovative genomics and phenomics techniques. INNOVAR developed two panels of durum and bread wheat representative of the European germplasm (250 and 280 cultivars, respectively). Additionally, UNIBO and international partners assembled the Svevo Durum wheat reference genome and a comprehensive Global Durum Genomic Resources including: the Tetraploid Global Collection (TGC: 1,856 tetraploids) and the Global Durum Panel (GDP: 1,033 varieties worldwide). All resources were genotyped with the Illumina 90K wheat SNP Array and characterized under a network of field trials for: (i) 52 DUS agronomic traits, (ii) phenology, agronomic, yield and quality traits, (iii) response to wheat diseases including Soil Borne Cereal Mosaic Virus (SBCMV). Haplotype-GWAS analysis on DUS traits revealed strong signals for all traits. Furthermore, genetic distances of INNOVAR durum wheat panels were compared to morphological distances derived from DUS traits, resulting in a good linear correlation value. Based on this comparison, genetic similarities values (GSVs) were obtained enabling us to identify highly similar varieties (low genetic and morphological distance) and highly different varieties (high genetic and morphological distances). These results allowed for a pre-screening analysis with varieties already distributed in the market. As to VCU, a major QTL on chr. 2B, named

QSbm.ubo-2BS=Sbm2, (R^2=60%) was fine mapped to a region of 1.5 Mb responsible for SBCMV resistance. We designed 21 KASP® markers defining haplotypes resistant/susceptible to SBCMV. The resistant haplotype spread in North American, French and northern Italian germplasm while highly susceptible alleles were identified in the Mediterranean region and CIMMYT germplasm. The gene content of the locus showed a clear enrichment for disease-response related genes. The development of informative KASP markers and candidate gene identification will enhance the information accuracy for variety selection in breeding programs and European CPVO registration protocols. Clearly, The Global Durum Genomic Resources provide unprecedented opportunities to exploit the tetraploid wheat diversity.

Acknowledgments

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Enhancing wheat salt tolerance using chitosan-based nanoparticles: a novel approach to mitigating oxidative stress and improving ionic balance

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Keywords: wheat, salt stress, chitosan nanoparticles, antioxidant enzymes, gene expression

Salt stress is a major environmental factor limiting wheat (*Triticum aestivum* L.) production, threatening global food security. This study investigates the potential of chitosan-proline (Cs-Pro) and chitosan-glycine (Cs-Gly) nanoparticles in mitigating salt stress effects in two wheat cultivars: Heydari (salt-tolerant) and Sepahan (salt-sensitive). Plants were treated with nanoparticles at 0, 200, and 400 mg L^{-1} concentrations under increasing salt stress levels (0, 200, and 400 mM NaCl). The results revealed that Heydari exhibited better adaptability to saline conditions, with Cs-Pro (400 mg L^{-1}) providing the most significant improvements. Key physiological and biochemical enhancements included higher relative water content (RWC), increased chlorophyll levels, and elevated proline accumulation, all contributing to improved stress tolerance. Oxidative stress markers, such as malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), were significantly reduced, while certain antioxidant enzyme activities were induced, particularly in Heydari. Additionally, Cs-Pro and Cs-Gly nanoparticles improved ionic balance by reducing Na^+ accumulation and maintaining a favourable Na^+/K^+ ratio. Molecular analyses showed the upregulation of stress-related genes (*TaADC*, *TaSAMDC*, *TaSPDS*, *TaPxPAO*, *TaSOS1*, *TaNHX1*) involved in ion transport and polyamine metabolism, further strengthening wheat's resilience. These findings suggest that chitosan-based nanoparticles represent a promising and sustainable approach to enhancing wheat salt tolerance and improving plant growth and productivity in saline environments. Further research is recommended to explore their broader applicability across different cultivars and agricultural settings.

Chromosome-scale M genome assembly and genotyping-by-sequencing accelerate gene introgression into wheat

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

Genebank accessions of allotetraploid *Aegilops biuncialis* ($U^bU^bM^bM^b$) together with their diploid progenitors, *Ae. umbellulata* (UU) and *Ae. comosa* (MM), display high genetic diversity and constitute a valuable source of new genes for resistance to diseases, abiotic stresses, and improved wheat grain quality. However, limited knowledge of their genomes and a lack of molecular tools remain major obstacles to mapping and transferring QTL-specific chromatin into durum and bread wheat.

We demonstrated that draft sequences of U and M chromosomes, flow sorted from diploid progenitors, facilitated marker development and the construction of a high-resolution segregating genetic map of *Ae. biuncialis*. These genomic resources enabled genome-wide association mapping of traits related to plant phenology, morphology, yield components, grain quality, and resistance to fungal diseases (Pm, Yr) in a diversity panel comprising 186 *Ae. biuncialis* accessions genotyped by DArTseq.

The newly generated chromosome-scale reference assembly of *Ae. comosa* MvGB1039 further improved the accuracy of read mapping from GBS libraries of wheat pre-breeding populations and enhanced high-throughput detection of alien chromatin. High-throughput genotyping of the wheat (Mv9kr1) × *Ae. biuncialis* (MvGB 642, 382) BC_3 populations, combined with automated cytogenetic screening (GISH, FISH), confirmed the presence of complete 1MS, 3M, 4M, 5M, and 6M chromosomes. Most U-genome chromatin was lost, with only fragments of 1U, 2U, 4U, and 7U chromosomes retained in some lines.

The new introgression and addition lines, together with chromosome-specific genomic resources, will facilitate the development of climate-resilient wheat cultivars and provide opportunities to clone agronomically important genes.

Acknowledgments
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Chromosome structure, cross-genome homoeology, and DNA markers in *Aegilops*: insights from single-gene FISH and chromosome flow sorting

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Keywords: goat grasses, *Ae. comosa, Ae. umbellulata, Ae. uniaristata*, Single-gene FISH, chromosome flow sorting, chromosome rearrangements, homoeologous relationships, molecular markers

Breeding climate-resilient and disease-resistant crops is hampered by reduced genetic diversity resulting from domestication and centuries of human selection. One approach to expand the gene pool is chromosome-mediated gene transfer from wild relatives via cross-hybridization. In wheat (*Triticum aestivum*), species from the *Aegilops* genus serve as valuable sources of novel genes and alleles. However, the evolutionary history of *Aegilops* and *Triticum* has led to extensive genomic diversification, particularly in the D-genome lineage, which diverged ~3 Mya. This diversification resulted in the formation of *Aegilops* species with C, D, M, N, S, and U genomes (Kilian et al., 2011). Phylogenomic analyses (Glémin et al., 2019; Tanaka et al., 2020) have suggested that species with C, M, N, and U genomes form a distinct group from those with D and S genomes, with the C genome of *Ae. markgrafii* and the U genome of *Ae. umbellulata* being closely related. Despite the potential of *Aegilops* species for gene transfer, the structural genome alterations and the lack of molecular tools to detect alien *Aegilops* chromatin hinder their effective utilization in wheat improvement. To address this challenge, we developed single-gene fluorescence *in situ* hybridization (FISH) maps for M- and U-genome progenitors (*Ae. comosa* and *Ae. umbellulata*), and we are currently mapping the N-genome progenitor (*Ae. uniaristata*). Using wheat cDNA probes, we identified 47 loci in *Ae. comosa* and 52 in *Ae. umbellulata*, based on 43 orthologous genes. Along with 18 additional genes, these genes were selected for mapping in *Ae. uniaristata* using the single-gene FISH method. Our findings revealed that M-genome chromosomes largely maintained collinearity with wheat, except for intrachromosomal rearrangements in 2M and a paracentric inversion in 6ML. While chromosomes 1U, 3U, and 5U of *Ae. umbellulata* retained collinearity with wheat, structural variations in 2U, 4U, 6U, and 7U indicated a closer similarity to the C genome of *Ae. markgrafii*. To develop molecular markers with precise physical positions on *Aegilops* chromosomes, we validated the single-gene FISH data in *silico* using DNA sequence assemblies from flow-sorted M- and U-genome chromosomes. Sequence similarity analysis confirmed 44 of the 47 mapped loci in *Ae. comosa* and 40 of the 52 in *Ae. umbellulata*. The identified polymorphic regions enabled the development of PCR-validated molecular markers. The single-gene FISH-based approach thus facilitated the creation of cytogenetically anchored PCR markers, compensating for the absence of a segregating map (Said et al., 2021). Our ongoing work on *Ae. uniaristata* (Fig. 1) has revealed additional inter- and intra-chromosomal rearrangements in the N genome, including translocations of the distal and proximal short arm regions of chromosomes 1N, 2N, and 3N to their respective long arms, as well as a paracentric inversion on chromosome 4N (Fig. 2). However, we observed a well-preserved wheat-*Ae. uniaristata* collinearity for chromosome 5N. FISH analysis of flow-sorted chromosomes (Fig. 3) confirmed a possibility to isolate N-genome chromosomes with purities ranging from 34% to 90%, though further optimization is needed to enhance purities in flow-sorted fractions. These advancements provide critical genomic resources that will facilitate the introgression of *Aegilops* genes into wheat as well as their cloning, supporting efforts to enhance wheat's adaptability and resistance to biotic and abiotic stresses.

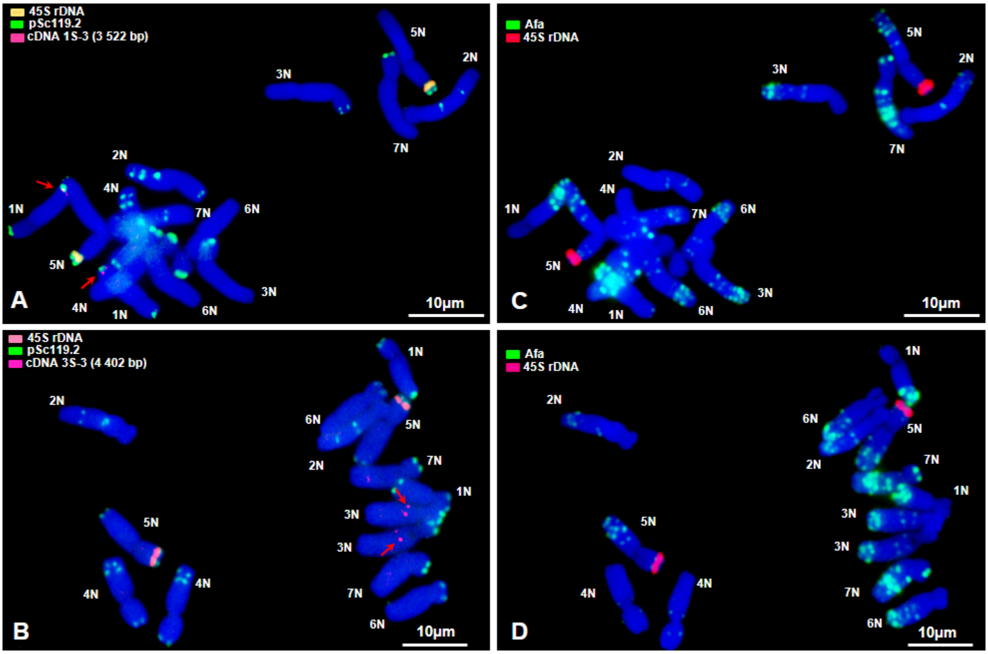

Fig 1. FISH with probes for 45S rDNA, *Afa* family, *pSc*119.2 repeat, and cDNAs (red arrows) on mitotic metaphase chromosomes of *Ae. uniaristata*. (A and B) FISH with 45S rDNA (orange), *pSc*119.2 repeat (green) and cDNA (red); (C and D) 45S rDNA (red) and *Afa* family repeat (green). The chromosomes were counterstained with DAPI (blue).

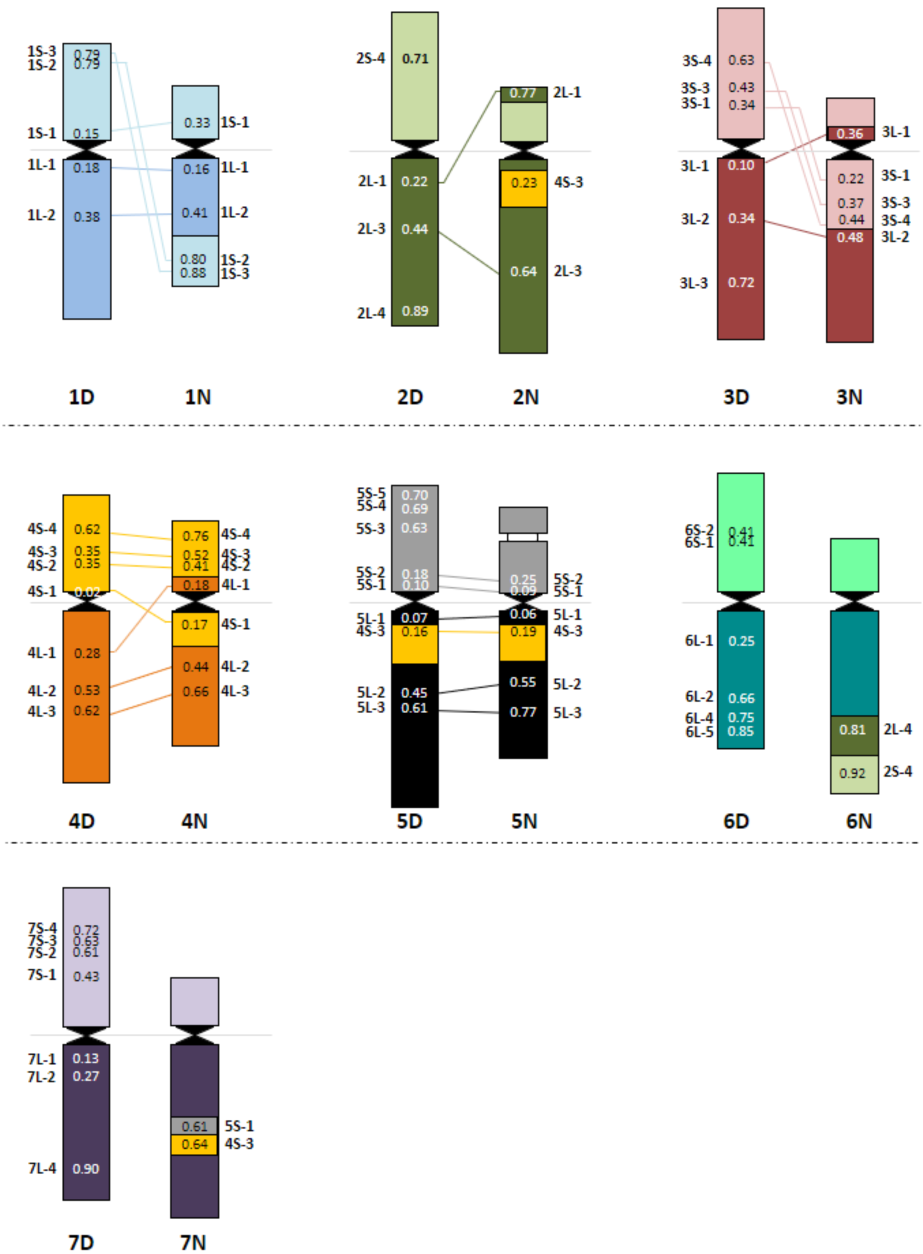

Fig 2. Chromosome organization of the *Ae. uniaristata* **N** genome (right) compared with the **D** (sub) genome of wheat (left). The average relative positions of cDNAs are shown on the chromosomes.

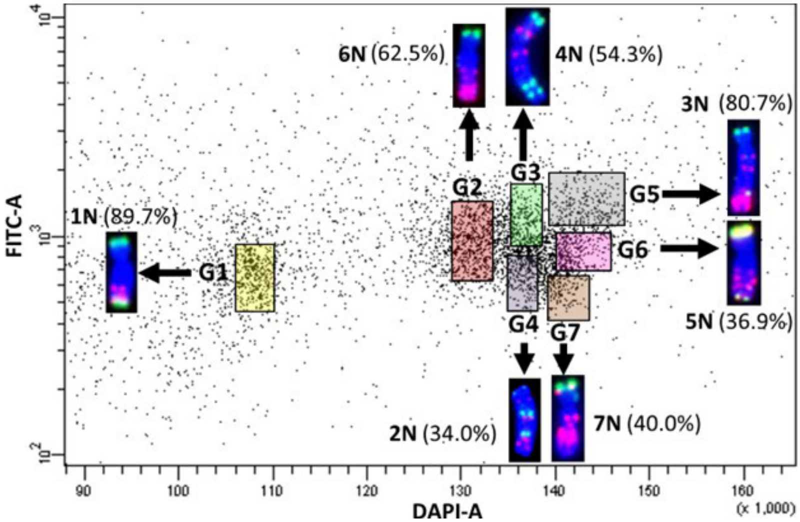

Fig. 3. Flow cytometric analysis of *Ae. uniaristata* chromosomes after fluorescence *in situ* hybridization in suspension (FISHIS) with FITC-labelled $(GAA)_7$ probe. Bivariate analysis GAA_7-FITC vs. DAPI enabled chromosome sorting at purities of 34 - 90%. Chromosomes were assigned to the colored regions (G1-G7) by FISH on sorted fractions using probes for 45S rDNA (yellow), *Afa* family (red), and *pSc119.2* repeat (green). Chromosomes were counterstained by DAPI (blue).

Acknowledgments

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References

1. Glémin, S., Scornavacca, C., Dainat, J., Burgarella, C., Viader, V., Ardisson, M., et al. (2019). Pervasive hybridizations in the history of wheat relatives. *Science Advances* 5, eaav9188. doi: 10.1126/sciadv.aav9188
2. Kilian, B., Mammen, K., Millet, E., Sharma, R., Graner, A., Salamini, F., et al. (2011). “Aegilops,” in *Wild Crop Relatives: Genomic and Breeding Resources: Cereals*, ed. C. Kole (Berlin, Heidelberg: Springer), 1–76. doi: 10.1007/978-3-642-14228-4_1
3. Said, M., Holušová, K., Farkas, A., Ivanizs, L., Gaál, E., Cápal, P., et al. (2021). Development of DNA Markers From Physically Mapped Loci in Aegilops comosa and Aegilops umbellulata Using Single-Gene FISH and Chromosome Sequences. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.689031
4. Tanaka, S., Yoshida, K., Sato, K., and Takumi, S. (2020). Diploid genome differentiation conferred by RNA sequencing-based survey of genome-wide polymorphisms throughout homoeologous loci in Triticum and Aegilops. *BMC Genomics* 21, 246. doi: 10.1186/s12864-020-6664-3

Can allelic variation at *HvCEN* and *VRN-H1* enhance barley yield stability under pre-anthesis heat stress?

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Keywords: barley, flowering-time genes, heat stress, yield components

Heat stress is a major abiotic factor limiting barley (*Hordeum vulgare* L.) yield, particularly when it coincides with critical reproductive stages. Understanding this threat and identifying genetic sources of resilience are essential for crop improvement. We conducted a field experiment in the Orria/Plaisant population to investigate the interaction between two key flowering-time genes, *HvCEN* and *VRN-H1*, per se and in response to heat stress. Four near-isogenic lines carrying contrasting allele combinations (HI and HII at *HvCEN*; *vrn-H1* and *VRN-H1-4* at *VRN-H1*) were evaluated for reproductive and agronomic traits under control and heat stress conditions. Heat stress was imposed pre-anthesis, using portable transparent polyethylene tents, which increased the day's ambient temperature by up to approximately 6 °C, while control plots remained under ambient conditions. Traits recorded included grain number, grain size, spikelet number, spikes per plant, spike fertility, thousand-kernel weight (TKW), and apparent fruiting efficiency. The *HvCEN* HI allele promoted a higher spike number per plant but was associated with lower TKW, leading to overall yield comparable to that of the HII haplotype. A similar compensatory pattern was observed for the two *VRN-H1* alleles. However, unlike *VRN-H1*, *HvCEN* HI conferred a markedly higher apparent fruiting efficiency relative to HII, attributable not to an increase in grain number per spike but to reduced rachis weight. The heat stress treatment affected dramatically yield components, although allelic effects on heat response were not as clear. These findings highlight the contrasting yet compensatory roles of *HvCEN* and *VRN-H1* in shaping barley yield components and provide new insights into their contribution to yield stability under pre-anthesis heat stress.

Acknowledgments

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T2: Environmental Adaptation

Induction of frost tolerance by different light spectra and low-temperature stimuli in wheat genotypes differing in vernalization alleles

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Keywords: wheat, vernalization, frost-tolerance, CBF regulon, metabolism, light regulation

The need of low but non-freezing temperature is a shared requirement for both cold acclimation and vernalization in winter cereals, indicating a potential connection between these physiological processes. Cold hardiness is mainly regulated by the CBF (C-repeat Binding Factor) regulon, which regulate the expression of downstream cold-responsive (COR) genes. In contrast the vernalization response mainly mediated by VRN (vernalization) gene network, with *VRN1* acting as a central regulator. A major issue with the expression of *VRN1* gene is its epistatic effect on CBF regulon, which leads to diminished freezing tolerance. Beyond temperature, light also serves as a crucial external factor influencing CBF gene expression, suggesting that light quality is integral to the regulation of cold acclimation. Importantly, light quality not only influences gene regulatory networks, but also has profound effect on plant primary and secondary metabolism. Alterations in light quality can modulate the accumulation of key cryoprotective metabolites including free amino acids, soluble sugars, and organic acids. However, the role of *VRN1* gene in modulating light quality induced frost tolerance remains unclear. In our study, we utilized reciprocal near-isogenic lines (NILs) generated by crossing the non-hardy, spring-habit cultivar ‘Manitou’ (*Vrn-A1*) with the cold-hardy, winter-habit cultivar ‘Norstar’ (*vrn-A1*), allowing direct comparison of *VRN1* alleles in common genetic background. Our aim was to examine the influence of winter and spring *VRN1* alleles (*vrn-A1*/*Vrn-A1*) on frost tolerance under different spectral illuminations. Plants were exposed to various spectral light treatments at 15 °C or 5 °C temperature, and subjected to freezing tolerance assays and cold-related gene expression analysis. Our results suggest that light-induced frost tolerance is not solely dependent on *VRN1* expression but significantly influenced by the genetic background. The application of monochromatic blue light effectively enhanced frost tolerance but only at 15 °C, particularly in genotypes with cold-sensitive background. Additionally, supplementing white light with far-red and blue light significantly improves frost tolerance regardless of *VRN1* expression. We also analysed metabolite accu-

mulation specifically in the original spring-habit 'Manitou' and its near-isogenic winter allele counterpart. The most dominant changes at both temperatures was observed under far-red supplementation, alongside the cold-induced increase of free amino acids and soluble sugars.

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The influence of far-red and blue light supplementations of white light on pre-hardening of winter barley leaves

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Keywords: blue light, far-red light, pre-hardening, frost tolerance, barley

Climate change introduces new challenges for the freezing tolerance of cereals. Milder winters and more erratic temperature swings are expected to diminish the effectiveness of cold acclimation and as a result, their ability to withstand freezing temperatures. Simultaneously, the frequency of sudden, extreme cold events is likely to increase, putting unacclimated or de-acclimated plants at risk of significant frost injury. Consequently, brief (lasting up to one week) cold exposures – termed as pre-hardening – along with factors like light quality, are expected to play a more significant role in the development of freezing tolerance in young winter cereals than in the past. Supplementing white light with far-red light (WFr) induces pre-hardening at 15°C and enhances the effect of cold at 5°C. Recent studies using monochromatic blue light have suggested that blue light may also improve the freezing tolerance of leaves. However, limited data in the literature predicts the negative interaction between far-red and high blue light proportions. In contrast, our recent work showed that adjusting WFr with violet-blue light (VB, λ_{max}=410 nm) more effectively reduced freezing injury in barley leaves than WFr alone, after one week at 5°C. Nevertheless, the blue wavelength specificity of this effect and its interaction with far-red induced changes remain unexplored.

Accordingly, in this study, the effect of different blue light supplementations of white light, VB and blue (B, λ_{max}=450 nm) were investigated with or without the presence of far-red light supplementation (W; W+VB_{410}; W+B_{450}; WFr+VB_{410}; WFr+B_{450}), on young winter barley 'Nure' leaves at different temperatures (15°C and 5°C) for 10 or 7 days, respectively. After the treatments, the expression key genes related to light signalling (e.g., photoreceptors, primary transcription factors such as 'Elongated Hypocotyl 5, HY5' and 'Phytochrome Interacting Factors, PIFs') and cold signalling (e.g., 'C-repeat binding factors, CBFs') were examined. Additionally, excised leaf segments were subjected to freezing temperatures. The analyses revealed that B_{450} supplementation negated the beneficial effects of WFr on the freezing tolerance of barley leaves, unlike B_{410} enrichment. Furthermore, W+B_{410} and W+B_{450} supplementations were unable to induce or enhance pre-hardening in the absence of far-red light. These results provide new insights into the interaction between blue and far-red light during the pre-hardening of barley leaves in mixed light environment.

Acknowledgments

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References

1. Caccialupi et al. (2023) *Cells.* 12(22), 2606.
2. Borbély et al. (2022) *Antioxidants.* 11(7), 131.
3. Ahres et al. (2023) *Plants.* 12(1), 40.
4. Ahres et al., (2025) *Environ. Exp. Bot.* 229, 106079.

Local adaptation and global prediction: GxE modelling of elite wheat across Europe

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Keywords: wheat, GxE, GxM, yield stability, nitrogen fertilisation, multiple-environment trials

Stagnating yields, rising food demand, and increasing restrictions on fertilizer and pesticide use present major challenges for wheat breeding. Climate change is expected to increase weather extremes, further threatening yield stability and food security. Assessing yield stability and dissecting genotype-by-environment (GxE) and -management (GxM) interactions requires multi-environment field trials with different treatments.

Here, we studied GxE and GxM interactions in 12 elite wheat cultivars across 12 locations spanning Europe's latitudinal and longitudinal breadth over three seasons. Varieties were selected for local agronomic merit. The Trials were grown in split-plot designs with three site-specific nitrogen levels (50%, 100%, 130% of the local optimum). Linear mixed-effects models were applied to assess GxE and GxM effects on 14 traits, including yield, yield components, and nitrogen efficiency traits.

All traits showed moderate to high heritabilities (H^2=0.50-0.97) across environments. Yield decreased under sub-optimal nitrogen, but did not increase with supra-optimal levels. Yield was positively correlated with stability (r = 0.8) and genotypes generally performed above-average in their target environments, indicating local adaptation. For all traits, substantial GxE but minimal GxM were observed (on average 40% and 2% of total genetic variance, respectively). The prevailing weather conditions were a main driver of GxE, which can be modelled by a factor analytic model incorporating observed meteorological data (Piepho & Blancon 2023). Using this approach to model GxE, genotype performance in unseen environments could be predicted with a median accuracy of r = 0.85, 0.84 and 0.84 for grain yield, grain weight and grain number, respectively.

Together, these results highlight the importance of environmental conditions and location specific adaptation for wheat performance. As demonstrated, a network of field-testing sites spanning a wide range of environments allows accurate prediction of genotype performance in specific environments within the tested range. This offers the opportunity for breeders to tailor varieties more accurately for particular environments without the need to test them in every single environment.

Acknowledgments
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References

1. Piepho H-P, Blancon J. 2023. Extending Finlay–Wilkinson regression with environmental covariates. Plant Breeding 142, 621–631.

Salinity stress response in durum wheat: new insight for the identification of key genes for tolerance

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Keywords: durum wheat, salt stress, tolerance, WGCNA, hub genes

Soil salinity is a major threat limiting crop productivity. To investigate molecular mechanisms of tolerance, four durum wheat recombinant inbred lines (RILs$_{F7:F8}$) with contrasting phenotypic responses to salt stress were selected based on germination rates, root and shoot length under stress conditions. Biochemical analyses further characterized these genotypes in terms of ion content and photosynthetic efficiency. Among them, genotype 158 displayed high tolerance, maintaining elevated germination rates and better physiological parameters under salt stress, 15204R showed marked sensitivity, with reduced germination and lower fitness, whereas 1502R and 15209 exhibit intermediate levels of tolerance.

Using in silico RNA-seq data, a similar number of differentially expressed genes (DEGs) was observed in each genotype. However, only a small subset of DEGs was shared, suggesting a highly genotype-specific transcriptional response to salt stress. The tolerant genotype modulated genes related to photosynthetic processes, while the sensitive one repressed genes with different functions, including ion homeostasis and reactive oxygen species (ROS). To identify key regulators involved in salt response, Weighted Gene Co-expression Network Analysis (WGCNA) was performed, leading to the identification of 35 distinct co-expressed gene modules. Among these, three were strongly associated with salt stress, whereas other two were associated with tolerance-related traits. Functional annotation of hub genes revealed several transcription factors, such as ERF and WRKY, and metabolic enzymes like PAL and PPO.

Altogether, the integration of phenotypic, biochemical, and transcriptomic data allowed the identification of key molecular pathways and candidate genes that may guide salt stress tolerance in durum wheat. These insights provide a valuable foundation for the development of stress-resilient wheat varieties through molecular breeding strategies.

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T3: Biotic Stress Response; Plant Microbe Interactions

Harnessing cereal volatilomes for mycotoxin risk assessment and fusarium resistance screening

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Keywords: fusarium head blight, wheat, volatile organic compounds (VOCs), mycotoxins, resistance screening, volatilome, DON, cereal breeding, HS-SPME-GC-MS, biomarker discovery

Fusarium head blight (FHB) of wheat, primarily caused by Fusarium graminearum and F. culmorum, remains a major threat to cereal production and food safety due to yield losses and crop contamination with trichothecene mycotoxins. While traditional resistance breeding and phenotyping rely on visual symptoms and mycotoxin quantification, recent advances highlight the diagnostic potential of volatile organic compounds (VOCs) emitted by infected plants.

In this study, we investigated the VOC profiles – volatilomes – of winter wheat cultivars differing in FHB resistance under monitored infection conditions. Volatile fingerprints were obtained from both green spikes (4–21 days after inoculation) and mature grain flours using HS-SPME-GC-MS. Multiyear field experiments revealed specific VOC markers, mostly sesquiterpenoids, that correlate strongly ($R^2 > 0.75$) with both *Fusarium* kernel infection and mycotoxin concentrations (DON and others), offering a non-destructive, early-stage risk assessment tool.

Moreover, cultivar-specific VOC patterns allowed discrimination between susceptible and resistant genotypes, supporting their application in resistance screening pipelines. The integration of volatilomic markers into breeding programs could significantly enhance selection efficiency, especially when coupled with traditional and molecular phenotyping.

Our findings emphasize the practical potential of cereal VOC profiling in mycotoxin risk forecasting and FHB resistance selection – advancing both biotechnology and breeding strategies for safer and more resilient cereal production.

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Spermine priming modulates antioxidant and stress responses in wheat under *Fusarium culmorum* seed infection

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Keywords: *Fusarium culmorum*, seed priming, spermine, stress markers, Triticum aestivum L., antioxidant defence

Fusarium root and base rot is caused by pathogenic fungi such as *Fusarium culmorum*. This disease represents a major threat to global cereal production. Usually to fight the disease, conventional chemical fungicides are used, however with the recent ecological regulations, new approaches for fighting Fusarium root and base rot disease are needed. Seed priming with environmentally friendly compounds is gaining attention for its ability to enhance plant resilience and growth. This study investigated the effects of seed priming with the polyamine spermine (5 mM), a naturally occurring plant growth regulator known to modulate stress responses. The experiments were performed on the Bulgarian wheat cultivar Sadovo-1 under a direct seed surface infection with *Fusarium culmorum*.

The infection resulted in a substantial decrease in plant fresh weight and height. At biochemical level, a significant increase was observed in key stress markers, including malondialdehyde, free proline, and electrolyte leakage. These changes were accompanied by an increase in non-enzymatic antioxidants, specifically free thiol-containing groups and total phenolics. The cellular response was further characterized by a significant increase in hydrogen peroxide content and the activities of antioxidant enzymes like superoxide dismutase and peroxidase, along with a strong decrease in catalase activity, indicating a disruption in the plant's primary antioxidant defense system.

Seed priming with 5 mM spermine proved to be an effective strategy to counteract these detrimental effects. The treatment successfully modulated the mentioned stress responses, alleviating the increases in stress markers and antioxidants and helping to restore plant growth parameters closer to control levels. This suggests that spermine-based seed priming could hold significant potential as a promising, eco-friendly method to protect Bulgarian wheat cultivar Sadovo-1 from *F. culmorum* seed infection.

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Biocontrol of aflatoxin B1 mycotoxin production under drought stress

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Keywords: mycotoxin, aflatoxin, atoxinogen, biocontrol, Aspergillus flavus, drought, corn

Climate change and global warming have intensified research into aflatoxin B1 contamination protection, as contaminated feed and food cause severe economic damage. Atoxigenic *Aspergillus flavus* isolates effectively reduce aflatoxin B1 contamination in corn, and biocontrol strategies have shown promise under controlled conditions. However, climate changes, particularly drought stress, make biocontrol effectiveness unpredictable in field applications, creating uncertainty about this protective strategy's reliability.

Therefore, we conducted an in vivo micro-plot experiment using Hungarian *A. flavus* isolates selected based on genome sequences and secondary metabolome analysis. One atoxigenic strain was applied to the soil, and one toxigenic strain was applied to the ear of the same corn hybrid [GEIX1771, Syngenta] under varying environmental conditions, examining starch and protein, total polyphenol content, mycotoxin contamination, and mould count in irrigated and non-irrigated conditions with different nitrogen supplementation.

Consequently, our results demonstrate that nutrient values responded to irrigation conditions independently of fungal inoculations. The atoxigenic *A. flavus* successfully reduced aflatoxin B1 through the repression of mould counts. Irrigation and the fungal treatments were negatively correlated, while total polyphenols positively correlated with irrigation. Thus, changing climate conditions significantly modify biocontrol organism effectiveness, requiring consideration in future aflatoxin management strategies.

T4: Phenotyping Technologies – IPPN CEPPG Session

High-throughput phenotyping of chickpea photosynthetic performance under combined drought and heat stress

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Keywords: chickpeas, biomass, quantum efficiency, drought, high temperature

Climate change has intensified the occurrence of drought and heat stress, posing significant challenges to global agriculture. In arid and semi-arid regions, these stresses often occur simultaneously, highlighting the need for climate-resilient crop cultivars with combined drought and heat (CDH) tolerance. Chickpea *(Cicer arietinum* L.*)*, a legume valued for its high nutritional content and ability to thrive under low-input conditions, is a key candidate for such improvement. Efficient phenotyping of chickpea plant genetic resources (PGR) is essential to assess the genetic variation available for breeding DH-tolerant cultivars. High-throughput phenotyping (HTP) systems offer a rapid and precise approach for evaluating dynamic traits related to DH tolerance. In this study, 200 single seed descent-derived chickpea genotypes from the INCREASE T-Core diversity panel (Rocchetti et al., 2022), consisting of an equal number of *kabuli* and *desi* types, were evaluated using an RGB image-based HTP system. Plants were subjected to CDH stress followed by a recovery phase under optimal conditions. The 61-day experiment spanned from early vegetative to early reproductive stages, with final yield parameters collected at maturity outside the platform. The study aimed to: (i) assess the impact of CDH stress on chickpea growth, physiological, and reproductive performance, and (ii) compare the responses of *kabuli* and *desi* types under CDH stress. Traits such as biovolume, projected area, plant height, fluorescence-based indicators, and color-related parameters were recorded using the HTP system, while yield traits and final plant height were measured manually at maturity. Preliminary results revealed that CDH stress significantly reduced growth performance, particularly biomass accumulation, plant height, and canopy area. The red-to-green color ratio exhibited considerable genotypic variation under stress, suggesting its usefulness as a selection trait for breeding. Desi type showed a higher proportion of brown and yellow pixels under DH stress compared to *kabuli*, potentially reflecting a stress protection strategy. Photosynthetic efficiency, measured by the operating efficiency of photosystem II (ΦPSII) and maximum quantum yield (Fv/Fm), declined under DH stress but recovered under optimal conditions.

Acknowledgments

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References

1. Rocchetti, L., Gioia, T., Logozzo, G., Brezeanu, C., Pereira, L. G., la Rosa, L. D., Marzario, S., Pieri, A., Fernie, A. R., Alseekh, S., Susek, K., Cook, D. R., Varshney, R. K., Agrawal, S. K., Hamwieh, A., Bitocchi, E., & Papa, R. (2022). Towards the development, maintenance and standardized phenotypic characterization of single-seed-descent genetic resources for chickpea. Current Protocols, 2, e371. doi: 10.1002/cpz1.371

From wild to brew: exploring root architecture in different coffee species under varying nutrient conditions

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Keywords: *Coffea* genus, climate change, root system, image analysis, diversity, nutrients

Improving root systems of fruit tree crops such as coffee is crucial to enhance water and nutrient uptake under altering temperature and rainfall patterns caused by climate change. Therefore, understanding root growth patterns and responses is key for breeding and managing coffee plants adapted to climate change and soil challenges. Emerging non-invasive imaging technologies now enable high-throughput root monitoring, opening new avenues for sustainable crop improvement.

To investigate differences in coffee root system architecture and its growth dynamics and plasticity, we employed the advanced rhizotron system integrating root and shoot imaging, located in IPK's unique PhenoSphere. Eight coffee genotypes—representing two cultivated (two *Arabica* types and one *Robusta* type) and five wild species of *Coffea* genus—were studied under two contrasting nitrogen (N) conditions of high and low levels, with ten replicates per genotype, over a period of three months. The existing deep-learning-based image analysis pipelines were adapted for coffee roots and shoots. Principal component analysis (PCA) of the 54 extracted root traits revealed distinct clustering by species. Similarly, PCA of the 36 shoot traits showed species-specific groupings with patterns reflecting contrasting N conditions. N supply had a notable impact on RSA, particularly on traits related to root biomass: most genotypes, exhibited a significantly reduced root biovolume under low-N conditions. Nitrogen availability also influenced shoot traits; shoot dry weight revealed reductions under low-N in all species, except for one species. We are currently analyzing the time-course data to determine the timing and progression of nitrogen effects across the different coffee genotypes. In an earlier test experiment using the large automated phenotyping system with the rhizopot system, roots were sampled for exploring differences in the transcriptome and microbiome with promising first insights. A new experiment is currently conducted on the same system to generate a combined in-depth data set of HTP data, microbiome and transcriptome data that will be used for integration of all data into predictive models that will be used to select resilient genotypes with desirable root traits and nitrogen use efficiency (NUE).

T5: Yield and Quality Improvement

Forward prediction of grain yield across early and advanced generations in winter wheat (*Triticum aestivum* L.) Using NIRS-based models

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Keywords: NIRS, models, forward prediction, grain yield, historical data

Near-infrared spectroscopy (NIRS) is a rapid, non-destructive, and low-cost phenotyping tool with strong potential to accelerate plant breeding. We evaluated NIRS-based models for forward prediction of grain yield in winter wheat, using historical phenotypic and spectral data across multiple years and breeding cycles. Two forward prediction scenarios were tested: a fixed-size training set and a cumulative training set spanning up to five years. NIRS-based predictions showed moderate to high predictive ability in F_5 and advanced lines across all models and scenarios (0.28-0.58). Machine learning methods such as Support Vector Machine and Random Forest performed competitively, while Ridge Regression was stable and effective. For F_4 generation lines, grown in unreplicated small plots without direct grain yield measurements, we predicted grain yield from their NIRS profile. Training sets consisted of F_5–advanced lines (e.g., 2015–2017) to predict the performance of F_4 nursery lines without direct grain yield measurements (e.g., 2018). The predictions were subsequently validated with the F_4-derived F_5 generation lines, field-tested in multi-location trials in the subsequent year (e.g., 2019), where still moderate predictive abilities were observed (0.22-0.31). Therefore, NIRS-based predictions can be practically applied to identify promising genotypes for subsequent selection stages (such as multi-location yield trials) at early breeding phases, even in the absence of grain yield data. This highlights the potential of this approach to support more efficient and data-informed selection decisions, particularly in breeding programs with limited genotyping resources.

Acknowledgments

We thank Lidea breeding company and their team for conducting field experiments, phenotyping and NIRS acquisition. We are also grateful to Hermann Gregor Dallinger, Jose Crossa, Renaud Rincent and Stephan Freitag for the many fruitful discussions when conducting this study.

References

1. Crossa J, Pérez-Rodríguez P, Cuevas J, et al (2017) Genomic Selection in Plant Breeding: Methods, Models, and Perspectives. Trends in Plant Science 22:961–975. https://doi.org/10.1016/j.tplants.2017.08.011
2. Dallinger HG, Löschenberger F, Bistrich H, et al (2023) Predictor bias in genomic and phenomic selection. Theor Appl Genet 136:1–16. https://doi.org/10.1007/s00122-023-04479-8
3. Rincent R, Charpentier J-P, Faivre-Rampant P, et al (2018) Phenomic Selection Is a Low-Cost and High-Throughput Method Based on Indirect Predictions: Proof of Concept on Wheat and Poplar. G3 Genes|Genomes|Genetics 8:3961–3972. https://doi.org/10.1534/g3.118.200760

Characterization of genetic diversity of bread wheat genotypes under water-limited conditions for their morpho-physiological, biochemical and quality-related attributes

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Keywords: drought stress, baking quality, cluster analysis, *Triticum aestivum* L.

Improvement of wheat crop for both yield and quality is one of the major tasks for scientists to feed the enormously increasing world population along with the drastically changing climatic conditions. This experimental study was designed to evaluate the existing wheat germplasm for its plasticity regarding physiological, morphological, biochemical, and quality-related traits under normal and drought stress conditions. The experimental material was comprised of 100 wheat genotypes including approved varieties, elite genotypes, ecotypes, and pure lines from different parts of the country. The experiment was then carried out under split-plot design with three replications and two treatments (i) Hundred wheat genotypes (ii) (a) normal and (b) water-limited conditions. The data obtained from several key agro-morphological, physiological, biochemical and grain quality-related traits were then subjected to statistical analysis and explanations. The results revealed that highly significant variations were present among wheat genotypes under both normal and water-limited conditions. The correlation coefficient analysis unveiled the strong positive correlation of grain yield with thousand-grain weight, number of grains per spike, net photosynthetic rate, stomatal conductance, superoxide dismutase and chlorophyll while the negative correlation with H_2O_2, plant height, grain protein and grain carbohydrate contents. The cluster analysis divided 100 genotypes into five groups based on their performance under both control and drought stress conditions. However, the genotypes were different in these groups while considering the control and water test treatments. The study showed that significant variations are already present in the cultivated germplasm which needs to be effectively used in wheat breeding programs involving sustainable yield and quality improvement.

Optimized cereal genotyping arrays based on haplotype structure, marker quality, and candidate genes

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Keywords: SNP genotyping, genotyping arrays, haplotype structure, marker-assisted selection, genomic selection, cereal (wheat, barley, rye, oat, triticale) crop breeding

SNP genotyping is widely used nowadays in cereal genetics and breeding, including for barley, rye, oat, Triticale, and hexaploid and tetraploid wheat. Examples of SNP applications include analyzing population structure and genetic relationships, performing marker-assisted selection and backcrossing, and identifying marker-trait associations (QTL, GWAS). Currently, SNP genotyping arrays allow for the simultaneous analysis of thousands of SNPs in a cost-efficient manner. SNP arrays have also become a major tool in genomic selection within routine cereal breeding.

We describe the continuous development and optimization of our cereal genotyping arrays, which are designed for genome analyses and genomic selection based on important criteria. One is marker quality, especially for polyploid species, including detecting genome-specific loci and clearly calling the three allelic states. The second criterion we considered when developing the array was detecting as many haplotype blocks as possible based on a genotyping database consisting of several hundred to thousands of varieties worldwide. With these datasets available, we selected one to three markers per identified haplotype block to obtain the maximum amount of information with these arrays. A detailed analysis revealed that these haplotype-specific markers are highly clustered towards the ends of cereal chromosomes, where most recombination events occur. To increase the utility of these arrays further, we added publicly available markers reported to be linked to specific traits in the literature.

Currently, we have genotyping arrays containing approximately 26k and 8k markers for hexaploid bread wheat and tetraploid durum wheat (excluding most of the D-genome markers); 16k and 5k for barley; 6k for rye; 8k for oat, and 32k for Triticale (combining the 26k wheat markers and the 6k rye markers). The larger SNP marker arrays for wheat and barley are optimized for detecting many haplotype blocks, while the smaller arrays can decrease costs per genotyped sample. These smaller arrays are widely used for wheat and barley breeding, including imputing markers based on the previous genotyping of parental lines with our larger arrays or other available genotyping arrays based on sets of common markers. Meanwhile, our arrays have been used for the genotyping of several hundred thousand lines from all over the world.

Molecular and genetic determinism of sorghum grain quality

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Keywords: sorghum, grain quality, starch, protein, protein digestibility, gene co-expression network, protoplast transient over-expressions, transcription factor, GWAS

Sorghum grains are rich in proteins and starch but exhibit low protein digestibility, limiting their use in food and feed. However, the genetic and molecular mechanisms underlying these traits remain poorly understood, particularly the genomic regions involved, as well as the structural genes and transcription factors (TFs) that regulate them, hindering efforts to improve sorghum grain quality. To address this, we adopted an integrated genomic and genetic approach. At the genomic level, we constructed a gene co-expression network using transcriptomic data collected during grain development (10 stages) over two years. In parallel, we quantified starch and protein content and measured protein digestibility. Two major gene co-expression modules were identified. The first was linked to the loss of protein digestibility, involving genes related to disulfide bond formation and modulation. The second contained most kafirin and starch metabolism genes, as well as orthologs of TFs known to regulate protein and starch accumulation in other cereal species. Functional assays performed in protoplasts for six TFs revealed a central role for SbPBF1a, SbPBF1b, and SbNF-YC13 in modulating the expression of genes involved in protein and starch biosynthesis. In the genetic approach, we used a diversity panel of 300 sorghum genotypes representing global genetic diversity. This panel was cultivated at two sites in France over two growing seasons. Protein, starch contents and protein digestibility were measured. Whole-genome sequencing (WGS) was performed for all genotypes. These biochemical and genotypic data were analysed through genome-wide association studies (GWAS) using both single and multi-locus models. Several significant SNPs were identified near genes previously associated with grain quality traits. Together, our work provides novel insights into the genetic basis and transcriptional regulation of protein and starch accumulation, as well as protein digestibility in sorghum grains. It also identifies regulatory and structural genes that could be targeted to enhance grain quality, thereby supporting the development of improved sorghum varieties with higher nutritional value.

Epigenetic regulation of microspore embryogenic potential in contrasting winter wheat genotypes: a multi-level molecular analysis

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Keywords: microspore embryogenesis, winter wheat, DNA methylation, epigenetic reprogramming

The ability of microspores to initiate embryogenic development under *in vitro* conditions is strongly genotype-dependent trait in wheat, limiting the practical application of doubled haploid (DH) technology in wheat breeding programs. Recent studies highlight the key role of epigenetic modifications -particularly DNA methylation - in regulating chromatin structure and gene expression during the reprogramming of microspores from the gametophytic to the sporophytic developmental pathway. Despite increasing interest in this field, the molecular basis of variation in embryogenic potential among different genotypes remains poorly understood - particularly the role of key epigenetic regulators such as *Ta*MET1, a major DNA methyltransferase responsible for maintaining methylation homeostasis in plant genomes.

The aim of this study was to identify epigenetic factors underlying genotype-dependent differences in microspore embryogenic potential in winter wheat. Particular attention was given to the regulation of the *Ta*MET1 gene, including its promoter methylation status and expression level, both under control conditions and following 5-azacytidine (AZC) treatment.

Anther cultures were established for two winter wheat genotypes exhibiting contrasting embryogenic potential, under both control and AZC treatment. Global DNA methylation levels were quantified by high-performance liquid chromatography (HPLC). Locus-specific DNA methylation analysis involved Sanger sequencing of promoter regions of selected genes potentially involved in the regulation of embryogenesis. In addition, the expression level of the TaMET1 gene, encoding a DNA methyltransferase type 1, was evaluated using quantitative PCR (qPCR).

Treatment with AZC influenced the efficiency of microspore embryogenesis induction in anther cultures. Molecular changes were found to correlate with the divergent embryogenic outcomes. Comparative analysis revealed distinct epigenetic profiles between the responsive and recalcitrant genotypes, including differences in global DNA methylation, promoter methylation, and *Ta*MET1 expression.

The results indicate that the analyzed epigenetic parameters may play a key role in differentiating the embryogenic response of microspores in winter wheat genotypes. 5-azacytidine treatment altered the cellular epigenetic context, potentially affecting the microspores' ability to reprogram toward the embryogenic pathway. The distinct epigenetic profiles observed between the responsive and recalcitrant genotypes support the existence of genotype-specific mechanisms of epigenetic regulation of totipotency, which may serve as potential targets for future efforts to enhance *in vitro* microspore embryogenesis in cereals.

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T6: Bioinformatics and Genome Editing

optRF: Optimising random forests for reliable genomic selection in wheat breeding

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Keywords: parameter optimisation, random forest, machine learning, non-determinism, decision-making, genomic selection

Random forest is a widely used machine learning method for genomic selection in wheat breeding due to its non-parametric nature, exceptional predictive performance, minimal input preparation requirements, and versatility in handling binary, categorical, count, and continuous response variables. Despite its popularity, a critical yet often overlooked characteristic of random forest is its inherent non-determinism. Repeated runs of random forest on the same data set can yield different prediction models. This variability is particularly problematic when the predictions are used to select the top-performing wheat lines.

Random forest works by constructing an ensemble of decision or regression trees and generating predictions through the aggregation of individual tree outputs. Even though the number of trees grown in a random forest can have a severe effect on the quality and stability of random forest, widely used implementations such as those in R employ a fixed default value of 500 trees. It is generally recognised that increasing the number of trees has only benefits for the quality of the model such as more stable and accurate results without overfitting. However, it is also known that the number of trees increases the computation time linearly, raising the practical question of how to optimally balance quality and computational efficiency.

The study presented here investigates the relationship between the number of trees and the corresponding stability and computation time in random forest models with a special focus on genomic selection. Our findings reveal that the relationship between stability and the number of trees is non-linear: stability improves steeply as the number of trees initially increases but reaches a plateau beyond which further gains are marginal. In contrast, computation time increases linearly, suggesting diminishing returns from excessively large forests. To address this trade-off, we developed the R package optRF, which models the relationship between the stability and the number of trees to determine the optimal number of trees. This adaptive approach allows users to achieve high stability and reliability without unnecessarily increasing the computation time. Our package is particularly beneficial for the application of genomic selection in wheat breeding where results from applying random forests are often impossible due to the large wheat genome and the small number of lines used as training population.

This work highlights the critical need for tuning key parameters in machine learning pipelines to ensure robust, reproducible outcomes—especially in the context of genomic selection for wheat breeding. Our study not only adresses the often overlooked issue of non-determinism but also provides a practical, user-friendly solution in form of the R package optRF. This tool empowers researchers and breeders to optimise predictive performance while managing computational costs, making random forest a more reliable choice for genomic selection.

Assessing an *in vivo* validation method for CRISPR-Cas9 RNA guides in durum wheat

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Keywords: genome editing, RNA guides validation, CRISPR-Cas9, durum wheat, tomato hairy roots

CRISPR-Cas9 technology has become one of the most extensively used methods for the introduction of precise modifications into plant genomes. This technique induces insertions and deletions (indels) in target genomic sequences through double-strand breaks followed by repair via the non-homologous end joining (NHEJ) pathway. Although numerous *in silico* tools are available to assist in the design of effective guide RNAs, *in vitro* and *in vivo* validations remain the most reliable approaches to confirm guide efficiency (D'Orso et al., 2024). In particular, *in vivo* validation systems better account for the biological context in which the CRISPR-Cas9 system operates. Moreover, some *in vivo* systems enable accurate assessment of off-target effects, which is particularly important for long-lived plant species, where back-crossing to eliminate unintended mutations may take several years. Given the considerable time and resource investment required for plant genome editing, a robust strategy for the selection of effective guides is essential.

In this study, we are evaluating an *in vivo* approach for validating guide RNAs intended for genome editing in durum wheat using a heterologous hairy root system in tomato (*Solanum lycopersicum*). This system allows to test the guides in a real biological context, although it does not provide information on potential off-target effects. We selected seven guides from a previous study in durum wheat (Camerlengo et al., 2020), that led to multiple editing events targeting the *CM3* and *CM16* genes, encoding amylase-trypsin inhibitor (ATI) proteins.

This method involves the cloning of each guide along with its corresponding target gene sequence into a single plasmid construct using the hierarchical Golden Gate cloning strategy resulting in seven final level-2 constructs. Each construct is then used to transform tomato cv. 'Micro-Tom' explants via *Agrobacterium rhizogenes* to induce hairy root formation. Each hairy root is subsequently genotyped using Sanger sequencing, as each root is expected to represent an independent editing event.

By comparing the outcomes with the editing events previously observed in durum wheat plants, we aim to assess the performance of this validation method in providing insights into the occurrence of genome editing, the types of indel mutations that can be generated, and the frequency of editing events. This study contributes to enhance CRISPR-Cas9 applications in durum wheat by refining the guide RNA selection process.

References

1. Camerlengo, Francesco, et al. "CRISPR-Cas9 multiplex editing of the α-amylase/trypsin inhibitor genes to reduce allergen proteins in durum wheat." *Frontiers in Sustainable Food Systems* 4 (2020): 104.
2. D'Orso, Fabio, et al. "Methods and techniques to select efficient guides for CRISPR-mediated genome editing in plants." A Roadmap for Plant Genome Editing. Cham: Springer Nature Switzerland, 2024. 89-117.

How does wheat regulate iron uptake and homeostasis amid genomic complexity?

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Keywords: wheat, biofortification, iron transporter, iron regulation, genome editing

Iron (Fe) is an essential nutrient for humans and plants. In developing countries, enhancing iron content in grains remains a prime objective to usher in micronutrient biofortification. The molecular network involved in Fe uptake and mobilization is nicely characterized in model plants such as Arabidopsis, whereas the knowledge of the molecular players in crops is in its infancy. Therefore, our detailed understanding of how plants sense, mobilize, and re-distribute Fe in edible tissues is essential. Bread wheat is an allohexaploid (*Triticum aestivum* L., AABBDD $2n=6x=42$) and evolved from the interspecific hybridizations of three distinct diploid species. Wheat grains are an appreciable source of nutrients, yet they have low levels of important micronutrients like Fe and Zinc. The work from our lab has provided an inventory of the molecular responses and signalling events involved in Fe mobilization and imparting Fe-deficiency tolerance. At the genome level, we observed an asymmetric heterogeneous expression of sub-genome homoeologous genes in roots that show dynamic changes with the temporal response during Fe deficiency. Specifically, we noted a coordinated and collective spatial response from each distinct cell-type when the root-tip transcriptome was overlayed with the previously known wheat root single-cell dataset. Additionally, using wheat mutant lines (generated by CRISPR/Cas9 and TILLING), our results provide the first insight into molecular and biochemical responses of hexaploid wheat under Fe deficiency. These studies provided the framework to investigate new resources and genes that could be of immense value in addressing the uptake, remobilization, and bioavailability of micronutrients in wheat.

Cereal genetic manipulation using RUBY: from wheat to its wild relatives

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Keywords: wheat transformation, RUBY reporter, genome editing, GRF4-GIF1 chimera, barley regeneration, stress tolerance, crop wild relatives

Efficient genetic transformation and genome editing in wheat remain challenging due to its large, polyploid genome and poor regeneration capacity. In this study, we present an integrated approach leveraging the GRF4-GIF1 chimera and the betalain-based RUBY reporter system to enhance transformation efficiency and facilitate genome editing in both wheat and barley. While GRF4-GIF1 significantly improved regeneration in wheat, it impaired regeneration in barley, necessitating the use of RUBY constructs without the chimera for successful transformation in barley. The RUBY system, through visible red pigmentation, enabled non-destructive, real-time monitoring of transgene expression. It was further employed as a visual marker for CRISPR/Cas9-based genome editing by targeting *CYP76AD1*. Knockout of *CYP76AD1* resulted in a loss of pigmentation and betalain-associated traits, including reduced resistance to leaf rust (*Puccinia triticina*) and salt stress.

Building on this platform, we are now applying RUBY to standardize transformation protocols in crop wild relatives (CWRs). Preliminary data in wild wheat (*Triticum dicoccoides*) supported the usefulness of RUBY as a robust visual marker for tracking transformation events and recovering transgenic lines in recalcitrant genotypes. Overall, this work underscores the broader utility of RUBY as a cost-effective, visually trackable tool to streamline genetic transformation and genome engineering in both cultivated and wild cereal species.

CBB8 – Poster

T1: Genetic Resources for Crop Improvement

Towards durable resistance in wheat: molecular identification of slow rusting and major resistance genes

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Keywords: diseases, molecular markers, multiplex PCR, resistance breeding, wheat

Leaf rust and other rust diseases caused by *Puccinia* spp. remain among the most significant threats to wheat (Triticum aestivum L.) production worldwide. Breeding for durable resistance is a crucial strategy to mitigate yield losses. While major race-specific resistance (R) genes can provide strong protection, their effectiveness is often rapidly overcome by pathogen evolution. In contrast, race-nonspecific "slow rusting" genes, such as *Lr34*, *Lr46*, *Lr67*, and *Lr68*, confer partial yet durable resistance, making them key components in sustainable breeding programs.

The aim of our study was the molecular identification of both slow rusting genes and major resistance genes in winter and spring wheat breeding materials, with a special emphasis on their application in marker-assisted selection (MAS). We analyzed over 2000 genotypes derived from multiple crossing combinations between donor and elite Polish breeding lines. Genomic DNA was isolated from seedling tissue and subjected to PCR-based analyses using gene-specific and SSR markers. Additionally, multiplex PCR assays were developed and optimized to simultaneously detect combinations of slow rusting and major resistance genes (e.g., *Lr19, Lr24, Lr26, Lr38*). Expression analyses of selected genotypes were conducted under controlled growth conditions to confirm the activity of resistance loci.

Molecular screening enabled the efficient identification of genotypes pyramiding slow rusting genes with effective R genes. The multiplex PCR protocols increased throughput and reduced costs compared to single-marker assays, while ensuring reliable detection. Several advanced breeding lines carrying favorable gene combinations were selected for further field evaluation.

The integration of molecular tools with classical breeding accelerates the development of wheat cultivars with durable, broad-spectrum rust resistance. The combination of slow rusting and major resistance genes represents a promising strategy for enhancing yield stability under changing environmental conditions and evolving pathogen populations.

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The synthetic hexaploid wheat as genetic resources for wheat leaf rust resistance

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Keywords: synthetic wheat, leaf rust, *Puccinia triticina*, *Aegilops tauschii*, molecular markers

The yield and quality of wheat are affected by both biotic and abiotic stressors. Ensuring healthy, toxin-free crops is essential for human health while simultaneously safeguarding the environment. Securing access to high-quality raw materials is a strategic measure essential for achieving the objectives of the Green Deal pact. In Romania, wheat rust diseases (leaf or brown rust, stripe or yellow rust, and stem or black rust) significantly affect yield and quality. This study concentrated on the accumulation and pyramiding of resistance genes to leaf rust from the synthetic hexaploid wheat E1 (Pandur x *Aegilops tauschii* ssp. *tauschii* var. *typica*-2472), which are infrequent or absent in the Romanian wheat germplasm, specifically *Lr21*(1DS) and *Lr32* (3D), alongside the adult plant resistance gene *Lr34* and the 1RS:1AL, rye translocation. This was achieved by crossing SHW-E1 with the DH wheat line B2-98 and conducting molecular marker assays. The 53 F3 elite lines were evaluated for leaf rust resistance gene detection using molecular markers and subjected to artificial inoculation throughout the 2024-2025 season. The molecular assay showed presence of the following genes combinations: T1RS:1AL + *Lr21* + *Lr32*; T1RS:1AL + *Lr21*+ *Lr34*; T1RS:1AL + *Lr21*; *Lr21*; *Lr34* and no genes. There were also elites with four genes combination but one of them is in heterozygous status. Based on the phenotyping observations from 2025 the following two gene combinations have conferred a good response to *Puccinia triticina* infection: T1RS:1AL + *Lr21* + *Lr32* and T1RS:1AL + *Lr21*+ *Lr34*. The results showed that synthetic wheat SHW-E1 line brings genetic diversity in wheat Romanian germplasm and can contribute to improve the wheat resistance to leaf rust. The main challenges in this case, when SHW is used as a genetic resource, are the ears morphology and non-free threshing trait. Furthermore, this study proves the value of MAS breeding strategy, for the acceleration of new leaf rust resistant cultivars development. The ongoing research, based on molecular and phenotyping results, will validate the best gene combination for wheat rusts resistance.

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Genetic analysis of the main historic Italian rice varieties: findings from the RISOLO Project

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Keywords: historical Italian rice varieties, awn, rice domestication, GAD1 gene, genetic characterization

Cultivated rice (*Oryza sativa* L.) traces its origins to domestication from wild rice (*Oryza rufipogon* Griff.), which naturally produces fewer grains per panicle and has longer grains compared to its domesticated counterpart. Additionally, wild rice features long awns, whereas cultivated rice typically exhibits short awns or lacks them entirely. These transformations mark key events in the domestication of rice. A crucial gene, GRAIN NUMBER, GRAIN LENGTH AND AWN DEVELOPMENT 1 (GAD1), governs these significant traits (Wang et al., 2019). Located on chromosome 8, GAD1 is predicted to encode a small secretory signal peptide belonging to the EPIDERMAL PATTERNING FACTOR-LIKE family. A frameshift insertion within gad1 disrupts conserved cysteine residues in the peptide, leading to a loss of function. This mutation is responsible for the characteristic cultivated rice phenotype, including an increased number of grains per panicle, shorter grain length, and the absence of awns (Liu et al., 2017).

The early 20th century was a pivotal era for rice cultivation in Italy, during which several traditional varieties formed the basis for modern rice farming. Grown predominantly in the fertile northern regions, these varieties played an essential role in shaping Italian culinary traditions. Each variety contributed distinct qualities, enhancing the diversity of Italian cuisine. Even today, these historical rice varieties are valued for their significance in Italy's agricultural and gastronomic heritage (Mongiano et al., 2018).

The origins of these traditional varieties can be traced back to the 19th century when rice from different parts of the world was introduced to Italy. The goal was to identify those best suited to the local environment and resistant to rice blast disease, which had begun devastating Italian rice fields in the mid-19th century. At that time, rice production was primarily based on a population known as "Nostrale."

In this work, 18 ancient rice varieties from the late 19th and early 20th centuries were studied. These varieties, cultivated in open fields and preserved in the Pavia Germplasm Bank, underwent genetic characterization using SSR molecular markers to assess redundancy among the collected accessions. Among them, certain varieties like Chinese Ostiglia and Novara retained awns—an undesirable trait that breeders actively selected against in the 20th century. These accessions are currently under molecular investigation to analyze the GAD1 locus in greater detail.

References

1. Liu, L., Li, Y., Wei, M., Wu, Z., Liu, F., Qiu, Y., Luo, J., Li, R. B., & Qin, B. X. (2017). The causal deletions in the second exon of An-3 closely associated with awn development and rice yield. *Genes & Genomics*, 39(11), 1205–1213.
2. Mongiano, G., Titone, P., Tamborini, L., Pilu, R., & Bregaglio, S. (2018). Evolutionary trends and phylogenetic association of key morphological traits in the Italian rice varietal landscape. *Scientific Reports*, 8(1), 13612.
3. Wang, T., Zou, T., He, Z., Yuan, G., Luo, T., Zhu, J. Y., Liang, Q., Deng, S., Wang, A., Zheng, H., Liu, L., Wang, P., Li, S., & Li, J. (2019). GRAIN LENGTH AND AWN 1 negatively regulates grain size in rice. *Journal of Integrative Plant Biology*, 61(10), 1036–1042.

Implementation of a system for identifying the purity of rye inbred lines in the rye breeding program of Danko Plant Breeding

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The idea of breeding hybrid rye is to increase the fertility of the obtained varieties compared to traditional population varieties. For this purpose, the phenomenon of heterosis is used, which involves crossing homozygous forms at loci that are key to fertility. Hybrid varieties are three-component hybrids, created by crossing a male sterile line with a complementary one, and then the fertility of the resulting sterile hybrid (single) is restored with a restorer line. In the breeding program, as well as in the production of the hybrid itself, it is very important to maintain the purity of male sterile lines and sterile single hybrids, especially in terms of the presence of fertile plants. As part of the project entitled: “New plant variety breeding - modern varieties of selected species of cereals and pea, based on innovative biotechnological methods” implemented under the Intelligent Development Operational Program 2014-2020, Professor Stojałowski from ZUT in Szczecin was commissioned to develop a system for identifying the purity of inbred lines of hybrid ryeA, by detecting the presence of other genotypes in them. A methodology was obtained describing the calculated optimal batch size of the tested material, the recommended DNA isolation method, the method of creating a collective sample, a set of 10 SSR markers with given sequences, the optimized composition of the reaction mixture and the PCR reaction conditions. The system was tested on a batch of plants of three male sterile inbred rye lines and three male sterile singles. Analog lines with the N cytoplasm variant and genotypes tested in parallel were used as the source of contamination. It was confirmed that the system could be useful for detecting foreign genotypes in hybrid components.

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Molecular and phenotypic characterization of wheat × *Dasypyrum villosum* hybrids

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Keywords: *Dasypyrum villosum*, introgression lines, SNP markers, disease resistance, stem rust, stripe rust

Wild *Triticeae* species related to wheat offer an excellent opportunity to enhance the genetic potential of cultivated wheat and to mitigate its genetic narrowing. *Dasypyrum villosum* (L.) (2n=14, VV), which belongs to wheat's tertiary gene pool, has adapted to diverse ecological conditions during a long evolutionary process. It is widely used to increase resistance to abiotic and biotic stresses, to improve yield and quality traits, and is also a subject in basic research aimed at deepening evolutionary and genomic studies.

Breeding lines developed at DAFNE (Department of Agriculture and Forest Sciences, University of Tuscia, Italy) from *T. aestivum* 'Chinese Spring' × *Dasypyrum villosum* hybrids (CSxV32, CSxV63, CSxV60, and CSxV58), and their progenies from crosses with the winter wheat lines 'Mv Emese' and 'AMP12', were studied under field conditions in 2023 and 2024, focusing especially on their disease resistance.

Some lines were assessed using SNP markers; the 25K Infinium analysis was carried out by TraitGenetics (SGS Institut Fresenius GmbH, Gatersleben) on 65 genotypes (6 controls and 59 samples). The plant material originated from small-plot field trials sown in 2023. Based on preliminary results – consistent with earlier studies – the highest number of wheat/*D. villosum* rearrangements involved wheat chromosome 6B and the V-genome chromosomes of *Dasypyrum villosum*, while potential introgressions were also identified on wheat chromosome 7A. The genotypes could be clustered according to SNP polymorphisms appearing on the chromosomes.

In recent years, the plants have been mainly evaluated for disease resistance [powdery mildew (*Erysiphe graminis*), leaf rust (*Puccinia triticina*), stripe rust (*Puccinia striiformis*)] under field conditions. Primary assessments revealed significant variability among the lines, with some exhibiting outstanding resistance to stem rust and stripe rust.

To identify individual chromosome introgressions, we are optimizing GBS (Genotyping-by-Sequencing) genotyping (Illumina NextSeq2000), which is now feasible due to the availability of the *D. villosum* reference genome. Using SNP (and shortly, GBS) data, we plan to analyze genotype–disease resistance associations with statistical and bioinformatic methods. This may enable the identification of resistance genes and the development of their markers, thereby contributing to the marker-assisted selection programmes.

In addition, we are investigating the compositional traits of the lines (protein, moisture, starch content) using FOSS (NIR) measurements, and determining seed physical properties (size, shape, color, thousand-kernel weight) with Marvin image analysis.

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Classification of tetraploid wheat genebank accessions with SSR molecular marker method

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Keywords: tetraploid wheat, genetic diversity, genebank collection, SSR marker, dendrogram

The National Centre for Biodiversity and Gene Conservation has a tetraploid wheat collection of hundreds of accessions. The aim of our experiment was to characterize these tetraploid genotypes by molecular biological methods and to determine their relative genetic distance. For our work, we used 164 *Triticum* accessions (AABB genome, n = 14) from the gene bank, which, based on our classical taxonomic observations, belonged to a total of eight tetraploid species as follows: *T. carthlicum* Nevski., *T. dicoccoides* (Körn. ex Asch. & Graebn.) Schweinf., *T. dicoccon* (Schrank) Schübl., *T. durum* Desf., *T. ispahanicum* Heslot, *T. militinae* Zhuk. et Migush., *T. timopheevii* (Zhuk.) Zhuk. and *T. turgidum* L. Furthermore, as a diploid control, a *T. urartu* Thum. ex Gandil (AA genome, n = 7) was also involved in the experiment. Polymerase chain reactions were performed with 15 SSR primer pairs. A total of 187 alleles were obtained, with an average of twelve per primer. The size of the multiplicated fragments was found between 51-473 bp. The fewest alleles were obtained by Xgwm4_4A, while the most by Xgwm302_7B (3 and 25, respectively). 80 percent of the primers had a PIC value higher than 0.7, the highest was 0.97 in case of Xgwm302_7B. Calculated Jaccard index revealed that several pairs of accessions belonging to the species *T. turgidum*, *T. timopheevii* and *T. durum* reached or exceeded the value of 0.9. Based on binary matrix, a dendrogram was created which separated the 165 wheat genotypes into 3 main groups. *T. carthlicum* were located on the first branch and *T. timopheevii* on the second one, while the third branch was further divided into 4 smaller sub-branches. Two of them were composed by only *T. dicoccon*, while the third and fourth sub-branches contained *T. durum* and *T. turgidum* samples, respectively. *T. dicoccoides*, *T. ispahanicum*, *T. militinae* and the diploid control *T. urartu* were scattered among the above mentioned groups. The Jaccard index based dendrogram and the morphological taxonomic results showed good agreement with each other, since accessions classified as same taxonomic category were located very close to each other on the branch diagram, for example *T. turgidum* L. conv. *turgidum* var. *falsejodorum* Flaksb.; *T. timopheevii* (Zhuk.) Zhuk. var. *timopheevii*; *T. dicoccon* (Schrank) Schübl. subsp. *dicoccon* conv. *dicoccon* var. *rufum*; and *T. dicoccon* (Schrank) Schübl. subsp. *dicoccon* conv. *dicoccon* var. *dicoccon*. The experimental plant material was composed by both spring-type and winter-type accessions – ears completely emerged in 56-73 / 170-221 days, respectively. The 15 SSR primer pairs were not efficient for separation along sowing time because both groups were scattered on the dendrogram.

However, we achieved our goal and proved that the tools of morphological taxonomy and the SSR-based PCR method complement each other well, and molecular biological studies are suitable for separation of a large number of gene bank samples. If we want to classify tetraploid wheats according to sub-species categories, or if we want to work with several hundred samples, it is worthwhile and necessary to include additional primer pairs.

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A genetransfer strategy based on durum wheat – *Aegilops comosa* amphiploid topcrossed with hexaploid wheat results in elimination of D chromosomes led to the production of homoeologous M(D) substitutions and translocations

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Keywords: interspecific hybridisation; *Aegilops comosa*; wheat-*Aegilops* introgression

Due to domestication and nearly eight thousand years of cultivation, wheat has developed a narrow genetic base, which hinders the identification of effective allele combinations and hampers breeding progress under changing environmental conditions. The gene portfolio can be extended using a crossing strategy providing favourable conditions to form wheat × alien chromosome addition, substitution and translocation lines. To utilize the gene pool of *Aegilops comosa*, the present study applied durum wheat-*Ae. comosa* amphiploids top-crossed (TC) by hexaploid wheat (Mv9kr1*ph1b*), a strategy exploiting monosomic conditions of D and M genomes in TC_1 to form cytogenetic stocks.

With the aim to test this hypothesis, amfiploids of durum wheat x *Ae. comosa* were top-crossed with *T. aestivum* (Mv9*kr1*). Sequential GISH (with M and D genomic probes) and FISH with probes pSc119.2, pTa71 and Afa family confirmed the theoretical chromosome composition of the amfiploids, TC_1 and TC_2 progenies.

While the expected genome structure of amphiploid and TC_1 generations was confirmed, consecutive *in situ* hybridization using D- and M-genomic, as well as DNA repeat probes on 52 TC_2F_1 lines, showed significant elimination of not only M- but also D-genome chromosomes. Differences in the elimination frequency seemed to be related to chromosome size, with an opposite tendency for the two genomes, as shorter M chromosomes (1M, 4M, 6M) were retained more frequently, while the shorter D chromosomes (1D, 4D) were predominantly eliminated. D-M rearrangements within group 1, 3, 4, and 6 chromosomes were dominantly homoeologous, with 5D/5M recombinations observed at the highest frequency. Besides monosomic introgressions, disomic substitutions 2M(2D) and 7M(7D), addition 6M, and translocation T6MS·6ML-6D were selected. Morphological characterization and yield components indicated the good compensation ability of these *Ae. comosa* chromosomes for the loss of those of wheat.

Relationships between the chromosomes size and their elimination from wheat × alien hybrid progenies were discussed. Based on the high level of homoeologous recombinations and substitutions, Topcrossing has proven to be an effective strategy for transferring alien chromosome segments from closely related diploid species into wheat. The newly developed wheat-*Ae. comosa* prebreeding materials represent potentially valuable genetic resources for wheat improvement.

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MicroRNAs as coordinators of hormonal networks in barley genotypes with impaired brassinosteroid and G-protein signaling

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Keywords: *Hordeum vulgare* L., hormonal crosstalk, miRNA-mediated regulation, phytohormones, signalosome, small RNA sequencing

Research on plant signalosome interactions, including the phytohormonal network, is an important area of systems biology, revealing molecular mechanisms of physiological processes leading to improved crops. Brassinosteroid (BR) signaling, with its multifaceted roles, is a major hormonal pathway whose influence on other phytohormones, including abscisic acid (ABA), is increasingly recognized. Another essential component of the plant signaling network is the heterotrimeric G-protein complex, with the Gα-subunit playing a pivotal role in transmitting signals, including those related to phytohormone perception. Despite growing interest, the mechanisms coordinating BR and G-protein signaling, as well as their crosstalk with phytohormones, remain poorly understood, especially in complex-genome monocots such as barley.

Recent research has emphasized the broad regulatory role of microRNA (miRNA) in plant functioning. Thus, in this study, we assumed that miRNAs participate in the coordination of hormonal signals associated with BR and G-protein pathways. Our research aimed to identify miRNAs affecting phytohormone action and interaction, and to assess the impact of disrupted BR signaling and Gα-subunit dysfunction on the miRNA profiles targeting various hormone-related genes in barley. We further examined whether ABA/BR application affects the behavior of specific miRNAs depending on BR and G-protein signaling.

To address this, we performed global small RNA sequencing on leaf samples from the reference barley cv. Bowman and its two near-isogenic lines (NILs), representing mutants deficient in BR and G-protein signaling due to the impairment of the BRI1 receptor (*uzu1.a*) and Gα-subunit (*brh1.a*), respectively, treated and untreated with exogenous abscisic acid and epi-brassinolide (epiBR). We identified a subset of miRNAs whose predicted targets were associated with metabolism and signaling of nine phytohormone classes and investigated their regulation status in our experimental system. Comparative analysis of the NILs versus the reference genotype revealed that, under control conditions, disruption of BRI1 had a stronger impact on hormone-related miRNA profiles than dysfunction of the Gα-subunit. However, epiBR application reversed this trend, inducing greater changes in the *brh1.a* mutant, primarily in auxin-related miRNAs. In turn, ABA treatment altered miRNA profiles only in the *uzu1.a* mutant, targeting genes associated with ethylene and strigolactone action. Overall, miRNAs showed limited responsiveness to ABA, but greater sensitivity to epiBR relative to control, with variation across genotypes. Notably, comparison of both hormonal treatments uncovered miRNAs with differential responses to ABA and epiBR in both *uzu1.a* and *brh1.a* mutants, although neither treatment alone significantly affected the abundance of these miRNAs relative to untreated plants. We identified a few miRNAs predicted to target genes involved in multiple hormone pathways, suggesting their potential role as coordinators of hormonal crosstalk.

Our findings provide new insights into the molecular architecture of the cereal signalosome by leveraging specific barley genetic resources, underscoring microRNAs as integral components of the hormonal regulome. These microRNAs represent potential targets for manipulating signal transduction pathways to improve crop performance amid global food security challenges.

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Exploring the useful properties of wheat (*Triticum aestivum* L.) crop wild relatives (CWRs) in breeding programmes

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Keywords: genetic diversity, crop wild relative (CWR), prebreeding, field tests, phenotyping, genotyping, GBS, FISH

The increasing negative consequences of climate change (e.g., drought, heat stress, emergence of new pests), and the shifting European political environment (EU Green Deal strategies promoting sustainable use of resources) necessitate further technical and methodological development of cereal breeding programs. Successful wheat breeding based on domestic genetic resources is also of considerable importance for the national economy and national security (food security). Crop wild relatives (CWRs) are the most important sources of genetic variance for cultivated species. Breeding and diversification are vital tools for food chains, and in this aspect, CWRs can play a key role in preserving diversity. Wild species related to wheat represent an almost inexhaustible reservoir of useful traits (and the genes behind them) that can increase the efficiency of wheat production, many of which have already been incorporated into modern wheat varieties in recent decades (e.g., Sr36, Lr19, Pm21). With the occurrence of extreme droughts in recent years, we will have to face them more and more frequently in the future, so the use of wild species may become more valuable in this area as well, since most of them have already adapted to these conditions in their original habitat (the Middle East). This research aims to introduce new selection procedures that can increase the efficiency of using prebreed material in breeding programmes.

For the tests, we use (primarily more developed) winter wheat accession lines carrying DNA fragments from wild relatives (*Triticum timopheevii, Triticum monococcum, Dasypyrum villosum, Agropyron glael, Aegilops biuncialis*) that were obtained from pre-breeding work. We test the plant material in small-plot field trials with three replicates and have also set up a greenhouse trial to test drought tolerance. We examine the most important morphological and agronomic traits, as well as the content and technological quality parameters of the harvested grain. In parallel with phenotyping, we perform FISH (fluorescence in situ hybridization) testing and optimize GBS (genotyping-by-sequencing) genotyping (Illumina NextSeq 2000), which allows us to identify foreign DNA fragments in the genetic background of wheat using the reference genome of wild relatives. We plan to introduce the GBS method into the breeding system in order to effectively track foreign DNA fragments carrying useful traits in subsequent generations of the wheat breeding program using targeted molecular markers.

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The effect of genotype and Zn and Se biofortification on the nutritional profile of wheat grain

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Keywords: wheat variability, nutritional quality, biofortification

The most important breeding objectives in cereal breeding are changing over time. From high yield as the most important trait, resistance to various biotic and abiotic stresses, in recent years, more attention has been given to quality traits such as grain protein content (GPC) and macro- and micronutrient content. Regarding these goals, it is essential to identify and explore the genetic variability and potential of old genotypes to increase macro- and micronutrient content and protein in grain. It is well-documented that wheat genotypes differ in their ability to adopt, translocate, and accumulate micro- and macronutrients in grain. Besides genotypic specificity for those traits, it is well known that successful agrotechnical measures for increasing essential micronutrients in edible plant parts include agronomic biofortification, which is often recommended as a cost-effective and rapid method for improving the concentration of desirable elements. This research aimed to examine the effect of genotype and Zn and Se biofortification on the macro- and micronutrient content. A total of nine wheat genotypes were selected and sown in a field experiment at the experimental station of the Faculty of Agrobiotechnical Sciences Osijek (FAZOS). The genotypes were selected based on the results of previous research carried out within the project WHEAT4GRASS, which includes genotypes that are part of the FAZOS cereal core collection. The experiment was designed as a completely randomized block design with three replicates and three treatments (T0 - control, T1 – 1.5 kg Zn ha^{-1} and 10 g Se ha^{-1} during the flowering stage and T2 - 1.5 kg Zn ha^{-1} and 10 g Se ha^{-1} applied 50% during the flowering, and 50% in the milky stage. The total concentrations of K, Ca, Mg, Mn, Fe, Zn, and Se in the grain were determined in samples after wet digestion, which was performed according to a standardized method [1]. The total concentrations of K, Ca, Mg, Mn, Fe, Zn, and Se were measured by the ICP-OES technique. Based on the results of the factorial ANOVA, we can conclude that genotype had a significant effect on the concentration of all examined elements. On the other hand, Zn and Se biofortification had a significant effect only on Zn and Se concentrations in grain, and in addition, a significant interaction of genotype and biofortification was found only for Zn and Se concentrations in grain. On average, as a result of biofortification, Zn concentration increased by 44% compared to the control treatment, while Se concentration in grain increased 7-fold in T1 and 5-fold in T2 compared to the control. Considering this, combining genotypes with a genetic predisposition for higher accumulation of essential elements with agronomic biofortification could be a promising approach in producing nutritionally dense grains.

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References
1. Kingston, H.M.; Jassie, L.B. Microwave energy for acid decomposition at elevated temperatures and pressures using biological and botanical samples. Anal. Chem.; 1986; 58, pp. 2534-2541. DOI: https://dx.doi.org/10.1021/ac00125a038

Identification of novel resistance loci to Septoria tritici blotch (STB) in winter wheat using Genome-Wide Association Mapping

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Keywords: genome-wide association study, haploblocks, Septoria tritici blotch, resistance, wheat

Septoria tritici blotch (STB) caused by the necrotrophic fungus *Zymoseptoria tritici* is one of the most important foliar diseases of wheat. To date, 23 major STB resistance genes have been identified. This number remains rather modest, which limits the possibilities of introducing effective resistance genes into breeding materials. Therefore, there is a need to search for new sources of resistance to the disease, especially among local or historical varieties that represent a much broader genetic variability than modern cultivars, and often originate from regions of common host-pathogen co-evolution, which increases the chances of identifying new resistance genes.

A genome-wide association study (GWAS) was conducted on a panel of 323 winter wheat cultivars, landraces and breeding lines from 43 countries. Genotyping was performed using the DArTseq platform. The phenotypic data were obtained by measuring the plant response to *Z. tritici* inoculation. The wheat panel was tested at the adult plant stage under polytunnel conditions in the years 2022-2024. Each year, plants with fully emerged flag leaves were inoculated with a mixture of four *Z. tritici* isolates, together covering a broad spectrum of virulence. The tests were evaluated 21 days after inoculation. Plants were assessed in terms of the percentage of flag leaf area covered with necrosis (%NEC) and pycnidia (%PYC). Precise determination of disease parameters was made using computer image analysis of infected leaves.

Missing genotypic data were imputed in R using the A.mat function, which applies an expectation maximization algorithm based on the multivariate normal distribution. A total of 3,746 SNP markers were used to define haploblocks, which were generated using Haploview 4.2 software. GWAS analysis was performed separately for each phenotypic dataset using the GWAS function of the rrBLUP package in the R environment.

In total, 2,479 haploblocks were created for the studied wheat collection. GWAS identified 17 haploblocks that were significantly associated with the analyzed disease parameters (%NEC and %PYC), distributed on 11 of the 21 wheat chromosomes, with some co-localizing with known major resistance loci, including *Stb6/StbSm3*, *Stb8/Stb13*, *Stb18* and *Stb19*.

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Genetic and morphological analysis of traditional maize varieties in Lombardy

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Keywords: *Zea mays* L., landraces, carotenoid content, genetic analysis, morphological characterization

The earliest recorded presence of maize (*Zea mays* L. subsp. mays) in Lombardy dates back to 1556. Over the centuries, maize adapted to local agroclimatic conditions and the needs of farmers, leading to the development of hundreds of traditional varieties across Italy. However, since the 1950s, these landraces have been progressively replaced by high-yielding hybrids. Research on biodiversity conservation indicates that approximately 90% of agricultural biodiversity has been lost in Northern Italy, while Southern Italy has experienced a decline of around 70%. Despite this, several traditional maize varieties continue to be cultivated in Lombardy, thanks to custodian farmers who maintain them on small farms under low-input agricultural systems. These landraces are primarily grown for family consumption or sold as specialty products linked to regional heritage (Landoni et al., 2024).

The project aims to recover maize varieties historically cultivated in Lombardy for human consumption, particularly for polenta, and to characterize them to preserve their varietal identity and encourage their continued use (Giupponi et al., 2020). Morphological and genetic characterization plays a crucial role in distinguishing different accessions, helping to prevent hybridization and extinction while enhancing their value. Additionally, the project will assess the carotenoid content in maize seeds, as these pigments possess strong antioxidant properties known to benefit human health. This nutritional aspect could serve as an incentive to revitalize the cultivation of these traditional varieties (Giupponi et al., 2021).

The project will present the genetic and morphological characterization of these maize accessions using SSR markers, with the objective of establishing their unique identities and investigating their phylogenetic relationships. These findings may contribute to develop maize populations with enhanced nutritional value and could provide insights into crop adaptation in response to ongoing climate change.

References

1. Giupponi, L., Pilu, R., Scarafoni, A., & Giorgi, A. (2020). Plant agro biodiversity needs protection, study and promotion: Results of research conducted in Lombardy region (Northern Italy). *Biodiversity and Conservation*, 29(2), 409–430.
2. Giupponi, L., Leoni, V., Colombo, F., Cassani, E., Hejna, M., Rossi, L., & Pilu, R. (2021). Characterization of “Mais delle Fiorine” (*Zea mays* L.) and nutritional, morphometric and genetic comparison with other maize landraces of Lombardy region (Northern Italy). *Genetic Resources and Crop Evolution*, 68(5), 2075–2091.
3. Landoni, M., Bertoncini, A., Ghidoli, M., Rossi, G., Cassani, E., Locatelli, S., Balconi, C., & Pilu, R. (2024). PGRFA management of outcrossing plants propagated by seed: From on-farm to ex situ conservation and some Italian maize case studies. *Agronomy*, 14, 1030.

Diversity and structure in Avena strigosa: a national collection perspective

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Exacerbating climate change poses a threat to global food security. Utilising plant genetic resources is one of the solutions implemented in order to resolve that issue. Main crops were developed through years of selective breeding and do not possess traits needed to adapt to the changing environment. Orphan crops however, have untapped genetic potential, which can be used for development of new, resilient varieties.

Avena strigosa i.e. bristle oat is an orphan crop which recently gained popularity due to its drought-resistant properties. This study aims to assess and characterise the genetic diversity of the 85 accessions of A. strigosa stored in the NCPGR i.e., the Polish Gene Bank, with particular consideration towards accessions with potential drought-resistant properties for further studies.

Single nucleotide polymorphisms (SNPs) were used in Principal Coordinate Analysis as a means to assess genetic diversity. The morphological diversity of the grains was assessed based on the Principal Component Analysis. Both analyses have shown significant genetic and morphological differences among the 85 studied accessions. The study revealed the relationship between the level of diversity of the studied populations and their geographical origin. Furthermore, accessions Sacavem (24) and Madrid (75) were chosen for future research and breeding. Further studies are needed in order to present a broader picture of the diversity among varieties of A. strigosa and to evaluate their full potential.

Water soluble fibers of different cereal species

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Keywords: breeding, cereals, dietary fibers

The fiber content of cereals can be divided into two main types: soluble (e.g. beta-glucan or water-soluble arabinoxylan (WE-AX)) and insoluble fiber, which affect the intestinal flora and digestion in different ways. In addition, soluble fiber helps to reduce cholesterol levels and slows down the absorption of carbohydrates. A special type of fiber with soluble and prebiotic effects is represented by fructans, which are oligosaccharides that are fermented in the intestine and can therefore cause complaints in those sensitive to them. Soluble fiber is also fermented in the intestinal flora and affects digestion, but its mechanism is not the same as fructans.

We examined the variability of water-soluble fiber (b-glucan/WE-AX) and fructan and protein content of forty winter wheat, barley and oat genotypes and their correlations.

The average thousand-grain weight (32.23, 42.96, 43.97%) and protein (12.45, 14.04, 15.8%) content, as well as fructan content (0.26, 0.94, 1.63%) in different cereals increased in the order of oats-wheat-barley. The average fructan content of oats was a quarter of that of wheat and a sixth of that of barley.

The average water-soluble fiber content increased in the order of wheat-barley-oats (0.76, 2.08, 3.11%), with a high degree of variability in the fiber content of barley. The thousand-grain weight had a negative effect on the protein content of oats and barley, while it had a positive effect on the WE-AX and fructan content of wheat. No significant correlation was found between water-soluble fiber (b-glucan, WE-AX) and fructan content in any of the species. However, we identified oat and barley varieties that, in addition to high water-soluble fiber content, had low fructan content. Thus, the OZFB24-24 and Mv-Imperial oat, and the MVHVNU25-22 and Mv-Fata barley genotypes.

Barley is not only a good source of fiber, but also a grain rich in fructans, so it is important to consider individual sensitivity to fructans, the amount of which varied between 1.1 and 2.5% in the total dry matter of barley, which is 20-25% of the total fiber content.

The fructan concentration of oats ranged between 0.19–0.38% in the dry matter, which means that it does not contain significant amounts of it. Therefore, oats are well tolerated by many people who are sensitive to it, such as those following the FODMAP diet. In addition, due to its protein composition, it can also be used in a gluten-free diet.

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Allelic variation in flowering genes linked to adaptative traits in Spanish bread wheat landraces

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Keywords: wheat adaptation, vernalization, climate change, photoperiod

Flowering time in wheat is largely determined by allelic variation in vernalization and photoperiod sensitivity genes, which regulate responses to temperature and day length. These genetic pathways have enabled wheat to adapt to diverse environments worldwide. However, rising global temperatures and reduced cold periods threaten the stability of winter growth types, making it essential to explore genetic diversity to secure adaptation under climate change.

The objective of this study was to better understand the genetic basis determining the adaptation of Spanish wheat landraces. A panel of 188 bread wheat landraces from Spain and 28 commercial varieties was evaluated. Phenotyping, focused on growth habit (spring vs. winter), days to heading (DH), and days to maturity (DM), was performed across three seasons. Genotyping was achieved with PCR and KASP markers targeting major vernalization and photoperiod genes (*VRN-A1, VRN-B1, VRN-D1, PPD-A1, PPD-B1*, and *PPD-D1*). Results showed that the absence of the *VRN-A1, VRN-B1,* and *VRN-D1* spring alleles is required to determine a winter growth habit. Winter genotypes reached heading (DH) in an average of 173 days compared with 148 days for spring genotypes. For maturity (DM), winter types averaged 196 days, whereas spring types matured in 176 days. Significant differences in DH and DM were also associated with allelic variation in *PPD-B1* and *PPD-D1*.

These findings provide new insights into the genetic basis of flowering time and growth habit in Spanish wheat landraces. The results highlight the value of this germplasm as a reservoir of adaptive diversity. Such knowledge supports the development of new wheat varieties better suited to current and future agro-environmental conditions, thereby contributing to food security in the face of climate change.

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T2: Environmental Adaptation

Response of winter wheat (*Triticum aestivum* L.) genotypes to heat and drought stress treatments under controlled conditions

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Keywords: abiotic stresses, stress priming, phenotypic variability, developmental phase specificity, association between drought and heat stress responses

Wheat crop growth and productivity are prominently affected by heat and drought, the severity of which depend on the occurrence, timing, intensity and on the combination of various stresses, but also on the genotype. Plant responses to each specific abiotic stress are complex traits and there is less information on how the genetic regulations of heat or drought stress responses are related to each other. Therefore, experiments were conducted under controlled environment to evaluate the performance of 27 winter wheat genotypes under both heat and drought stress conditions, separately; each stress consisted of three treatments namely, control, single stress applied at booting stage and double stress applied at first node appearance and then again at booting stage. Data were collected on various morphological and grain yield related traits were subjected to different statistical analyses including higher order analyses such as principal component analysis, and stress tolerance indices. Averaged over the 27 wheat genotypes, both heat and drought stresses significantly decreased reproductive tiller number, and grain number in the main and in the side ears, that led to significant grain yield loss, in spite of a small increase in the thousand kernel weight. The negative effects of the two stresses were similar in magnitude; in single stress treatment grain yield was reduced to 57.2% of the control due to drought, while to 61.1% due to heat. On average, the double stress led to similar (in the case of drought: 57.2% vs 57.3%) or stronger yield reduction (in the case of heat: 61.1% vs 50,8%), indicating that the priming effect was not detectable for either stress. There was however large diversity in the stress responses of wheat cultivars, and genotypes with better stress tolerances were identified via the various stress tolerance indices. Under heat stress, cultivars 'KWS-Scirocco' (D), 'Nuo-maizi' (CN), 'Cutter' (USA) and 'Ellvis' (D) proved to be the best, while under drought conditions, these were 'Valoris' (F), 'KWS-Scirocco' (D), 'Balada' (CZ) and 'Roane' (USA). The correlation between the drought and heat stress induced grain yield reductions of the individual wheat cultivars were not significant ($r = -0.23^{ns}$), underlining the basic differences in the genetic and physiological processes induced by the two types of stresses. The overlapping occurrence of some specific genotypes ('KWS-Scirocco') between the more tolerant groups of both stresses however may indicate the possibility of combining the genetic components of better drought and heat stress tolerance in one genotype. The current study proved that the identified genotypes are

potential sources to be used in various crop improvement programs. Further studies are required to depict the bases of the genetic determination of drought and heat stress tolerance in wheat.

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Genome-wide association mapping for yield traits under heat stress in a highly diverse spring wheat population

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Keywords: Genome –wide association study (GWAS), heat stress, Single nucleotide polymorphism (SNP), Minor allele frequency (MAF)

Heat stress is one of the major issues that limits wheat crop production across its different growth stages, and it drastically impairs growth and lowers grain yield. A set of 193 highly diverse wheat genotypes was examined under normal (N) and heat stress (HS) conditions. Yield traits: plant height (PH), spike length (SL), grain number/spike (GN), number of spikelets /spike (NS), grain yield/spike (GY), thousand kernel weight (TKW), peduncle Length (PL), peduncle diameter (PD) and peduncle weight (PW) were measured. The analysis of variance revealed high genetic variation among the genotypes for each trait under each treatment. High heritability estimates were observed for each trait under each condition. High positive correlation between spike and peduncle traits indicates the important role of peduncle characteristics in enhancing spike traits under heat stress. Genome–wide association study (GWAS) was carried out for all traits using SNP markers produced by the 25k SNP Illumina Infinium Array. This study provided very important novel genes associated with heat tolerance in wheat for all traits.

Dissecting peduncle contributions to spike productivity in wheat under contrasting water regimes

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Keywords: *Triticum aestivum* L., peduncle weight, peduncle diameter, distinct SNPs, genetic association

The peduncle stem plays a critical role in resource allocation and water transport to developing grains under normal conditions. Exploring peduncle traits and their relationships with spike productivity under drought stress may provide valuable insights into the mechanisms that mitigate yield loss in wheat. To this end, a panel of 198 highly homozygous and diverse spring wheat varieties was evaluated under normal and drought conditions across two growing seasons. Peduncle traits length (PUL), diameter (PUD), and weight (PUW) along with spike traits (spike length, number of spikelets per spike, grain number per spike, grain yield per spike, and thousand kernel weight) were assessed. PUW and PUD, but not PUL, were consistently and significantly associated with spike traits and grain yield under both conditions. In addition, total soluble carbohydrates (TSC) were analyzed in contrasting genotypes for peduncle traits, and correlation analysis revealed a highly significant association between TSC and spike traits across both environments and years. Genome-wide association study (GWAS) results indicated that peduncle and spike traits are governed by distinct genetic mechanisms, as no stable SNP markers were shared between the two groups. However, the identification of contrasting SNPs between genotypes with extreme peduncle traits revealed a key marker located within a gene model encoding a protein highly expressed in both peduncle and spike tissues. Comparisons between cultivars with low versus high peduncle trait values, particularly PUW and PUD, demonstrated that high-value cultivars exhibited superior yield-related traits under both normal and drought conditions.

Impact of drought stress on seed storage protein composition in einkorn (*Triticum monococcum*) genotypes

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Keywords: einkorn, abiotic stress, storage proteins

In recent years, rapidly changing climate conditions -rising temperatures, drought, and increased CO_2 - have significantly affected grain yield, storage protein content and composition, and food safety, therefore substantially intervening in the final quality of the product as well. In this study, four einkorn genotypes (*Triticum monococcum* ssp. *monococcum*), MVGB770, MVGB1177, MVGB748, and MVGB787, were examined regarding the effects of individual elevated atmospheric CO_2 levels (650 ppm), drought stress during anthesis, grain-filling stages, and their combination on mature grain storage proteins. Under controlled climate conditions, crop safety, climate adaptability, and bread-quality-related protein fractions were assessed.

Changes in the size- and hydrophobicity-based distribution of the total proteins of the wholemeal samples were monitored using SE- and RP-HPLC, respectively. The impact of water scarcity on seed storage protein subfractions and relative protein content differed in severity depending on the phenophases.

Yield, assessed by thousand kernel weight (TKW), varied by genotype and drought timing. Under control conditions, TKW ranged from 18.7 to 28.2 gg, depending on the genotype. Elevated CO_2 increased TKW in MVGB 748, MVGB770, and MVGB787 (by 13.5%, 10.1% and 2.5%), but reduced it in MVGB1177 (by 9.7%). Drought at anthesis resulted in a yield loss of 9–36.1% in three genotypes, whereas MVGB770 showed a 6.4% yield improvement under the same conditions. Drought during grain filling caused a reduction of 14.9–80.6% depending on the genotype.

Elevated CO_2 levels led to a 20.6–41.4% increase, while drought stress during anthesis resulted in a 16.8–67.4% rise in protein content. The combination of elevated CO_2 and drought during grain filling caused the most significant increase—between 34.6% and 71.5%—depending on genotype. The most prominent and consistent increases were detected in ω-gliadins, with concentrations rising between 26.2% and 161.2%, depending on genotype and treatment conditions. In three of the four genotypes, the gliadin content of the wholemeal flour increased at a higher rate (20 – 80 %) than that of glutenins (14.6 – 100.2 %), resulting in a deterioration in the glutenin-to-gliadin ratio. Considerable variability was observed in the alterations of gliadin subfraction levels across einkorn genotypes under drought stress. The data indicate that drought resilience among einkorn genotypes is highly genotype-dependent and influenced by the timing and developmental stage at which stress exposure occurs. These findings contribute to a deeper understanding of einkorn's stress tolerance mechanisms and its potential adaptability to changing climatic conditions.

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Characterisation of auxin transport-related *HvACT1* and *HvGNOM* in barley

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Keywords: barley, auxin, miRNA, heat, cold

Barley (*Hordeum vulgare* L.) is an essential cereal crop for food, feed, and brewing, yet its productivity is increasingly challenged by climate change. To understand the molecular basis of stress adaptation, this study investigates two barley genes, *HvGNOM* and *HvACT1*, with putative roles in temperature stress responses. *HvGNOM* encodes a guanine nucleotide-exchange factor involved in vesicle trafficking and auxin transport, while *HvACT1* encodes an actin protein critical for cell growth and intracellular trafficking. Preliminary results showed that *HvACT1* transcript levels increased under cold conditions, whereas *HvGNOM* expression remained unchanged, consistent with observations in Arabidopsis thaliana. However, the expression of our target genes was upregulated after 6 h of heat stress (30°C). Root elongation assays in Arabidopsis *act8-2* mutants (*ACT8* is a homologue of *HvACT1*) further supported *HvACT1*'s involvement in cold stress responses. Ongoing work includes generating transgenic Arabidopsis and barley lines overexpressing these genes, assessing their effects on auxin homeostasis, and evaluating tolerance to cold and heat stress. Additional experiments will explore miRNA-mediated regulation of these two genes and auxin transport. MiRNA sequencing was achieved after 6 hours of cold and heat treatment in the 10-day-old shoots. The expression of 44 miRNAs was altered following combined heat and cold treatments: 37 miRNAs responded to heat stress, 7 to cold stress, and 9 were influenced by both stressors. Target prediction is ongoing, after which auxin transport- and signalling-related genes will be selected. The interactions between these miRNAs and their predicted targets will be validated using the 5′ RACE system. Expected outcomes include a functional characterisation of *HvGNOM* and *HvACT1*, providing insights into auxin regulation and temperature stress tolerance. These findings could inform breeding and genetic engineering strategies for barley.

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Comprehensive evaluation of root development and drought tolerance in winter wheat cultivars under different environmental conditions

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Keywords: drought stress, root development, abiotic stress tolerance, integrated analysis

As a result of climate change, droughts are becoming more frequent and severe, making research and breeding programs aimed at developing drought-tolerant wheat varieties a priority to improve yield stability. Root development and architecture play a crucial role in drought tolerance, as they fundamentally determine the efficiency of water uptake and, by acting as reservoirs of water and nutrients, enabling plants to survive prolonged dry periods until the end of the generative phase.

At our institute, we investigated the root development of cereals using various experimental approaches and systems. Since each method has its own limitations, a combined application of different methods provides a more complete picture of the root development characteristics.

In this study, we examined root development and drought tolerance in four winter wheat cultivars: Aura (Romania), Mv Kolompos (Hungary), Feng-you 3 (China), and Mv Verbunkos (Hungary). Based on our previous results, Aura and Mv Kolompos can be considered drought-tolerant, while Feng-you 3 and Mv Verbunkos are considered drought-sensitive genotypes.

Root development was analysed in three experimental systems. First, the early root growth of seedlings was studied under hydroponic conditions, where osmotic stress was simulated using polyethylene glycol (PEG). Then, plants were grown in PVC tubes 75 cm in height and 11 cm in diameter, filled with graded sand. In this system, drought was simulated by complete water withdrawal from the onset of stem elongation, with well-watered controls maintained throughout the experiment, while plants received half-strength Hoagland solution as nutrient supply. At the wax ripening stage, roots were washed out of the sand and analysed using the WinRHIZO Plus software. Finally, under field conditions, at a sowing density of 450 plants/m², root biomass and total root surface area were determined from soil samples.

All three methods revealed significant differences among the cultivars. The reduction in root length and surface area induced by the PEG treatment was consistent with the data obtained in the sand-tube experiment at the wax ripening stage. At the same time, our results confirmed that large root biomass alone is not directly associated with drought tolerance. This is exemplified by Mv Verbunkos, which developed high root biomass under control conditions but showed a substantial reduction of its root system under stress. Thus, the drought-induced change in root length and surface area may be a suitable indicator for assessing drought tolerance in wheat cultivars.

Acknowledgments

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Effects of ambient temperature on the daily expression patterns of the main circadian and photoreceptor genes of bread wheat

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Keywords: ambient temperature, daily gene expression patterns, circadian and photoreceptor genes, hexaploid wheat

The interactions between the plant circadian and photoreceptor genes have already been significantly explored in *Arabidopsis* and many homologous genes have been described in hexaploid wheat, however a scant information is available on the interactions among these gene families, and on the effects of ambient temperature on the expression patterns of them. The aim of this research was that to study the daily expression patterns of the major circadian clock (*CCA1*, *PRR95*, *TOC1*, *LUX*, *ELF3*, *CO* and *GI*) and photoreceptor genes (*PHYA*, *PHYB*, *PHYC*, *CRY1* and *CRY2*) in three winter wheat cultivars with different plant developmental patterns under two controlled environmental conditions (optimal 18 °C and supra-optimal 25 °C) applied at long photoperiod (16 h). The daily expression of the circadian genes showed a definite diurnal activity irrespective to the environment and genotypes. The expression of the *CCA1* gene in the early cultivar (’Mv Toborzó’) has nearly doubled at 18 °C. The same tendency was observed in the activity of the *LUX* gene (nearly a three times increase) in the late heading genotypes (’Tommi’ and ’Charger’). The average expression level of *PRR95* gene has doubled in ‘Mv Toborzó’ and ‘Charger’, it has tripled in ’Tommi’ genotype at 18 °C. The daily average expression level of *ELF3* in the early cultivar showed a five and ten-times increase compared to the late genotypes. In case of the late genotypes ’Charger’, a two times higher average expression level of the *GI* gene was observed at 18 °C, in contrast, the *CO1* gene showed three-five times higher peak expression at 25 °C compared to the other two wheat genotypes. The gene expression the *PHYA*, *PHYB* and *CRY1* genes was significantly influenced by the environment, while the daily dynamics of *PHYC* and *CRY2* genes were determined by the genotype and environment. Approximately, ten times increase in the daily expression patterns of *PHYA* and *PHYB* genes was observed at 18 °C in all the examined winter wheat cultivars. A stronger interaction was identified between *PHYC* vs. *ELF3*, *PHYC* vs. *CRY1*, *LUX* vs. *TOC1*, and *LUX* vs. *GI* genes, while a negative interaction was observed between CCA1 vs. LUX genes in both ambient temperature levels. At 25 °C, positive interactions between *PRR95* vs. *LUX*, *PRR95* vs *GI*, and *ELF3* vs. *CRY1* genes were observed, while a negative interaction was identified between *LUX* vs. *CRY2*. These results might support the deeper understanding of the effects of the abiotic environmental factors on plant developmental patterns determining both the environmental adaptation and grain yield formation in wheat.

Acknowledgments
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Developing an EMS mutant population for dissecting the genetic components of ambient temperature sensing in winter wheat (*Triticum aestivum* L)

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

Temperature is a fundamental and important environmental signal in plant regulatory processes, performing a number of functions. Although a vast amount of information has been collected on both low-temperature vernalisation and the heat stress responses during and after flowering, much less is known about the role of temperature as a central regulator of plant development or the composition of the genetic network involved in temperature sensing. In order to understand the molecular basis of ambient temperature sensing in wheat, the potential of chemical mutagenesis with ethyl methane sulfonate (EMS) was used as a tool to identify untapped genes and characterize gene function. For this purpose, we chose Mv Toborzó winter wheat, the plant development of which significantly fastens (especially the beginning and speed of intensive stem elongation) when the ambient temperature is higher than 18°C, under flowering inductive conditions. One thousand seeds of Mv Toborzó was treated with 0,7% EMS solutions leading to 90% mortality. At the end, 13 M_0 plants were able to flower, ten of which proved to be fertile producing between 1 to 29 grains per plants. The vernalized M_1 progenies (altogether 129 plants) of the 10 fertile M_0 plants were then raised under controlled climatic conditions optimal for flowering induction (16 h photoperiod and constant 18°C ambient temperature). Their developmental characteristics including changes in plant height, in number of leaves and tillers were regularly monitored every five days and the timing of first node appearance (ZD31), booting (ZD49) and heading (ZD59) was also recorded. After ripening, the grain yield related traits were also measured. Significant variations in all the traits were detected within and between the 10 M_1 families and in comparing with the donor genotype, as well. 8.5% of the mutant plants (11) were significantly later than Toborzó throughout the complete developmental period. In addition, 53 plants (41.1%) with various disturbances in the beginning and in the progress of intensive stem elongation were also identified. Interestingly, we found 7 mutants that were significantly faster than Toborzó in the timing of fully developed flag leaf stage (ZD39), but that earliness however disappeared by booting stage. Plants of the M_2 progeny will be raised again at 18°C under long photoperiod and individuals with plant development significantly slower than the parental cultivar will be selected and phenotypically further characterised in the M_3 - M_4 generations, but already under 25°C temperature environmental conditions. At that stage, comparative DNA sequencing of the most interesting mutants with the donor genotype is planned to be carried out.

Acknowledgments

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Changes of the enzymatic defence system by melatonin root application during recovery after drought of the Bulgarian wheat cultivar Gines

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Keywords: *Triticum aestivum* (L.), drought, recovery, melatonin, antioxidant enzymes, gene expression

Drought affects agricultural crop production due to its multilevel negative impacts on plants, including alterations in antioxidant defence system. Melatonin is a natural substance in plants that could positively modulate the antioxidant defence system. A five-day deficit of soil moisture was imposed to young wheat (*Triticum aestivum* L.) plants of the Bulgarian cultivar Gines, after which normal water supply was resumed. Twenty-four hours later, plants were supplemented with a 75 µM melatonin solution added to the irrigation water. The activity of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POX) and transcript profiling of genes coding for enzymes scavenging reactive oxygen species (viz. SOD, glutathione peroxidase (GPX), CAT, and ascorbate peroxidase (POD1)) were assessed in wheat leaves on the second (2D-R) and fourth day (4D-R) of the recovery period. Drought provoked a substantial increase in activity of CAT, up-regulation of *CAT3* and *GPX* transcripts, and down-regulation of *POD1* transcript on 2D-R. Transcripts of *CAT3*, *CATA*, *GPX* and *SOD-Fe* were up-regulated, while a severe decrease in enzymatic activity of SOD and expression of *SOD-Cu-Zn* was observed at the 4D-R. When applied after drought, melatonin stabilized the enzymatic activity of POX and SOD along with up-regulation of *SOD-Cu-Zn*, *SOD-Fe*, which is assisting the recovery of the Bulgarian wheat cultivar Gines from water shortage.

Acknowledgments
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How does supra-optimal temperature affect the development of bread wheat varieties with different vernalization requirements?

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Keywords: vernalization, supra-optimal temperature, developmental phases, hexaploid wheat

The saturation rate of vernalization requirement (which fundamentally determines the length of the vegetative phase), depends on both temperature and the duration of cold exposure. However, as a result of global climate change, an increasing frequency of relatively mild winters is being observed in temperate regions, which may reduce the adaptability of plants that require cold treatment. The main objective of our experiment was to investigate the effect of supra-optimal temperature (constant 25°C, 16 h daylength) for a short period (2 weeks) after vernalization on the developmental dynamics of 19 wheat cultivars with different plant developmental diversities. The varieties used in the study underwent cold treatment for 30, 45, or 60 days. Following this, they were exposed to supra-optimal temperature before being transferred to a greenhouse (16–20°C, 16-hour photoperiod). As controls, we used plants that had undergone the same cold treatments and were kept only in the greenhouse. The plants were regularly monitored, and the following consecutive phenophases were defined: ZD31 (appearance of the first main stem node), ZD37 (appearance of the flag leaf), ZD39 (full expansion of the flag leaf), ZD49 (booting stage), and ZD59 (heading). Based on the principal component analysis of the data matrix comprising the five plant developmental parameters and the six treatments applied to the 19 wheat varieties, distinct groups with different developmental dynamics can be observed in the quadrants (Q1–Q4) defined by the x- and y-axes. While genotypic variance among genotypes was relatively low in Q1–Q3, it was significantly higher in the fourth quadrant (Q4). All three genotypes found in Q2 were spring types, whereas all photoperiod-insensitive, winter-type genotypes included in the experiment were classified into Q1. Furthermore, most photoperiod-sensitive, winter-type varieties were found in Q3 and Q4. Comparing the developmental dynamics of the genotypes under each treatment, it can be concluded that the time required to reach full heading (ZD59) was shortest for genotypes with a spring growth habit (Q2), followed by photoperiod-insensitive, winter-type varieties (Q1). This correlation was observed in all six treatments. The length of the intervals between plant developmental stages was also strongly influenced by the treatments, and this was a group-specific phenomenon. The interval between the appearance of the first main stem node and the appearance of the flag leaf (ZD3731) exhibited one of the highest levels of variability across groups and treatments. The ZD3731 values of the photoperiod-insensitive genotypes classified in Q1 remained relatively stable regardless of the treatments. In contrast, in spring genotypes (Q2), a two-week warm period (25 °C) shortened this interval, regardless of the duration of vernalization. However, in late-heading genotypes (Q4), the interval increased significantly, with the extent of the increase becoming more pronounced as the cold treatment period was shortened.

Acknowledgments
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Physiological and phytohormonal responses to drought stress in glaucous and glossy barley lines

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Keywords: drought stress, earliness, phytohormones, recovery, stomatal density, stress tolerance.

Drought stress initiates a complex chain of events beginning with reduced soil water availability, leading to impaired water uptake and consequent physiological adjustments. Barley plants with early or late heading times exhibit distinct responses to drought, with early-heading genotypes generally showing quicker adjustments to stress conditions. Additionally, barley lines with different wax coating characteristics, such as glaucous or glossy phenotypes, may have varying abilities to retain water, influencing their stress tolerance. The concentration of phytohormones, such as abscisic acid (ABA), salicylic acid (SA), and gibberellic acid (GA_3), plays a crucial role in modulating plant responses to drought, with ABA being particularly important in regulating water loss and stress adaptation. As soil moisture declines, the initial response in barley is characterized by changes in leaf water status, prompting a rise in endogenous abscisic acid (ABA) levels. This study explores the response to drought stress in two glossy and two glaucous barley lines, derived from cross combinations between the Bowman eceriferum (cer) mutant, a reference variety known for its strong wax coating, Lubuski – a Polish cultivar, and Cam/B1/CI08887//CI05761 (CamB) – a Syrian breeding line. DH lines were exposed to two irrigation regimes—well-watered (70% FWC) and severe drought (20–30% FWC)—under controlled conditions for 10 days. Leaf samples collected at mid-stress, end-stress, and recovery were analyzed for chlorophyll, flavonoid, anthocyanin, and nitrogen indices (Dualex), stomatal density (SEM), and key phytohormones (ABA, SA, GA_3, GA_6) *via* UPLC-HESI-HRMS/MS.

The plants exhibited variation in selected parameters, including the leaf chlorophyll index and flavonol indices, depending on the treatment conditions. Glaucous (waxy) genotypes, owing to their thicker epicuticular wax layers, demonstrated enhanced water retention, reflected in more stable chlorophyll and nitrogen balance indices under drought. Glossy phenotypes, in contrast, were generally more prone to pigment degradation and stress-related metabolic shifts. Additionally, early-heading genotypes appeared to initiate stress response mechanisms more rapidly than late-heading ones, suggesting a temporal advantage in adjusting to declining water availability. This included more prompt alterations in phytohormone levels and pigment-related indices, potentially conferring greater resilience during the initial phases of stress. Upon rehydration, physiological recovery varied among the genotypes, further underscoring the interplay between structural traits (e.g., wax coating, stomatal density), temporal growth patterns, and dynamic hormonal regulation. Drought in barley triggers a cascade: water deficit alters leaf water status, induces ABA-driven hormonal changes, leading to stomatal closure and metabolic adjustments. These are modulated by genotypic traits such as waxiness, stomatal architecture, and heading time, ultimately determining the plant's ability to maintain physiological function and recover post-stress. These findings provide insights into the physiological mechanisms and phytohormonal modulation underlying drought resistance in barley, with potential applications for breeding drought-tolerant cultivars.

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Climate change and the challenges to wheat breeding in Bulgaria

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Keywords: winter wheat, ideotype, climate change, stress factors

Current situation and aims: Food supply safety is directly related to the climate changes. The long phenological development of winter cereals, including wheat, implies different types of stress, which determine the stability of production. The challenges are on different levels and include both the applied agronomy practices and an adequate varietal structure. The main purposes of the breeding programs are related to development of more adaptable varieties and possibilities for realization of their productivity potential. In the recent years, the tendencies in the regional breeding of wheat are in development, while others are being changed. These are related to specificity of the phenological development, the parameters of the desired ideotype, and many of them are determined by the specific requirements of the market and the consumers. The aims of this investigation were: identification of the stress types and their frequency in the production of wheat within a 10-year period; evaluation of the productivity potential of cultivars under risky environment; characterization of the level and variation of main economic traits under stress.

Methods: The subject of study were common winter wheat cultivars investigated in competitive varietal trials at Dobrudzha Agricultural Institute (DAI). Under field conditions, the level and variation of key parameters outlining the breeding value of the genotypes were studied during ten vegetative growth seasons. They were developed by the method of combinative breeding after long-term multiple selection in hybrid populations. Their pedigree involved parental components from different geographic origin characterized by specific combination of traits.

The values of the meteorological factors were taken from a meteo cell localized on the territory of DAI.

Key results: The comparison to a long-term period revealed several major tendencies: severe drought in summer and impossibility for quality pre-sowing tillage of soil; high mean daily temperatures during the autumn-and-winter period impeding the plants from entering into a dormant stage; late spring frosts causing different levels of damage; frequent droughts during different stages of the phenological development of the plants. At the level of biotic stress, more frequently were observed mass occurrences of pests, which are vectors of viral diseases. Yellow rust was also with high frequency of occurrence.

Conclusions: In comparison to the previous breeding program of wheat, the researches were directed toward developing varieties with higher number of productive tillers and balance between the yield components; maintaining a stable level of cold resistance preventing dehardening at the end of the winter months, high drought tolerance; developing varieties of intensive type with high biological potential; developing "efficient" varieties which are able to realize high mean productivity under limited use of pesticides and fertilizers.

Abiotic stress tolerance of winter common wheat varieties, created at the Dobrudzha Agricultural Institute, northeastern Bulgaria

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Keywords: winter common wheat, climate change, drought tolerance, cold resistance

Abiotic stress significantly affects plant growth and common wheat production worldwide. Dynamic temperature conditions and uneven distribution of precipitation during the growing season are major risk factors reducing yield. The uncertainty of climate models leads to the need to create high-yielding and at the same time stress-tolerant wheat varieties. The aim of the investigation was to monitor the drought tolerance and the resistance to low temperatures of the winter common wheat varieties created at the Dobrudzha Agricultural Institute and to accent the priorities in creating new ones suitable for climate change in northeastern Bulgaria. The study included 44 genotypes created in the period 1990-2024, distributed in 4 sub-periods, respectively 1990-2000 (10 varieties), 2001-2010 (15 varieties), 2011-2019 (11 varieties) and 2020-2024 (8 varieties). Drought tolerance was tested in a greenhouse between 2 and 13 years. Data on the drought tolerance coefficient and the MSTI stress tolerance index of the samples were summarized. Cold tolerance was determined by growing and hardening the plants under natural conditions and their artificial freezing in cold chambers in the tillering stage. The level of resistance to low temperatures was compared with that of cold tolerance standard varieties. Results of a 6-year test (2014-2019) for 36 samples and a 2-year test (2023-2024) for the newly created varieties were presented.

A large variety has been established in terms of tolerance to abiotic stress. Part of the genotypes combined high drought tolerance and a level of cold resistance suitable for the requirements of the region. The varieties with a coefficient of drought resistance close to (90-100%) and exceeding (over 100%) the coefficient of the standard with high drought tolerance had 45% of the total number. Genotypes with a high level of cold resistance between the standards Mironovskaya 808 and Bezostaya 1, and between the standards Bezostaya 1 and No. 301 had 38.6% and 36.4% of the tested samples, respectively.

Cultivar differences in plant height plasticity and stability in wheat (*Triticum aestivum*)

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Keywords: wheat, height, stability, plasticity, cultivar, environment

Plant height is one of the most important agronomic traits in wheat, because it is related to intra-plant and plant-to-plant competition and to lodging resistance**.** We analysed the variation of plant height in fifteen winter wheat cultivars tested in thirty-four yield trials conducted in Southern and Western Romania, during 2021-2024. Most of the large observed variation was due to environmental factors, but cultivars and G*E interaction were also significant sources of variation. Plant height was not correlated with grain yield in the analyzed trials and cultivars. However, there was a positive trend of higher yields being associated with taller plants induced by favorable environments (averaged over cultivars) and a negative relationship between cultivar yields and heights averaged over trials.

The studied cultivars showed major differences in plant height averaged over all trials, as well as in minimum and maximum recorded height. Minimum recorded plant height of cultivars was not correlated with the maximum height (figure 1). This allowed identification of cultivars with high minimum height and below average maximum height (especially cultivars *FDL Columna* and *Izvor*). Our results suggest that better plant height stability might be associated with better drought resistance during the stem elongation period. With increased drought frequency and unpredictable water supply associated with climate changes**,** selecting cultivars with higher minimum height under stress, lower height plasticity (smaller slope of Finlay Wilkinson regression) and better height stability (smaller s%) should be an important breeding goal.

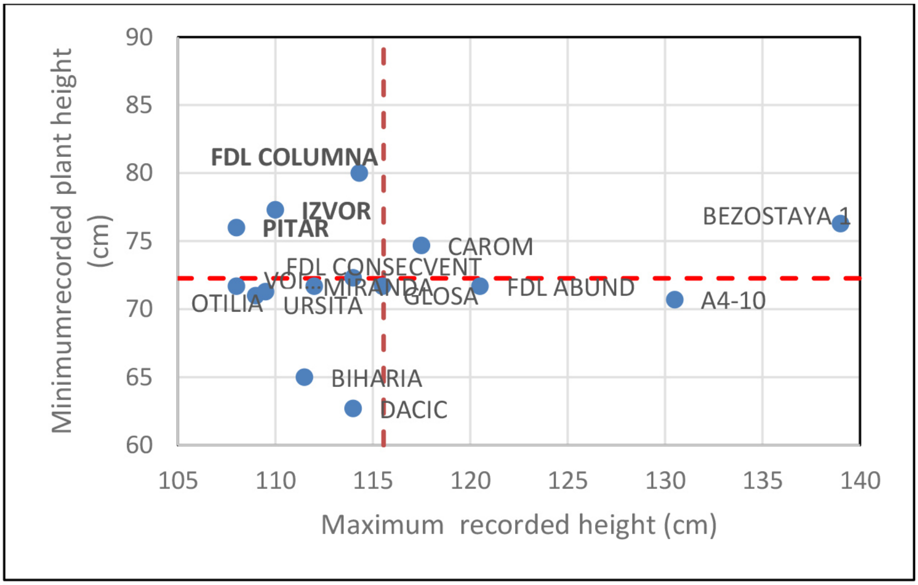

Figure 1. The relationship between minimum and maximum recorded plant height of tested cultivars

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Melatonin root application modifies the enzymatic defence system during recovery after drought in the Bulgarian wheat cultivar Fermer

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Keywords: wheat, drought, melatonin, antioxidant enzymes, gene expression

Drought is a ubiquitous stress factor affecting agriculture worldwide due to its negative impact on various aspects of plant physiology. Investigations on ecologically friendly approaches for mitigating the adverse effects of water shortage in plants have indicated melatonin as a promising, naturally occurring and highly potent antioxidant that could modulate the antioxidant defence system. Young seedlings of the Bulgarian wheat (*Triticum aestivum* L.) cv. Fermer were exposed to a five-day soil drought, after which the irrigation was restored. One day later, plants were root-supplemented with a 75 µM melatonin solution. The activity of superoxide dismutase (SOD), catalase (CAT) and guaiacol peroxidase (POX) along with gene expression of *SOD-Cu-Zn, SOD-Fe*, *SOD-Mn*, *CATA*, *CAT3*, *GPX*, and *POD1* in the leaves were assessed on the second (2D-R) and fourth day (4D-R) of recovery. Drought increased measured enzymatic activities and transcripts of *GPX* on 2D-R, which was in parallel with notable down-regulation of *SOD-Fe*, *SOD-Mn*, *CATA*, and *POD1* transcripts. Drought induced an opposite tendency at 4D-R. Slight changes in *SOD-Cu-Zn* and *CAT3* transcripts were observed due to water shortage during recovery. Obtained results evidenced that post-drought application of melatonin stabilized (SOD) or increased (CAT, POX) enzymatic activities during recovery and provoked substantial time-dependent up-regulation of *SOD-Fe*, *GPX* and *CAT3* transcripts, supporting plant recovery.

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Functional characterization of HvHY5 transcription factor in barley

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Keywords: light spectrum, light signalling, HY5, barley

It has been shown in previous studies that altering the quality of light can influence stress tolerance of plants, which increases the urge to investigate their light signalling pathways. For example, the supplementation of white light (W) with far-red (WFr) or Fr plus violet-blue (WFr+VB) light improved the freezing tolerance of barley or wheat. Gene orthologs that are well-studied in Arabidopsis could have similar or different functions in other plant species, which make their characterization desirable in crop plants. Barley is one of the most important crops; however, most of its light signalling elements lack functional characterisation. Elongated Hypocotyl 5 (HY5) is a key transcription factor in Arabidopsis that transmits both low temperature and light signals. It could bind to the promoter of more than 9000 genes, out of which it regulates more than 1100. The aims of this study are to rescue the *hy5* mutant (SALK) phenotype in Arabidopsis via the heterologous expression of HvHY5, to characterise the protein function, and to monitor its gene expression in barley.

We found that the 'long hypocotyl phenotype' of Arabidopsis *hy5* mutant was rescued by HvHY5, among other mutant phenotypes. The protein-protein interaction ability of HvHY5 with AtHY5 interaction partners was also investigated. The characterised HvHY5 gene showed light- and cold-inducible expression in barley sprouts and young barley plants, respectively. These results represents an important step toward understanding light signalling in barley.

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References

https://doi.org/10.3390/antiox11071311
https://doi.org/10.3390/plants12010040
https://doi.org/10.1016/j.agrformet.2020.108041

Root development of winter wheat (*Triticum aestivum* L.) varieties

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Keywords: abiotic stress tolerance, drought stress, adaptability, genetic diversity

Climate change, which is leading to more frequent and severe droughts, highlights the importance of research and breeding aimed at identifying and producing better drought-tolerant wheat lines to increase food security. One of the most important components in surviving the drought period is the dynamics of root development and the shape of the root structure, which helps the plant through more efficient water absorption and also functions as a water and nutrient reserve until the end of the generative phase. In our experiments, the root development of 12 winter wheat genotypes with different origins and abiotic stress tolerance was investigated under controlled environmental conditions in sand tubes and under real field conditions.

In the sand tube system, vernalized seedlings were planted in PVC tubes (11 cm diameter and 75 cm height) containing classified KH-30 sand under controlled environmental conditions in greenhouse chambers. The plants were grown till the early dough stage (BBCH 83) using the parameters of the spring–summer climate program. Plants were watered three times per week with half strength Hoagland's solution. In addition to the well-watered control treatment, drought was simulated by complete water withdrawal for 21 days when plants reached the first node appearance (BBCH 30) developmental stage. At the end of the experiment, the entire root system was carefully washed out of the sand and root parameters (root length and diameter) were measured using the WinRHIZO Plus root scanner and software (Regent Instruments Ltd, Canada). Root length density (cm cm^{-3}) and specific root length (m g^{-1}) were calculated from the measured parameters.

In the sand tubes, water withdrawal at BBCH 30 resulted in an increase in total root length, but significant differences were observed among varieties. Root length increased significantly (198.7%) on average in the drought tolerant genotypes (Aura, Roane and Mv Kolompos), while a reduction in total root length was observed in drought sensitive varieties such as Mv Verbunkos (36.7%) and Ellvis (-13.5%) compared to the well-watered control. Overall, a large variability in root length was observed as a result of water withdrawal, but a consequent increase in root diameter and root surface area was noted.

The field trial was carried out in the field trial nursery of the Agricultural Institute in Martonvásár during the growing season 2023/2024. The plants were cultivated according to the conventional local agronomic practice. When the genotypes reached the BBCH 83 stage, soil samples were collected in triplicate from the 6 m^2 plots using soil cores from 0-80 cm soil depth. The soil core was divided into 20 cm subsamples. Samples were dried and roots were carefully collected using 1 millimetre mesh sieves. Root parameters were determined using the WinRHIZO system. Significant differences in root length were observed between varieties. Mv Verbunkos and Ellvis had the longest root systems (1149 and 913 mm, respectively), while Roane has only 469 mm of roots in the soil core. Differences were observed in the vertical distribution of the roots because in the case of some genotypes similar root length were observed in the upper (0-40 cm) and lower (40-80 cm) soil layers (Aura, Ellvis and Fengyou 3) while 71% and 64% of the total root length can be found in the upper soil layer for Roane and Mv Verbunkos, respectively.

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T3: Biotic Stress Response; Plant Microbe Interactions

A novel approach to mitigate Fusarium root and base rot: spermine seed priming in wheat

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Keywords: stress marker content, antioxidant defence system, *Fusarium culmorum,* seed pre-treatment, spermine, wheat

Seed priming with ecologically safe compounds like polyamines is emerging as a promising strategy to alleviate different biotic stresses in plants, and is especially interesting in wheat against *Fusarium culmorum* infection. This study investigated the protective effect of spermine (5mM), a polyamine known to modulate plant stress responses, on the wheat cultivar Sadovo-1 when the pathogen was introduced through soil contamination.

The plants infected with *F. culmorum via* soil exhibited significant decrease in fresh weight and height. At a physiological level, soil contamination caused an increase in stress markers malondialdehyde, hydrogen peroxide, free proline, and electrolyte leakage. These changes were accompanied by elevated levels of non-enzymatic antioxidants like total phenolics and free thiol-containing groups. The plant's enzymatic response included a significant rise in the activities of antioxidant enzymes peroxidase and superoxide dismutase. A particularly interesting finding was the slight, almost negligible, increase in catalase activity.

Crucially, 5 mM spermine seed priming proved effective in regulating these stress-induced changes. The treatment mitigated the negative effects on plant growth and biochemical parameters, suggesting it could beneficially modulate the plant's defense mechanisms. These findings indicate that spermine priming could be a viable approach to reduce the detrimental impact of *F. culmorum* soil contamination on the Bulgarian wheat cultivar Sadovo-1.

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Monitoring pathogen occurrence and host resistance in Martonvásár cereals

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Keywords: leaf rust, yellow rust, powdery mildew, leaf spot, breeding

The objectives of this study were to examine the resistance of cereal varieties and lines and occurrence of pathogens under field conditions near Martonvásár over three different growing seasons (2022/2023, 2023/2024 and 2024/2025). Wheat yield and yield loss (%) was were measured separately for each fungicide-treated and untreated replicated plot after harvesting at 15% water content.

In the 2024/2025 growing season, in addition to the pathogens identified in previous years, conidia of *Pyrenophora tritici-repentis* (anamorph: *Drechslera tritici-repentis*) were sporulated on 80% of the leaves showing symptoms of leaf spot in wheat, durum wheat, triticale and spelt. A severe yellow rust epidemic in the first year, leaf rust in the second year, and the higher incidence of powdery mildew in the third year provided a good opportunity for the selection of resistant lines. According to our observations, approximately 50% of the tested cereal varieties and lines exhibited good resistance to yellow rust in 2023 and in 2024, respectively.

Field assessments revealed that certain genotypes responded to the infection with localized necrosis, effectively restricting pathogen development. In 2023, 36% of bread wheat and 75% of durum wheat genotypes displayed good resistance to leaf rust. In 2024, despite the high pathogen pressure, durum wheat maintained excellent resistance, while 28-52% of the bread wheat genotypes and more than half of the barley genotypes exhibited tolerance to leaf rust. In 2025, rust fungi appeared only sporadically at the end of the season. Nearly 10% of wheat, and 30% of triticale varieties and lines were completely symptomless against powdery mildew, while around 20% of durum and 10-20% of barley varieties and lines showed resistance to the pathogen.

Based on the results of the analysis of variance, yield was significantly influenced by year, management and genotypes. Year had the strongest effect on yield ($p < 0.001$), followed by management and genotypes, both also highly significant. Significant two-way interactions (year × management, year × genotype, management × genotype) indicated that management and genotypic performance varied across years, whereas the three-way interaction was not significant. The average wheat yield over the three years was 7.6 t ha^{-1}, with the highest yield recorded in 2025. Average yield loss was 9.8%, but in susceptible genotypes losses reached 43% in 2023 and 37.5% in 2024.

An important goal of the European Union's "Farm to Fork" strategy announced as part of the “Green Deal” is to reduce the overall use and risk of chemical pesticides by 50% by 2030. The European The Commission considers it a priority to promote the use of integrated plant protection management methods, with resistance breeding being one possible approach. Our results highlight the key role of host resistance in disease management, which is a cost-effective and sustainable method for controlling cereal diseases and making food systems more economical and environmentally friendly.

Acknowledgments

The research was supported by the TKP2021-NKTA-06 project, provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund.

Investigation of salicylic acid and jasmonic acid regulated gene expression induced by Pyrenophora teres f. teres infection in barley

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Keywords: barley, *Pyrenophora teres* f. *teres*, Jasmonic acid, Salicilic acid, ORA59 transcription factor

Plant hormones have a key role in defence mechanism against plant pathogenes.

The APETALA2/Ethylene-Responsive Transcription Factor (AP2/ERF) family is one of the main regulators of plant responses to both biotic and abiotic stress responses. ORA59 transcription factor is the member of this family, whose expression increased upon pathogen attack in Arabidopsis. It is under the regulation of three hormones: JA and ET stimulate, while SA inhibits. The barley *ORA59* gene was identified based on Blast searches (HORVU4Hr1G000700.2 in Plant Ensembl), it showed 69% AA identity to the Arabidopsis protein. We investigated the role of *ORA59* gene in defence mechanism against *Pyrenophora teres* f. *teres* (PTT) infection in barley, because it has not been studied, yet.

Ora59 mutant Golden Promise barleys were generated by CRISPR/Cas9 system that were more susceptible than the wild type ($p<0.05$) to the infection caused by the necrotrophic fungus *Pyrenophora teres* f. *teres* (PTT) . In order to understand the function of the *Ora59* gene in resistance to PTT, cv. Arda a resistant variety was involved to the study. The results of transcriptome sequencing show that the expression of several important gene functional groups (GO-terms), such as defense response and response to biotic stimulus, differed on day 0 between GP and Arda, as well as GP and Ora59 mutant. The more sensitive genotypes started with lesser function of these pathways. The activity of several differentially expressed genes (DEGs) also differed between genotypes and in response to infection. The expression of the Ora59 gene increased in all tested varieties until the second day after inoculation. It was significantly ($p<0.001$) higher in resistant varieties than in susceptible ones. Interestingly, high gene expression was also observed in the Ora59 mutant. According to literature data, the Arabidopsis Ora59 gene enhances the expression of effector genes against pathogens. In relation to the barley homologue, it also exerted similar, positive regulation on on the following TF genes, their expression was lower in the Ora mutant than in the wild-type GP and several of them were also significantly expressed in the resistant Arda. Two WRKY TFs, and the RING-H2 finger protein ATL60 relative expression was higher in the wild type (2.79-fold) and the resistant Arda variety (8-fold) than in the susceptible varieties (0.80-fold) till day 7 after PTT infection. However, this difference, had disappeared to 15^{th} day, suggested the role of these TF in the early stages of infection. The expression of the AP1 TF gene increased continuously from 0-15 days in the resistant variety Arda (120-fold), while the expression level was low in the susceptible varieties (0.4-fold). Expression of a chloramphenicol acetyltransferase gene was also higher in wild-type GP than in the Ora mutant in the whole studied period. This gene presumably plays a role in the later stages of defense as well. In contrast the inhibitory effect of Ora59 on some genes were also observed. A lipase gene, which was most highly expressed in the Ora mutant was barely expressed in the resistant varieties. It suggested, that Ora59 may have regulated a gene relevant for PTT defense.

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Impact of Fusarium infection on the storage protein composition of bread wheat cultivars

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Keywords: Fusarium spp. infection, wheat, storage proteins, SE- and RP-HPLC

In the examination of the impact of climate change-induced abiotic stress factors, in agreement with findings from other research groups, our previous study revealed that drought and heat stresses resulted in significant changes in the seed storage protein content and composition of bread wheat cultivars (*Triticum aestivum*). In addition to abiotic environmental conditions, biotic stressors, such as infection by Fusarium spp. are recognized as risking food- and health security, and adversely influence the composition of grain storage proteins.

In this study the seed storage protein composition was investigated in two bread wheat cultivars, including a susceptible ('Mv Suba') and a moderately resistant variety ('Mv Kolompos') to Fusarium infection. The experimental plot was treated at anthesis with an equal mixture of isolated spores from *Fusarium graminearum* and *F. culmorum*. The concentration of the spore suspension was 50 000 conidia/ml and the total applied amount was 200 ml/m^2. From the early flowering stage until the entire crop had flowered, the spray inoculation was performed three times within a week. Total protein extraction from seeds for SDS-PAGE was used to detect the changes in the protein profile caused by the applied biotic stress treatment. SE- and RP-HPLC analyses were performed on the wholemeal samples to determine the storage protein composition and to monitor the alterations in techno-functional attributes (e. g., glutenin-to-gliadin ratio (Glu/Gli), unextractable polymer protein percentage (UPP%)) related to the Fusarium spp. seed infection. In parallel, changes in the immunoreactive characteristics of the extracted proteins were investigated using enzyme linked immunosorbent assay (ELISA) method, including the commercially available Ridascreen® Gliadin R5 (R-Pharma) and AgraQuant® Gluten G12 (Romer Labs) antibody-based test kits.

The results revealed that seed infection with F. graminearum and F. culmorum generated significant changes in storage protein content and composition. A decrease by 18-23% compared to control was observed related to thousand kernel weights, whereas crude protein content increased by 2-12% compared to control. Total amounts of polymeric glutenin proteins showed a severe reduction by 96% and 90% in the susceptible and the moderately resistant varieties, while the monomeric gliadins increased by 57% and 45%, respectively. These changes led to deteriorated Glu/Gli by 97.5% (in the susceptible cultivar) and by 93% (moderately resistant cultivar) compared to their controls. However, higher values (by 6-15%) were found related to UPP%, this does not mean superior quality due to the significant drop in glutenin quantities. ELISA analyses resulted in reduced R5- (by 51% and 31%) and G12 epitope content (by 40% and 29%) in the susceptible and moderately resistant cultivars, respectively.

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Antifungal potential of coumarin derivatives against biotrophic pathogens in cereals

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Keywords: agrochemicals, coumarins, fungal pathogens

Fungal diseases caused by obligate biotrophs, including *Blumeria graminis* and rust fungi (*Pucciniales*), are among the most serious constraints on cereal cultivation worldwide. These pathogens occur annually and lead to significant yield losses, while their ability to rapidly adapt and develop resistance to conventional fungicides reduces the long-term effectiveness of chemical control. The increasing challenges posed by fungal infections necessitate the search for new, effective, and environmentally safe plant protection strategies. Natural compounds such as coumarin and its derivatives have attracted considerable attention due to their antifungal properties and favorable biodegradability, making them promising candidates for the development of novel fungicides. The present study evaluated the inhibitory potential of coumarin derivatives against major biotrophic cereal pathogens. Their biological activity was assessed in vitro using host–pathogen assays, where leaves of susceptible cereal varieties (oat, wheat, triticale, barley) were placed on agar medium enriched with coumarin derivatives, inoculated with fungal spores, and incubated for 10 days. The degree of infection was then quantified. The results demonstrated diverse antifungal activity of the tested compounds, with most derivatives showing significant inhibitory effects. Importantly, a group of derivatives with broad-spectrum activity was identified, providing a foundation for further research toward the development of new, efficient, and environmentally friendly agrochemicals.

The crown rust resistance gene *Pc51* in oat has been mapped to chromosome 1D

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Keywords: fungal diseases, DArTseq genotyping, *Avena sativa*, gene mapping

Crown rust caused by *Puccinia coronata* Corda f. sp. *avenae* (P. Syd. & Syd.) (Pca), is one of the most destructive fungal diseases of oats worldwide and represents a major constraint to oat production. Breeding disease-resistant oat cultivars is the most effective strategy for controlling the spread of crown rust and preventing potential epidemics. Currently, over 100 crown rust resistance (Pc) genes have been identified in oat, however, the chromosomal locations of most of these genes remain unknown.

The objective of this study was to genetically map the position of the *Pc51*, a race-specific seedling crown rust resistance gene that confers effective resistance at all growth stages. Dominant allele of the crown rust resistance gene were derived from a subline separated from the Iowa X270/X434 line, obtained by crossing *A. sterilis* Wahl No. 8 × *A. sativa*. *Pc51* came from *Avena sterilis* accession., originally collected in Israel. Physiological tests of crown rust response were conducted, and segregation ratios in the population derived from a cross between Pc51 differential line and the crown rust susceptible cultivars 'Kasztan', 'Bingo' and 'Kanota' confirmed monogenic inheritance of the resistance gene.

Linkage analysis to identify a map location for *Pc51* was performed in bi-parental mapping population genotyped with DArTseq™ markers generated by Illumina next-generation short-read sequencing. Markers linked to *Pc51* were mapped to chromosome 1D of the *Avena sativa* cv. Sang reference genome, within the region spanning 0,87 Gb and 4,44 Gb [1]. Based on SNPs identified in the DArTseq sequences derived from this chromosomal interval, allele-specific markers were developed. These newly developed markers represent valuable tools for marker-assisted selection in oat breeding programmes and may facilitate the pyramiding of multiple resistance genes.

References

1. Avena sativa, Oat Sang v1 genome assembly - https://wheat.pw.usda.gov/jb?data=/ggds/oat-Sang

Genomic insights into barley–powdery mildew interactions: identification of resistance-linked loci

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Keywords: *Blumeria hordei*, DArTseq, *Hordeum vulgare*, linkage mapping

Cultivated barley (*Hordeum vulgare* L.) is one of the world's most important crops in terms of cultivation area and yield, with Poland ranking seventh in Europe for barley acreage. However, fungal diseases such as powdery mildew, caused by the pathogenic fungus *Blumeria hordei*, pose a serious threat to both yield quantity and quality. Powdery mildew reduces the photosynthetic capacity of infected leaves, thereby impairing overall plant growth and productivity. A single infection site can produce up to 100,000 spores, which can infect neighbouring plants and be dispersed over hundreds of kilometres by air currents. Intensive chemical protection promotes the emergence of fungicide-resistant pathogen races. Therefore, the development and use of resistant cultivars are key components of sustainable plant protection strategies. Although more than 58 resistance genes have been identified to date, their genetic basis remains poorly understood.

The aim of this study was to identify loci associated with barley resistance to powdery mildew. We used landraces cultivated in regions with traditional agricultural practices, which have undergone limited breeding selection and therefore retain broad genetic diversity. These landraces represent a valuable potential source of resistance for breeding programs. Here, we present results for the barley single seed line 826-1 (obtained from ICARDA LBY 37570 ICB 120704 I accession), which was selected based on a previous GWAS analysis of pathogen response. An F_2 mapping population was developed from this line and genotyped using the DArTseq platform (Diversity Arrays Technology, Ltd., Australia). Linkage mapping enabled the localization of a resistance-associated locus on the short arm of chromosome 1H. This region is rich in known Ml-resistance genes; nevertheless, the identified locus appears to be novel, providing new insights into the genetic basis of resistance.

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Genetic diversity analysis of spelt wheat (*Triticum spelta* L.) genotypes using DArTseq technology

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Keywords: spelt wheat, genetic diversity, DArTseq technology, SNP markers, association mapping, disease resistance

Spelt is an ancient cereal increasingly valued in organic farming due to its natural resistance to biotic stress and tolerance to adverse environments. It is also a valuable resource for breeding resistant varieties. In this study, 27 spelt genotypes were characterized using DArTseq technology, which enabled simultaneous analysis of SilicoDArT and SNP markers. Out of 96,136 markers, 16,712 met quality criteria and were used for genetic similarity and association mapping. A dendrogram distinguished four main genotype groups, while association mapping revealed over 2,600 markers significantly linked to the virulence of Blumeria graminis f. sp. tritici. Notably, SilicoDArT 7492586 and SNP 1126088 showed strong associations with resistance to three of five pathogen isolates. Several chromosomal regions (1D, 3D, 5B, 6A) were identified as resistance loci, confirming the polygenic nature of the trait. The results highlight the high genetic variability of spelt and the potential of DArTseq for identifying resistance markers. These markers can support marker-assisted breeding, particularly in programs focused on ecological and organic farming.

Durable powdery mildew resistance of 'Mv Hombár' – a new insight

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Keywords: winter wheat, *Pm* gene donor/source, marker assisted breeding, SNP, *Blumeria graminis* f.sp. *tritici*

Presence of powdery mildew in Hungarian wheat fields is a common phenomenon, that can lead to a certain degree of yield loss every year, depending on the level of susceptibility of wheat cultivar produced and agrotechnical routines. The resistance breeders' aim is the creation of varieties carrying their self-defense mechanisms genetically, to gain effective protection without application of fungicides. 'Mv Hombár' is a winter wheat variety with outstanding powdery mildew resistance. Durability of the resistance is proven as the cultivar was registered in 2004, and can be produced without visible mildew symptoms in field to date. The resistance is effective along the whole vegetation period, with slight hypersensitive reaction only present in seedling stage.

Study of genetic background of resistance in 'Mv Hombár' was implemented by our research team earlier (Komáromi et al. 2006). The primary aim of the present work was the fine mapping of *PmHo* gene for the purpose of creating effective tools for marker assisted selection. Utilization of a gene present in parental background that is completely suitable for local agricultural conditions can have the highest value for breeders. Eighty lines of the 'Ukrainka/Mv Hombár' biparental population was chosen for detailed genetical evaluation, with regard to their marker pattern in key positions revealed in our previous work. Genetic analysis was carried out by Illumina Infinium 25K Wheat Array (SGS Institut Fresenius GmbH, Traitgenetics Section, Germany).

Out of the 7750 SNP markers proved to be heterogeneous in the population, after filtering out the identical ones, 1610 markers served as basis of the analysis. Linkage map construction resulted in the coverage of 2260 cM altogether, representing all wheat chromosomes and chromosome arms. It means that the resolution of the genetic map became approximately doubled with the SNP genotyping in comparison with our previous study. The more detailed map containing regions remained hidden earlier could lead to a new discovery. As a result of QTL mapping a major genomic region was identified on the long arm of chromosome 7A. Dominance of the locus was demonstrated with the analysis of infection data originated from field experiments and greenhouse trials, too. The connection of this region of 7AL to powdery mildew resistance is proved both on seedlings and adult plants, with LOD-values in the range of 26 and 31. The explained phenotypic variance was around 80% in all examined disease datasets.

Acknowledgments

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References

1. Komáromi, J., Jankovics, T., Fábián, A., Puskás, K., Zhang, Z., Zhang, M., Li, H., Jäger, K., Láng, L., Vida, G. 2016. Powdery mildew resistance in wheat cultivar Mv Hombár is conferred by a new gene, *PmHo*. Phytopathology 106: 1326–1334.

Virulence analysis of Polish isolates of the fungus *Zymoseptoria tritici* causing Septoria tritici blotch

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Keywords: Septoria tritici blotch, *Zymoseptoria tritici*, virulence

Septoria tritici blotch (STB) is one of the most significant leaf diseases of wheat and may cause up to 50% yield loss under favorable conditions. It is considered a high-risk pathogen due to its high potential for adaptation and substantial effective population size. It is estimated that over 70% of the fungicides applied annually to cereal crops in Europe target STB. This high selection pressure can be devastating, as STB has been reported to develop resistance to various classes of fungicides within a single growing season. Similarly, resistance in the host plant can also be overcome in a short period of time. Therefore, continuous monitoring of the pathogen population structure and its virulence against host resistance genes is crucial for developing resistant wheat varieties and promoting sustainable agricultural practices.

The presented study used single-pycnidial isolates of the fungus *Zymoseptoria tritici*, derived from leaves with STB symptoms, collected in various locations in Poland between 2020 and 2024. The virulence profile was determined based on the reaction of the differential set to infection with the fungal isolate. The differential set was composed of 23 wheat varieties/lines containing know *Stb* resistance genes. The experiments were conducted under controlled environment on seedlings. Tests were evaluated 21 days after inoculation. Plants were assessed in terms of the percentage of second leaf area covered with necrosis (NEC) and pycnidia (PYC). Precise determination of disease parameters was made using computer image analysis of infected leaves.

The tested *Z. tritici* isolates exhibited high diversity in terms of both disease parameters. Among the varieties/lines of the differential set, the M3 Synthetic (W-7976) line (containing genes *Stb16q* and *Stb17*) exhibited the highest levels of resistance throughout the years, with a maximum NEC value of 8% and no pycnidia formation observed for any of the tested isolates. Most of the tested isolates were virulent against varieties/lines containing genes *Stb1*, *Stb4*, *Stb5*, *Stb6*, *Stb7* and *Stb9*.

Assessing stripe rust (*Puccinia striiformis* f. sp. *tritici*) population structure using molecular methods

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Keywords: MARPLE, SSR, yellow rust, pathogen

Yellow rust caused by the fungus *Puccinia striiformis* f. sp. *tritici* (Pst), may induce severe yield losses under favorable conditions. It is considered one of the most important pathogens of wheat. The risk of the decreasing quantity of yield depends on the frequency and speed of spreading of new fungus races. Knowledge of the pathogen population structure is one of the key elements of resistance breeding and sustainable agriculture.

In this study 48 single uredinium Pst isolates obtained from wheat and triticale from four localizations in Poland in year 2025 were assessed. DNA from Pst isolates was extracted using CTAB method. Assessment of the isolates was carried out using two methods: analysis of SSR profiles for 19 loci (Ali et al. 2011) and MARPLE (Mobile And Real-time PLant disEase; Radhakrishnan et al. 2019).

The SSR method can be easily implemented in most laboratories using existing equipment and is a robust tool used to characterize isolate races. MARPLE allowed us to obtain much more data and information about the isolates, however it takes up more resources, both in consumable reagents as well as computing power and data disc storage.

The *P. striiformis* population in Poland was diverse. Both methods allow us for rapid identification of known races or new variants. Monitoring of the pathogen population and knowledge of its genetic structure is very important for crop protection and resistance breeding.

Early defence gene activation in Oat–*Puccinia coronata* interactions

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Keywords: oat, *Puccinia coronata*, gene expression, defence-responsive genes

Oat (*Avena sativa* L.) breeding in Poland has been conducted since the late 19th century, and currently, according to FAO statistics, Poland is among the world's top four oat grain producers. Despite the annual release of new oat varieties, most remain susceptible to crown rust caused by *Puccinia coronata* f. sp. *avenae*, leading to major yield losses. Fungicide use is common to prevent crown rust development, but breeding for resistance is a more sustainable approach and in line with the European Green Deal. Effective resistance breeding requires understanding molecular defence mechanisms, still poorly characterised in oats. This study compared the expression of selected defence-related genes in an oat line carrying the *Pc39* resistance gene and the susceptible cultivar Kasztan during incompatible (resistant) and compatible (susceptible) interactions with *P. coronata*. Gene expression was analysed at six time points post-inoculation, covering key pathogen development stages. Both genotypes responded rapidly, with changes visible as early as 8 hpi, and peak expression for many genes was between 16–24 hpi. *PR8* and *POD* showed the highest induction in both genotypes regardless of interaction type. *PAL* and *NADPH RbohD* had slightly higher expression in Pc39 during compatible interaction. *CAT*, *PR1-GLUC* and *PR4* were markedly higher in Pc39 during incompatible interaction, indicating their potential as resistance markers. Identifying such genes may support early diagnosis of resistance and marker-assisted selection. Expanding analyses to other *Pc* genes could reveal universal defence-responsive genes and guide breeding strategies. Insights from these findings may also enable targeted enhancement of immune responses using genetic engineering.

Mycotoxin profiles of Tritordeum grain after artificial spike inoculation with *Fusarium culmorum* W.G. Smith

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Keywords: Tritordeum, *Fusarium head blight*, multi-mycotoxin analysis, artificial inoculation

The responses to artificial spike inoculation with *Fusarium culmorum* (DON chemotype) were compared in 11 Tritordeum breeding lines, two durum wheat cultivars and one naked barley cultivar. Inoculation led to a significant decrease in spike weight, kernel weight per spike, and one kernel weight (by 18%, 28%, and 16%, respectively). Durum wheat responded most strongly to inoculation. The content of 31 mycotoxins differed considerably in the grain of 11 Tritordeum lines. Artificial inoculation induced a significant increase in the total concentration of trichothecenes (9902 vs 558 μg/kg in non-inoculated control) and other Fusarium toxins (40207 vs 3250 μg/kg in non-inoculated control). The content of three Alternaria toxins was not significantly modified by inoculation. The principal component analysis (PCA) of all fungal metabolites supported the discrimination of control and inoculated grain, and the results were used to divide the examined Tritordeum lines into two groups with different mycotoxin concentrations and profiles. The analyzed Tritordeum lines responded differently to artificial spike inoculation, which suggests that lines with a low propensity to accumulate Fusarium toxins in grain can be selected from the existing gene pool. The study also demonstrated that Tritordeum grain accumulates significantly smaller amounts of mycotoxins than durum wheat grain.

Acknowledgments

This research is the result of a long–term study carried out at the University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Genetics, Plant Breeding and Bioresource Engineering, topic number 30.610.007-110.

Natural products as biochemical defenses in biotic stress response: insights from *Toxocarpus brevipes*

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Plants are continuously exposed to a variety of biotic stress factors, including bacterial, fungal, and insect attacks. A fundamental survival strategy against these stresses is the synthesis of secondary metabolites that act as chemical defenses. These phytochemicals not only protect plants from pathogens but also play important ecological roles and represent promising scaffolds for drug discovery.

This study focuses on the phytochemical composition and biological activities of *Toxocarpus brevipes*, a lesser-known medicinal plant traditionally employed in West African ethnomedicine. Leaf and stem extracts were subjected to chromatographic separation and spectroscopic characterization (GC–MS, FTIR, NMR) to identify bioactive metabolites. The antimicrobial and antioxidant properties of the isolates were further assessed using conventional assays complemented by novel electrochemical sensor-based screening.

Results indicate that alkaloids, flavonoids, and terpenoid derivatives from *T. brevipes* exhibit strong antibacterial activity, particularly against Gram-positive pathogens, while also displaying high antioxidant capacity. These findings highlight their potential ecological role in defending the plant against microbial invasion and oxidative stress. Moreover, they provide chemical evidence that natural products are central to plant biotic stress response.

Susceptibility of durum wheat to infections caused by *Fusarium graminearum, Blumeria graminis* f.sp. *tritici* and *Zymoseptoria tritici* in Poland

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Keywords: *Triticum durum*, mycotoxins, fungal diseases

Great challenge in the durum wheat production is its high susceptibility to fungal pathogens. The aim of this study was to identify spring cultivars of durum wheat that are less susceptible to *Fusarium graminearum* infections and the accumulation of deoxynivalenol (DON) in grain, as well as infections caused by *Blumeria graminis* f.sp. *tritici* and *Zymoseptoria tritici*. Two experiments were established in different locations in Poland. In 2018-2019, symptoms of *Fusarium* head blight were noted sporadically, whereas in 2020, the disease was endemic in both experimental sites. In 2020, symptoms of *Fusarium* leaf blight were observed in southern Poland, and symptoms of STB were noted in the northern part of the country. The severity of powdery mildew was low, and disease symptoms were observed mainly on lower leaves in all cultivars. Fhb2, Stb1, Stb2, Stb3, Pm36, and Pm41 alleles were identified in the tested cultivars. Cultivar IS Duragold grown in a greenhouse was least susceptible to primary infection by *F. graminearum* and the growth of fungal hyphae on the rachis. After inoculation, DON was accumulated in spikelets in all cultivars, in particular Duramant (1582 mcg/kg) and Durasol (1455 mcg/kg). Most suitable for cultivation were cultivars IS Duragold and IS Duranegra.

Acknowledgments
This research is the result of a long-term study conducted at the Department of Genetics, Plant Breeding and Bioresource Engineering and Department of Entomology, Phytopathology and Molecular Diagnostics Faculty of Agriculture and Forestry of the University of Warmia and Mazury in Olsztyn (research topics 30.610.007-110 and 30.610.011-110).

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T4: Phenotyping Technologies – IPPN CEPPG Session

Image-based classification of breeding trial plots using the ENVI software

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Keywords: UAV, small plots, cereals, ENVI, classification methods

Automated field phenotyping platforms offer a powerful means of continuously and accurately measuring key phenological traits throughout the plant breeding process. High-frequency, high-resolution monitoring of plant growth and development enables deeper insights into genotype–phenotype relationships, including gene-regulated yield potential and pathways of environmental adaptation.

While the use of drones in agriculture has become routine, their application in plant breeding and research still faces many challenges. One of the primary tasks is to identify appropriate image processing methods for interpreting the vast amount of digital image data generated during each flight/survey. These methods are essential for effectively distinguishing between varieties, detecting infection hotspots, and identifying the presence of abiotic and biotic stress-factors.

The aim of our work is to identify a classification method capable of distinguishing between different species and varieties in multispectral images, and eventually between healthy and stressed (abiotic/biotic) plants. To this end, we started the "Drone" experiment in the breeding nursery of the Cereal Breeding Department of HUN-REN ATK in Martonvásár. The experiment included eight winter wheat varieties with varying sensitivity levels, one durum wheat, one triticale, and one winter barley. When selecting the varieties for the field trial, it was important to include both disease-susceptible and tolerant genotypes.

Flights were conducted during two phenological phases, and phenotypic data were recorded continuously throughout the growing season. Orthophotos were stitched together, NDVI maps were created, and four different classification methods were run in the ENVI software using masked experimental plots. We evaluated the resulting images and statistical data (error matrix, commission-omission classification errors).

The Maximum Likelihood Classification algorithm yielded the highest accuracy percentage (67.07%). The Minimum Distance Classification and Mahalanobis Distance Classification metrics had similar accuracy values (45.84% and 53.28%, respectively), while the lowest accuracy was observed with the Parallelepiped Classification method (30.96%). Despite the fact that the diagonal values of the error matrix for the Parallelepiped Classification often do not indicate correct pixel classification, we still consider it worth further testing in areas affected by stress.

Acknowledgments

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T5: Yield and Quality Improvement

Naked and hulled oat (*Avena sativa* L.) breeding in Lithuania

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Keywords: naked oat, hulled oat

Oat breeding in Lithuania was started more than 100 years ago. Until the 20th century, only hulled oat varieties were bred. However, in 2001, the first cross with Czech naked variety Abel was made. Consequently, the first Lithuanian naked oat variety Mina DS was registered in 2010. Today we have 4 hulled varieties and 3 naked oat Lithuanian varieties in the Lithuanian National List of Plant Varieties. Hulled varieties Viva DS was registered in 2015, Frekula DS in 2020, Svaja DS in 2023, Simer DS in 2024. Naked varieties Milija DS and Agoda DS were registered in 2021 and 2022. The newest naked variety Vainius DS was registered in 2025.

Naked oat usually has lower yield and higher protein, fat content and hectolitre weight than hulled oats. The average yield in 2024 was 3.04 t ha^{-1} for hulled oats and 1.63 t ha^{-1} for naked oats in oat variety testing trial at Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture. It was 53.62 percent lower than that of hulled oat. However, protein content and hectoliter weight were significantly higher in naked oats. The average protein content was 13.84 percent for hulled oats and 18.19 percent for naked oats, and the hectoliter weight was 47.5 and 60.0 kg/hL, respectively.

Naked varieties Mina DS, Milija DS and Agoda DS were tested at Lithuanian Plant Variety Testing Stations in 2020. According to the data, the best yielding variety was Mina DS. The average yield of Milija DS was 93.13 and the yield of Agoda DS was 92.43 percent comparing with Mina DS. However, varieties Milija DS and Agoda DS had lower husk percentage. It was 5,73 percent for Mina DS, while 3.60 and 1.03 percent for Milija and Agoda. The new varieties also had better TGW and hectolitre weight. TGW of Mina DS was 26.66, whereas Milija DS and Agoda DS had 28.30 and 28.82 g, respectively. Hectolitre weight was respectively 60.33, 64.30 and 66.80 kg/hL. All varieties had high fat and protein content. Mina DS had 7.33 percent of fat and 16.03 percent of protein, Milja DS respectively 7.57 and 15.23 and Agoda DS 7.27 and 15.27.

The newest naked oat variety Vainius DS shows high yield under ecological growing practices and high (often higher than 20 percent.) protein content.

CRISPR-Cas9 mediated knock-down of HvNAC94 transcription factor reveals its regulatory role on HvKCX1 gene expression in barley

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Keywords: CRISPR/Cas9, transcription factors, stress regulation, Barely, *HvNAC94*, *HvCKX1*

Transcription factors (TFs) play a key role in gene expression regulation and, consequently, in plant development and stress response. In our previous study, we have shown that TaNAC transcriptions were potentially involved in the regulation of members of the *TaCKX* gene family. The barley (*Hordeum vulgare* L.) *HvNAC94* transcription factor is hypothesized to regulate the *HvCKX1* gene, which is involved in major physiological processes. In the present study, we used Yeast One-Hybrid (Y1H) assays to demonstrate the direct interaction of *NAC94* with the *CKX1* gene promoter and thereby validate its role as a potential transcriptional regulator. To further investigate the functional relevance of *HvNAC94*, we performed CRISPR-Cas9 mediated gene knock-down in barley plants and were successful in generating T_0 transformants. Transformation was verified by PCR analysis, and knock-down efficiency of the gene was up to 91%, as verified by sequence data analysis. The results indicate a competent gene editing process that allows detailed functional analysis to be performed. Plants are currently in the T_0 generation, and further molecular and physiological characterizations will be carried out to disclose the downstream effects of *HvNAC94* knock-down on the expression of *HvKCX1* gene and the plant phenotype. The forthcoming data will educate us about the *HvNAC94* regulation network and its role in the adaptation regulation of barley stress. This study demonstrates the ability of CRISPR-Cas9 technology to decipher gene function in barley and points to HvNAC94 as a key regulator of *HvKCX1* expression. Our findings will be useful in the investigation of transcriptional regulation in cereals and can have implications for crop improvement programs.

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CAPS-based insights into barley's genetic potential

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Keywords: crop productivity, cultivation, DNA marker, genetic variation, genomic-assisted selection, *Hordeum vulgare* L., molecular breeding

Barley is one of the most important cereal crops worldwide, playing a critical role in global food security, livestock feed, and industrial applications. Its adaptability to diverse environmental conditions and relatively short growing cycle have made it a model species in both agronomic and genetic studies. Traditional selection for desirable agronomic traits is labor- and time-consuming, relying on breeders' evaluation. Recent advances in molecular genetics and genomics have significantly accelerated the pace of barley improvement, enabling the development of cultivars with enhanced yield potential, stress tolerance, and grain quality traits. One of the key approaches driving this progress is marker-assisted selection (MAS) based on DNA markers. Therefore, the aim of this study was to develop molecular markers using the Cleaved Amplified Polymorphic Sequences (CAPS) system targeting selected yield components for the rapid and efficient screening of large breeding germplasm collections as potential MAS tools.

To address this objective, selected CAPS markers were designed based on the sequence of genes of interest and tested on a pool of 56 different barley genotypes to investigate previously uncharacterized genetic polymorphisms. Only primers exhibiting 100% efficient PCR amplification were applied, confirming the broad applicability of these primers across diverse germplasm. The PCR products were then digested with specific endonucleases to distinguish SNP allelic variations in all tested genotypes. For example, we identified genotypes carrying different SNP allelic variants (T/C) in the *HORVU.MOREX.r3.7HG0658720* gene, which is involved in determining plant height. Breeding barley with reduced plant height is advantageous, as it lowers the risk of lodging and improves shading around the plant due to lower-set leaves, thereby reducing water evaporation from the soil. The use of this marker in breeding could therefore facilitate the rapid selection of barley genotypes with reduced height. In addition to plant height, we identified sequences suitable as CAPS markers for estimating other phenotypic traits, such as the number of grains per spike (three sequences) and thousand grain weight (two sequences). Our findings demonstrate that CAPS analysis can reveal the functional impact of sequence polymorphisms on phenotype and productivity. Functional SNP allelic variants may encode proteins with altered efficiency, as nonsynonymous SNPs change the amino acid sequence of the encoded protein, potentially leading to measurable phenotypic differences.

CAPS analysis enabled high-resolution mapping of genetic diversity and the development of robust, high-throughput genotyping platforms for precise identification of favorable alleles. By revealing novel allelic variants, it provides critical insights into the genetic architecture of key agronomic traits, facilitating precision breeding strategies that accelerate the development of elite barley cultivars tailored to specific agro-ecological conditions and resilient to current and future climatic challenges.

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Variation of grain yield and grain protein content in random recombinant wheat lines from a cross between contrasting parents

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Keywords: wheat descendent lines, protein %, yield, regression GY/GPC

Obtaining productive wheat lines with high grain protein content is the goal of most breeders in the word. Two contrasting wheat parents for these important trais, grain yield and grain protein content, namely UNITAR and AMURG, were crossed. UNITAR is an advanced breeding line, obtained from two Romanian mutant lines, M1 Izvor/M1 00628G34 and was used as yielding parent, while AMURG is an advenced breeding line obtained from Murga/F03124G//Pitar which was used as highest quality parent. MURGA is a Mexican variety containing *Aegilops tauschii* and PITAR is a Romanian variety with very good quality properties. The high grain protein percent from AMURG line might be due to the presence of genes introgressed from *Aegilops tauschii* (from MURGA variety), possibly complemented with favorable genes present in the Romanian cultivar PITAR.

One hundred-forty $F_{5,3}$ lines were randomly extracted from this cross and tested on plots during two seasons, 2023-2024 and 2024-2025, along with the two parents, replicated five times. The correlation between grain yield and grain protein content in the two parents was significantly negative, with all AMURG plots having higher grain protein but lower yields than UNITAR plots. There was no correlation between grain yield and protein concentration in lines randomly extracted from the cross UNITAR/AMURG. Many lines overyielded the higher-yielding parent UNITAR but no line had higher protein concentration than AMURG. The aim of this research was to highlight some wheat lines with higher grain yield than the highest-yielding parent, UNITAR, while having improved protein concentration.

Silage-focused triticale breeding in Hungary

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Keywords: triticale, silage, biomass yield, variety trial

The youngest of the cultivated small grain cereals is triticale (×*Triticosecale* Wittm.), which was created as an artificial hybrid of wheat and rye at the end of the 19th century. As result of the intensive breeding carried out since then – in which Árpád Kiss from Hungary played a pioneering role – secondary hexaploid triticale varieties were created, that are now cultivated on nearly 4 million hectares worldwide. It stands out among other cereals with its abiotic stress tolerance and its huge biomass and grain yield, which is why it has become one of the most popular cereal crops on marginal arable lands, and is even used for grazing in many countries. As a result of climate change, increasingly larger areas will be characterized by more extreme growing conditions, so the role of triticale is expected to increase in the future. Silage corn, which forms the basis of the huge amount of fermented plant biomass feed required by farms keeping ruminants, is being displaced from more and more arable lands affected by climate change, and one of the most suitable cereals (along with rye) to fill this gap is the drought-tolerant triticale.

The joint proposal of Hungarian triticale breeders based on preliminary research results brought to life the green biomass-focused variety qualification procedure that can be requested in addition to/instead of the grain yield-focused variety qualification of the species in the official state variety tests of NÉBIH (National Food Chain Safety Office, Hungary), which entered their sixth year this year. A consensus has also emerged among breeders that the green biomass yield should be examined in the most optimal plant development stage in terms of nutritional quality (i.e. booting stage, BBCH45-47). NÉBIH also uses this method, the plants are cut at a dry matter content of approx. 20% and their quality is examined: the Net Energies for Meat Production, Living and Milk Production (MJ/kg dry matter) are measured, as well as their sum and the value per hectare (Energy yield: GJ/ha).

Thanks to this certification system, the first dual-purpose triticale variety in Hungary ('GK Trivita') was born at the beginning of 2024, and a year later the first triticale variety for silage, 'Mv Eridanus' was registered. The dry matter yield of this latter variety (7.27 t/ha out of 40.54 t/ha green yield) significantly exceeded the average of the standard varieties ('Mv Sámán', 'Dimenzio', 'Hungaro') by 13.1% and its Energy yield (111.33 GJ/ha) by 11.5% in the three-year (2022-2024) state variety trials of NÉBIH.

From the point of view of seed production, it is an important issue that the grain yield of a variety that performs so outstandingly in green biomass yield should also be competitive (not tested by NÉBIH). Therefore, the selection of silage triticale breeding lines at Martonvásár is also based on grain yield, which is carried out every year in a small-plot, three-replication experiment set up at two production sites using the standard varieties included in the grain yield focused national variety tests ('Mv Talentum', 'GK Maros'). Based on the results of the past 6 years, it can be stated that grain yield of ‘Mv Eridanus’ is on average only 6% lower than the average of standard varieties, even more, it exceeded them in half of the years (2021, 2023, 2024). Based on our results, ‘Mv Eridanus’ has all the characteristics that could make it a successful mass feed producing winter triticale variety in Hungary.

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Impact of plant protection and fertiliser regimes on the performance of winter wheat

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Keywords: *Triticum aestivum,* plant protection cost, nitrogen input, tillage systems, crop rotation

Soft wheat (*Triticum aestivum*), particularly winter wheat, is the main cereal crop in Latvia, with areas increasing by over 30% in the past decade. While pesticide use is often studied separately, in practice management measures interact, affecting resource allocation, including nitrogen fertiliser. This study evaluated the winter wheat performance regarding yield and protein content under diverse pesticide inputs and nitrogen rates, considering different crop rotations and tillage systems at practical farm conditions.

Field survey was conducted on 150 fields across Latvia. Data from 2020 were selected for 55 farms with winter wheat fields within the 2018–2022 period due to representative weather conditions. Winter wheat was grown in diverse rotations: 7% monoculture, 36% wheat–rapeseed, and 56% in rotations with more than two crops. Analyses considered low (≤150 kg N ha^{-1}) and high (>150 kg N ha^{-1}) nitrogen inputs, and conventional versus reduced tillage. The total pesticide cost per tonne of yield (EUR), the percentage share of pesticide costs, and pesticide inputs (EUR ha^{-1}) by category (fungicides, herbicides, insecticides, growth regulators, seed treatments, and desiccants) were evaluated.

Winter wheat yields were significantly higher under high nitrogen input. However, in **43%** cases conventionally tilled fields under high nitrogen inputs, the applied nitrogen did not achieve expected yields based by the Latvian Rural Advisory and Training Centre Crop technological models*. Grain protein content significantly increased under high input, averaging ~13%. Herbicides and fungicides accounted for a higher percentage share of pesticide applications across all management regimes, indicating their dominant contribution to overall pesticide input. Expenditures on herbicides, fungicides, and growth regulators were significantly higher under high-input of N fertiliser level.

Higher yields and improved grain quality require increased N fertiliser input and thus higher pesticide input. Economic outcomes are determined by the balance between crop performance, and input intensity, production costs, emphasizing the importance of optimal management decisions for efficient winter wheat production.

Acknowledgments

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References

1. Latvian Rural Advisory and Training Centre. Crop Technological Models. Unpublished data of the EIP-Agri project “Development of electronic farm management system” (No. 18-00-A01612-000018).

Evaluation of yield and qualitative performances of winter two-rows barley genotypes under nitrogen fertilization conditions and climatic variability

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Keywords: two-rows barley, yield, seed size, thousands grain weight, protein content, starch content

Optimization of nitrogen fertilization in the context of increasing climatic variability represents a determining factor in maximizing production and improving the quality of winter barley. The present study aimed to evaluate the response of different winter two-rows barley genotypes created at National Agricultural Research and Development Institute to nitrogen fertilization under contrasting climatic conditions and to evaluate yield and qualitative performances over the medium term.

Ten winter two-rows barley genotypes consisting of 5 registered varieties (Artemis, Gabriela, Diana, Ileana and studied Check) and 5 perspective lines (DH 403-12, DH 417-12, DH 425-3, DH 425-4, F8-114-10) were analysed for three consecutive years (2023-2025) with distinct climatic conditions. The experiment was conducted in Southeast of Romania plain on cambic chernozem soil organized in a bifactorial design, with two nitrogen fertilization levels: unfertilized treatment (0 kg urea/ha) and fertilized treatment (100 kg urea/ha). Five parameters were determined for each genotype: grain yield (kg/ha), thousand grain weight (TGW, g), assortment - representing the proportion of seeds with diameter > 2.5 mm from 100 g sample (%), protein content (%), and starch content (%). Climatic conditions were continuously monitored from sowing to harvesting recording rainfall and temperatures for each month of the experimental period.

Nitrogen fertilization determined a significant average yield increase of 812 kg/ha (+13.4%), from 6067 ± 711 kg/ha in the unfertilized treatments to 6879 ± 856 kg/ha in the 100 kg N/ha treatments, but with considerable annual variability influenced by climatic conditions. The year 2023, characterized by severe drought during autumn sowing in 2022, (59.5 mm rainfall below multiannual average of the same period), recorded the smallest fertilization effect (+437 kg/ha), while 2025, with optimal spring conditions and rainfall closer to multiannual average, showed the maximum effect (+1,627 kg/ha). Yield ranged between 4475 and 9,168 kg/ha, demonstrating both genetic diversity and environmental impact. Genotypes with the highest performances under fertilization conditions were the studied Check (7442 kg/ha), Gabriela (7233 kg/ha), and Diana (7204 kg/ha), maintaining stability across all experimental years. Assortment (seeds > 2.5 mm) varied between 25.96% and 99.7% from 100 g sample, with significant improvement determined by nitrogen fertilization (+9.83%), from 77.88% in the unfertilized variant to 87.71% with 100 kg N/ha. Genotypes with superior seeds size performance were DH 425-3 (93.99%), DH 417-12 (93.91%), and DH 425-4 (93.9%), while some genotypes such as Diana showed particularly favourable response to fertilization (+21.75%). Thousand grain weight showed variations between 34.65 and 62.9 g, with superior performances in genotypes DH 425-4 (48.35 g) and DH 425-3 (47.88 g), highlighting positive correlation with seed size and the importance of these parameters for commercial quality, especially for malting industry. Protein content varied between 9.5% and 20.5%, with maximum values recorded for Ileana (16.06%), DH 417-12 (14.4%), and DH 425-4 (13.97%), being influenced by both genotype and fertilization × climatic conditions interaction while starch content ranged between 53,8 % (F8-114-10) and Ileana variety 66,2 %.

The identified genetic variability and genotype × environment × fertilization interaction confirm the necessity of adaptive selection and differentiated fertilization strategies based on forecasted climatic conditions, yield and quality objectives.

Molecular and genetic determinism of sorghum grain quality

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Keywords: sorghum, grain quality, starch, protein, protein digestibility, gene co-expression network, protoplast transient over-expressions, transcription factor, GWAS

Sorghum grains are rich in proteins and starch but exhibit low protein digestibility, limiting their use in food and feed. However, the genetic and molecular mechanisms underlying these traits remain poorly understood, particularly the genomic regions involved, as well as the structural genes and transcription factors (TFs) that regulate them, hindering efforts to improve sorghum grain quality. To address this, we adopted an integrated genomic and genetic approach. At the genomic level, we constructed a gene co-expression network using transcriptomic data collected during grain development (10 stages) over two years. In parallel, we quantified starch and protein content and measured protein digestibility. Two major gene co-expression modules were identified. The first was linked to the loss of protein digestibility, involving genes related to disulfide bond formation and modulation. The second contained most kafirin and starch metabolism genes, as well as orthologs of TFs known to regulate protein and starch accumulation in other cereal species. Functional assays performed in protoplasts for six TFs revealed a central role for SbPBF1a, SbPBF1b, and SbNF-YC13 in modulating the expression of genes involved in protein and starch biosynthesis. In the genetic approach, we used a diversity panel of 300 sorghum genotypes representing global genetic diversity. This panel was cultivated at two sites in France over two growing seasons. Protein, starch contents and protein digestibility were measured. Whole-genome sequencing (WGS) was performed for all genotypes. These biochemical and genotypic data were analysed through genome-wide association studies (GWAS) using both single and multilocus models. Several significant SNPs were identified near genes previously associated with grain quality traits. Together, our work provides novel insights into the genetic basis and transcriptional regulation of protein and starch accumulation, as well as protein digestibility in sorghum grains. It also identifies regulatory and structural genes that could be targeted to enhance grain quality, thereby supporting the development of improved sorghum varieties with higher nutritional value.

Multivariate analysis of molecular mechanisms associated with yield in common maize *Zea mays*

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Keywords: corn, yield, next-genertion sequencing (NGS), association mapping, physical mapping, molecular markers

The answer to the needs of the agricultural sector in the 21st century is the implementation of biological progress in agricultural production, which allows for the improvement of food production and quality while improving the degree of utilization of inputs on chemical and technical means of production. Biological progress in the corn breeding and seed industry is unique in terms of the social and ecological aspects of innovation. It influences agricultural productivity and adapts cultivated corn varieties to market requirements and changing climatic conditions, but does not do so at the expense of the environment. Modern corn breeding around the world is based on a wide range of research techniques in the field of molecular genetics. Thanks to these technologies, we can identify regions of the genome associated with various phenotypic traits, including yield, which is fundamental to understanding and manipulating these regions. Therefore, the aim of the study was to identify new molecular markers coupled with candidate genes that determine grain yield, kernel germination and early vigor of corn, thanks to the use of next-generation sequencing, association and physical mapping.

As a result of the analyses, 20 molecular markers were identified that were related to the germination capacity of grains and the vigor of seedlings. Of these, 6 markers were located inside genes. In 3 cases, according to literature reports, these genes may affect grain germination and vigor in maize (cinnamoyl-CoA reductase 1, WAT1, eukaryotic translation initiation factor 3 subunit c). In the next stage of research, a total of 36 molecular markers related to crop structure features and yield were identified. Of these markers, 11 are located inside genes. In 5 cases, according to the latest literature reports, these genes are responsible for the yield (RNA polymerase II transcriptional coactivator KELP, aspartate aminotransferase, sucrose transporter I, arabinosyltransferase arad I, hydroxyproline o-galactosyltransferase). All identified, statistically significant molecular markers can be used in the maize breeding process to select parental components for crossbreeding. This will shorten the breeding cycle and at the same time save material resources.

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Barley grain composition profile of released cultivars over seven breeding decades

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Keywords: barley, cultivars, quality grain traits, breeding

Barley breeders' efforts have been focused on particular breeding paths regarding the quantitative and qualitative potential of a cultivar and the end-use of the grains: malt, beer, animal feed, and human nourishment.

In this study, two years (2018 and 2019) are used to analyse the main biochemical grain traits of twenty-seven barley cultivars (six-row and two-row) released by the National Agricultural Research and Development Institute Fundulea between 1968 and 2023. Seven traits, namely neutral detergent fibre (NDF), ash content (ASH), crude fibre content (CF), acid detergent fibre (ADF), hemicellulose (HCELL), protein (P) and starch (S) content, were used to characterise the chemical grain composition.

An FT-NIR spectrometer TANGO with OPUS software was used to measure these characteristics on the whole grain flour. The results were expressed on a dry weight basis and statistically processed using the PAST program, version 4.02. The average, minimum, maximum, standard deviation and coefficient of variation were calculated. The Pearson coefficient of correlation was used to determine the degree of variation between the analysed traits.

According to analysis of variance ($p < 0.05$ level), year, cultivar and year × cultivar interaction had a significant effect on the studied traits. The data analysis of the obtained results revealed considerable differences among cultivars in terms of row type (six rows or two rows), and due to the influence of the year.

Acknowledgments

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Yield and hectolitric weight variability evaluation of some winter wheat cultivars in the South-East of Romania

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Keywords: no-till, minimum till, rotation, crop debris, yield, HW

In the latest, more farmers from Romania intend to use the seeders for heavy soil for direct seeding (no-till), minimum till or conventional till to obtain high yield and good quality of wheat grains.
In this paper are presented the obtained results of some winter wheat Romanian cultivars, under no-till and minimum till conditions.

Two experiments were performed in the field of Conservative Agriculture at the National Agriculture Research and Development Institute Fundulea, Romania, during the 2023-2024 year, under no-till and minimum till conditions, using in rotation two different previous plants (soybean and maize).

The first objective of this study is to see if the different types of soil preparation influenced the yield and hectolitric weight (one of the important wheat seed quality parameters).

In the context of dramatic changes in climatic conditions over the last years, the second objective of this study was to find a solution to increase the yield and quality of wheat by testing different cultivars under different technologies used in conservative agriculture.

Analysis of variance revealed a significant influence of cultivars, no-till method and previous plant (soybean) on the yield and HW under no-till conditions. In the case of the minimum till method, it must be underlined that the influence of cultivar, method and cultivar x method interaction was significant on the yield after maize as the previous plant, and only cultivar and method significantly influenced HW.

This way, the study of different genotypes provided details about the reactions regarding the level of yield and the values of HW and the best results were obtained by all cultivars (Glosa, Otilia, Pitar, Ursita, Voinic, FDL Abund, FDL Columna, and FDL Consecvent) under no-till conditions (direct seeded). The highest yield and value of HW were registered by the FDL Columna cultivar under both conditions and previous plant. According to the results, after at least two testing years, the recommendations for farmers will be elaborated.

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Identification of molecular markers linked with Minolta b* value and gluten index in a winter durum wheat biparental mapping population

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Keywords: *Triticum turgidum* ssp. *durum*, yellow pigment, gluten strength, molecular mapping

The chromosome regions determining the Minolta b* value and gluten index were examined in a biparental mapping population. Parental lines with extremely high and low Minolta b* values were selected to create the population. The high yellow pigment parent was the durum wheat line 'PWD1216' (Saatzuch Donau GmbH, Probstdorf, Austria), and the low one was the 'MvTD10-98' from Martonvásár. The gluten index of the lines also proved to be diverse.

RAPD, AFLP, SSR and DArT markers were used to create the linkage map necessary to identify the chromosome regions linked to the Minolta b* value and gluten index. After excluding redundant and non-Mendelian markers, the final version of the linkage map was created using 454 markers. The mapping was performed using JoinMap 4.0 software. The 14 chromosomes of durum wheat contained a total of 31 linkage groups. The chromosomes were composed of 1 (1A, 1B, 4B), 2 (2A, 4A, 6A, 6B, 7B), or 3 (2B, 3A, 3B, 5A, 5B, 7A) linkage groups. The total length of the map was 1550.1 cM. After compiling the map, the chromosome regions linked to the Minolta b* value and the gluten index were identified by QTL analysis using MapQTL5 software. The calculations were performed based on the average values as well as on the annual data.

In the 'PWD1216/MvTD10-98' population, the two technological quality traits were controlled by a complex genetic background. QTLs determining the Minolta b* value or gluten index were identified on 12 of the 14 durum wheat chromosomes, but based on the average values, loci controlling both traits can be found on chromosomes 1A, 1B and 5A. In the case of Minolta b*, the QTLs located on chromosomes 3B and 7A were found to have significant effects in the vast majority of years, but the loci mapped to chromosomes 1B, 2B, 3A, 3B, 4B, 5A and 5B also appeared in several years and in the mean of the years. The gluten index was basically determined by the homeologous chromosomes 1 in most years, but for this trait there was also a region on chromosome 5A whose effect could only be identified in two years.

For Minolta b*, four markers and one for gluten index were extremely closely linked to the QTL determining the traits, and only three markers were located more than 5 cM away from the position with the highest LOD value. Phenotypic variance was determined by several QTLs with a greater than 10% effect. For Minolta b*, the region with the strongest effect was located on the long arm of chromosome 7A (29.6%), followed by QTL identified on chromosomes 3B (12.8%) and 1B (10.8%). Gluten index was essentially determined by a locus located on chromosome 1B, which explained 62.4% of the total phenotypic variance observed in the population. The location of the qGI_1B locus with the strongest effect on gluten index, is similar to the Glu1-B1 locus encoding HMW glutenin subunits. In these cases, a causal relationship can be clearly demonstrated. The 7+8 pattern characteristic of the parent ‘MvTD10-98’ was identified in all lines with strong gluten structure, while the same subunit composition as the parent ‘PWD1216’ was present in all lines with low gluten index.

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Identification of heritable androgenic capacity determinants via crossbreeding

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Keywords: rye, androgenesis, trait transfer, selection marker, androgenesis capacity

Contemporary rye breeding, carried out through hybrid breeding, involves crossing homozygous male-sterile maternal lines with paternal lines that restore fertility. This approach generated a strong heterosis effect in the F1 generation. Traditionally, homozygous lines are derived through several years of intensive inbreeding and enforced self-pollination to overcome rye's natural self-incompatibility. The incorporation of homozygous doubled haploid (DH) lines has become an integral part of recent plant breeding strategies at some stage of variety development (Marulanda et al., 2016). The use of androgenesis process offers the potential to significantly shorten the rye breeding cycle in a highly competitive environment among European breeding companies. However, limited knowledge of the genetic mechanisms underlying androgenesis in rye restricts the application of this method to in both *in vitro* cultures and genetic manipulation. The question arises as to whether an androgenic line can pass this ability to its offspring, and consequently to valuable breeding lines? Following many years of research onto androgenesis in different cereal plants we have demonstrated that the capacity for androgenesis is species dependent. In this context, rye is one of the most recalcitrant species, next to oats.

The experiments presented tested twenty rye genotypes to identify lines with minimal *in vitro* androgenic response. Of these, fourteen were able to induce androgenic structure (AS) formation, but only in limited quantities, ranging from one to twenty-seven AS. In contrast, the androgenic control line produced 1,306 AS under the same conditions. These studies led to selection of lines that did not respond to reprogramming cells of gametophytic pathway to form AS under the applied experimental conditions. One such non-responsive line was crossed with a highly androgenic line (8J), and the ability of the resulting progeny to undergo androgenesis was assessed. Control lines (the parental lines used in the cross) confirmed their negative (line 12S) or positive (line 8J) predisposition to induce androgenesis. Among 80 progeny plants, more than 70% showed the ability to induce androgenesis. Notably, 14% of these plants exhibited a higher capacity for androgenesis than the positive control line (8J).

These results suggest that the ability to undergo androgenesis can be transmitted by crossbreeding, although this trait is not uniformly inherited across all genotypes.

With a goal to identify a genetic marker for androgenesis capacity in rye, we created an F2 population derived from crossing lines with a contrasting capacity for this trait. We again characterised this generation based on their androgenesis capacity, allowing a selection of contrasting individuals for a transcriptomic study. Thus, we were able to identify transcription factors, chromatin re-modellers, and other regulatory genes that differed significantly in their expression levels between the plants. We will use these candidates to design PCR reactions (markers) specific to variants that correlate with the androgenic phenotype.

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References
1. Marulanda JJ et al (2016) Theor Appl Genet 129:1901-1913 doi:10.1007/s00122-016-2748-5

T6: Bioinformatics and Genome Editing

Effect of *HvCKX9* gene knockout on plant growth and yield-related traits in barley

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Keywords: cytokinin, cytokinin dehydrogenase, CRISPR-Cas9, gene knockout, barley

Cytokinins (CKs) are a class of plant hormones that play an important role in a wide range of developmental and physiological processes. CK levels are tightly regulated by enzymes encoded by several multigene families, in which individual members tend to have tissue- and developmental-specific expression patterns.

Previous studies have shown that changes in the expression levels or knockout of specific CK metabolic genes in crop plants can significantly affect plant agronomic traits and modulate their response to various stresses. Our main research targets are genes encoding isopentenyl transferase (IPT) and cytokinin oxidase/dehydrogenase (CKX) – the key enzymes responsible for biosynthesis and degradation of CKs, respectively. These enzymes are considered to be major regulators of CK levels in plants.

The aim of this study was to investigate the function of the *HvCKX9* gene in barley by generating loss-of-function mutants using the CRISPR/Cas9 system. The CRISPR construct was introduced into immature embryos via *Agrobacterium*-mediated transformation, resulting in edited T_0 plants with an efficiency of approximately 54%. From the gene-edited T_0 plants, T_1 generation individuals were grown for further analysis.

From each plant, a pooled sample of 8-10 flag leaves and spikes at 4 and 7 days after pollination (DAP) were collected. Subsequent steps will include the analysis of expression levels of selected *HvCKX* and *HvIPT* using RT-qPCR, as well as CK quantification *via* LC-MS/MS in the collected samples. Agronomic parameters such as plant height, the number of spikes per plant, spike length, the number of grains per plant, thousand grain weight, and leaf width will be measured. These data will be subjected to correlation analysis to explore relationships between measured parameters and phenotypic traits.

Preliminary data indicate that *HvCKX9* gene expression is specifically higher in leaf blades, either in the vegetative, reproductive, and grain-filling stages. We expect that altering HvCKX9 activity will modify CK metabolism during plant development, thereby affecting growth and yield-related traits. The results of this study will provide valuable information on the role of the *HvCKX9* gene in barley growth and productivity and give insights for future breeding strategies.

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Ectopic expression of wheat *TaCbf14* and *TaCbf15* genes in barley induces cold tolerance and reveals trichome- and anthocyanin-associated responses

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Keywords: CBF transcription factor, cold stress, barley transformation, trichome, anthocyanin

Previous studies have established that the transcription factors CBF14 and CBF15 play a central role in the acquisition of freezing tolerance in wheat (*Triticum aestivum* L. ssp. *aestivum* cv. 'Cheyenne'). These genes were cloned and ectopically overexpressed in spring barley (*Hordeum vulgare* L. cv. 'Golden Promise'), resulting in enhanced frost tolerance (Soltész et al., 2013). Transcriptome profiling via RNA sequencing revealed widespread transcriptional reprogramming, with several hundred differentially expressed genes in transgenic lines. In addition to improved cold tolerance, an unexpected trichome phenotype emerged. The morphology of these trichomes was characterized using scanning electron microscopy, while the underlying molecular mechanisms were explored through in silico analyses. Functional annotation using Gene Ontology and interrogation of the TAIR (The Arabidopsis Information Resource) database identified approximately 75 trichome-associated genes showing altered expression levels. Promoter analysis further suggested the presence of putative cis-regulatory elements potentially mediating cold- and dehydration-inducible expression in these genes.

Recently, prolonged exposure of transgenic lines to low temperatures resulted in pronounced anthocyanin accumulation, indicating the activation of additional stress-responsive metabolic pathways. In light of these observations, future efforts will focus on uncovering the architecture regulating cold-induced anthocyanin biosynthesis. Specifically, we aim to investigate the potential role of a possible MBW (MYB–bHLH–WD40) transcriptional complex in eliciting this response in the context of our transgenic barley model.

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References

1. Soltész, A., Smedley, M., Vashegyi, I., Galiba, G., Harwood, W., & Vágújfalvi, A. (2013). Transgenic barley lines prove the involvement of TaCBF14 and TaCBF15 in the cold acclimation process and in frost tolerance. Journal of Experimental Botany, 64(7), 1849–1862. https://doi.org/10.1093/jxb/ert050