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Oral Presentations Abstract Book

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Rajeev K Varshney and Anu Chitikineni

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Preface

The International Wheat Congress (IWC) 2024 brought together global experts to discuss key advances in wheat research, innovations, and challenges in the field.

Welcome to the IWC 2024 Oral Presentations Abstract Book. This compilation features oral presentation abstracts submitted by our esteemed authors, whose contributions greatly enriched the Congress. We are delighted to present the work of these authors, showcasing a diverse array of research and insights related to wheat science.

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This collection stands as a testament to the dedication and hard work of our participants. Although some authors were unable to submit their abstracts, we acknowledge and appreciate their efforts and contributions to the Congress.

We hope you find this book both informative and inspiring, as it reflects the remarkable work presented at IWC 2024.

Thanking you.

Editors
Rajeev K Varshney, &
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Paper #52

Spike-branching wheat: Hype or Hope?

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Over the course of domestication and breeding, tremendous progress is achieved in improving wheat grain yield primarily by increasing number, fertility, and size of floral organs, while mostly preserving the overall spike architecture, where the grain-bearing spikelets are directly attached to the rachis in a distichous pattern. However, slow rates of yield increase in the past two decades suggests a “yield plateau” even in some of the highly productive agroecosystems worldwide.

Conceivably, introducing radical changes in the inflorescence architecture might offer new paths for furthering yield potential and overcome yield barrier. Here, we explored the physiological and genetic basis of grain yield determination in ‘Miracle-Wheat’ (*Triticum turgidum* convar. *compositum* (L.f.) Filat.) that deviates from the standard developmental programme, producing a non-canonical spike with lateral branches.

Notably, the Miracle-Wheat accessions are landraces with an exceptionally high number of spikelets because of spike-branching, but suffers from reduced grains per spikelet and grain weight, thus impeding its use in wheat breeding. To address this limitation, we investigated the effect of source-sink strength on such trade-offs by analyzing a large population comprising 385 RILs derived from the spike-branching landrace TRI 984 and CIRNO C2008, an elite durum (*T. durum* L.) cultivar.

Multi-year field experiments identified favorable allele combinations viz., *branched head*^{t} *3* (*bh*^{t}*-A3*), a new modifier locus for spike-branching and *grain protein content* (*gpc-B1*) that delayed senescence, which together improved grain number and grain weight in the spike-branching recombinants.
Furthermore, a set of promising spike-branching RILs were selected for yield trails in two diverse environments alongside leading local varieties. Overall, our study provides insights on mitigating grain yield trade-offs in Miracle-Wheat and propose spike-branching as a new breeding target to enhance yield gains in wheat.

#264

Integrating uas phenomic data into the texas a&m winter wheat breeding program

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The Texas A&M AgriLife Research Wheat Improvement Program for the Texas High Plains develops high yielding, drought tolerant, insect and disease resistant winter wheat (*Triticum aestivum*) varieties with high end use quality for rain-fed and irrigated production systems. Unoccupied aerial system (UAS) technology is revolutionizing precision agriculture, offering a cost-effective method for collecting high-spatiotemporal data in plant breeding nurseries.

Previous ground-based spectral reflectance research quantified genotypic variation in biomass for high-throughput phenotyping, which progressed to UAS-based visual and multispectral imagery data collection throughout our eight month long growing season. UAS-based phenomic data from these contrasting environments provide valuable insights on biomass production and grain yield that strengthens the breeding selection process. UAS-based red, green blue (RGB) and multispectral phenomic data was collected on 15,450 breeding plots in both rain-fed and irrigated nurseries during the 2018-2023 growing seasons, totaling 358,750 observations.

Geospatial data products are processed from raw UAS images by the Texas A&M Corpus Christi Digital Agriculture Hub. Spectral reflectance indices (SRIs) including Normalized Difference Vegetation Index (NDVI), Normalized Difference Red-Edge Index (NDRE), and Excess Green Index (ExG) are calculated and physical traits such as canopy cover and canopy height extracted.

Traditional breeding selection criteria of grain yield, test weight, heading date, plant height, forage biomass, early season vigor, and breeder's ag score were compared to UAS-based predictions of grain yield. Correlation between SRIs and grain yield varied by year based on timing and amount of precipitation received during the growing season. In general, irrigated grain yield correlated well with NDVI during boot stage through grainfill. Canopy volume predicted forage biomass and early season vigor, which are important traits for dual-purpose wheat in the Southern High Plains.

#515

Harvesting hope: The east african wheat transformation

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The East African Wheat Breeding Pipeline, initiated in 2022 through a joint program by CIMMYT and KALRO, strives to enhance wheat varieties for regional food security. This integrated breeding pipeline, a collaboration between CGIAR and NARES Partner, focuses on accelerated breeding, employing a 3-year Rapid Generation Bulk Advancement scheme and data-driven selection for informed breeding decisions. It aims to develop and release improved wheat varieties adapted to East African conditions, addressing gaps in yield potential, disease resistance, and climate resilience.

The pipeline utilizes an expanded testing network across Kenya and Ethiopia, driving impactful breeding programs for enhanced farm productivity. It tackles diseases like rust, fusarium, septoria, and wheat blast, particularly challenging in Kenya. Climate-adaptive varieties are developed, tested in marginal rainfed environments experiencing heat and drought stress.

The collaboration's success with KALRO, spanning four decades, includes the Mexico-Kenya shuttle breeding approach, releasing over 200 varieties and mitigating stem rust threats. Strategic partnerships with Agventure address critical gaps, facilitating yield testing and identifying high-performing lines.

Investments in KALRO's infrastructure and capacity building through training and shared resources improve yield potential. A recent training course covered essential topics in wheat breeding and research. Supporting initiatives include plant health, market intelligence, and seed equality, emphasizing the importance of regional collaboration, knowledge sharing, and strategic investments for addressing critical challenges in wheat production.

#188

Switches in homoeolog expression bias in hexaploid wheat are associated with cis-variation

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Differences in the relative level of expression of homoeologs, known as homoeolog expression bias (HEB), is widely observed in allopolyploids including wheat. While the evolution of homoeolog expression bias through hybridisation has been characterised, on shorter timescales the extent to which homoeolog expression bias is preserved or altered between generations remains elusive. In wheat breeding programmes, these inter-generational changes in homoeolog expression bias may influence significant traits including flowering time and spike development.

Using two biparental mapping populations consisting of 50 F_6 lines we identified over 5,000 triads exhibiting substantial variation in HEB. We measured the relative distance between HEB of F_6 lines and their parents in these 5,000 triads. One cross exhibited a higher prevalence of triads with notably divergent HEB from a single parent, whereas the other cross predominantly displayed divergence from both parents. To understand, if these trends are associated with genetic variation between the parental wheat varieties, we performed variant calling and discovered over 80,000 SNPs.

These SNPs revealed direct associations with HEB in 800 triads and cis- variation was strongly associated with switches in HEB between F_6 lines. Furthermore, we investigated whether there are specific genomic loci that contribute to this inheritance. eQTL analysis of more than 200 F_6 lines, revealed over 7,000 cis- and 2,000 trans- loci that likely contribute to the inheritance of HEB in wheat. Notably, 95 of these eQTL loci were shared across F_6 lines derived from distinct biparental crosses, indicating a common genetic framework influencing HEB across populations.

Our study highlights that homoeolog expression bias is heritable and can alter rapidly within a few generations, and this may contribute variation within wheat breeding programmes.

#186

Why stabilizing grain proteins can help modern wheat agriculture

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It is a global challenge to enhance yield while still maintaining grain-quality attributes such as grain protein content in wheat breeding programme due to the negative correlation between grain yield and grain protein content. Protein abundance in wheat grain is determined by the relative rates of protein synthesis and protein degradation occurring during grain development.

The stability of any given protein during grain development defines its N sink strength in the formation of mature grain. Through combining *in vivo* ^{15}N stable isotope labelling and in-depth quantitative proteomics, we have measured the turnover rates and N sink strength of 1400 different types of proteins during wheat grain development.

Our data show that approximately 20% of total grain ATP production is used for grain protein biogenesis and maintenance, and nearly half of this energy budget is invested exclusively in storage protein synthesis. We demonstrate that wheat storage proteins are not as stable as we traditionally thought, and about 25% of newly synthesized storage proteins are turned over during grain development rather than stored, causing a 20% loss of N sink on a grain basis and a 6.6% loss of NUE on applied nitrogen basis.

Our approach to measure protein turnover rates at proteome scale reveals how different functional categories of grain proteins accumulate, calculates the ATP cost and N sink strength of each protein as it accumulates during wheat grain development and identifies the most and the least stable proteins in the developing wheat grain. This can provide a new perspective on targets in quality wheat breeding through improving N sink strength and NUE by stabilizing storage proteins that turnover rapidly. Success in this pursuit could also help negate the inverse relationship between grain yield and grain protein content by making grain protein accumulation cheaper in terms of cellular energy costs.

#279

Gene editing of upstream open reading frames to investigate novel iron biofortification strategies in wheat

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Iron (Fe) deficiency affects 30-50% of the world's population. Genetic biofortification of staple crops is a promising strategy for improving human nutrition, but the number of effective precision breeding targets for Fe biofortification is small.

Recent studies have shown that 50% of genes in some eukaryotes possess short (<100 amino acid long) upstream Open Reading Frames (uORFs) located within the 5' leader sequence (LS). These uORFs can be translated and function as negative regulators by downregulating the translation of the primary ORF (pORF). We aligned publicly available rice (*Oryza sativa* L.) ribo-seq datasets and transcriptomes to identify non-canonical translation within the 5' LS of important Fe homeostasis genes.

A dual luciferase assay (DLA) was used to determine whether 5' LS translation indicated from ribo-seq datasets corresponded to uORFs that repressed the pORF. We identified two positive regulators of the Fe-deficiency response that contained a repressive uORF region: IDEF1 and IDEF2. The IDEF2-uORF peptide was highly conserved among monocots and a mutation series in the 5' LS of the bread wheat (*Triticum aestivum* L.) *TaIDEF2-A1* gene demonstrated variable pORF derepression. To fine-tune *TaIDEF2* expression in bread wheat, we utilised CRISPR-Cas9 gene editing to target and mutate the *TaIDEF2*-uORF homoeologs in cv.

Fielder with the aim to increase expression of the *TaIDEF2* pORF and activate the plant's Fe deficiency response. Three T_1 mutations in the *TaIDEF2-A1*-uORF and *TaIDEF2-D1*-uORF homoeologs have been identified and are predicted to inhibit the uORF's repressive function.The effect of *TaIDEF2*-uORF mutations on plant growth under Fe deficiency and grain nutrition is now being assessed via hydroponic and glasshouse studies.

Together these findings highlight the potential of manipulating uORFs to achieve precise control over gene expression in plants using CRISPR-Cas9 gene editing.

#465

The platinum-quality svevo reference genome assembly: A new tool for advanced plant breeding

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The reference durum wheat genome (Svevo Rel.1) was previously sequenced and assembled using a short-read sequencing approach and recently an international consortium was established to achieve a Platinum-quality reference genome (Svevo Rel.2), fulfilling the ultimate requirements for contiguity, completeness and correctness. PACBIO HiFi long read 35X sequencing was coupled with Bionano Optical Mapping, producing 259 Hybrid scaffolds (N50 = 112.3 Mb) that were ordered by Hi-C data into 14 contiguous pseudomolecules spanning 10.4 Gb.

The new assembly was aligned with Svevo Rel.1, and discrepancies were manually checked. The Svevo Rel.2 is available in the GrainGenes database. A complete and accurate gene annotation was then obtained by coupling Illumina RNA-Seq and Nanopore Isoseq sequencing of 58 samples representing a range of tissues and developmental stages from Svevo plants grown under optimal conditions as well as exposed to different biotic, abiotic, and nutrient stress. Immunity receptor genes containing LRR and storage protein gene families have been manually curated.

The expression of 68,154 high-confidence genes has been integrated into a transcriptome atlas (https://bar.utoronto.ca/), together with more than 100,000 low-confidence, TE-related or long non-coding genes. The entire QTLome of durum wheat consisting of around 7,000 loci identified by linkage or association mapping for a range of traits (phenology, biotic stress resistance, abiotic stress tolerance, grain yield and components, grain quality) has been anchored on the new genome.

The analysis of the QTL data allowed us to identify QTL clusters containing loci confirmed across multiple tetraploid panels and populations, and phenotyping experiments, and some clusters co-locate with known master gene regulators. The Svevo Rel.2 allowed us to resolve a specific region previously part of the unanchored chromosome sequence known to be involved in durum wheat domestication. One of the two loci controlling the free-threshing (tenacious glumes) trait, the *TG1* locus, was physically assigned to a region of chromosome 2B unassembled in Svevo Rel.1. Notably, a large inversion encompassing *TG1* distinguishes the free threshing from the tenacious glume phenotypes.

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#363

Unveiling the mechanism of the Sr62 wheat tandem kinase

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Tandem kinases (TKs) have recently emerged as crucial determinants of resistance in wheat and barley against various pathogens. In this study, we investigated the mechanism of Sr62^{TK}, which imparts broad-spectrum resistance to stem rust. We identified *Sr62*^{NLR}, an NLR-encoding gene, as a genetic interactor of *Sr62*^{TK} and demonstrated its requirement for *Sr62*^{TK} function.

Additionally, we cloned the corresponding fungal AvrSr62 effector from stem rust isolate Pgt21-0 and showed its interaction with Sr62^{TK}. Alphafold predictions suggest that a β-finger in the N-terminal kinase domain of Sr62^{TK} contributes to Sr62^{TK} homodimerization and AvrSr62 binding. We found that different orthologous NLR genes from wheat can support the Sr62^{TK}-mediated immune response, both in wheat protoplasts and in *N. benthamiana* leaves, upon detection of the AvrSr62 effector. These insights enhance our understanding of the mechanisms underlying TK-mediated resistance, paving the way for targeted crop protection strategies.

#160

Unveiling the mechanism of the Sr62 wheat tandem kinase

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These insights enhance our understanding of the mechanisms underlying TK-mediated resistance, paving the way for targeted crop protection strategies.

#126

Discovering the *aegilops* genomes

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The *Aegilops* genus comprises 11 diploid, ten tetraploid and four hexaploid species. Belonging to all three gene pools of wheat, these species represent a largely untapped genetic diversity for its improvement. To create a resource for exploiting this diversity, we sequenced, assembled and annotated the genomes of diploid *Aegilops* species with the C, M, N, S and U genomes. Sequencing was done using PacBio HiFi and assemblies were scaffolded with Bionano optical maps and genetic maps. Annotation was performed using evidence based IsoSeq and RNASeq data as well as *ab initio* approaches.

The high-quality assemblies have N50 values ranging from 48 to 665 Mb with BUSCO scores ranging from 97.8 to 98.5. Comparative analyses of genomes revealed chromosome-scale rearrangements across and within species. Using strict criteria, 59-72K high-confidence (HC) genes were identified for each genome. These HC gene estimates exceed previously reported estimates for other *Aegilops* genome annotations, a likely consequence of the high-quality and depth of the IsoSeq and RNASeq data.

Phylogenetic analysis of the single-copy core genes of all diploid *Triticum* and *Aegilops* species assemblies released to date revealed the B-lineage of *Ae. speltoides* and the D-lineage of all other *Aegilops* species, including all remaining Sitopsis and the genomes sequenced in our study. The polyploid *Aegilops* species represent various combinations of the diploid genomes. To understand their evolution, we sequenced them using a combination of technologies.

While having a higher number of scaffolds than the diploid assemblies, these assemblies have N50 values of 116 Mb to 1.4 Gb and BUSCO scores of 94.5-100, which largely suffice to investigate chromosomal rearrangements and transposable element evolution. This comprehensive *Aegilops* genome assembly database is expected to be a useful resource for broadening the genetic diversity of wheat.

#545

Short-season winter wheat: A new strategy to build resilient and diverse cropping systems

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Long-term trends in rising temperatures and extreme weather events during the crop season will dictate how we grow crops. It is therefore urgent to identify resilient varieties and alternative cropping systems better adapted to changing climate.

The increased frequency of drought events and weed infestations at normal planting time has driven wheat farmers to delay planting. Wheat growers in the US Southern Great Plains have recognized the necessity for short-season winter wheat varieties adapted to late planting without delaying harvest or losing yield. Delaying planting of full-season wheat from October to December results in yield losses of about 50% and harvest delays of 10 days or more.

According to producers, delaying wheat harvest by five days is problematic when planting a subsequent summer crop. Furthermore, farmers are interested in diversifying income using double-cropping systems with summer crops, driving demand for short-season wheat varieties. The overall objective of this research is to determine the best management practices (BMPs) that simultaneously maximize grain yield, protein concentration, and nitrogen use efficiency of late-sown wheat.

Results will be presented from multiple field experiments conducted using multi-location trials in Oklahoma to evaluate the performance of short-season versus commonly grown full-season varieties under different agronomic management including planting date, planting density, and nutrient management.

This research addresses pressing climate challenges and will help producers thrive at late-planting date and double-cropping systems across multiple winter wheat-growing regions.

#427

Faststack: an ai-guided breeding for wheat genetic improvement

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As a standard practice, parents for crossing are selected based on their performance per se or their breeding value. A significant challenge for elite wheat breeding germplasm is to increase rates of genetic gain without exhausting the genetic diversity. This is particularly challenging when a combination of quantitative traits (yield, grain quality, heat-tolerance) are considered simultaneously.

A new optimal haplotype selection strategy – called ‘FastStack’ that uses evolutionary computing, a type of AI optimisation technique, was developed at UQ, in partnership with LongReach, to first identify and then stack desirable chromosome segments in the shortest possible time. In our pilot study to improve wheat yield, we used genetic algorithm in a large commercial data set comprising more than 40,000 genotyped breeding lines which have been tested over multiple years (2010–2018) and locations across Australia.

The objective function of the algorithm predicts optimal crosses that most efficiently stack complementary haplotypes for yield; and generated offspring from the digital twin is advanced through speed breeding which allows a rapid turnaround of several generations and breeding cycles per year.

For the first time, validation trials (2022–2023) showed top performing breeding lines have considerably better yield than existing varieties. Selected lines are also now being further advanced (2024) and/or used as new parents to introgress stacks into LongReach elite germplasm.

With the success of our pilot study, LongReach is now in a position to integrate the AI-guided optimal haplotype stacking strategy within their breeding program. To expedite this development further, we are also currently developing a multi-trait approach on how to stack desirable haplotypes for grain quality and heat-tolerance into high yielding backgrounds.

#469

Thermally safe flowering window for wheat: Learning from data to inform breeding targets

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Australia's climate has warmed by 1.47 ± 0.24 °C since 1910, accompanied by a decline in cropping season rainfall in the southwest, particularly at sowing, stalling water-limited wheat yields (Hochman et al., 2017). Within the restrictions imposed by water availability, farmers try to minimise direct damage by frost and heat to yield, by choosing planting dates and cultivar combinations that lead to flowering within a thermally safe window. In this study we used historic weather records, knowledge of genotypic variation in phenology and a simulation model (APSIM NextGen) to investigate three topics.

Firstly, we asked if there had been historic changes in the thermally safe flowering window, in length and timing of occurrence. Using weather records in a 5km x 5km grid covering the cropping belt, we investigated the trends in the occurrence of the last day of frost (90th percentile, $T<0°C$) and first day of heat (20th percentile, $T>32°C$). We show that a significant proportion of the wheatbelt is experiencing later frosts, advancing by ca. 1 day year^{-1} or more since 1970, extending a previous study (Zheng et al., 2015). At the same time, the first day of heat, is uniformly occurring earlier across the wheatbelt, by half a day or more per year. This has led to a significant shortening in the length of the thermally safe window in most regions. Secondly, we asked how historic changes in weather have impacted on the yield of cultivars of different phenology.

To this end, APSIM NextGen was used to simulate phenology and water limited yield of ca. 200 cultivars in sowing dates between 1st April and 29th July in the whole wheat belt. Across maturity groups and regions, the total cycle length is significantly shorter and yield potential has declined over time, with shorter cycles more impacted than long ones.

Finally, we characterized frost and heat patterns in terms of frequency and intensity during the critical period for yield formation (300°Cd before and 120°Cd after flowering) to analyze the likelihood of success of avoidance and sensitivity reduction targets.

Taking together all this evidence, we argue that as crop phenology is already a management tool to minimize losses (Hunt et al., 2019), future research should focus on addressing mechanisms to reduce the sensitivity to extreme temperatures during the critical period. Choosing frost as an example, we discuss to what degree sensitivity to frost would need to change during the reproductive period to impact significantly on yield loss and the boundaries of the thermally safe window for a given risk level.

#168

Innovative strategies for advancing wheat genetic improvement utilizing synthetic

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Over the past three decades, the International Maize and Wheat Improvement Center (CIMMYT) has let the way in developing and utilizing synthetic hexaploid wheat (SHW) to facilitate the transfer of genes from *Ae. tauschii* and durum wheat to bread wheat. Despite the generation and characterization of numerous SHWs, accurately forecasting their phenotypic performance has proven challenging, especially when relying solely on the performance of the parental lines.

To address this challenge, we investigated methods originally designed for predicting traits in intraspecific hybrids and applied them to the context of allopolyploids, specifically the cross of durum wheat with *Ae. tauschii*. We successfully predicted the phenotypes of SHWs for three significant quantitatively inherited diseases in wheat: tan spot, Septoria nodorum blotch and spot blotch.

Genomic prediction models, incorporating both pedigree information and non-linear kernels, surpassed the predictive capabilities of models relying solely on linear kernels. Consequently, we have initiated the implementation of recurrent, early-generation genomic selection for crosses between SHW and elite spring wheat lines from CIMMYT. This strategic deployment aims to generate multiple layers of pre-breeding populations and novel candidate lines, enriched with novel allelic variations from the SHW crucial for enhancing quantitative disease resistance.

#44

Wheat spike development and its impact on grain number per spike

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In wheat, the transition of the inflorescence meristem to a terminal spikelet (IM->TS) determines spikelet number per spike (SNS), an important grain yield component. Simultaneous loss-of-function mutations in the wheat *SQUAMOSA* MADS-box genes *VRN1*, *FUL2* and *FUL3* result in a reversion of spikelets to tillers subtended by leaves. Mutations in individual *vrn1* or *ful2* genes increase *SNS*, whereas *vrn1 ful2* combined mutants have an indeterminate IM.

These increases are associated with a combination of a delay in the IM->TS transition and changes in the rate of lateral spikelet development. By contrast, loss-of-function mutations in *LEAFY* or *WAPO1* reduce SNS, mainly by reducing the rate of spikelet formation. LFY and WAPO1 interact physically and genetically with each other, with *lfy wapo1* showing a similar reduction in SNS as the single mutants, indicating that they jointly regulate the number of spikelets per spike. Single-molecule fluorescence in-situ hybridization revealed that *LFY* and *WAPO1* are expressed together at the early stages of spike development but not at later stages.

This result suggests that the formation of the LFY-WAPO1 complex at the initiation of spike development is sufficient to alter the rate of spikelet formation. The spatial transcriptomics also showed that he down-regulation of *LFY* and up-regulation of the *SQUAMOSA* MADS-box genes in the distal part of the developing spike during the IM->TS transition, supporting their opposite roles on spikelet development. *LFY* and *WAPO1* also play an important role in spikelet development.

Loss-of-function mutations in either gene result in the downregulation of similar floral organ identity genes and result in similar floral defects in lodicules, stamens and pistils. At the lemma primordia stage, *LFY* is expressed at high levels in a nest-shaped region distal to the lemma primordia and proximal to the *WAPO1* expression domain.

We hypothesize that the overlapping expression domains of *LFY* and *WAPO1* facilitate interactions between their encoded proteins, providing important spatial information for the developing florets. Finally, we describe *LFY* and *WAPO1* natural alleles with significant effects on SNS and identify allele combination that maximize SNS for wheat improvement.

#123

Temperature and photoperiod interaction: Releasing aerial branching in wheat

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Across the world, wheat varieties are sown which are intimately adapted to their environment and growing season. Climate change poses a threat to wheat productivity by modifying the duration of the growing season, determined by the coordination of temperature and photoperiod. While climate change will affect temperatures, photoperiod cycles will remain the same.

Our aim is to understand the mechanisms governing temperature and photoperiod integration in wheat and elucidate how they impact the genetic pathways that coordinate shoot architecture. Shoot architecture is the result of synchronized adjustments in the development and determinacy of shoot meristems and branching patterns. Unlike related species, wheat lacks aerial branching, meaning that each tiller yields a single inflorescence/spike.

We recently observed that a wheat landrace releases aerial branching consistently under higher temperature conditions and UK natural daylength. This suggests that the axillary meristems that would typically be dormant were derepressed under these conditions. To discover the mechanism underlying this unique phenomenon, we are using genetic approaches to identify the causal gene(s) and have performed RNA-sequencing analyses on derepressed and dormant axillary meristems of this landrace. We are combining these approaches with spatial transcriptomics analyses in the aerial branching meristems.

We aim to identify candidate genes which will be functionally validated through genome editing. With this research, we hope to contribute to our mechanistic understanding of the genetic and phenotypic responses of wheat under future climate conditions.

Ultimately, we aim to deploy this knowledge to future proof wheat cultivars to a changing environment.

#485

Wheat yield progress Nw Mexico: A bellwether for lower latitude yields around the world.

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The farm yield (FY) of irrigated wheat in the Yaqui Valley of NW Mexico has risen from 2 to 7 t/ha from 1960 to 2019, due to efforts of CIMMYT, and Mexican researchers and farmers. This progress has been and remains influential for yield progress involving disease resistant wheat globally below around latitude 35°.

Fischer et al (2022) divided this Yaqui Valley period into three consecutive 20 year subperiods for detailed study. FY was corrected for annual FY fluctuations related to such fluctuations in minimum temperature (Tmin, yield response -7%/°C), and the small upwards 60 yr trends in Tmin and CO2, to get accurate linear technology trends. For each consecutive 20-y period, this was 4.2, 0.5, and 1.6 % p.a.

Knowing the relative area and potential yield (PY) of varieties grown each year allowed calculation of a variety yield index for the Valley, which increased by 1.0, 0.5, and 0.7% p.a. over the successive 20 year periods. By difference, agronomic + socioeconomic factors therefore contributed 3.2, 0, and 0.9% p.a., respectively. The large 1960-79 figure was boosted by the only N x variety interaction, large and positive.

Agronomic progress itself was largely due to steadily higher N and P fertilizer rates, better weed control, and improved planting and water management (raised bed system); in the final period there was probably also more skillful agronomic management. However, over the 60 years cropping system diversity has declined.

The depressed agronomy+ progress in 1980 to 1999 was largely due to the withdrawal of Government support to the small-farmer *ejido* system and to prices. Since 2019, FY corrected for temperature has not increased, nor has the variety yield index, with progress in breeders' plots no better than 0.5% of current PY. Have we reached the limit?

Fischer, T., Ammar, K., Ortiz Monasterio, I., Monjardino, M., Singh, R., Verhulst, N. 2022 Sixty years of irrigated wheat yield increase in the Yaqui Valley of Mexico: past drivers, prospects and sustainability. Field Crops Research 283, 108528.

#538

Unravelling the genetic basis of heat tolerance in wheat

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Wheat (*Triticum aestivum L.*) is the most prominently grown crop in the world that is pivotal in human nutrition. Its production requires at least ~2.5% annual increase by 2050 to meet the nutritional demands of the surging population. However, global warming and climate change gravely threaten wheat production worldwide, resulting in significant yield reductions.

Heat stress affects a plethora of physiological mechanisms in plants, such as photosynthesis, redox reactions, protein and starch synthesis, and source-sink translocation, leading to deterioration in grain quality and quantity. Adaptation to the climate scenarios of the future is critical to meet global food security in the coming years. Assessing the genetic variation underlying the traits of interest is the key for such adaptation. With this preamble, our study aims to explore the variation, and identify the candidate genes and superior haplotypes associated with the heat tolerance-component traits in a panel of 345 diverse wheat genotypes which include cultivars and landraces.

One season field trial data in Northam, Western Australia depicted significant differences for all the traits among these genotypes. Days to flowering and flag leaf chlorophyll content had the most effect on biomass and grain yield. In the upcoming field trials, unmanned aerial vehicle (UAV)-mounted multispectral sensors will be used for high-throughput field phenotyping (HTFP) of staygreen and agronomic traits in timely and late-sown wheat to assess the genotypic heat stress response. Also, the effect of heat on fertilization will be quantified by spikelet fertility measurements.

Further, candidate genes and superior haplotypes associated with heat tolerance will be identified through genome-wide association studies (GWAS), followed by validation of the candidate genes using expression analysis and functional marker assays.

In essence, this study will not only identify superior heat tolerant genotypes possessing better staygreen character, spikelet fertility and yield, but also the markers underlying the heat tolerance-component traits, thus providing a resource base for the breeding industry in Australia and elsewhere in the world for developing climate-smart varieties for the future.

#148

Genetic variation for mineral uptake and partitioning in the watkins germplasm collection

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Wheat is an important source of mineral micronutrients for human and livestock nutrition. It is well-established that commercial wheat cultivars exhibit low genetic diversity compared to older varieties, including landraces. Exploiting the A. E. Watkins landrace cultivar collection, a historical germplasm collection dating back a century, we aim to elucidate novel genes controlling the accumulation of essential minerals in wheat grains and identify germplasm for breeding programmes.

The set of 821 Watkins lines, recently sequenced, were grown in two successive years in replicated field trials at Rothamsted in the UK. In addition, selected biparental populations (Paragon x Watkins lines) and NAM panels focused on variation in specific traits were also grown in multiple years and subject to QTL or GWAS analysis. Grain and straw yield were recorded and subsampled for mineral analysis by ICP-AES, as well as grain weight, size and shape.

This enabled total mineral offtake, partitioning from grain straw (mineral harvest index) as well as grain content and concentration to be determined. In addition, in season phenotyping recorded growth and developmental stage. Data will be presented showing the diversity of mineral uptake and partitioning between grain and straw along with variation in phenotype, allowing insights into mechanistic control mineral uptake and distribution. The entire population data is being subjected to GWAS analysis, which may indicate novel loci are involved in these traits, and by exploiting the genomic sequence information, novel candidate genes.

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#240

Interactive effects of eco$_2$ and mycorrhization on plant performance are host-species or cropping pattern dependent

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Introduction: Almost all elevated atmospheric CO$_2$ (eCO$_2$) studies have focused on plant aboveground characteristics under mono-cultures, whereas limited considerations of interactions with arbuscular mycorrhizal fungi (AMF) under intercropping, let alone under the same, yet unrealistic, daytime/nighttime CO$_2$ concentrations.

We thus addressed how auto-simulated field daytime and/or nighttime eCO$_2$, AMF and N$_2$-fixing/non-N$_2$-fixing intercropping could interactively affect plant biomass production and major physiological characteristics.

Methods: Non-N$_2$-fixing winter wheat (*Triticum aestivum* cv. Yunmai) and N$_2$-fixing fababean (*Vicia faba* cv. 89-147) inoculated with AM fungus (*Funneliformis mosseae*) were grown in mono-cultures and intercropping in soil (Eutric Regosol) filled pots inside environmentally controlled glass-made chambers. Those chambers had the same growth conditions (nitrogen-fertilization, AMF inoculation, etc.), except CO$_2$ concentrations: ambient CO$_2$ (ACO$_2$, 400ppm daytime/450ppm nighttime), daytime elevated (DeCO$_2$, 550ppm/450ppm), nighttime elevated (NeCO$_2$, 400ppm/600ppm), and daytime/nighttime elevated (D+NeCO$_2$, 550ppm/600ppm).

Plant physiological parameters were examined. Results: Regardless AM-mycorrhization, root and shoot biomass and total soluble sugar were significantly increased, but tissue NPK, leaf gas exchange, total chlorophyll, soluble protein and free amino acids were significantly decreased under eCO$_2$ in mono-wheat, mono-fababean or wheat/fababean intercropping. Significantly different responses of plant growth parameters (tissue N, total chlorophyll, soluble protein and free amino acid) to eCO$_2$ patterned as: DeCO$_2$ > D+NeCO$_2$ > NeCO$_2$ > ACO$_2$, while to AM-mycorrhization as D+NeCO$_2$ > DeCO$_2$ > NeCO$_2$.

Conclusions: Mycorrhization and/or eCO$_2$ have varied interactive effects on plant biomass production and physiological characteristics, no matter whether plants were grown in mono-cultures or intercropping. Such interactive effects are probably plant host-species or cropping pattern dependent.

#285

Beyond the genome: A pan-transcriptome for *aegilops tauschii*

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Wild relatives of bread wheat (*Triticum aestivum*) hold enormous potential for improving agronomic and disease resistance traits in elite wheat cultivars through introduction of novel alleles, and also serve as a platform to explore the natural diversity across the *Triticeae*. This diversity allows investigation into how important traits may be influenced by genetics. The comparison to the gene pool in cultivated wheat, which has been limited due to population bottlenecks introduced during polyploidization and domestication, underscores the importance of the genetic diversity in its wild relatives for maintaining resilience and adaptability.

Recently, the Open Wild Wheat Consortium (OWWC) assembled the genomes 46 *Aegilops tauschii* accessions (the progenitor of the hexaploid wheat D genome), with a special emphasis on three diverse lineages: TA10171 ((Lineage 1), TA1675 (Lineage 2), and TA2576 (Lineage 3). We generated comprehensive RNA-Seq data for these accessions using five tissue types: seedling leaf, flag leaf, root, shoot, and inflorescence; in order to perform *de novo* gene annotation for this pan-genome.

Now, we complement earlier analyses of the genetic diversity exhibited between these lineages by assessing their transcriptomic diversity. This assessment aims to understand how differential gene expression might influence phenotypic diversity and to identify links between transcriptomic regulation and previously identified features such as structural variation between chromosomes or differences in gene content and copy number.

Differential expression of orthologous genes between *Aegilops tauschii* lineages may provide further insight into the regulation of the D genome in bread wheat which is known to be partially dependent on the content of the A and B genomes, a factor which cannot be captured using solely genomic approaches.

#477

Nitrogen management and agronomic performance in winter wheat

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The conventional practice of applying nitrogen (N) without adequate information regarding the amount and timing of crop N requirements can result in excessive N fertilizer use, leading to increased greenhouse gas emissions and groundwater contamination. The objective of this research was to improve N management by monitoring plant N status in order to apply N fertilizers at optimal amounts based on site and crop-specific N requirements.

A split-plot design experiment with two winter wheat cultivars (Main plots) and three spring application N rates [Split plots – 0, 100 (recommended rate) and 150 kg/ha] was conducted at the Harrow R&D Centre of Agriculture & Agri-Food Canada in 2021-2022 and 2022-2023. Agronomic, spectrometric, and photosynthetic data were collected at up to three wheat growth stages, and soil and plant N contents were determined at two growth stages. In 2022, 25R34 had significantly lower protein content and test weight, earlier heading, higher seed mass, plant height, and winter survival compared to Pro81. In 2023, 25R34 had significantly lower test weight, earlier heading, higher grain yield and seed mass compared to Pro81.

Lodging was only observed in 2022, and 25R34 showed significantly higher lodging compared to Pro81 particularly at the 100 and 150 kg/ha N rates. In both years, protein content and plant height significantly increased with higher applied N. However, the effect of nitrogen treatments varied between years for several traits suggesting they were influenced by factor(s) other than N fertilizer. At harvest in 2022, Pro81 had significantly higher grain carbon (C) and N contents than 25R34.

Tests of φPSII, which measures the proportion of light utilized by Photosystem II once the leaf is already exposed to light, showed that 25R34 reached a plateau at ~90 kg/ha N; whereas, Pro81 was able to use a higher amounts of light energy in PSII even at the 150 kg/ha N suggesting that Pro81 has a higher capacity for nitrogen use than 25R34.

Further data analysis will investigate associations between soil and plant N contents and hyperspectral data. Hyperspectral sensors may provide a method to monitor in-season plant N status which is integral to implementing precision agriculture because, in addition to crop fertility status, hyperspectral data can provide early indication of plant nutrient and water stresses that may impact yield potential.

#383

Amylose content of wheat flour noodles effects post prandial satiety and glycaemic response in healthy adults

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The aim of the study was to investigate the effect of consumption of high amylose noodles on satiety and postprandial glycaemic response. Satiety was mean fullness over 2-hour periods measured by monitoring changes in feelings of fullness and calculating the total area under the curve.

Methods: Twelve healthy young adults were recruited to a repeated measure randomised, single-blinded crossover trial to compare the effect of consuming noodles (180g) containing 15%, 20% and 45% amylose. Fasting blood glucose concentrations were taken from capillary blood samples with postprandial blood glucose concentrations measured at 15, 30, 45, 60, 90 and 120 minutes.

Results: Subjects consuming high amylose noodles made with flour containing 45% amylose reported greater satiety and significantly greater blood glucose levels at 15, 30 and 45 min (5.5 ± 0.11 mmol/L, 6.1 ± 0.11 mmol/L and 5.6 ± 0.11 mmol/L; $P = 0.01$) compared to subjects consuming low amylose noodles with 15% amylose (5.8 ± 0.12, 6.6 ± 0.12 mmol/L and 5.9 ± 0.12 mmol/L).

The total area under the blood glucose concentration curve after consumption of high amylose noodles with 45% amylose was 640.4 ± 9.49 mmol/L/min, was 3.4% lower than consumption after low amylose noodles with 20% amylose (662.9 ± 9.49 mmol/L/min), $P = 0.021$. Subjects reported greatest satiety 15 minutes after consumption for all noodle formulations with high amylose noodle consumption inducing the highest satiety. Satiety in subjects consuming noodles made with flour containing 45% amylose had the greatest satiety area under curve to 120 minutes.

Conclusion: Noodles made from high amylose wheat flour increase satiety and attenuate postprandial glycaemia in healthy young adults as characterised by the significantly lower blood glucose concentration and 3.4% reduction in glycaemic response.

#491

Increasing micronutrient retention and bioavailability through processing and genetic improvement

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Every year, millions worldwide suffer from iron (Fe) and zinc (Zn) deficiencies, leading to conditions like anemia and stunting. Biofortifying staple crops, such as wheat, is an efficient means to combat these micronutrient deficiencies. However, increasing Fe and Zn concentration in wheat grains could prove ineffective if processing methods diminish their content or bioavailability, or if antinutritional components like phytic acid (PA) hinder micronutrient absorption.

Thus, this project aimed to investigate the impact of various wheat processing methods on Fe and Zn retention and bioavailability (estimated through PA/Zn or PA/Fe molar ratio) in zinc-enriched wheat lines. Additionally, it sought to explore the genetic regulation of PA which could be an additional potential target to enhance biofortification effectiveness.

To conduct these analyses, 60 biofortified elite spring bread wheat lines were evaluated. These lines were milled at three extraction rates (70%, 85%, and 100%) and processed into chapatis and pan-bread. Monitoring Fe, Zn, PA content, and phytase activity throughout the production process revealed that milling significantly influenced Fe, Zn, and phytic acid variations. However, neither pan-bread nor chapati production adversely affected Fe and Zn concentrations. Fermentation and phytase activation in bread rendered the micronutrients more available than in chapatis with the bread obtained using 85% flour extraction rate being associated with the lowest PA/Zn (7.04) or PA/Fe (6.08) molar ratio, indicating an increased bioavailability of such micronutrients.

Overall, these findings confirm biofortification's efficacy in increasing Fe and Zn intake and underscore the importance of employing specific wheat processing techniques to maximize micronutrient consumption and bioavailability.

To better understand the genetic control of phytic acid content variation and evaluate the possibility of breeding for a reduced PA concentration, an additional set of 199 advanced spring bread wheat lines grown under both optimal conditions and reduced irrigation across two consecutive years were evaluated. Wide variation in phytic acid (ranging from 0.41 to 0.84 g/100g) was observed.

Although a strong correlation across environments could not be observed (r = 0.16 - 0.42), a strong QTL associated with significant variations in PA content in one unique environment was identified on chromosome 5BL, explaining more than 50% of the observed variation.

Further studies are required to validate the significance of this genomic region and elucidate the factors driving these changes. Nevertheless, selecting biofortified lines with reduced phytate content appears to be a promising approach to boost biofortification effectiveness.

#96

Exploring basal spikelet infertility through spatial transcriptomics

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Our work explores why the short branches (spikelets) situated towards the base of the wheat inflorescence (spike) produce fewer and smaller grains than those in the centre. The basal spikelets initiate first in development but soon lag behind the central spikelets, ultimately showing higher rates of floret abortion and giving wheat spikes their lanceolate shape. Uncovering the genetic network governing this phenomenon of basal spikelet infertility could facilitate breeding improved spike architecture, with the potential for higher per-spike, and perhaps per-plot, productivity.

To investigate this process, we generated a semi-spatial transcriptomic timecourse of micro-dissected central and basal spike sections collected at five stages of early spike development (Waddington W2-5). We utilised NILs differing at the VRT-A2 locus to allow us to exclude downstream targets of this well-characterised spike architecture gene. We found that even at W2, over 7,000 high confidence gene models, including 547 transcription factors, were differentially expressed between the central and basal sections.

After filtering for expression patterns of interest and against the rice literature, we identified *MOF1/MFS2* and *SEP1-6* as candidates for further study. Both promote the transition from spikelet meristems to floral meristems in rice and, intriguingly, are more strongly expressed in the centre versus the base of the early wheat spike. Given that floret abortion is determined by floret maturity two weeks pre-anthesis, we hypothesise that accelerating the spikelet-to-floral meristem transition in basal spikelets could reduce abortion and raise their productivity.

To test this, we aimed to boost the expression of our candidate genes specifically in the basal spikelets at W2-2.5. We developed a custom promoter by identifying genes with the desired expression pattern, then selecting putative proximal regulatory regions using tissue-specific ATAC-seq data. This promoter was shown to confer basal spikelet-specific expression to a tdTomato reporter and we are now generating additional transgenics to mis-express our candidate floral transition regulators.

We have also begun exploring spike development with fully spatial transcriptomics. Our semi-spatial dataset was used to inform selection of a 300-gene panel for VizGen MERFISH, a highly multiplexed form of fluorescence in-situ hybridisation providing subcellular resolution of single transcripts. MERFISH transcript counts correlate well with expression values from the semi-spatial RNA-seq (ρ=0.67±0.04 SD), indicating that the data can be used quantitatively as well as qualitatively. In addition to validating findings from our semi-spatial study, the data is proving useful for generating new hypotheses on the genetics of spike development and basal spikelet infertility.

#283

High throughput phenomic and multi-omic analysis of the pan wheat cultivars reveal complex response plasticity to moderate drought stress

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In recent years, more than 15 high-resolution wheat genome sequences have been made available to the wheat community by the International Wheat Genome Sequencing Consortium and the 10+ Wheat Genomes Project. The wealth of genomic resources coupled with extensive phenotypic information and in-depth multi-omics analyses allows us to understand the intricate relationships between yield potential, seed nutritive and anti-nutritive component accumulation and broad adaptation to abiotic stresses such as drought.

Using high-throughput phenomics, proteomics, metabolomics profiling and micronutrient composition analysis, we monitored cultivar-specific variation in the grain composition of nine cultivars from the pan-wheat collection and the underlying mechanism for their ability to cope with moderate drought applied at the stem elongation stage.

We showed that significant variations in phenotypic traits in the vegetative and reproductive tissues were primarily manifested in significant changes at metabolome and micronutrient levels in the grain. However, less than 10% of the quantified grain proteome was affected, suggesting a minimal impact of moderate vegetative stress on grain macromolecular composition.

Integrated analysis of the multiple -omics datasets revealed that each cultivar employs a mixture of coping mechanisms. These mechanisms are related to stress escape, avoidance and tolerance by plants increasing intrinsic water use efficiency, controlling respiration and increasing photosynthetic activity, which together maintain grain yield and protein composition under water deficit conditions. Detailed analysis of proteins associated with nutrient reservoir activity reveals a potentially novel function of sulphur-rich proteins in abiotic stress adaptation.

Our analysis reveals cultivar-specific variations and the impact of moderate vegetative phase drought stress on grain quality, providing insights into bread wheat's -omics dynamics, including protein accumulations and sub-genome distributions, and valuable resources for wheat improvement beyond the reference genome.

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#339

Candidate loci and evolutionary insights on yellow rust resistance in an asian wheat nam population

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The global spread of aggressive races of yellow rust (*Puccinia striiformis* f.sp. *tritici*) poses a significant threat to wheat production worldwide. To address this challenge, genetic loci associated with yellow rust resistance were identified, and the role of the near-Himalayan region in the conservation and evolution of resistance loci was explored in this study.

A nested association mapping (NAM) population (1060 recombinant inbred lines (RILs)) was utilized. These RILs were derived from the common paternal parent Norin 61, crossed with a diverse set of maternal lines, which comprises 13 traditional and modern Asian bread wheat varieties originating from around the Himalayan Mountains, China, and Japan. The overlap between the origins of these varieties and the proposed origin of the yellow rust pathogen adds interest to this study. Moreover, most of the Asian germplasm exhibits significant yet underrepresented genetic diversity.

Extensive field trials spanning two years and two locations were conducted for both parental lines and Recombinant Inbred Lines (RILs) under intense artificial disease pressure created through the establishment of artificial epidemics.

A combined mapping approach for the whole NAM population was employed to identify Quantitative Trait Loci (QTL) in each environment, implementing IBD-based mixed models specific for multi-parent populations (statgenMPP). The analysis is based on a consensus map that spans 3,699 cM, including 3757 SNP markers with unique map positions. Our findings underscore the prevalence of yellow rust resistance within traditional wheat varieties situated in high-disease pressure areas south of the Himalayan mountains and in the lowlands of China.

The detection of two well-known rust resistance genes (Lr34/Yr18 and Lr67/Yr46) validates our results and their respective origins in China and Pakistan. Additionally, the identification of two potentially novel resistance genes on chromosome 3D and 5B presents promising candidates for integration into breeding programs.
By utilizing diverse wheat varieties in the NAM population originating from key geographic regions, we have gained valuable insights into the geographic distribution and evolution of yellow rust resistance.

#147

Molecular diversity in wheat - fungal pathogen interactions as a basis of novel breeding strategies

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Race-specific resistance (*R*) genes have played an important role in breeding against obligate biotrophic pathogens that cause diseases such as powdery mildew or rust in wheat. However, such genes are frequently overcome after being introduced into agricultural use. We have recently found that novel *R* genes can be efficiently identified in landraces held in genebanks based on geographical and environmental information. Landraces from Turkey, Iran and Pakistan have revealed a sursprising diversity of powdery mildew resistance genes.

Given the adaptive potential of fungal pathogens, we are also actively developing strategies to improve the use of race-specific *R* genes in wheat breeding and agriculture:

First, we want to diversify the molecular basis of disease resistance beyond NLR-based immune receptors. We have identified a novel chimeric kinase-MCTP protein encoded by the *Pm4* race-specific *R* gene. Notably, the *Pm4* gene has recently been found to confer resistance not only to powdery mildew, but also to wheat blast, opening exciting new avenues of research to develop combined resistance to two pathogens. In addition to the non-canonical Pm4 immune receptor, we recently identified the WTK4 tandem kinase as the product of a race-specific, powdery mildew resistance gene. Besides characterizing Pm4 and WTK4 function, we have developed an innovative mutagenesis approach in powdery mildew. We have identified the pathogen factors AvrPm4 and AvrWTK4, recognized by Pm4 and WTK4, respectively. Thus, there are successful initial attempts to understand the molecular basis of non-NLR based, race-specific wheat immunity.

Second, we are exploring several approaches to improve NLR-based resistance: one of them relies on the monitoring of avirulence genes to predict, based on pathogen-derived information, the potential durability of genes newly introgressed into wheat from wild species or wheat crop relatives.
We propose that the identification and monitoring of avirulence gene diversity in pathogen populations becomes an integral part of introgression breeding to ensure effective and durable resistance in wheat. Moreover, the knowledge of the wheat-fungal pathogen “interactome” promises to support breeding strategies for increased durability of resistance. We are also combining transgenic, overexpressed *NLR* genes to improve the durability of resistance.

Such combined (“pyramided”) genes have shown very good, long-term resistance in the field and represent promising new genotypes for breeding. Finally, we present a recent approach broadening NLR receptor specificity which was achieved by the improved molecular understanding of avirulence protein-NLR interactions.

#43

History of the bread wheat d genome

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Since the dawn of agriculture, bread wheat cultivation has been tightly interwoven with human history, culture, and migration in the Fertile Crescent and beyond. The suggested birthplace of bread wheat comprises an area along the southern shores of the Caspian Sea. This is where a hybridization between a tetraploid wheat and Tausch's goatgrass (*Aegilops tauschii*) created bread wheat, the most widely cultivated crop species today. Previous reports indicated that an *Ae. tauschii* accession belonging to lineage 2 (L2) was the main contributor of the bread wheat D genome.

Here, we analyzed comprehensive *Ae. tauschii* genomic resources generated by the Open Wild Wheat Consortium to shed light on the composition, origin, and evolution of the bread wheat D genome. We used a *k*-mer based approach (IBSpy) to resolve the haplotype composition of the bread wheat D genome at a fine, 50-kb resolution.

We found that the bread wheat D genome represents a tapestry of haplotypes from different *Ae. tauschii* lineages and subpopulations, with only ~75% of the bread wheat D genome originating from *Ae. tauschii* L2 accessions from the southern Caspian Sea region. In particular, we reveal the dynamics of introgressions from the genetically distinct and geographically restricted *Ae. tauschii* lineage 3 (L3) into the bread wheat D genome. *Ae. tauschii* L3 is geographically restricted to modern-day Georgia and contemporary elite bread wheat cultivars contain around 1% of L3 introgressions. These L3 genomic segments are comparable to the Neanderthals' genetic footprints in the human genome. Our analyses revealed a wide range of L3 introgression patterns across bread wheat landraces, with a cumulative size of L3 segments spanning a total of 660 Mb (16%) of the bread wheat D genome.

Our study revealed a complex evolutionary history of the bread wheat D genome. Following its emergence in the southern Caspian Sea region, bread wheat dispersal was accompanied by extensive gene flow from different *Ae. tauschii* populations. Although the proportions of alternative haplotype blocks in individual wheat cultivars are often low, the different segments can accumulate to considerable lengths across various wheat genotypes. These findings raise important questions about the adaptive potential of alternative haplotype blocks for wheat improvement.

#360

Health-promoting and technological properties of novel durum wheat materials and derived foods

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Whole wheat is a rich source of fibers and bioactive phytochemicals with health-promoting properties showing significant protection against chronic non-communicable diseases. There is evidence that wheat polyphenols are bioactive dietary agents able to prevent, delay, or even reverse chronic inflammation while supporting tolerance and tissue repair in a variety of inflammatory diseases, including intestinal disorders [1].

In the present study we characterized the phenolic acid profile and health-promoting potential of a newly developed high-amylose durum wheat genotype, obtained by tilling from cv. Svevo [2], and of Faridur, a durum wheat cultivar with soft kernel texture also derived from cv. Svevo by introgressing the wildtype *Pina* and *Pinb* from common wheat [3]. These materials and derived products were characterized through HPLC-DAD analyses to assess the phenolic acids profile and the associate antioxidant capacity (TEAC).

The elevated content of amylose in Svevo HA induced an increase of polyphenol content and TEAC especially in semolina and bread compared to the same products derived from Svevo wild type. We also characterized the effects of Svevo high amylose phenolic extracts (Svevo-HAPE) on endothelial activation, the first obliged step of the atherosclerotic process, analyzing the antioxidant and anti-inflammatory properties and the underlying mechanisms of action.

To this aim, human microvascular endothelial cells (HMEC-1) were treated with Svevo-HAPE (1-10 μg/mL) for 2 hr and then stimulated with the pro-inflammatory cytokine *tumour necrosis factor*-α to simulate the inflammatory milieu. In HMEC-1, endothelial-leukocyte adhesion, the expression of endothelial inflammatory mediators, intracellular reactive oxygen species (ROS) levels and the activation of nuclear factor (NF)-κB were evaluated by multiple assays.

The results showed that under inflammatory conditions, Svevo-HAPE suppressed the adhesion of leukocytes to endothelial cells and the related expression of endothelial inflammatory markers, being mediated by a reduction of NF-κB activity and intracellular ROS levels.

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#352

Establishing a robust long-term field screening site for the identification of frost tolerance in wheat

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Frost-after-spike-emergence (FASE) is a major production risk to global grain production. Assessing germplasm for its performance under frost stress under field conditions poses significant challenges. Field climate conditions are characterised by variability and unpredictability, making it challenging to decipher Genotype x Environment interactions unless the environments are thoroughly characterised. Establishing and characterising a long-term field screening site to phenotype large populations of wheat to search for tolerance to the reproductive effects of frost on wheat and other grain crops is of the utmost priority for wheat growers.

To establish a site in the Mediterranean Wheatbelt environment of Western Australia several key criteria must be met:
1) the site needs to be frost prone but able to give discriminating levels of damage;
2) the site must be as uniform as possible in elevation and soil type;
3) it must be flat but have enough local topography for cold air masses to pool consistently over the trial area;
4) it must have access to a large volume of water for irrigation for early sown (often dry sown) crops;
5) be situated close to a major city or town to service the site with research scientists, technical staff, contractors and casual labour for assistance with field measurements.

Agronomically the site needs to be managed to ensure uniform frost impacts with bulk crops sown early around the trial area to lead to early canopy closure and remain until after the frost window, while managing the rotational impacts of the site.

The Dale Research Site was established in 2016 and began from its humble beginnings with water supplied by two trailing knocker sprinklers, four firefighting pumps and one dam. From 2017 is was developed into a 40ha sight, with eight irrigated 75 x 360 m blocks that irrigated by a 70 m lateral irrigator, bore water and pumping systems backed up by a 16GL storage.

Two onsite weather stations and a significant number of Tinytag temperature loggers. The site to date has: screened ~1,200 of wheat germplasm for frost tolerance; evaluated biological and chemical treatment of ice-nucleating bacteria (2019 to 2024); demonstrated the power of a distributed temperature sensing network (DTS) (2017 to 2018); showed impact of crop nutrition (N, P & K) and canopy density and plant height on frost damage in wheat; been used for studies into the impact of ice nucleation activity in crop plants via stationary and drone field thermography (2017, 2018, 2020); yield studies under frost conditions (2016 to 2019), development of field-based frost controls to study yield impacts of frost (2017 to 2019).

The Dale Research Site has proven to be a reliable field phenotyping site for abiotic stress research over the last decade. Further investment into the establishment of similar sites for abiotic stresses is required to increase the throughput of germplasm to enhance abiotic stress tolerance in wheat crops globally.

#66

A phase-separated protein hub modulates resistance to fusarium head blight in wheat

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Fusarium head blight (FHB) is a devastating fungal disease of wheat worldwide. *Fhb1*, a widely used genetic locus of FHB resistance, is successfully applied to wheat breeding across all major wheat-producing regions. The promising candidate gene of *Fhb1*, *TaHRC*, includes susceptible (*S*) and resistant (*R*) alleles that are genetically associated with FHB symptoms, the encoding proteins of TaHRC-S and TaHRC-R differ only in their first 21 residues, yet their exact functions in FHB response remain unknown.

Here, we show that two TaHRC alleles opposingly drive liquid-liquid phase separation (LLPS) within a proteinaceous complex, determining FHB susceptibility or resistance. TaHRC-S exhibits stronger LLPS ability than TaHRC-R, which is governed by a cysteine variation at the N-terminus.

We further show that TaHRC recruits a class of proteins with LLPS potentials characterized by their intrinsically disordered regions, referred to as an “HRC hub” within the plant nucleus. TaHRC-S coalesces with hub components via strong LLPS, while TaHRC-R comparatively suppresses the formation of such condensates. Function of the key hub member TaSR45a, a serine/arginine-rich splicing factor, is dependent upon this TaHRC-driven condensate state, which in turn differentially directs alternative splicing, switching between susceptibility and resistance to wheat FHB.

These findings reveal a mechanism for FHB spread within a spike and shed light on the roles of complex condensates in controlling plant disease.

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#205

Comparisons of phenotypic vs genomic selection on yield in us winter wheat from mutiple environments

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Modern breeding programs are accumulating large historical datasets as annual genotyping and field trials are routinely stored in databases. These investments in phenotyping and genotyping can be leveraged far after the year they are evaluated. Field locations year to year can have very different environmental factors that have a significant impact on performance.

Breeders try to control extreme environments by performing multi-environment trials and evaluating lines across many different environmental conditions and across years to best understand performance and stability. Multi-environment genomic prediction models can be a great tool for predicting genotype performance in specific historical environments, or to leverage sparse testing designs to optimize field resources. The difficulty lies in the unreliability of local climate year to year. Our research suggests that a sparse testing design is the best way to leverage shared genomic information to get the best prediction accuracies.

However, publicly available climate information can be used to inform new or unknown environments. Using a gaussian distance kernel to model environmental relatedness generally outperformed using a kinship kernel to inform predictions.

Evidence suggests that the climate data up until mid-season can be a useful tool to provide breeders with in-season predictions without a significant drop in overall PA. Climate and soil characteristics are only part of what make up the total environmental effects, and prediction accuracies can be influenced by other factors such as unusual biotic stresses.

Comparisons of advancement and discard decisions between breeder phenotypic selection and model predicted genetic merit were summarized.

#162

De-novo annotation of the wheat pangenome reveals complexity and diversity of the hexaploid wheat pan-transcriptome

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Wheat is the world's most extensively cultivated crop covering over 215 million hectares annually. However, breeders are challenged to increasing production by approximately 60% within the next four decades to satisfy the needs of a growing global population. As part of the 10+ Wheat Genomes Project, genomes from 15 wheat cultivars were recently sequenced and assembled to enhance our understanding of genetic diversity and selection within the wheat pan-genome.

Our study contributes a wheat pan-transcriptome, featuring de novo annotation and differential expression analysis for nine of these cultivars across various tissues and whole seedlings sampled at dusk/dawn. Leveraging these de novo annotations, we uncovered genes absent from the Chinese Spring reference, identified cultivar-specific genes, and calculated the core and dispensable genomes.

Moreover, our expression analysis revealed conservation in the expression of a substantial core set of homeologous genes, alongside notable alterations in subgenome homeolog expression bias among cultivars. Co-expression network analysis elucidated the impact of sub-genome homeolog expression divergence and unveiled tissue-associated cultivar-specific expression profiles.

This comprehensive work not only provides a vital resource for the broader wheat community but also sheds light on the diversity in gene content and expression patterns among global wheat cultivars.

#511

Do wild species help to improve the quantitative traits of wheat?

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The durum wheat community developed a long-term collaborative project to switch durum wheat from orphan to crop for the future. The Wheat Initiative Durum wheat expert working group, CIMMYT, ICARDA and public and private partners worldwide joined efforts towards deciphering, cataloguing, and making available the huge amount of untapped diversity of tetraploid wheat germplasm including 11 T. turgidum subspecies. Tetraploid genetic diversity was shaped by exposure to diverse environmental conditions, pathogen and human selection, thus providing valuable alleles for resilience to abiotic and biotic stresses.

The Svevo Platinum reference sequence was used as a framework to study diversity and assemble a comprehensive, highly diverse Global Durum Genomic Resource (https://wheat.pw.usda.gov/GG3/global_durum_genomic_resources) including the Global Tetraploid Collection (GTC with 1,856 accessions, Maccaferri et el. 2019, Nature Genetics) and the Global Durum Panel (GDP with 1,033 accessions, Mazzucotelli et al. 2020, Frontiers). Joint molecular haplotype analysis of tetra- and hexa-ploid haplotype diversity led to characterization of genomic and demographic structure including haplotype transmission from Triticum dicoccoides to T. dicoccum to durum and bread free-threshing forms.

Based on these results, we assembled the Tetraploid wheat Core Collection (TCC; 432 accessions), effectively capturing most of the diversity in tetraploids. TCC was used to develop the tetraploid wheat pangenome which includes a golden level of 40 accessions PACBIO25X sequenced plus Bionano Optical mapping and Hi-C, a silver level (PACBIO10X) of 100 accessions, and up to 432 accessions in total for the bronze level.

The Global Durum Genomic Resource is currently under distribution and phenotypic evaluation. Phenotypic characterization started by targeting: (i) phenology, (ii) resistance to main wheat diseases, (ii) root system architecture, (iii) grain Yield components, spike fertility, and grain quality. GWAS allowed to discover loci relevant for wheat breeding and haplotype-tagging KASP markers were developed based on a highly efficient custom-made KASP primer design pipeline.

Main results will be presented and discussed, including the identification and tracing of favorable alleles. The Global Durum Genomic Resource is available at UNIBO, CREA and ICARDA. Genomics data are accessible on GrainGenes and will be continuously updated.

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Wheat fungal diseases, as wheat rusts (yellow rust-YR caused by *Puccinia striiformis*, leaf rust- LR by *P. triticina,* stem rust-SR by *P. graminis -Pgt*), and Septoria Tritici Blotch (STB, by *Zymoseptoria tritici*) pose significant threats to durum wheat production. Qualitative and quantitative genetic resistances are available in the tetraploid wheat germplasm, but not yet fully utilized by breeding due to lack of research and pre-breeding. Curated germplasm collections are key to identify favourable alleles for resistance through association mapping. Previously, large collections of tetraploid wheat have been established, the Global Durum Panel and the Global Tetraploid Wheat Collection. Selected sub-panels of them were characterised for resistance to rusts and STB in different multi-environment networks with natural/artificial infection or in controlled conditions with single *Pgt* races.

For LR and YR, 500 cultivars and 200 landraces were tested in 8/10 locations x years environments in the Mediterranean region and Argentina; STB resistance was looked for in 500 landraces and 250 varieties assessed for two years in Italy. A panel of wild emmer was characterised for SR and LR resistance to single races, to deploy their short LD decay for high resolution mapping.

Population structure revealed differentiated frequency of resistance among populations. For all diseases, GWAS was performed using high density array-based SNP datasets and fitting both single/multi-locus mixed linear models. 8 QTLs for YR and 16 QTLs for LR resistance were significant in at least two and three environments, respectively, with some significant in a wide range of environments (*i.e.* QYr.ubo-1B.1 effective in six out of eight environments and three QTLs for LR, on 6B and 7B, effective in six out of ten environments), as well as loci for SR resistance to multiple races. Some loci co-locate with known genes (i.e. QYr.ubo-1B.1 with *Yr64*), other look novel resistance sources. Haplotype analysis was conducted on the most effective QTLs within the germplasm diversity and are being targeted through haplotype-tagging KASP® markers, as valuable contribution towards molecular-assisted breeding.

Lastly, a rare resistance gene for leaf rust on chromosome 1B with complementary effect with a resistance locus on 7B has been mapped at high resolution in a domesticated emmer accession, thanks also to the availability of its whole genome sequencing as part of the tetraploid pangenome initiative. Upon leaf infection, the transcriptional response of some candidate genes is suggestive of their involvement in the leaf rust resistance.

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#664

Unlocking the secrets of female fertility in wheat for improved hybrid seed production

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One major challenge to the success of hybrid wheat is the low outcrossing rates of the maternal parent, partly due to the short functional life span of the floral stigma. Despite their importance in seed production, the post-anthesis viability of unpollinated stigmas has been overlooked. Thus, research to understand the dynamics of stigma survival in wheat could be key to improving the efficiency of hybrid seed production.

To first address this, we have developed a phenotyping approach for the quantification of female floral traits of field-grown plants by combining light microscopy and machine learning. The application of this approach reveals (1) the presence of distinct stigma growth patterns among cultivars; (2) this trait to be robust across different environments and; (3) the existence of a common underlying developmental programme. Secondly, we examined the seed set capacity of a subset of six male sterile (MS) cultivars, each varying in the onset of stigma deterioration.

To mimic a hybrid seed production scenario, MS cultivars were grown during two consecutive field seasons, and open pollination was allowed up to 15 days after flowering of the female parent. Detailed analysis of the temporal and spatial distribution of hybrid seed set along the MS spike showed that grain production remained remarkably stable during the pollination window tested.

Contrary to previous assumptions within the hybrid wheat community, our findings challenge the notion that stigma longevity poses a significant limitation to hybrid wheat seed production.
We, therefore, conclude that breeding efforts should prioritise the study of other female traits, such as enhanced access to airborne pollen.

#551

The application of short-read and long-read rna sequencing and high-resolution phenotyping to analyse wheat-pathogen interactions

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Pathogen attacks elicit dynamic and widespread molecular responses in plants. While our understanding of plant responses has advanced considerably, given the complexity of the wheat genome, there is much to characterise to understand the biotic stress-driven phenotypes and their impact on plant physiology. By assessing the progression of yellow spot disease in wheat, we have shown that calcium is uniquely manipulated in susceptible wheat, and the asymptomatic regions surrounding the lesions are desensitised to follow-up infection.

A follow-up comparison of calcium re-distribution in four wheat genotypes infected with yellow spot disease found that the re-distribution of calcium depends on how the disease develops based on the environment and pathogen load. It is independent of the major wheat susceptibility gene (*Tsn1*), which suggests that the pathogen manipulates other interactions and results in similar disease outcomes. To further characterise this phenotype, the team investigated the potential of other interactions that the pathogen can manipulate or deploy that result in the disease phenotypes we see.

For this, a large wheat experiment was established in the glasshouse with wheat genotypes that are rated resistant to very susceptible to yellow spot. The molecular analysis comprising both short- and long-read RNA sequencing was combined with high-resolution phenotyping to associate the mineral nutrient redistribution and fungal growth to construct pathways leading to disease resistance and susceptibility.

I will present our recent findings using this layered approach and its potential in identifying novel traits.

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The caige program – A valuable source of genetic resistance to several wheat diseases

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Wheat disease patterns will vary with a changing climate presenting new challenges for wheat scientists. Under this scenario, the identification of resilient wheat germplasm that combines both high yield and adequate disease resistance will be vital.

The CAIGE (CIMMYT-AUSTRALIA-ICARDA Germplasm Evaluation) concept has proven to be an effective way for Australian cereal breeders, pre-breeders and pathologists to optimize the use of wheat genetic resources from the wheat programs of the Consultative Group on International Agricultural Research (CGIAR).

The Australian Grains Research and Development Corporation (GRDC) provides partial support to this multi-partner collaborative program with international, and Australian national partners including public pathology programs, universities and private breeding sector companies. CAIGE, led by the University of Sydney, coordinates the selection, importation and quarantine (provided by the Australian Grains Genebank), multiplication, and assessment of the international crop germplasm (bread wheat, durum wheat and barley) in Australia, including concurrent testing for important foliar and soil borne pathogens. CAIGE manages the information generated using a relational database and disseminates the information through our website (caigeproject.org.au).

CAIGE aims to provide a basis for local Australian scientists, breeders, and pre-breeders to optimise selection decisions. More than a dozen valuable high yielding wheat lines have been identified across the main cereal cropping regions of Australia and have confirmed multiple pathogen resistance to three or more pathogens including the rusts (stripe, leaf and stem), *Septoria nodorum* blotch (both leaf and glume), *Septoria tritici* blotch, yellow spot (tan Spot), powdery mildew, and soil borne pathogens including root lesion nematode (*Pratylenchus thornei* and/or *P. neglectus*) and/or crown rot (*Fusarium pseudograminearum*). Likewise, CAIGE serves as platform to identify multiple disease resistant parents for the CGIAR breeding pipelines.

It is likely that many of these disease resistances could be novel as the imported materials generally have genetic diversity not present in Australian germplasm. Use of known KASP disease resistance markers for several pathogens have inferred novel resistances for some of the lines. Further work to explore the novelty of these resistances is planned in collaboration with the Australian and international partners. These lines provide significant value to the Australian breeding community and may also prove to be useful for other programs around the world, as may the CAIGE model of germplasm exchange and collaboration.

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#8

Optimizing wheat root traits for carbon sequestration: A multidisciplinary approach from controlled environment to field

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Developing resilient wheat varieties with optimized root systems is essential for ensuring food security and mitigating climate change impacts. This study aims to investigate crucial root traits, including root biomass and rooting depth, with the overarching goal of improving carbon sequestration through developing wheat with larger and deeper root systems.

Our methodology employs a multiscale phenotyping approach, integrating greenhouse experiments utilizing a cylinder method and a turface semi-hydroponic system. Leveraging state-of-the-art software such as SLEAP and RhizoVision Explorer, these experiments facilitate precise quantification and analysis of root traits. To enrich genetic diversity and uncover novel genetic determinants associated with improved root traits for carbon sequestration, collaborative efforts with the International Maize and Wheat Improvement Center (CIMMYT). Utilizing the Elite Diversity Panel International Experiment (EDPIE), featuring 150 genetically diverse wheat lines heat- and drought-adapted, our investigation has unveiled significant variation in early root traits for root biomass, total root length, and surface area traits.

A genome-wide association study (GWAS) has pinpointed two Single Nucleotide Polymorphisms (SNPs) linked to shoot biomass. Intriguingly, a robust correlation has been observed between root biomass and shoot biomass, alongside total root length, underscoring the interconnection of these traits. While from the GWAS one gene is identified, the other remains unknown. However, both appear to play a role in enhancing drought tolerance and increasing yield. Further validation is required to confirm these findings.

From the turface semi-hydroponic system, a subset of 19 genotypes exhibiting the most divergent root/shoot traits were subjected to field root coring in Obregon (Mexico), complemented by remote sensing data obtained through Unmanned Aerial Vehicles (UAVs). Moreover, correlations between root and shoot traits measured in field conditions and early root traits assessed through high-throughput screening methods will be investigated to pinpointing reliable proxies measured at early stage for mature root traits under natural field conditions.

This comprehensive approach accelerates phenotyping and selection processes for root traits crucial in the development of wheat varieties with root systems optimized for stress resilience and increased carbon sequestration for a more sustainable agriculture.

#82

High-throughput image-based phenotyping to predict crop performance in new

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Predicting crop performance for new environments and new genotypes is becoming increasingly important in plant breeding. Anticipating future performance is essential in the face of changing climate. Traditionally, multi-environment trials (MET) have been combined with statistical models to infer and predict genotype performance in different environments.

However, MET data are typically not available for most breeding stages. High-throughput phenotyping offers an alternative perspective to monitor genotype-by-environment (GxE) interactions. The state of a phenotype in a given environment can be viewed as the accumulated response of its genotype to environmental covariates. Modern measurement techniques allow to capture both - environmental covariates and phenotypes - with high temporal and spatial resolution.

Here, we provide two use cases that demonstrate the potential of exploring the temporal dimension of plant development in breeding.

(1) Temperature is a major driver of plant development in the stem elongation phase of winter wheat. We measured the height development of 352 European cultivars over four years to quantify genotype-specific *per se* temperature responses. Our results indicate that breeders have influenced the temperature response through co-selection for phenology in the past. In addition to the known major genes related to vernalization, photoperiod, or dwarfing, a genome-wide association study (GWAS) revealed additional unknown loci associated with temperature response. Predictions of winter wheat phenological stages (jointing, heading, and senescence) with cultivar-specific temperature responses explained GxE interactions better than thermal time.

(2) Intermediate traits, such as response parameters, are expected to be more stable across environments than target traits, such as yield. In a second study, we hypothesized that phenomic selection (PS) may allow selection for genotypes with advantageous response patterns in a defined population of environments. A set of 45 winter wheat cultivars was grown at 5 year-sites.

A trained PS model was able to predict overall yield performance, yield stability, and grain protein content with state-of-the-art accuracy. In summary, observing phenotype development over time at a few sites has the potential to reduce the need for large METs.

However, the processing of dense time-series data is resource and knowledge demanding.
As an outlook, we give insights on end-to-end (deep) learned image-based prediction approaches that have great potential to facilitate such research.

#241

Pre- and post-anthesis heat waves impact on yield under field conditions

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The detrimental impact of high temperatures on crop yields has been extensively documented for over a century. Heat waves, brief periods of high temperatures, have been recognized for the past four decades, but in the last years are gaining more attention as (i) they are expected to become more frequent and severe due to climate change, and (ii) the penalty imposed by them on crop yield are different in mechanisms triggered and larger in magnitude when normalised per degree increase in average temperature, than when comparing crops grown under lower or higher temperatures throughout.

Various experimental approaches exist to determine/quantify the effects of heat waves, none being perfect. Portable polyethylene tents, when placed over plots, offer a cost-effective and convenient solution, particularly for larger plots and fields lacking electricity access. Here we summarised results from a number of field experiments in which heat wave treatments were imposed determining the effects on key physiological and morphological determinants of grain yield when applied either at pre- or/and post-anthesis.

Analysis of data from 18 experiments conducted over 7 years in NE Spain, involving across experiments 10 different genotypes under irrigated field conditions, reveals that crop yield is notably more affected by pre- than by post-anthesis heat waves. This trend remains consistent across various background conditions, locations, and genotypes. Depending on the experiment and particular heat wave treatment imposed, grain number reduction ranged from 10 to 73 %, primarily occurs through grain abortion and mortality of floret primordia.

Conversely, reduction in grain weight was between 5.5 to 10 %, does not appear to be linked to changes in source strength, but due to a direct effect on reducing potential grain weight *per se*. It is urgent to establish a framework for further progressing in develop both management and breeding strategies to enhance resilience under these extreme climate events.

#276

Socio-economic impact of improved wheat varieties at farmers' field in India – An experimental approach

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Seeds of improved crop varieties are pivotal in modern agriculture since they serve as a key channel for technology (variety) delivery. Despite concerted efforts to foster improved wheat varieties in India, the seed and varietal replacement rates remain low. Frontline demonstrations (FLDs) have been advocated and practiced to alter this trend.

It is a popular extension method wherein the improved varieties (treatment) are demonstrated at the farmers' field and compared with the farmers' choice of variety (control). FLD aims to harness yield potential and allows the farmers to learn the 'package of practices' associated with the new technology through experts, which is echoed in the profitability and welfare of the farmers.

In this context, we evaluated the socio-economic impact of the recently released improved wheat varieties using an experimental approach. The seeds of the newly released five wheat varieties (DBW 187, DBW 222, DBW 327, DBW 332, and HI 1605) were offered under the scheduled caste sub-plan program (SCSP) to the SC farmers and demonstrated at their farm during 2022-23 crop season. A total of 539 farmers were selected randomly across five wheat-growing regions.

Yield gain analysis using the survey data from 26 collaborating centers indicated a significant difference ranging from 3.08% to 24.04% between treatment (improved wheat variety) and control (farmers' choice of variety). Yield gain was higher in Rajasthan, followed by Jammu & Kashmir and Uttar Pradesh. Among the demonstrated varieties, 'Directorate Bread Wheat 187' (short as DBW 187), demonstrated in Sriganganagar (Rajasthan), registered the highest yield gain (24.23%). In contrast, the wheat variety 'DBW 327' recorded the highest yield (7000 kg/ha) in the Sangrur district of Punjab. Gross margin analysis corroborated the positive effect of demonstrations with an incremental profit estimated at ₹ 8310 (~100US$) per hectare, leading to increased welfare.

Further, a profit function using the regression approach has been fitted to assess the impact of the demonstration using wheat-growing regions as a binary variable along with other farm-specific socio-economic factors. Regression analysis indicated that the profit levels are determined by the wheat grain price and the region wherein the variety is demonstrated.

Escalating input cost was a major constraint in adopting the improved wheat variety. Our experimental approach confirms the positive socio-economic impact of adopting technology in the farmers' field. Such demonstrations must continue to improve the socio-economic status, especially in socially backward communities, and cover regions where the adoption rate is substantially low.

#544

Understanding starch granule formation in wheat for improved nutritional and functional quality

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Starch is vital for humankind as a major source of calories in our diets, but we still do not fully understand the mechanism of starch biosynthesis in plants. Starch is synthesised in amyloplasts of the wheat endosperm as insoluble, semi-crystalline starch granules. Wheat and other Triticeae are unique in that they have a bimodal distribution of starch granules in the endosperm, containing large, lenticular “A-type” granules, and small spherical “B-type” granules.

These arise from a distinct spatiotemporal pattern of granule initiation during grain development, where A-type granules are initiated in each amyloplast at early developmental stages (by approx. 6-8 days post anthesis (dpa)), followed by B-type granule initiation at 15-20 dpa. We recently discovered that A- and B-type granule initiations occur via distinct biochemical mechanisms.

The initiation of A-type granules is mediated by Starch Synthase 4 (SS4), while B-type granules are initiated by the plastidial alpha-glucan phosphorylase (PHS1). Wheat mutants defective in SS4 produce highly aberrant A-type starch granules, while mutants defective in PHS1 have a dramatic reduction in the number of B-type granules. In addition to these proteins that control granule initiation, we discovered that the structure of the amyloplast plays an important role in determining the number and morphology of starch granules in the endosperm.

Using this knowledge, we can now manipulate granule size and shape in wheat to control the relative amounts of A- and B-type granules, and create diverse granule morphologies that resemble those typically found in other crops (e.g., rice and maize).

The discovery of these gene targets for modulating starch granule structure has major implications on improving the nutritional and functional qualities of starch.

#38

Controlling homologous and homoeologous recombination in bread wheat to enhance the development of original and productive wheat

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Crop improvement relies on the production of crosses between the most relevant accessions and the screening of the progeny for the most powerful descent gathering the best combination of genes of agronomical interest. Reshuffling of the genetic material between individuals and/or between species occurs through meiotic recombination with the formation of crossovers (COs).

Meiotic recombination is a fundamental process for all sexual eukaryotes that is required to produce balanced gametes and therefore is essential to the fertility of species. Over the past decade, considerable progress has been made in deciphering meiotic recombination in plants. However, much of that work has focused on diploid model species and thus largely ignored possibilities of practical exploitation and/or disregarded the special features of crop genomes like polyploidy.

As an allo-polyploid species, bread wheat (*Triticum aestivum* L.; $2n = 6x = 42$; AABBDD) must face two levels of control for recombination, between homologues and between homoeologues. Homologous recombination is a rare event (one CO/chromosome arm/meiosis) that locates in small regions (hot-spots) that are unevenly distributed along the chromosomes.

In bread wheat, this reduces drastically the genes' admixture in the pericentromeric regions that nevertheless bear ~50% of the genes. In addition, negative linkages between antagonistic genes are often almost impossible to break.

Finally, introgression of DNA fragments from related species that are frequently used to introduce biotic and abiotic resistance genes, fully blocks recombination leading to undesired linkage drag. Here, our latest results regarding understanding and improvement of recombination in bread wheat will be presented as well as their impact on breeding strategies.

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#9

Wheat breeding and scaling in Africa: challenges and opportunities from ICARDA'S perspective

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Associated with increasing population and urbanization, demand for wheat in Africa increases through the years with the current average annual consumption of 75 million tons. The continent produces only 25 million tons on a total area of 10 million hactares. Hence, Africa imports 55 million tons of wheat at a cost of 20 billion USD on an annual basis to cover the gap between demand and supply.

The productivity of wheat in Africa is very low (less than 2.5 t/ha) principally due to abiotic (heat, drought) and biotic (rusts, septoria, etc) stresses in addition to poor infrastructure, mechanization and other socio-economical limitations. The wheat breeding program at ICARDA develops high yielding wheat genotypes with resistances to these major stresses using classical and molecular approaches.

A modified shuttle breeding program involving two cycles of elite x elite crosses (simple crosses, F1top crosses while increasing F1s) and two cycles in the field (winter and summer seasons) at Merchouch station (Morocco) and Kulumsa station (Ethiopia) has been deployed. Using such breeding schemes, it takes only 4 years from crossing to distribution of elite genotypes to national programs through international nurseries. Genomic selection is applied for stage 1 trials at F6.

Elite genotypes at F7 are evaluated across key locations:

Wadmedani (Sudan)for heat tolerance, Merchouch (Morocco) for drought tolerance, Sids (Egypt) and Terbol (Lebanon) for yield potential and Kulumsa (Ethiopia) for resistance to diseases (rusts, septoria).
Yield levels of the top yielding elite spring bread wheat genotypes ranged up to 6t/ha at Wadmedani station of Sudan under extreme heat stress, 7t/ha at Merchouch station of Morocco under terminal moisture stress (260 - 300 mm) and 11 t/ha at Sids station in Egypt under optimum conditions.

Significant MTAs have been identified for heat and drought tolerance, resistance to rust and septoria, and nutritional qualities including iron and Zinc. Pedigree analysis showed that resistance sources for heat and drought tolerance in such elite wheat germplasm were introgressed from synthetic wheats and wild relatives mainly *T. dicoccoides*. Annually, ICARDA distributes 300 elite spring bread wheat genotypes to its partners through international nurseries.

In the last 10 years alone, more than 70 bread wheat varieties of ICARDA origin have been released by National Agricultural Research System (NARS) in the CWANA and SSA regions. Deployment of heat tolerant wheat varieties have been carried out in SSA and significant impact has been achieved in Ethiopia, Sudan and Nigeria. The heat tolerant irrigated wheat production in Ethiopia started 5 years ago and has reached 1 million ha in 2023 with average productivity of 4 t/ha.

Establishment of innovation plat forms (IP), cluster farming, strong partnership among stake holders such as national/regional governments, extension departments, national and CGIAR research centres, donors (CGIAR, AfDB, Agri-Banks), farmer unions and private sector miller’s associations, etc. was instrumental for achieving such a big milestone and setting the beginning of green revolution in Ethiopia. Deployment of such approach at scale could potentially transform wheat production and ensure food security in Africa.

#452

Breeding nutrient-dense, climate resilient and biotic/abiotic stress resistant/tolerant wheat varieties for western Himalayas of India

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Bread wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. The crop is considered crucial for ensuring food security and doubling farmers income in Kashmir valley. Therefore, efforts are being made by us to breed wheat varieties that will suit Kashmir valley.

Our broader wheat breeding objectives including

- (i) breeding early maturity wheat varieties that can fit into rice-wheat crop rotation,
- (ii) (ii) breeding stripe rust and foliar blight resistant wheat varieties,
- (iii) (ii) breeding wheat varieties tolerant to cold/freezing,
- (iv) (iv) breeding wheat varieties resistant to army worm and cereal leaf beetle infestation (two devastating insect pests of wheat in Kashmir valley).

In addition, efforts are being made to pyramid several traits/gene together through molecular breeding approaches. As a partner in several national and international wheat breeding programs, we have procured more than 10,000 wheat genotypes including 2 core sets, 7 mini-core sets, 3 mapping populations, released wheat varieties, advanced breeding lines, landraces and evaluated them for a variety of traits including biotic (stripe rust, leaf blight, army worm and cereal leaf beetle) and abiotic stresses (cold/freezing), nutrition, pre-harvest sprouting, lodging, growth, phenology, yield and yield contributing traits. The analysis of trait data led to the identification of most promising/candidate genotypes for almost all the important targeted traits. The candidate genotypes are being used in different breeding programs for the development of climate resilient, nutrient dense and disease resistant next-generation wheat varieties.

All the core sets and mini-core sets have been genotyped with 35K SNP genotyping platform. The trait data recorded in different environments over years and genotypic data has been sued together to identify genes/QTLs for these targeted traits through genome-wide association study (GWAS). Several important genes/QTLs/associated markers discovered for stripe rust, leaf blight, lodging, nutrition, pre-harvest sprouting tolerance, army worm, and cereal leaf beetle will prove useful in wheat molecular breeding programs world-wide.

We have also used multi-omics approaches including transcriptomics, metabolomics, lipidomics, proteomics and metabolomics for understanding the mechanism involved in cold tolerance, stripe rust resistance and cereal leaf beetle resistance using contrasting wheat genotypes.

#197

Do wild species help to improve the quantitative traits of wheat?

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Wheat-related wild species have been recognized as an important genetic resource for wheat improvement, and many accessions are maintained in genebanks. Useful qualitative genes, such as disease resistance, have been found in these wild species and used in breeding programs. However, there is insufficient evidence that these exotic germplasm can provide useful quantitative traits such as yield performance and abiotic stress tolerance.

The morphology of wild species, with small seeds in hard and brittle spikelets, is so different from that of cultivated wheat. Thus, it may be difficult to evaluate the "agronomic traits" of the wild species. Therefore, instead of evaluating the wild species themselves, we adopted a strategy of first developing a highly diverse wheat population by introducing various alleles from many accessions of wild species, and then evaluating the agronomic traits by the wild alleles in the wheat genetic background.

We used 43 primary synthetic hexaploid wheat (SHW) lines with common A and B genomes and D genomes from 43 Aegilops tauschii accessions covering its intraspecific diversity. We crossed and backcrossed the SHWs with a Japanese commercial cultivar "Norin 61" (N61), mixed the BC1F2 seeds and self-pollinated for several generations to fix the alleles. We named this diverse population in the N61 genetic background “multiple synthetic derivative (MSD) population”. We then selected 400 genotypes from the MSD population and genotyped each plant with genome-wide molecular markers, which was named "MSD Panel".

Field evaluation of the MSD population for heat and drought tolerance in Sudan, followed by GWAS, revealed a number of molecular markers for traits associated with tolerance to both stresses. Our intensive and systematic phenotypic and genotypic studies clearly indicated that wild species harbor positive alleles to improve stress tolerant quantitative traits. However, the direct use of such alleles in breeding programs remains challenging, unless the additive and epistatic effects of these alleles have been validated.

Therefore, using molecular markers for the stress-adaptive traits, we are producing near-isogenic lines (NILs) using MSD lines with favorable alleles as donor parents and N61 or other elite cultivars as recurrent parents.

#516

Dissecting the durum wheat QTLome for drought-adaptive trait

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Ensuring durum wheat resilience to abiotic stress under climate change requires a holistic approach to effectively dissect and leverage the genetic components, including the QTLome, of drought resilience, a complex trait. We extensively assessed the UNIBO Durum Panel, a GWAS panel including 248 elite durum wheat (*Triticum turgidum* L. ssp. *durum* Desf.) varieties and advanced lines from worldwide, for drought-related traits under both field and controlled conditions.

Response to high-temperature growing conditions and terminal stress was also assessed for two years in Maricopa station, Tucson, Arizona. Phenotypic characterization included two Unmanned Aerial Vehicles (UAVs) and a ground-based platform used to measure Normalized Difference Vegetation Index (NDVI), associated to biomass development, leaf chlorophyll content (SPAD), leaf rolling and dry biomass under terminal drought stress, Chlorophyll fluorescence (ChlF) assessed through LIFT sensor, leaf relative water content (RWC), osmotic potential (ψs), osmotic adjustment (OA), chlorophyll content (SPAD), and leaf rolling (LR).

All physiological data were adjusted for heading date as a covariate. Global R^2 was high, due to optimal experimental conditions, and equal to 89.6% for UAV-based NDVI and 72.3% for active OA. Notably, a high positive correlation ($r = 0.78$) between active OA and RWC was found under severe drought conditions
The QTLome was mapped based on the Illumina 90K SNP array. In total, 15 QTL hotspot clusters were identified for NDVI and other drought-adaptive traits, where a higher OA capacity was positively associated with RWC and/or SPAD, and negatively with LR, indicating a beneficial effect of active OA on the water status of the plant. The comparative analysis with the results of 14 previous field trials conducted under varying water regimes showed concurrent effects of five OA QTL cluster hotspots on normalized difference vegetation index (NDVI), thousand-kernel weight (TKW) and grain yield (GY).

Gene content analysis of the eight major QTL hotspots/clusters revealed the presence of several candidate genes, including bidirectional sugar transporter SWEET, rhomboid-like protein and DREB1. Our results support active OA as a valuable proxy for marker-assisted selection (MAS) aimed at enhancing drought resistance in wheat.

In parallel, the same materials extended to the full Global Durum Genomic Resource (GDGR, https://wheat.pw.usda.gov/GG3/global_durum_genomic_resources) were characterised for root growth angle (RGA), a relevant trait to access residual water stored in deep soil layers. RGA ranged from 13.0 to 160.8˚ with a h^2 of 0.82.

Genome-Wide association study (GWAS) pinpointed 7 and 13 QTLs in cultivars and landraces, respectively. Three major QTLs on chr. 2A, 6A and 7A were consistently found, accounting for a total of 23.5% and 14.5% R^2 in cultivars and landraces, respectively. *QRGA.ubo-6A.2* on chromosome 6A showed the largest R^2 effect (13.4% in cultivars and 8.6% in landraces) and, most importantly, overlap with a QTL cluster already identified for OA/RWC and NDVI in Maricopa.

The narrow haplotype is frequent in North American, French and ICARDA '70s cultivars. In contrast, the RGA-6A shallow haplotype was predominant in CIMMYT'60-SVEVO and CIMMYT'80-ALTAR_C84 lineages. RGA-contrasting cultivars based on the cumulative effects of chr. 2A, 6A and 7A QTLs are being assessed for their effects on yield and drought resilience at CIMMYT, ICARDA and the University of Queensland. Haplotype-tagging KASP® markers have been developed and candidate genes have been mined based on genomic and transcriptomic resources available. The most recent achievements in cloning *QRGA.ubo-6A.2* will be presented.

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#208

Wheat rust resistance genes: R we there yet?

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Our lab studies the resistance of wheat to its major diseases with a focus on the rusts (www.wulfflab.org). Over the last decade we have developed methods and resources for accelerated resistance (*R*) gene discovery and cloning in wheat and its wild relatives. I will provide an update on our expanded *Aegilops tauschii* sequence-configured resource which includes 500 re-sequenced lines and 46 high-quality genomes developed under the aegis of the Open Wild Wheat Consortium (www.openwildwheat.org). In collaboration with the group of Simon Krattinger we have used this resource for *R* gene cloning and to elucidate wheat domestication.

To our surprise, many of the wheat *R* genes that we cloned turned out to encode kinase fusion proteins (e.g. *WTK4*, *Sr43*, *Sr62*, *Rwt4* and *Lr39*). Focusing on *Sr43* and *Sr62*, we have collaborated with the group of Peter Dodds to identify and clone genetic interactors and used these to elucidate kinase fusion protein mechanism.

The wealth of cloned wheat rust *R* genes presents exciting opportunities for engineering resistance into elite wheat cultivars. However, we know precious little about the resistance spectrum of most of these genes, a prerequisite for the judicious design of multi-*R* gene stacks.

Therefore, we are using transgenesis to develop a true isogene library of cloned rust *R* genes that can be used to faithfully test the resistance spectrum of individual genes. We hope these efforts will support ongoing work to engineer super wheat strains with durable triple rust resistance.

#21

Editing of host susceptibility factor genes increased wheat yellow mosaic virus resistance without yield penalty

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Plant viruses account for almost 50% of the pathogens responsible for emerging and re-emerging plant diseases worldwide. Plant RNA viruses usually encode few proteins, and these success of viral infection depends on the deployment of host cell machineries, including host-encoded virus-compatible proteins called susceptibility factors (*S* genes).

Modification of S genes causes loss of susceptibility or recessive resistance. The yellow mosaic disease of wheat, caused by *wheat yellow mosaic virus* (WYMV) that transmitted by the soil-borne plasmodiophorid *Polymyxa graminis*, is a lasting and disaster threat in several countries of East Asia. Breeding for resistance varieties is the only optimal strategy for virus control.

By taking advantage of the former studies in barley that reported the susceptibility factor genes *HvPDIL5-1* and *HveIF4E* against BaYMV/BaMMV, both in genus of *Bymovirus* where WYMV is present, the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technology was deployed to edit the orthologous genes *TaPDIL5-1* and *TaeIF4E* in hexaploid wheat.

Simultaneous knockout of three homoeoalleles at either *TaPDIL5-1* or *TaeIF4E* resulted in complete WYMV resistance, while the single- and double-mutants remained susceptible (yellow discoloration and delayed growth).These approved that the recessive resistance in polyploid species was blinded by functional redundancy of the homoeoalleles.

In addition, knockout of either of both genes is likely able to impair viral hijacking of the host-encoded partners in the photosynthesis pathway. No penalty in *TaPDIL5-1* edited lines was observed, whereas an increase on plant height and delayed heading date were observed in *TaeIF4E* knockout lines. Several species in the genera *Triticum* and *Aegilops* were found showing the variations on WYMV accumulation, suggesting the occurrence of WYMV resistance in wheat progenitors and relatives.

Collectively, these results demonstrate a strategy to recover recessive resistance genes against viruses in hexaploid wheat by identifying the susceptibility genes in its diploid progenitors (e.g. *T. urartu* and *A. tauschii*) or relatives (e.g. *A. speltoides*, *T. monococcum* and *H. vulgare*), followed by manipulation of their homoeologues in transformable elite varieties via genome editing.

#423

Unleashing genetic potential: Battling fusarium crown rot in Australia

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Fusarium crown rot (FCR), caused primarily by *Fusarium pseudograminearum*, is a growing threat to wheat production in semi-arid regions worldwide. Its prevalence has surged in conservation cropping systems in recent years due to frequent cereal rotations and stubble retention. While several loci conferring FCR resistance have been identified in wheat, previous studies suggested interference from factors like plant height and heading time.

To remove the effects of such characteristics and accurately assessing effects of these loci, near isogenic lines (NILs) have then been generated for several of them. Facilitated by transcriptome profiling, markers tightly linked several of these loci have been obtained by analysing NIL-derived populations. With the assistance of the diagnostic markers developed, these loci were pyramided, and the results showed gene pyramiding can be effective in enhancing resistance to this disease in wheat. However, only limited number of resistant loci have been identified and additional resistant sources of resistance are urgently needed.

Our current efforts focus on introducing *Fhb7* gene into Australian wheat and combine it with other loci which are already in Australian breeding pipelines. This gene showed significant promise, with backcross lines in 2023 single-row field trials showing up to 11.4% higher grain yield under FCR pressure compared to lines lacking the gene. Similarly, disease severity differed significantly with the lines possessing Fhb7 showing less severe symptoms. Breeding lines with five loci (on chromosomes 2B, 2D, 3B, 5D and 7D (Fhb7), respectively) assessed in 2023 showed that the genotypes with Fhb7 and 3B locus had the highest grain yield in the presence of FCR followed by the lines with all five resistant loci, which are significantly higher than the lines without any of these loci.

In addition, our recent findings suggest a potential link between FCR resistance and drought tolerance. Analysis of RNA-seq from one NIL pair targeting the 2D locus in wheat that showed significant differences in FCR resistance and drought tolerance, revealed that similar regulatory frameworks were activated in response to both stresses.

Future use of drought tolerance genes in breeding program may potentially provide resistance to this disease in wheat. Furthermore, we at CSIRO successfully cloned the first gene conferring FCR resistance in barley. Investigating the potential of transferring this resistance to wheat through gene editing represents an exiting avenue for further improving FCR resistance. Taken together, the battle against FCR necessitates a multi-pronged approach.

#489

Overcoming genotypic dependency and bypassing immature embryo in wheat transformation by using morphogenic regulators

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Traditional genetic transformation of wheat relies on specific genotypes and immature embryo, which seriously impedes progress in wheat functional genome research. Recently, a class of morphogenic regulators (e.g., *BABYBBOM*, *WUSCHEL2*, *GRF4/GIF1*, *WOX5*, etc.) has been identified to improve the efficiency of genetic transformation of crops. We developed a wheat genetic transformation mythology to reduce the cycle of genetic transformation to 54 days.

A systematic comparison of the effects of multiple regeneration factors in wheat transformation revealed that the four regeneration factors, *BBM/WUS* & *GRF-GIF*, could achieve a transformation efficiency of up to 55.4% when co-transformed with the tested strains. In order to reduce the negative effect of regeneration factors, we compared the isolation efficiency of the latter when the test strain was co-transformed with *BBM/WUS* and *GRF-GIF* strains at 5:1 and 10:1 ratio, respectively, and found that the transformation efficiency was unaffected but the elimination efficiency was significantly increased to 95.5% with 10:1 ratio.

Meanwhile, visual isolation of regeneration factors could be achieved by expression pLTP2::*DsRed* cassettes with morphological regulators together. We tested the robustness of expressing the four morphogenic regulators simultaneously in multiple genotypes and the results all showed that this combination could greatly promote genetic transformation efficiency and editing efficiency in wheat.

Surprisingly, by combining the four morphogenic regulators, we succeeded in obtaining transgenic lines with approximately 13% efficiency with mature embryo from both Fielder and Kenong199 genotypes. This method greatly reduces cost and time needed for transformation in wheat.