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 Workshop 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.

You may like to cite abstracts from this book with the following citation:
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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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GRDC GRAINS RESEARCH & DEVELOPMENT CORPORATION

iWC 3RD INTERNATIONAL WHEAT CONGRESS

Workshop 1: From Reference to Pan: A New Era in Wheat Genomics

Sunday, 22 September 2024

Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 1, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Dr Manuel Spannagl, Helmholtz Center, Germany** **Dr Angela Juhasz, Edith Cowan University, Australia**	
09:00 – 09:10	Introduction	
09:10 – 09:30	Epigenomic dynamics during synthetic wheat formation	**Dr Maxim Messerer** Helmholtz Center Munich, Germany
09:30 – 09:50	A high-resolution map of evolutionary constraint in bread wheat using Triticeae genomes	**Dr Fei Lu** Institute of Genetics and Developmental Biolo CAS, China
09:50 – 10:10	IWGSC 2.0: Implementing the Wheat Diversity Project	**Dr Joshua Stein** International Wheat Genome Sequencing Consortium
10:10 – 10:30	Progress towards a haplotype pangenome for the wheat stem rust fungus and new surveillance strategies	**Miss Eva Henningsen** Australian National University, Australia
10:30 – 10:50	**Tea/Coffee break**	
10:50 – 11:10	A study of Aegilops genomes: towards understanding their evolution and potential for wheat improvement	**Ms Hamna Shazadee** University of Ottawa, Canada
11:10 – 11:30	Understanding the bread wheat genome: from ESTs to graph pangenomes	**Prof Dave Edwards** The University of Western Australia, Australia
11:30 – 11:50	Towards developing Australian wheat pangenome	**Dr Vanika Garg** Murdoch University, Australia

11:50 – 12:10	Einkorn Wheat Genomics: Genomes Pangenomes and Beyond	**Dr Vijay Tiwari** University of Maryland, USA
12:10 – 12:15	**Closing remarks by** Co-Chairs	

Workshop1 September 22, 2024, 9.10-9.30

Epigenomic dynamics during synthetic wheat formation

Maxim Messerer1,*, Neil McKenzie2, Angela Juhasz3, Richard Horsnell4, Yuxuan Lan5, Heidrun Gundlach1, Laura Gardiner5, Nadia Kamal1, Daniel Lang1, Azahara Martin2, Sara Goodwin6, W. Richard McCombie6, Michael Regulski6, Rob Martienssen6, Graham Moore2, Klaus F X Mayer1, Anthony Hall5, Manuel Spannagl1,7, Michael Bevan2

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Introducing new genetic variation from DD genome progenitors of hexaploid wheat into breeding programmes is a key part of wheat improvement due to the relatively narrow base of DD genome variation in current breeding populations. The addition of a large, closely related DD genome to a tetraploid AABB genome to form a stable hexaploid AABBDD genome provides an interesting opportunity to analyze epigenetic states and interactions of homoeologous genes and repeats in their diploid, tetraploid and hexaploid contexts.

We show that gene expression, DNA methylation, chromatin accessibility and interactions, and histone modifications are highly dynamic in newly formed hexaploid lines, with the DD genome undergoing higher levels of changes than the AA and BB genomes.

We are establishing how these changes may lead to stable gene regulatory and expression patterns. In the case of dominant, suppressed and balanced triad formation in leaf, developing grain and meiotic tissues, new expression patterns are rapidly established, many of which mirror in part those seen in stable hexaploids, with meiotic patterns being the most conserved at each ploidy level and grain development the most dynamic.
Gene methylation had a very minor influence on gene expression changes. Some regulatory networks during grain development have the same overall topology at each ploidy level but expand to incorporate new genes. Other storage protein networks incorporate DD genome transcription factors into a more dominant control of gluten genes, while some networks remain independent and are unaffected by ploidy changes.

Repeated elements, specifically the abundant and well-annotated full-length LTR classes, show decreased methylation, increased expression and chromatin accessibility as they age. Only a few young fl-LTR elements exhibited high expression levels of full-length transcripts, indicating potential transpositional activity.

Approximately 9 elements were differentially expressed upon hexaploid formation, and only three of these were associated with loss of DNA methylation, indicating that LTR elements appear to be mainly quiescent during wheat synthetic formation.

This suggests that epigenomic and genomic interactions are the primary mechanisms integrating the activities of newly formed hexaploid wheat genomes.

Workshop1 September 22, 2024, 9.30-9.50

A high-resolution map of evolutionary constraint in bread wheat using Triticeae genomes

Lipeng Kang[1,2,3,#], Zhiliang Zhang[1,2,3,#], Jiayu Dong[1,2,3], Yafei Guo[1,2,3], Jun Xu[1,2,3], Song Xu[1,2,3], Jijin Zhang[1,2,3], Zelin Niu[1,2,3], Aoyue Bi[1,2,3], Daxing Xu[1,2,3], Xuebing Qiu[1,2,3], Liping Jiang[1,2,3], Xinyue Song[1,2,3], Beirui Niu[1,2,3], Bingjie Zhu[1,2,3], Yiwen Li[1], Jing Wang[1,2], Changbin Yin[1,2], **Fei Lu**[1,2,3,4,*]

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Understanding the function of genomes is of paramount importance in biology. Comparative genomics has emerged as an invaluable tool decrypting the code of life, offering potent metrics of evolutionary constraint to pinpoint functional elements in the genome.

The tribe Triticeae encompasses major cereal crops, such as bread wheat, barley, rye, and their wild relatives. Not only being economically important, but also Triticeae exhibits tremendous diversity that facilitates comparative genomic studies for bread wheat.

Here, we sequenced and assembled genomes of 18 Triticeae species using PacBio HiFi sequencing. By integrating 60 genomes of other species across Poaceae, we developed the first generation of evolutionary constraint map of bread wheat (CMap 1.0), which captures the selective footprints shaped over the past 100 million years. Using the genome conservation score, we identified 2.8 million conserved non-coding sequences (CNSs) showing regulatory functions.

Further analyses revealed that CNSs are crucial in wheat adaptation across both micro and macro evolutionary scales. Given the changing climate and the pressing need for crop adaptation, our findings are expected to significantly benefit genetic research and breeding for bread wheat and many other crops.

Workshop1 September 22, 2024, 9.50-10.10

IWGSC 2.0: implementing the wheat diversity project

The International Wheat Genome Sequencing Consortium[1], **Joshua C. Stein[1]**, Victor Llaca[2], Kevin Fengler[2], Elodie Belmonte[3], Vincent Pailler[3], Hugo Zalzale[4], Leonardo Pereyra-Bistraín[4], Clément Debiton[3], Véronique Gautier[3], Pauline Lasserre-Zuber[3], Sophie Bouchet[3], John P. Hamilton[5], Pierre Marin[3], Isabelle Lhommet[3], Hélène Rimbert[3], Michael Alaux[6], Charles Poncet[3], Pierre Sourdille[3], C. Robin Buell[5], Frédéric Choulet[3], Moussa Benhamed[4], Kellye Eversole[1]

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The International Wheat Genome Sequencing Consortium continues its mission to accelerate bread wheat improvement through a strategy of increasing community coordination in the generation and access to genomic tools and resources. This approach led to the first complete genome sequence and annotation for cv. Chinese Spring, now a key reference resource in wheat biology, genetics and breeding (current release IWGSC CS RefSeq v2.1).

In Phase II, the IWGSC will take advantage of dramatically improved sequence and assembly methods to update the CS RefSeq and produce 12 additional reference sequences that represent the extent of world-wide diversity in hexaploid wheat (*Triticum aestivum* L. $2n = 6x = 42$, AABBDD). Previous high-density genotype studies identified 8 foundational phylogeographic haplogroups among 632 landrace accessions conserved at the INRAE Biological Resources Center (Balfourier et al., 2019). Those selected for this study exhibit minimal admixture and encompass thousands of years of genetic differentiation due to localized dissemination and cultivation throughout regions of Europe, Northern Africa, and Southern and Eastern Asia.

These highly diverse and ancestral gene pools are largely absent in modern cultivars, thus providing an untapped reservoir for adaptive traits with potential value in future breeding. Long-read sequencing, optical mapping, and chromosome conformational capture sequencing will enable chromosome scale reference assemblies approaching platinum quality for each accession. This project will sustain the role of the IWGSC CS RefSeq as a community-curated resource by precisely mapping existing gene annotations to the new reference, thus providing tractability with cumulative years of research literature and online knowledgebases.

To enable evidence-based gene annotation and to identify accession-specific or novel genes, this project will additionally develop transcriptomic sequences from each accession using multiple tissues and developmental stages. Finally, this project aims to represent the diversity in these and other emergent publicly available reference sequences using a Practical Haplotype Graph (Bradbury et al., 2022).

This compact storage method for pangenome data also provides tools for efficient and accurate genotype imputation for trait-dissection and breeding applications. These products will empower the wheat community to strategically incorporate underutilized germplasms to meet current and future challenges in bread wheat research and crop improvement.

This project is funded by NSF Award #2322957, the European Research Council ERC (project 3Dwheat #101044399), INRAE Plant Biology and Breeding department, and the IWGSC.

Workshop1 September 22, 2024, 10.10-10.30

Progress towards a haplotype pangenome for the wheat stem rust fungus and new surveillance strategies

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Wheat stem rust, caused by the fungal pathogen *Puccinia graminis* f. sp. *tritici* (*Pgt*), ranks highly as a threat to global food security. After the emergence of Ug99 (race TTKSK), which represented the first major international outbreak of stem rust since the widespread deployment of *Sr31*, other unrelated *Pgt* strains have also emerged and caused epidemics in east Africa and Europe.

The combination of Hi-C chromatin contact sequencing and long-read DNA sequencing led to the first nuclear haplotype phased genome assemblies for rust fungi, (wheat stem rust isolates Pgt21-0 and Ug99). Following these advances, we have generated haplotype-phased genome references for two important *Pgt* races TKTTF (ETH2013-1) and TTRTF (ITA2018-1) which have spread through east Africa and Europe. These isolates contain novel nuclear haplotypes designated G, H (ETH2013-1), I and J (ITA2018-1) which are highly divergent.

A comparative analysis showed that ETH2013-1 is one of two distinct lineages that emerged in Ethiopia in 2012 and share the same pathotype (TKTTF) but are highly divergent in genome sequence. Importantly, a potential nuclear exchange of the H nuclear haplotype involving the ETH2013-1 lineage and another lineage represented an isolate (IR-06) collected in Iran in 2015, was detected.

This observation suggests that, as observed for Ug99, nuclear exchange events may be behind other global stem rust outbreaks. The ETH2013-1 and ITA2018-1 reference genomes were mined for alleles of known avirulence (*Avr*) genes and their virulence genotypes at these *Avr* loci defined based on functional analysis of recognition of the encoded effector proteins by the corresponding wheat *Sr* genes.

We propose a genomics-driven approach to global rust surveillance with a focus on *Avr* genotypes to better anticipate the emergence of isolates that overcome widely used resistance genes.

Workshop 1 September 22, 2024,10.50-11.10

A study of *Aegilops* genomes: towards understanding their evolution and potential for wheat improvement

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Wheat plays a vital role in global food security, serving as a staple food crop worldwide. However, challenges like climate change and growing populations demand innovative solutions to enhance its productivity. Wild relatives, such as *Aegilops* species, represent a largely untapped reservoir of genetic diversity that could benefit wheat's adaptation and resilience.

However, understanding and exploiting this genetic diversity is challenging partly due to our limited knowledge of *Aegilops* genomes and the challenges associated with transferring beneficial traits free of the deleterious ones. Here, we sequenced the genomes of four diploid and 14 polyploid *Aegilops* species using advanced technologies, such as Oxford Nanopore, Illumina, PacBio, and Hi-C. This work complements our other assemblies of the remaining diploid species, with the overarching goal of developing a publicly available database of reference genomes of all *Aegilops* species.

High-quality genome assemblies with N50 values ranging from 116 Mb to 1.9 Gb and BUSCO scores of 95.8-99.1 were produced for all 18 genomes. These assemblies will be analyzed to identify structural variations and transposable elements, shedding light on the evolution of the *Aegilops* genus across ploidy levels. This comprehensive collection of *Aegilops* assemblies and annotations represents a valuable resource for wheat improvement.

Novel gene isolation and genome editing strategies will likely play key roles in the identification and the rapid transfer of useful genetics from *Aegilops* to *Triticum* species with the ultimate goal of enhancing the resilience and productivity of wheat, particularly in the face of environmental challenges.

Workshop 1 September 22, 2024,11.10-11.30

Understanding the bread wheat genome: from ESTs to graph pangenomes

Professor David Edwards, University of Western Australia

DNA sequencing technology continues to advance and support the development of improved varieties. It has been 20 years since capillary-based Sanger sequencing was applied to a transcriptome resource for wheat functional genomics. This identified genes and where they were expressed, and importantly provided a resource for large scale single nucleotide molecular marker discovery at a time when these markers were starting to replace simple sequence repeat markers.

Ambitious plans were developed to sequence the whole genome of wheat, with the variety Chinese Spring selected due to the availability of cytogenetic stocks; and chromosome arm specific BAC libraries, sequenced using the Sanger method decided as the approach. As next generation DNA sequencing technology advanced, it was becoming feasible to shotgun assemble genomes using this data. Whole genome sequencing was first attempted using Roche 454 technology, and the sequencing of isolated chromosome arms was attempted using Illumina paired reads. The successful sequencing of the gene content for 7DS and ordering of these genes based on synteny with other grasses in 2011 demonstrated that it was possible to assemble initial sequence-based maps for wheat.

Chromosome 7DS was quickly followed by 7BS in 2012 and 7AS in 2013, permitting comparison of gene contents between these homoeologous chromosome arms. The general approach was adopted by the IWGSC to sequence the remaining arms to produce the first rough draft of the wheat genome in 2014. As technology developed it became feasible to shotgun the whole genome of bread wheat to provide an improved reference in 2018.

By 2016 a significant amount of gene presence/absence variation was found in plant genomes, and it was becoming understood that a single reference cannot represent the species, leading to the construction of pangenomes. An earlier Australian project in 2012 had generated low coverage sequencing of 16 bread wheat varieties for diversity analysis, and this data was reused to construct the first bread wheat pangenome in 2017 using the iterative assembly approach and Chinese Spring as the base reference. This highlighted the huge difference in gene content between Chinese Spring and modern varieties.

As it becomes both cheaper and easier to sequence wheat genomes, with significant improvements in their assembly quality, many more genomes are being sequenced and compared. These high-quality assemblies were used to make the first wheat graph pangenome in 2022, providing a reference for genome analysis that represents modern germplasm. This reference, together with the sequencing of large diversity sets allows us to understand the genomic basis of many important wheat traits and identify genes and haplotypes that can be used for further crop improvement.

Workshop 1 September 22, 2024,11.30-11.50

Towards developing Australian wheat pangenome

Vanika Garg[1], Annapurna Chitikineni[1], Dion Bennett[2], David Tabah[3], Scott Sydenham[4], Tristan Coram[5], Dini Ganesalingam[3], Bertus Jacobs[4], Jon Wright[6], Anthony Hall[6], Chengdao Li[1], Xue Yong Zhang[7], Kadambot HM Siddique[8], Manuel Spannagl[9], Martin Mascher[10], Rajeev K Varshney[1]*

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Bread wheat (*Triticum aestivum* L.) is one of the most widely cultivated and consumed crops worldwide. Wheat production must increase significantly to meet the food demands of the rapidly growing population. The Australian wheat industry, which exports 65–75% of its production to over 50 countries, must enhance productivity to remain competitive internationally.

Modern genomics approaches, such as pangenomics, can assist in identifying and cataloguing superior haplotypes for traits that enhance crop productivity. Wheat genome analysis is challenging due to its hexaploid nature and large genome size (approximately 16 Gb). Despite these challenges, the International Wheat Genome Sequencing Consortium (IWGSC) has delivered genome assemblies for 10 wheat varieties, using NRGene technology.

Recent advances and reduced costs in genome sequencing technologies have popularised long-read sequencing for de novo genome assemblies. Consequently, research groups internationally, including those in Canada, the USA, the UK, China, Germany, France, and Saudi Arabia, are developing pangenomes for their wheat varieties. In collaboration with key industry stakeholders, we selected 10 representative Australian wheat varieties to generate PacBio's HiFi reads and Hi-C sequencing data.
This data will be processed to develop chromosome-length assemblies, which will be aligned against the publicly available reference genome. The identified variations will be integrated to create a

comprehensive graph-based pangenome. This pangenome will provide an exhaustive overview of genetic variation within the species, capturing both common and rare alleles.

These detailed genetic insights will enable the effective mining and utilisation of functional genetic diversity, facilitating the development of improved wheat varieties tailored to specific market needs and environmental conditions.

Workshop 1 September 22, 2024, 11.50-12.10

Einkorn wheat genomics: genomes pangenomes and beyond

Vijay Tiwari

University of Maryland College Park, USA

Wheat an essential crop for human civilization faces a threat from several biotic and abiotic challenges. Genetic improvement of wheat against such threats is urgently required for the global food security. Genetic improvement of wheat requires identification of new genes and alleles and their fast-paced integration into the elite cultivars.

Bread wheat has a narrow genetic base due to domestication bottleneck and rare hybridization events that were resulted in the evolution of bread wheat. Einkorn wheat a diploid ancient grain wheat belongs to the first set of domesticated crops. With simple diploid cereal genome (2n=2x=14; ~5Gb; AA) and untapped genetic diversity in its germplasm, einkorn wheat presents an excellent model system for gene discovery and deployment for wheat improvement.

We integrated germplasm resources and genomics tools to develop high quality genomics resources for einkorn wheat. Four reference level genomes are already assembled, and work is in progress to develop a pangenome of most diverse einkorn genomes.

These genomic resources have resulted in identification of new genes and alleles against wheat diseases such as leaf rust, powdery mildew and fusarium head blight.

Workshop 2: Domesticated and Wild Relatives: Challenges and Opportunities in Breeding

Sunday, 22 September 2024

Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 2, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Dr Upinder Gill, North Dakota State University, USA Dr Surbhi Grewal, University of Nottingham, UK Dr Gurcharn Sing Brar, University of Alberta, Canada**	
09:00 – 09:10	Introduction	
09:10 – 09:30	Genetic control of leaf trichome variation in the wild wheat *Aegilops tauschii*	**Ms Andrea Gonzalez Munoz** King Abdullah University of Science and Technology, Saudi Arabia
09:30 – 09:50	*Aegilops tauschii*: A treasure Trove of genetic Variation for Nitrogen Use Efficiency in Wheat	**Dr Parveen Chhuneja** Punjab Agricultural University, India
09:50 – 10:10	Exploring novel sources of rust resistance from wheat wild relatives and wheat genetic/genomics resource development	**Dr Upinder Gill** North Dakota State University, USA
10:10 – 10:30	Introgression of stripe rust resistance from spelt to bread wheat is complicated by resistance suppressors	**Mr Jujhar Singh Gill** The University of British Columbia, and University of Alberta, Canada
10:30 – 10:50	**Tea/Coffee break**	
10:50 – 11:10	Root system architecture and transcriptome reprograming regulated by	**Dr Tamar Krugman** University Haifa, Israel

	nitrogen-deficiency tolerance QTL introgressed from wild emmer	
11:10 – 11:30	Alteration of homoeologous gene expression by allopolyploidization: An example of cold response	**Dr Moeko Okada** Niigata University, Japan
11:30 – 11:50	Unlocking the treasure trove of pathogen resistant genes from *Thinopyrum* chromosome 7E	**Dr Hongwei Wang** Shandong Agricultural University, China
11:50 – 12:10	High-throughput evaluation of unknown rust resistances in genetic resources of wheat	**Dr Albrecht Serfling** Julius Kuehn Institute, Germany
12:10 – 12:30	Accelerating identification of resistance to Septoria tritici blotch disease in Tunisia	**Dr. Sarrah Ben M'Barek** Regional Field Crops Research Center of Beja, Tunisia
12:30 – 12:40	Open Wild Wheat Consortium (OWWC) phase III	**Dr Gurcharn Singh Brar** University of Alberta, Canada **A/Prof Brande B.H. Wulff** Associate Professor, King Abdullah University of Science and Technology
12:40 – 12:45	**Closing remarks by** Co-Chairs	

Workshop2 September 22, 2024, 9.10-9.30

Genetic control of leaf trichome variation in the wild wheat Aegilops tauschii

Andrea González-Muñoz[1], Catherine Gardener[1], Ana B. Perera[1], Chia-Yi Hu[1], Konstanze D. Laquai[1], Harryson Orjuela[1], Open Wild Wheat Consortium, Brande B. H. Wulff[1]

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Aegilops tauschii, the D genome progenitor of bread wheat, has a broad natural genetic diversity useful to improve stress tolerance traits in bread wheat through breeding and genome engineering. Trichomes are specialized structures on the aerial parts of the plant that can provide protection against pests, pathogens and drought and radiation stress, among others. Causal genes or alleles for trichome variation in wheat have not been cloned, possibly due to a complex genetic regulation of this quantitative trait.

We focused on trichome-regulating gene discovery in *Ae*. *tauschii* using a sequenced diversity panel of 493 non-redundant accessions and a genomic resource of 46 high-quality genome assemblies generated through the Open Wild Wheat Consortium (www.openwildwheat.org; Cavalet-Giorsa et al., 2023). We phenotyped the diversity panel for leaf edge trichome density and performed *k*-mer-based genome-wide association studies (*k*GWAS).

Significantly associated *k*-mers were mapped to multiple reference assemblies, revealing variation and novelty in the linkage disequilibrium (LD) blocks detected in comparison to using a single reference. LD blocks in chromosome arms 4DS, 4DL and 5DL were found predominantly associated in *cis* when mapped to accessions with high leaf edge trichome density and contain expressed candidate genes controlling trichome variation in this wild wheat relative.

These findings in *Ae. tauschii* correlate with quantitative trait loci described previously in rye (*Secale cereale*), barley (*Hordeum vulgare*) and the bread wheat D genome, suggesting a potential genetic conservation of this trait across Triticeae.

We will discuss these findings and our progress towards candidate gene isolation.

Cavalet-Giorsa, González-Muñoz, Athiyannan et al., (2023). Origin and evolution of the bread wheat D genome, bioRxiv, https://doi.org/10.1101/2023.11.29.568958.

Workshop2 September 22, 2024, 9.30-9.50

Aegilops tauschii: A Treasure Trove of Genetic Variation for Nitrogen Use Efficiency in Wheat

Parveen Chhuneja, Nitika Sandhu, Satinder Kaur

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Enhancing nitrogen-use efficiency (NUE) in crops is crucial for sustainable agriculture, as it reduces the need for synthetic nitrogen fertilizers, which are both costly and environmentally damaging. Efficient nitrogen use improves crop yields and stability under varying environmental conditions. This is particularly important for wheat, which is grown on more than 240 million hectares globally, as improvements in NUE can significantly impact overall nitrogen consumption.

In this study, we employed a multi-pronged approach to explore genetic variation for NUE in wild wheat *Aegilops tauschii*, the D-genome donor of common wheat. A panel of 135 *Ae. tauschii* accessions, sequenced under the Open Wild Wheat Consortium, was evaluated for root architecture and agromorphological traits under two nitrogen levels. Association mapping using 36,373,307 SNP markers identified 69 and 51 marker-trait associations for traits under N60 and N120 conditions, respectively.

Expression profiling of the putative candidate genes provided valuable insights for enhancing NUE through marker-assisted selection in wheat breeding programs using *Ae. tauschii* accessions with high NUE. A second strategy involved mining genetic variations for NUE in backcross introgression line libraries (BILs) derived from two in-house developed synthetic wheats. These BILs were phenotyped for NUE and yield traits at three differential nitrogen levels in two environments. The libraries were genotyped with 9,474 SNP markers using the 35K Axiom® Wheat Breeder's Array. Significant phenotypic variation was observed across 22 traits, and 65 marker-trait associations (MTAs) were identified, linked to various biological processes and yield traits.

Nitrate transporters are key genes for nitrogen metabolism, especially in cereal crops, due to the significant costs associated with nitrogen fertilizers. Four nitrate transporter gene families are known: NPF, NRT2, CLC, and SLAC/SLAH. *In silico* analysis identified 20 root-specific, 11 leaf/shoot-specific, and 17 grain/spike-specific genes in the hexaploid wheat genome. These genes were collocated to marker-trait associations related to nitrogen use efficiency identified in previous studies. However, the diversity of nitrate transporter (NRT2) genes in wild species is less understood. Novel allelic variations in 18 NRT2 genes were also mined from the genome of 306 *Ae. tauschii* accessions, identifying an average of 15 SNPs per gene, including those causing missense mutations or stop codons. Four to six haplotypes for these genes were generated and are being correlated with NUE.

These findings from multiple studies offer promising avenues for developing nitrogen-efficient, high-yielding wheat varieties by utilizing selected donors and the NUE-associated genes/alleles identified in this research.

Workshop2 September 22, 2024, 9.50-10.10

Exploring novel sources of rust resistance from wheat wild relatives and wheat genetic/genomics resource development

Upinder Gill[1]

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Wheat is an important cereal crop, providing stable sustenance for billions of people worldwide. The narrow genetic base of cultivated hexaploid wheat is one of the critical constraining factors that makes it prone to a range of biotic and abiotic stresses. Globally, plant diseases and pests cause approximately 21.5% yield losses in wheat annually, potentially escalating global food insecurity.

Additionally, climate change is bringing new challenges, such as the emergence of novel and more adaptive pathogen isolates. Wheat wild relatives (WWRs) are crucial in providing many beneficial stress resilience traits for wheat improvement. Evaluation of some of the WWRs, including *Aegilops umbellulata* and *Ae. geniculata*, *Ae. comosa*, *Ae. sharonensis*, *Ae. peregrinum*, and *Ae. neglecta* identified strong resistance against multiple wheat rusts, and the genetic and genomic characterization of the resistance sources are at advanced stages.

For *Ae. umbellulata*, we have generated a telomere-to-telomere genome assembly of an accession "PI 554389", containing resistance against multiple wheat diseases, including wheat rusts. The novel resistance genes are being characterized using biparental genetic mapping, association analysis, and transcriptomics approaches.

Similarly, introgression sizes have been precisely estimated for leaf and stripe rust resistance, such as *Lr56/Yr38*(*Ae. sharonensis*), *Lr59* (*Ae. peregrinum*), and *Lr62/Yr42*(*Ae. neglecta*). Characterization of rust resistance genes and genetic/genomic resources developed for these WWRs will be presented.

Workshop 2 September 22, 2024,10.10-10.30

Introgression of stripe rust resistance from spelt to bread wheat is complicated by resistance suppressors

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Wheat stripe rust is among the five priority one wheat diseases in Canada. Each infection cycle produces trillions of *Puccinia striiformis* f. sp. *tritici* (*Pst*) spores, which accumulate mutations that threaten to evade host recognition and overcome disease resistance. Resistance bred into new cultivars is often defeated within a few years under high disease pressure. For this reason, novel sources of stripe rust resistance are needed to limit yield losses, which can reach 60% in epidemic years. Bread wheat relatives, like spelt wheat, are reservoirs of resistance genes that can be mined for improving elite cultivars.

Two spelt wheat lines, 'CDC Silex' and '10Spelt17', have shown non-race-specific near immunity at the adult plant stage over multiple field seasons in British Columbia and in Saskatchewan, and against all tested *Pst* races in controlled environment growth chambers. Both cultivars showed susceptibility to all tested *Pst* races at the seedling stage, indicating that the resistance is only expressed at the adult plant stage.

The adult plant resistance in the spelt wheat lines is novel as no adult plant resistance (APR) gene has been identified in spelt and the resistance phenotype confers near immune response unlike all known APRs in wheat. Through allelism test, we proved that both the spelt wheat lines carry same resistance gene. When the spelt wheat lines were crossed with 'Avocet' (a susceptible bread wheat), the resulting F2 and F2:3 populations from each resistant spelt did not show expected Mendelian segregation ratios for stripe rust resistance. We observed severe segregation distortion to the susceptible phenotype.

Interestingly, when crossed with various CWRS (Canada Western Red Spring) elite cultivars, both resistant spelt lines generated susceptible F1 progeny, however, the F1 from 'CDC Silex' and 'CDC Origin' (susceptible spelt wheat) were stripe rust resistant.

To map the resistance loci in 'CDC Silex' and to develop DNA markers associated with the resistance, we performed BSAseq on a bi-parental F2 population of 'CDC Silex' and 'CDC Origin'. We have generated a high-quality genome assembly of 'CDC Silex' which was used to anchor polymorphic

SNPs between resistant and susceptible F2 bulks and develop KASP markers linked to the genomic region conferring resistance.

We identified a region on 2D chromosome that controls the resistance phenotype. By developing KASP assays, we have fine-mapped the region. Additionally, by looking at haplotype of the locus in spelt/bread wheat RILs, we have attempted to identify susceptibility factor/suppressor of resistance.

Workshop 2 September 22, 2024,10.50-11.10

Root system architecture and transcriptome reprograming regulated by nitrogen-deficiency tolerance qtl introgressed from wild emmer

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Nitrogen (N) is an important macronutrient that supports wheat growth and development, and its deficiency is one of the main factors causing abiotic stress. N-deficiency caused by extremely low- or high-water availability, is regarded as an agro-climatic stress that critically affects wheat productivity. Nitrate (NO_3^-) and ammonium (NH_4^+) are the primary N sources in agriculture; their uptake and assimilation are tightly controlled. In this study, we evaluated the response to severe N-deficiency (0.37 µM NO_3^- and NH_4^+) for 26 days, in the N deficiency tolerant introgression line (IL99) that carries a GPC QTL (*QGpc.huj.uh-5B.2*) derived from wild emmer wheat (WEW), as compared with its susceptible recipient bread wheat cultivar Ruta.

We identified in IL99 significant changes in 16 root system architecture (RSA) traits, as compared with Ruta (e.g., increased steep angle frequency, reduced shallow angle frequency, increased total root length, number of root tips in the lower part of root area, average root orientation, maximum number of roots, and root tips/root length ratio), along with five vegetative traits. The changes of RSA traits shifted root growth towards longer, deeper, and steeper root orientation, enabling nutrient acquisition from deeper soil layers and a larger soil volume. This mode of adaptability by RSA traits modifications to irregular distribution of N in the soil is a key determinant of IL99 performance.

Comparative transcriptome analyses at the whole plant level showed that IL99 also has distinct gene expression reactions to NS, including more upregulated genes in the leaves and the roots (3.14 and 4.45 times more) than in Ruta. GO and KEGGs pathway enrichment analyses identified unique pathways in IL99, such as phenylpropanoid biosynthesis, glutathione metabolism, and plant hormone signal transduction and metabolism. Specific genes in these pathways can be regarded as candidate genes for NS tolerance, including protein kinases, signaling and metabolism of plant hormones (JA, IAA, GA, ABA, and Ethylene), nitrogen recycling, carbohydrate metabolism, transcription factors, and amino acid metabolism.

The unique transcriptome reprogramming and whole plant-level morphological modifications identified in IL99 provided valuable insights into the mechanisms for improved N-use efficiency and N stress tolerance.

These sophisticated molecular adaptations conferred by the introgression of a QTL from WEW, recalibrated gene networks, enzymatic nodes, hormonal crosstalk, and resource allocation under stress mitigation. As a result, IL99 exhibits better vigor than the cultivar, which struggles under N limitations.

These insights hold great promise for enhancing crop productivity and resilience in nitrogen-limited environments.

Workshop 2 September 22, 2024,11.10-11.30

Alteration of homoeologous gene expression by Allopolyploidization: an example of cold response

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Polyploidization is a common phenomenon found in both plants and animals, and is considered an important evolutionary driver. However, the contribution of polyploidization to biological evolution and environmental adaptation is still debated. Wheat serves as a typical example of how polyploidy expands habitats compared with their parental species.

In this study, to reveal how the expression patterns of each homoeologous gene located on the A, B, and D genomes were altered by allopolyploidization, we conducted RNA-seq analysis of leaf samples from three hexaploid wheat (*Triticum aestivum* L. cv. 'Chinese Spring' (CS), 'Arina *Lr*For' (Ar), and 'Norin 61' (N61)), one tetraploid wheat (*Triticum turgidum* ssp. *durum* Desf.) cv. 'Langdon' (Ldn), an artificial tetraploid obtained by removing the D genome from CS (Tetra-Chinese Spring, TCS), two accessions of *Aegilops tauschii* Coss., and two synthetic hexaploid wheat lines obtained by crossing between Ldn and the two *Ae. tauschii* under both control and cold conditions.

The seeds of N61, Ldn, and the two accessions of *Ae. tauschii* were obtained by the National BioResource Project-Wheat (Kyoto University, Japan). TCS, Ar and synthetics were supplied by Dr. Hisashi Tsujimoto, Dr. Beat Keller and Dr. Yoshihiro Matsuoka, respectively.

We examined the change in the ratio of maternal (AB) to paternal (D) expression. For accurate homoeologous expression analysis, we used EAGLE-RC software (Kuo et al. 2020) to distinguish the RNA-seq reads into the A, B, and D homoeologs. First, we evaluated how the expression ratio of the AB and D genomes changed after the cold treatment.

Homoeologs that showed differential expression ratios (differentially expressed homoeologs, DEHs) accounted for approximately 1% of all genes and included cold-responsive genes. Second, we compared the expression ratios between synthetic and virtual synthetic wheat created by combining the count data of Ldn and *Ae. tauschii*. Virtual synthetics have more DEHs than synthetics, indicating that the regulation of gene expression occurs through the interaction of three homoeologous genes of ABD in the hexaploid background.

To evaluate the direct effect of allopolyploidization on homoeologous expression patterns, we compared DEHs among virtual synthetics, synthetics, and cultivars. The number of DEHs decreases with each passing generation.

In summary, our findings suggest that allopolyploidization allows allopolyploid species to adapt to novel environments, not only by combining their partial expression patterns, but also by obtaining new expression patterns of genes that are necessary for a particular situation.

Workshop 2 September 22, 2024, 11.30-11.50

Unlocking the treasure trove of pathogen resistant genes from *Thinopyrum* chromosome 7E

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Through decades of research, it has been established that a highly valuable cluster of resistance genes, comprising *Fhb7*, *Lr19*, *Sr25*, *Sr43*, *Bdv2*, and others, is located at the termini of chromosome 7E from different Thinopyrum species.Utilizing wheat-Thinopyrum substitution lines to construct mapping populations, we successfully cloned the Fhb7 gene, which confers broad resistance to Fusarium diseases, including Fusarium head blight (FHB) and crown rot (CR).

Further studies revealed that Fhb7 can broadly detoxify trichothecene toxins through de-epoxidation, thereby highlighting its potential in combating FHB and reducing deoxynivalenol (DON) contamination in wheat breeding.

Additionally, we cloned the well-known leaf rust resistance gene Lr19, which encodes a CC-NLR receptor protein capable of directly and/or indirectly recognizing avirulent genes in leaf rust, thereby initiating a broad-spectrum resistance.Based on genetic mapping of this gene cluster, Fhb7, Lr19, and Sr25, along with others, have been independently introgressed into various wheat backgrounds, demonstrating improved resistance without penalizing wheat grain yield.

Furthermore, by overcoming the linkage with the 7E-psy gene, we have pyramided double or triple resistance genes, creating a selection model for deployment in multiple resistance breeding.

Workshop 2 September 22, 2024, 11.50-12.10

High-throughput evaluation of unknown rust resistances in genetic resources of wheat

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Two of the most important fungal pathogens in wheat, leaf rust (*Puccinia triticina*) and stripe rust (*Puccinia striiformis*), develop races through mutation that lead to the breakdown of vertical resistances, benefit from climate change and increasing CO_2 content in the atmosphere. During evaluation projects, which include phenotyping at different environments distributed over Europe, around 15,000 wheat genotypes and genetic resources from the secondary gene pool with regard to their resistance against those rusts have been investigated.

To take the diversity of rust populations into the account, racial composition by virulence/avirulence pattern were investigated genetically and by wheat differential sets. Around 30 races in populations were detected. Only 13 out of 54 leaf rust resistances (*Lr*-genes) and 9 out of 33 stripe rust resistances (*Yr*-genes) are effective but as mostly vertical resistances vulnerable to be broken down. Therefore, a core collection of 800 genetic diverse wheat genotypes was selected to identify effective and more durable resistances reliably by a high-throughput phenotyping system.

The heritability across seedling high-throughput investigations and field trials could be estimated with h^2=0.77 and between field trials in six environments with h^2=0.7 (stripe rust) and h^2=0.92 (leaf rust). Based on on 49,646 markers from SNP-chip analysis, GBS approaches and genome sequencing data together with BLUES based on phenotyping data, GWAS was performed. Six highly significant new leaf rust and four stripe rust associations in genome regions, not associated with known resistances could be identified.

Already known resistance loci containing *Yr*-genes and *Lr*-genes were excluded from analysis based on marker information from NILs, e.g. *Yr10* on chromosome 1B, *Yr17* on chromosome 2B or *Yr6* on chromosome 7B, and *Lr22b* on chromosome 2D. A subset of 200 genotypes, registered in last 50 years in Germany was investigated under elevated CO_2 concentrations. The results gave hint, that infection pressure by rusts is increased and some of commonly used vertical resistances are less effective under an elevated CO_2 concentration.

This should be taken into account for future analysis of resistances. From the investigated secondary gen pool in a *T. monococcum* accession, a prehaustorial, race independent nearly non-host resistance as a candidate for a future durable resistance in wheat could be identified. The genetic background is a berberine bridge enzyme-like protein and differs from the vertical resistance based on *NBS-Lrr* genes, common in wheat.

Genotypes carrying such unknown resistances are now usable as breeding material together with closely linked or gene specific markers to accelerate the breeding process of resistant elite cultivars in the future.

Accelerating Identification of Resistance to Septoria Tritici Blotch Disease in Tunisia

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Tunisia is a key durum wheat producer in this region but is also the largest per capita durum wheat consumer in the world. The wheat-based production system in Tunisia is facing several challenges in keeping up with the growing demand to ensure food security, as the supply is largely reliant on imports, which currently account for over 75% of total needs. Septoria tritici blotch disease (STB) caused by *Zymoseptoria tritici,* is a particularly serious threat to Tunisian wheat production. Control of STB in Tunisia relies largely on fungicides and a few resistant cultivars although neither currently provides adequate levels of disease control. Faced with this situation, durum wheat breeding programs launched intensive work in search for novel sources of resistance. Up to date, Septoria resistance genes were characterized mostly on bread wheat while very few were recently identified on durum wheat. CIMMYT in partnership with the Institution of Agricultural Research and Higher Education (IRESA) and the National Institute of Field Crops established a Septoria precision phenotyping platform initially supported by CRP Wheat (CGIAR Research Program on Wheat) promoting the search for resistance to *Z. tritici* in close cooperation with stakeholders within Tunisia and National Agricultural Research System of North Africa as well as advanced research Institutions and Universities in Italy, France, Canada and United Kingdom.The platform is part of a global network of precision fieldbased wheat phenotyping, where selected locations at key environments host platforms that generate data on prioritized traits, fostering global germplasm and data exchange. Currently, the platform screens up to 20.000 accessions annually on average at two distinct hot spot locations representing two different climatic zones, complemented with artificial inoculation. Reliable data was obtained on a large number of lines/accessions and good novel resistance sources were identified. The development of molecular markers is under progress to effectively breed for STB resistance from the Mexico-based program. In addition, a collection of USDA Mediterranean durum wheat accessions has been phenotyped for resistance to STB and Tan spot, genotyped using the 90K array, and subsequent genome-wide association analysis (GWAS) was conducted to identify genomic regions associated with resistance to the latter diseases.

Workshop 2 September 22, 2024, 12.30-12.50

12:30 – 12:40	Open Wild Wheat Consortium (OWWC) phase III	**Dr Gurcharn Singh Brar** University of Alberta, Canada **A/Prof Brande B.H. Wulff** Associate Professor, King Abdullah University of Science and Technology

No Abstract Provided

Workshop 3: Trait Mapping

Sunday, 22 September 2024

Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 3, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Dr Ratan Tiwari, ICAR- Indian Institute of Wheat and Barley Research, India Prof Meixue Zhou, The University of Tasmania, Australia**	
09:00 – 09:10	Introduction	
09:10 – 09:30	Optimizing QTL mapping strategies: leveraging prior germplasm and genotypic assets for adult plant rust resistance	**Dr Laura Ziems** The University of Sydney, Australia
09:30 – 09:50	Genome association analysis for identifying leaf rust resistance loci in wheat	**Dr Liang Wang** Huazhong Agricultural University, China
09:50 – 10:10	Genome-wide association studies to identify QTLs/genes for fusarium head blight resistance in Indian durum wheat	**Dr Sundeep Kumar** Indian Council of Agricultural Research (ICAR) - National Bureau of Plant Genetic Resources, India
10:10 – 10:30	The less is more concept applies to root architecture for high-yielding wheat in Australia	**Dr C. Mariano Cossani** South Australian Research Institute, Australia
10:30 – 10:50	**Tea/Coffee break**	

10:50 – 11:10	Physiological traits associated with drought tolerance and yield in a winter wheat population (TX1112)	**Dr Qingwu Xue** Texas A&M AgriLife Research, USA
11:10 – 11:30	Multi-omic characterisation of seed dormancy and reserve mobilisation in wheat cultivars with late maturity amylase	**Dr Utpal Bose** Commonwealth Scientific and Industrial Research Organisation (CSIRO) AG&F and Edith Cowan University, Australia
11:30 – 11:50	Improving wheat for climate change resilience: discovery of QTL/genes and markers associated with heat tolerance	**Dr Sachin Kumar** Chaudhary Charan Singh University, India
11:50 – 12:10	Studying QTL-by-environment interaction pattern for yellow rust resistance in wheat	**Miss Jiaojiao Wang** Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany
12:10 – 12:30	Linking genebank genomics to the wheat pan genome	**Dr David Chisanga** Agriculture Victoria, Australia
12:30 – 12:50	High-resolution mapping of quantitative trait loci for Type-I FHB resistance in winter wheat cultivar 'Everest'	**Dr Umara Sahar Rana** University of Agriculture Faisalabad, Pakistan
12:50 – 12:55	**Closing remarks by** Co-Chairs	

Workshop 3 September 22, 2024, 09.00 – 09.30am

Optimizing QTL mapping strategies: leveraging prior germplasm and genotypic assets for adult plant rust resistance

Laura Ziems[1*], Urmil Bansal[1], Peace Kankwatsa[2] Robert Park[1], Davinder Singh[1]

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Generating populations for mapping quantitative trait loci (QTL) is typically a time consuming and expensive process. Where possible pre-existing germplasm and genetic data should be revisited to extract additional value through both assessments for new traits and or exploring alternative mapping strategies. In wheat biotic stress resistance genetics, there is often a standard susceptible genotype that is used to map genes donated from resistant parents.

This occurs for several reasons, crossing compatibility and reliability of the line, agronomically desirable background for delivery to breeders etc. As reference parents are continuously adopted, they become well characterised and more likely to be selected in the future. This results in the development of numerous bi parental populations with a common parent, designed to map the same foliar disease. These populations can be combined and assessed together as a nested association mapping population.

The aim of this study was to identify novel 'modifiers' enhancing durable APR genes. Three (F7) wheat stripe rust (WYR) mapping populations; ARZ/AvocetS [n = 146], West Savla 68/AvocetS [n = 110] and Kazouria Talian/AvocetS [n = 97] assessed for adult plant resistance (APR) to stripe rust in 2018 (employing pts. 150, 134, 239) and 2019 (pts. 110, 150, 134, 198) were further investigated. A total of 4,966 tGBS AgriBio markers positioned on the IWGSC Chinese Spring assembly v1.0 were available.

The genotypic diversity of the population was assessed and clustering into distinct families was observed. AvocetS, the common parent among the families is a stripe rust susceptible, whereas resistance donors are known carriers of APR to WYR; ARZ and Kazouria Talian carrying *Yr46* (4D) and West Savla 68 carrying *Yr18* (7D). The donors were observed presenting low infection types across both years of assessment with the prevalent most virulent pathotypes.

Three approaches; bi-parental mapping (QTL cartographer), genome wide association analysis (rrBLUP) and nested association mapping (StatgenMPP) were used to analyse the populations. Both known (*Yr18* and *Yr46*) and novel genes for APR were detected. KASP markers were developed for the novel region on 5A ~673-677 Mbp consistently detected across the three strategies.

This study highlights the potential for optimizing QTL mapping processes by revisiting pre-existing germplasm and genetic data, thus maximizing the value of previous findings and investments.

Workshop 3 September 22, 2024, 09.30-09.50

Genome association analysis for identifying leaf rust resistance loci in wheat

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Wheat is one of the most important food crops worldwide with ranking second based on the total production annually. Wheat leaf rust is one of the important factors affecting the global wheat yield, which can reduce wheat yield by about 50% during the epidemic period.

Breeding resistant wheat varieties is the most economical, environmentally friendly and effective way to control wheat leaf rust. In the present study, we identified a total of 27 stable leaf rust resistance loci at seedling stageby GWAS analysis of 569 wheat germplasms, of which six may be the known resistance genes *Lr10*, *Lr16*, *Lr37*, *Lr73*, *Lr79* and *Lr80*. Interestingly, one locus on chromosome 2BS was mapped in all three leaf rust isolates and it was located nearby the known leaf rust resistance gene *Lr16*.

Thus, we used the *Lr16* monogenic line Thather+*Lr16* to cross with the Chinese susceptible wheat variety Yangmai 18 to develop an F_2 population with 3459 plants. Five polymorphic SSR molecular markers were selected for genotyping of the population, and combined with the phenotypic against leaf rust isolate 18054, a molecular marker *SSRN.N* was co-segerated with the phenotype.

Combined GWAS and linkage analysis, the transcription factor *TaWN* was identified as a candidate gene for *Lr2BS*, and its function was verified by using VIGS and transgenic techniques. In addition, the interaction between *TaWN* and *TaGAPDH-4B* was confirmed by yeast two-hybrid(Y2H) method and firefly luciferase complementation experiment (LCA). We also developed the gene based molecular marker for *Lr2BS* in breeding leaf rust resistant wheat varieties.

Workshop 3 September 22, 2024, 0950.10.10

Genome-wide association studies to identify qtls/genes for fusarium head blight resistance in Indian durum wheat

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Fusarium Head Blight (FHB), caused predominantly by Fusarium graminearum and other Fusarium species, is a devastating fungal disease affecting wheat worldwide. It causes significant yield reductions, with severe outbreaks causing yield losses of up to 50% or more. Moreover, FHB-infected grains are often contaminated with mycotoxins, particularly trichothecenes such as deoxynivalenol (DON) and its derivatives. These mycotoxins not only reduce grain quality but also pose serious health risks to humans and livestock if consumed. This disease poses a significant threat to both durum wheat (*Triticum turgidum* L. subsp. *durum*) and bread wheat (*Triticum aestivum* L. subsp. *aestivum*), presenting a considerable challenge for wheat breeders worldwide.

Due to climate change, Fusarium head blight has become a serious threat to durum wheat in central India. Due to the complex nature of pathogen, it is very difficult to control the disease. Identification of resistance genes/QTLs for effective FHB resistance could greatly enhance our ability to breed durably resistant varieties. In spite of some notable achievements, the incorporation of resistance QTLs from non-adapted sources into commercial durum cultivars has faced challenges.

Factors such as the extended breeding process, linkage drag, or the suppression of resistance in durum backgrounds have hindered the release of commercial durum cultivars with these QTLs. Consequently, there is a growing preference for utilizing existing FHB resistance within durum cultivars as a more efficient strategy for quickly introducing durum wheat cultivars with improved resistance to the market. In view of the above, we performed a 90K SNP genotyping assay on an association panel of 285 diverse durum wheat genotypes including Indian wheat landraces.

These genotypes were evaluated for FHB resistance during 2020-21 and 2021-22 under controlled polyhouse conditions at IARI, New Delhi while, same set of lines were evaluated for FHB resistance under natural field conditions at IARI Regional Station, Wellington (Tamil Nadu) during 2021 and 2022.

Association analysis using three different multi-locus GWAS models (MLMM, FarmCPU and BLINK) identified a total of 17 significant SNPs which were identified on the threshold –log10 (H&B P-value) >0.05. These were mainly distributed on chromosomes 2A (3), 2B (2), 3A (1), 5B (3), 7A(1) and 7B (1) and some unknown markers were also identified. Four SNPs *viz*., wsnp_Ex_c16577_25095267, Kukri_rep_c115699_270, Ku_c7467_446 and Kukri_c9898_769 were consistently detected in most of the models and multiple environments.

Identified QTLs were further used for ontology analysis of the associated genomic regions to identify the potential candidate gene's functions. Some highly favorable alleles were identified from the 4 different environment studied. Identified candidate genes and highly favourable alleles showed their role in FHB resistance directly or indirectly.

The information generated in this study will be of potential value for improving FHB resistance in wheat cultivars using marker-assisted selection.

Workshop 3 September 22, 2024, 10.10 – 10.30

The *less is more* concept applies to root architecturE for high-yielding wheat in Australia

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Genetic yield gain of wheat in Australia since the 1960s is around 0.6% yr^{-1}. Selection pressure for yield and agronomic adaptation has changed crop traits related to the efficiency in the use of water, radiation, and nitrogen.

In this work, we report changes in root biomass and in its vertical distribution associated with selection for yield and agronomic adaptation. We sampled roots at anthesis using DNA as a proxy for biomass in a field experiment combining 4 wheat cultivars released during the last 55 years and 4 environmental conditions resulting from the combination of water and N availability. Yield ranged between 3.1 and 4.9 t ha^{-1}. Root biomass at the deeper soil layers (60-100 cm) related negatively to yield (R^2=0.31, P<0.03) and year of release (R^2=0.5, P<0.003).

Root biomass at deeper layers related negatively with water use efficiency (R^2=0.47, P<0.004), nitrogen use efficiency (R^2=0.28, P<0.04), and soil moisture at flowering (R^2=0.27, P<0.04). The proportion of roots allocated to deeper layers in relation to topsoil (10-30 cm) declined with year of release (R^2=0.63, P<0.0003).

Our findings in the field support previous work in rhizo-boxes and indicate that modern wheat cultivars adapted to winter rainfall environments produce higher yields with less root biomass in deeper soil layers.
This partially accounts for changes in water and nitrogen use efficiency with implications for breeding and crop management.

Workshop 3 September 22, 2024, 10.50-11.10

Physiological traits associated with drought tolerance and yield in a winter wheat population (TX1112)

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Hard red winter wheat (HRWW) is a major crop in the U.S. Great Plains. Texas A&M AgriLife Research has a productive winter wheat breeding program. Our cultivars (TAM prefix) are widely grown in both dryland and irrigated systems in the HRWW region and have been used as parental germplasm by wheat breeding programs worldwide.

Two of our most popular cultivars, TAM 111 and TAM 112, have been cultivated over one million hectares annually. Although TAM 111 and TAM 112 have both been documented with drought tolerance characters, the physiological mechanisms of tolerance and yield determination are not fully understood. A population (TX1112) containing 124 $F_{5:7}$ recombinant inbred lines (RILs) was developed from the cross of TAM 112/TAM 111. The objective of this study was to identify physiological traits related to drought tolerance and yield in TX1112 population.

The population was grown in 13 environments with combinations of years (2011-2017), locations, and soil water regimes (dryland, limited irrigation, and full irrigation) in the Southern Great Plains. Traits evaluated included heading date, plant height, biomass at maturity, harvest index (HI), three major yield components (spikes/m^2, seeds/spike and thousand-kernel weight, TKW), some early-stage traits, carbon reserve remobilization, and canopy temperature during grain filling.

Wheat grain yield varied largely among environments and genotypes. Water regime is the main factor affecting yield among the environments, in which yield ranged from 0.33 Mg/ha under severe drought to up to 7.12 Mg/ha under full irrigation. In general, plant height is positively correlated to yield, and early heading lines had yield advantage under drought.

Although both biomass and HI contributed to higher yield in genotypes, biomass is more related to yield than HI under drought conditions. Biomass at maturity explained 80% of yield variation under drought. Biomass at anthesis is also correlated to yield. At high water level, yield is related to spikes/m^2 and seeds per spike, not related to TKW. Under drought stress, all three yield components contributed to higher yield. Among the early-stage traits, biomass, NDVI and canopy height at jointing stage were positively correlated to yield.

In addition, there was a strong negative relationship between yield and canopy temperature during grain filling under drought.

Workshop 3 September 22, 2024, 11.10-11.30

Multi-omic characterisation of seed dormancy and reserve mobilisation in wheat cultivars with late maturity amylase

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Wheat quality defects, such as pre-harvest sprouting (PHS) and late maturity amylase (LMA), directly impact growers' profitability. Unlike PHS, LMA is a genetic defect present in specific wheat genotypes, with high expression of one or more alpha-amylase subtypes triggered by cold stress and temperature shock during the post-anthesis period. Both PHS and LMA, are complex traits involving interactions between genotype and environment, and the underlying interactions between genome and phenome are poorly understood.

Here, we selected five wheat cultivars representing resistant, intermediate, susceptible and control genotypes of LMA expression and performed transcriptomics across two-time points (dormant and 48h germination) and collected global proteome data across three-time points (dormant, 2 h imbibed and 48 h germination). The comparative analysis of dormant seeds between constituent expresser and LMA-resistant varieties showed the 710 upregulated and 1403 downregulated genes (Fold change=2; Adj. p value=0.05), where the upregulated genes were associated with galactose metabolism and increased ion transporter activity, evidencing the initiation of vacuolation and α-amylase activity. While the comparison between the constitutive expresser and intermediate genotype showed minimal changes.

Notably, the resistant cultivar retained the functions associated with nutrient reservoir activity, while the constitutive and intermediate expresser cultivars were high in carbohydrate metabolism and positive regulation of nutrient levels during the 48h germination period. The comparative analysis of the proteomics dataset between the wheat cultivars showed enrichment of peroxidase, antioxidant and oxidoreductase activities in the resistant varieties, suggesting their role in blocking starch catabolism and hydrolase activities at the 0, 2 and 48 h times compared to the constituent LMA expresser genotype.

The Weighted Gene Network Correlation Analysis on the proteomics dataset reveals that the LMA-resistant cultivars have a significantly higher abundance of endopeptidase inhibitors, peroxidases and nutrient reservoir proteins, explaining the role of protease inhibitors and the peroxidases in protecting the nutrient reservoirs in the seed.

We found a module (Pearson r=0.76) that positively correlates transcriptomics and proteomics analysis with seed germination and time (h). This module is overrepresented by genes and proteins involved in starch and sucrose metabolism and fatty acid degradation, which provide energy and carbon skeleton for seedling establishment.

The identification of a higher abundance of antagonistic regulatory proteins and the decrease of carbohydrate metabolism events associated with LMA-resistant varieties further enhances our understanding of additional mechanisms that regulate starch breakdown, hydrolase activities and nutrient mobilisation beyond the expression of alpha-amylase expression within the dormant seed.

Workshop 3 September 22, 2024, 11.30-11.50

Improving wheat for climate change resilience: discovery of QTL/genes and markers associated with heat tolerance

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Development of heat tolerant wheat varieties has assumed importance due to frequent episodes of high temperature stress. To understand the genetic architecture of important agronomic traits under heat stress, we used a doubled-haploid (DH) mapping population (177 lines) derived from the cross of a heat-sensitive cultivar (PBW343) and a heat-tolerant genotype (KSG1203).

This population was evaluated in alpha-lattice design with three replication for 11 agronomic traits under timely (optimum), late (mild heat stress), and very late sown (heat stress) environments at two locations namely Meerut (latitude 28° 58' 19.8588'' N; longitude 77° 44' 30.678'' E) and Lucknow (latitude 26° 53' 48.9696'' N; longitude 81° 5' 54.8268'' E) located in Indo-Gangetic Plain (IGP) region for three years (2018-2020); totalling 15 environments.

Best linear unbiased estimates for each trait and a sequencing based genotyping (SBG)-SNP genetic map comprising 5,710 markers were used for composite interval mapping of QTLs. The identified 66 QTLs were integrated into a physical map (5,710 SNPs; 14,263.4 Mb) of wheat. Each of the 66 QTLs (20 novel QTLs) explained 5.3–24.9% of the phenotypic variation.

Thirteen stable QTLs each with high PVE were recommended for marker-assisted recurrent selection (MARS) for optimum and heat stress environments. Selected QTLs were validated by their presence in high yielding DH lines. Three QTLs for 1000-grain weight were co-localized with known genes *TaERF3-3B*, *TaFER-5B*, and *TaZIM-A1*; a QTL for grain yield was co-localized with *TaCol-B5*, and gene *TaVRT-2* was associated with some of the QTLs for spike traits.

Specific known genes for several traits including thermostability, enhanced grain yield etc. were co-located with the QTLs. Furthermore, 61 differentially expressed candidate genes for heat tolerance in plants that encode 28 different proteins were identified.

The RT-qPCR analysis for 13 genes exhibited their role in heat tolerance at three grain development stages (10, 15, and 20 days post anthesis) in parental genotypes grown under normal and heat stress conditions. Further, Kompetitive allele specific PCR (KASP) markers for three major/stable QTLs were developed for exercising MARS.

Noteworthy aspect of the current study is the endeavour to validate the QTLs identified for MARS by examining their marker allele composition in the five top yielding DH lines each in timely, late and very late sowing environments.

Overall, present study reported stable QTLs, promising candidate genes, novel KASP markers and superior DH lines for heat tolerance, providing genomic resources to assist breeding for the development of high-yielding and heat-tolerant wheat germplasm and varieties.

Workshop 3 September 22, 2024, 11.30-11.50

Improved genome-wide association mapping approaches to understand genetic-by-environment interactions

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The investigation into genotype-by-environment interaction (G × E) has gained prominence owing to its theoretical and practical importance in understanding the genetic architecture of complex traits. Utilizing data from diverse environments to perform genome-wide association studies (GWAS) provides a convenient means to study G × E.

However, conducting GWAS with multi-environment data is challenging, mainly due to the absence of a systematic framework. In this study, we developed a GWAS pipeline that efficiently tests the main effects of markers across environments, the specific effects of markers in each environment, and the interaction effects between markers and environments. In addition, it is capable of handling both additive and dominance effects.

As an application, the established model was used to dissect the pattern of marker-by-environment interactions of yellow rust (YR) resistance in wheat with a dataset comprised of 5,243 hybrids and their 597 parents from 3 different experimental series and 19 environments.

We found that markers with additive effects showed stronger G × E interaction and explained high proportion of phenotypic variance. Additionally, QTLs with dominant effects were detected across more environments.

Workshop 3 September 22, 2024,12.10-12.30

Linking genebank genomics to the wheat pan genome

David Chisanga[1], Kenny Yu[1], Don Isdale[1], Mahdi Rahimi[1], Kerrie Forrest[1], Debbie Wong[1], Matt May[2], Josquin Tibbits[1,3], Sally Norton[2], Gabriel Keeble-Gagnère[1], Matthew Hayden[1,3]

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Plant genetic resources (PGRs) hold immense potential for crop improvement, offering valuable genetic diversity to address current and future production constraints. Modern genomics now allows the genetic potential of PGRs to be unlocked even faster. To fully harness the potential of PGR genomic data, pangenomes are needed to capture the full range of genetic variations across a plant species. There is also the need for digital tools to efficiently manage and leverage this wealth of information.

The Australian Grains Genebank (AGG) Strategic Partnership between Agriculture Victoria and the Grains Research and Development Corporation (GRDC) is transforming the AGG from a traditional seedbank into a future-oriented bio-digital resource center. This includes genetically characterizing its PGR collection, of which about 50 000 are wheat. Digital tools to rapidly connect PGR data to existing and new crop pangenomes are also being developed to facilitate the utilization of PGRs in research and breeding.

Here, we demonstrate how DNA fingerprints for PGRs can be cross-referenced with chromosome segments from pangenomes using *Brioche* and *Pretzel*. *Brioche* efficiently maps genomic markers to reference genome assemblies, while *Pretzel* – an interactive webtool for navigating multi-dimensional datasets from the scale of the nucleotide to pangenome – allows these mappings to be visualized, crucially linking the results to genebank genomics data.

Our approach empowers researchers and breeders to explore the relationship between PGR genetic variation and agronomically important traits. By bridging the gap between genotypic data and pangenome representations, *Brioche* and *Pretzel* accelerate the translation of PGR genomic characterization data into tangible and useful information for research and breeding.

Workshop 3 September 22, 2024, 12.30-12.50

High-resolution mapping of quantitative trait loci for type I FHB resistance in winter wheat cultivar 'Everest'

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Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schw), remains a pervasive menace to global wheat production, inflicting severe yield losses and compromising grain quality. Host plant resistance is the most effective and sustainable strategy against this pathogen. In this study, leveraging the high-throughput capacity of genotyping-by-sequencing (GBS), the intricate genetic architecture of type I FHB resistance was unraveled using regionally adapted cultivars.

To identify native quantitative trait loci (QTLs) governing type I resistance, a population of 181 recombinant inbred lines (RILs) derived from the 'Overland' × 'Everest' was genotyped using single nucleotide polymorphism (SNP) markers derived from GBS. Phenotypic assessment for type I resistance was conducted through meticulous greenhouse experiments, wherein the initial infection was induced via spraying spore suspension over wheat spike and resistance was evaluated by monitoring the initially infected spikelets in the spike before progression of disease symptoms within the spike.

Seven QTLs on chromosomes 1AL, 3BL, 4BS, 4BL, 6AL, 6BL, 7AS, and 7BL, contributing significantly to type I resistance in greenhouse conditions were identified. Notably, cultivar 'Everest' exhibited the presence of resistance alleles across these loci, with exceptions observed on chromosome arms 4BS and 6BL, where the donor cultivar 'Overland' contributed the alleles. This elucidates 'Everest' as a promising reservoir of native genetic resources for type I FHB resistance.

Furthermore, the selected SNP markers tightly linked with these QTLs were converted into robust Kbiosciene competitive allelic PCR (KASP) assays which hold immense potential to be used in marker-assisted breeding for FHB resistance in wheat.

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Workshop 4: Phenotyping of Complex Traits

Sunday, 22 September 2024

Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 6, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: A/Prof Bettina Berger, University of Adelaide, Australia** **Prof Nicola Pecchioni, CREA Council for Agricultural research and Economics, Italy** **Prof Zhong-Hua Chen, Western Sydney University, Australia**	
09:00 – 09:10	Introduction	
09:10 – 09:30	Supporting Wheat Breeding in Canada with High-throughput Phenotyping	**Dr Adam Carter** Crop Development Centre, University of Saskatchewan, Canada
09:30 – 09:50	Phenotyping chloroplast traits to screen for salt tolerance in wheat	**Dr Jayakumar Bose** Western Sydney University, Australia
09:50 – 10:10	Variability in root morphological traits and drought tolerance in winter wheat	**Dr Yinglong Chen** The University of Western Australia, Australia
10:10 – 10:30	Advancements and future prospects in wheat phenotyping	**Dr Alexis Comar** Hiphen, France
10:30 – 10:50	**Tea/Coffee break**	

10:50 – 11:10	Designing climate-smart wheat	**A/Prof Karine Chenu** The University of Queensland, Australia
11:10 – 11:30	Building the OZ wheat genome-to-phenome platform for the wheat research community	**Dr Jessica Hyles** CSIRO, Australia
11:30 – 11:50	Identifying tolerant wheat varieties under heat and drought stresses at anthesis	**Dr Jingjuan Zhang** Murdoch University, Australia
11:50 – 12:10	Spectral-genomic chain-model approach enhances wheat yield components prediction under mediterranean climate	**Mr Roy Sadeh** Hebrew University, Israel
12:10 – 12:15	**Closing remarks by** Co-Chairs	

Workshop 4 September 22, 2024, 09.00-09.30

Supporting wheat breeding in Canada with high-throughput phenotyping

Adam Carter[1], Andrii Fatiukha[1], Harmeet Singh Chawla[2], Steve Shirtliffe[1], Ian Stavness[3], Andrew Sharpe[4], Curtis Pozniak[1]

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Plant breeding requires phenotyping for multiple traits throughout the growing season in large, multilocation field trials. Unoccupied aerial vehicles (UAVs) equipped with sensors offer potential to assist breeding programs in data collection from these field trials. However, approaches to best use UAV-based imaging to support field trial evaluation remain underexplored.

To initially determine the potential of UAV-based imaging to support in-field phenotyping, a diverse hexaploid wheat (*Triticum aestivum* L.) nested-association mapping (NAM) population consisting of 1160 recombinant inbred lines was evaluated at three locations in two years. UAV-based multispectral imaging was conducted at 10-15 timepoints throughout the growing season at approximately weekly intervals. Image features, including spectral summary statistics, spectral indices, and texture features, were extracted from plot images at each timepoint.

Models of varying complexity ranging from simple linear regression to gradient boosted decision trees were assessed for prediction accuracy of breeding-relevant traits on test sets. LASSO regression models trained on image feature sets were able to predict days to heading (mean $R^2 = 0.76$), days to maturity (mean $R^2 = 0.84$), plant height (mean $R^2 = 0.70$), and grain yield (mean $R^2 = 0.64$) within testing environments more accurately than all other tested models. Cross-environment predictions were also evaluated, and a combination of image feature and genomic prediction models led to higher prediction accuracies for grain yield than either model alone (mean $R^2 = 0.39$). Image-based prediction models were then applied to durum wheat (*Triticum turgidum* L. var durum) breeding population field trials evaluated in four environments.

High within-environment LASSO regression prediction accuracies for grain yield (up to $R^2 = 0.88$) indicated the potential for high-throughput phenotyping of complex traits in breeding populations. Genome-wide association mapping of image features from the NAM population provided insight into the heritable information image features were capturing. The texture feature energy detected a marker-trait association near the locus of the wheat height gene *Rht-B1* and was associated with the presence of lodging in one environment.

This work is being built on to determine potential applications for high-throughput phenotyping to support the evaluation of breeding populations at earlier stages of the breeding program, and for traits which are difficult to manually measure including response to drought and heat stress.

Workshop 4 September 22, 2024, 09.30-9.50

Phenotyping chloroplast traits to screen for salt tolerance in wheat

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Salt stress severely inhibits photosynthesis in wheat, affecting growth and yield. Wheat generally excludes sodium and chloride ions from photosynthetic tissue to cope with salt stress. However, keeping salt away from photosynthetic tissue comes with additional energy costs to fuel salt exclusion mechanisms and the synthesis of organic compatible solutes resulting in reduced overall growth.

To minimise the energy demand and to improve overall salt tolerance in wheat, germplasms exhibiting enhanced photosynthesis while accumulating salt inside the leaf tissue must be identified. In this study, we screened for variations in chloroplast traits and functions in several cultivars of bread wheat *Triticum aestivum* (AABBDD) and its relatives *Aegilos tauschii* (DD), *T. monococum* (AA), *T. durum* (AABB) by exposing whole plants to 150mM NaCl.

We measured changes in chloroplast shape, chloroplast number per cell, chloroplast sodium ion accumulation (using Na-selective CoRoNaGreen dye), chlorophyll content and overall photosynthetic performance.

Our findings suggested that maintaining chloroplast shape and exerting greater control over sodium accumulation inside the chloroplasts are key to maintaining leaf greenness and enhanced photosynthesis in wheat under salt stress.

Workshop 4 September 22, 2024, 09.50-10.10

Variability in root morphological traits and drought tolerance in winter wheat

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Understanding root system morphology in bread wheat is critical for identifying root traits to breed cultivars with improved resource uptake and better adaptation to drought stress and other abiotic stresses. Variability in root morphological traits at early vegetative stages was examined among 184 bread wheat genotypes originating from 37 countries grown in a semi-hydroponic phenotyping system, followed by validation study in rhizoboxes, early-season drought, and terminal drought experiments in soil-filled columns.

The phenotyping experiment showed that, at the onset of tillering (Z2.1), phenotypic variation existed for both shoot and root traits. Of the 41 measured traits, 24 root traits and four shoot traits had larger coefficients of variation ($CV \geq 0.25$). Strong positive correlations were identified for some key root traits (i.e., root mass, root length) and shoot traits ($P \leq 0.05$). Strong relationships between performance traits with functional traits suggest their linkage to plant growth and fitness strategies.

The validation experiment in rhizoboxes (1.0 m deep) showed that genotypes with large root systems had 25% more leaf area and biomass than those with small root systems, which presumably reflects high canopy photosynthesis to supply the demand for carbon assimilates to roots. Phenology, particularly time to anthesis, was associated with root system size. Early-season drought delayed phenology, but there was recovery of root and shoot biomass at anthesis. The restricted water supply at sowing reduced leaf water potential, stomatal conductance, leaf photosynthetic rate, shoot and root biomass.

At maturity, early season drought reduced grain yield more in small-rooted Tincurrin than large-rooted Bahatans-87. Terminal drought reduced stomatal conductance, leaf photosynthesis, and transpiration rates faster in Bahatans-87 than Tincurrin. Terminal drought reduced grain yield in both cultivars, more so in Bahatans-87 than Tincurrin due to a reduction in grain number and grain size in Bahatans-87 and grain size in Tincurrin.

These studies demonstrated phenotypic variability in root system morphology in wheat genotypes at the tillering stage in a semi-hydroponic phenotyping system, which maintained their size ranking at booting when grown in soil.

Phenotypic differences and trait correlations among some interesting root traits may be considered for breeding wheat cultivars with efficient water acquisition and better adaptation to dryland environments.

Workshop 4 September 22, 2024, 10.10-10.30

Advancements and future prospects in wheat phenotyping

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As HIPHEN approaches its 10th-anniversary milestone in 2024, it stands as a leading service provider in the digital phenotyping domain, catering to the diverse needs of the Agro-Industrial sector worldwide. Our clientele includes seed breeders, universities, and agro-processing firms.

This presentation aims to review the evolving objectives and significant achievements of the phenotyping community in wheat, particularly leveraging emerging technologies such as Artificial Intelligence (AI). Utilizing concrete examples spanning all breeding stages that HIPHEN helped implement across the globe, we will illustrate how digital measurements add tangible value. This will encompass plot quality assessment, yield correction, phenomic prediction, and phenological-based breeding strategies.

Additionally, we will explore the array of measurement tools available to breeders to enhance assessment accuracy, ranging from full High Throughput Phenotyping platforms such as the one recently implemented in ICARDA or UM6P (Morrocco), ground vehicles (PhenoMobile®) such as one implemented in France with ARVALIS, drones (UAVs), satellites, handheld devices, to even smartphones.

An emphasis will be placed on how the use cases and technologies can combine to maximize value. This talk will also provide insights into emerging technologies/solutions that will be readily available in the near future.

Workshop 4 September 22, 2024, 10.50-11.10

Designing climate-smart wheat

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Abiotic stresses limit wheat production in major production regions worldwide. With climate change, increases in CO_2 concentration, temperature, evaporative demand and rainfall variability are projected to heavily impact different crop processes and their complex interactions. Abiotic stresses experienced by wheat crops in Australian production systems, their changes in recent decades and their future projections were characterized using crop modelling. Changes of climate in recent decades have caused significant impacts on wheat crops due to an increase in post-flowering heatwaves, drought and even frost stress.

In the future, to optimize productivity, projections indicate that growers should sow earlier or plant earlier-maturing cultivars (Collins and Chenu 2021, *Climate Risk Management*, 100300). However, despite such agronomic adaptations, heat and drought are expected to remain important factors limiting grain yield.
Combining physiological and modelling approaches, key adaptive traits such as transpiration efficiency and stay-green were identified (e.g., Casadebaig et al 2016, *Plos One* 11, 1-27; Christopher et al 2016, *Journal of Experimental Botany* 67, 5159-5172). The value of such traits varies spatially (across studied production regions) and temporally (current vs future projected climates) (e.g. Collins et al 2021, *in silico Plants* 3).

New phenotyping approaches have been developed to screen reliably for such traits (e.g. Ullah et al 2023, *European Journal of Agronomy* 144, 126757; Chenu et al 2018, *Journal of Experimental Botany* 69, 3181-3194), and wheat populations were screened (Christopher et al 2021, *Field Crops Research* 270, 108181).
The results, including advances in physiological and genetic adaptations will be discussed with a view to promoting increased grain yield in a context of changing environments.

Workshop 4 September 22, 2024, 11.10-11.30

Building the OzWheat genome-to-phenome platform for the wheat research community

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Accelerated genetic improvement of wheat is imperative to meet the demands of a growing global population in an era of changing climate. We established the OzWheat genome-to-phenome (G2P) platform to support wheat research and pre-breeding, by providing an understanding of complex traits and genetic targets for breeding. Through pedigree information, a panel of 285 lines was curated to represent global genetic diversity and important landraces, parents and cultivars from Australian wheat breeding programs.

This panel was used to generate a large data resource, including expansion to new genetic diversity. Data such as genome-wide sequence haplotypes, multi-environment gene expression variants and trait measures from multi-year field and controlled conditions, provide a high-resolution platform for gene and allele discovery.

We demonstrate the power of the OzWheat G2P platform to identify important genetic variants, and development of data visualisation tools to ensure ongoing accessibility of the platform. This provides the wheat research and breeding community a valuable resource to support cultivar development into the future.

Workshop 4 September 22, 2024, 11.30-11.50

Identifying tolerant wheat varieties under heat and drought stresses at anthesis

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Terminal heat and drought stresses have greatly affected wheat production since the high temperature and dry weather are in company with the stages of wheat flowering and grain filling. Global warming enhances the importance of improving the tolerance of heat and drought stresses in wheat for sustaining wheat industry.
For quantifying the impact of the heat and drought stresses and understanding the genetic basis of the tolerant genotypes, the heat and drought stresses were applied for 345 wheat genotypes for five consecutive days at anthesis in a heat chamber. Substantial genotypic differences in heat and drought tolerant levels were found.

The grain weight loss was arranged from 0 to 91% while the average was 52%. The tolerant lines showed almost equal flag leaf area and similar chlorophyll content of the flag leaf to the control plants, whereas, in general, the flag leaf area went down 21% due to the narrowed down leaf width by 20%, and the chlorophyll content of the flag leaf was decreased by 40%.

On average, the plant height was shortened due to the great reduction of the peduncle length under the stress treatment while the effective tiller number, the biomass and spike weight per plant were significantly reduced. The large loss of grain weight per plant was caused by the large deduction of thousand grain weight and grain number per spike. Especially, the seed setting rate was greatly decreased.

However, the tolerant genotypes with no or less grain weight deduction did not show the decrease of the leaf chlorophyll content and the shrink of the flag leaf area, which indicates the tolerant mechanisms in response to the heat and drought stresses.

Under the stresses, the large osmotic potential within the plants induced by small molecules, eg. proline, polysaccharides etc. may contribute to the tolerant levels, and the high remobilization efficiency of the stem water soluble carbohydrate is strongly suggested as another tolerant mechanism for those tolerant genotypes.

Further GWAS analysis are highly recommended to be carried out in this population for identifying the candidate genes in those mechanism pathways. In addition, the flag leaf area (in particular, leaf width), and the leaf chlorophyll content are suggested as valuable indicators for heat and drought tolerant genotype screening.

Workshop 4 September 22, 2024, 11.50-12.10

Spectral-genomic chain-model approach enhances wheat yield components prediction under mediterranean climate

Roy Sadeh[1], Roi Ben-David[2], Ittai Herrmann[1], and Zvi Peleg[1]

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In light of the climate change that jeopardizes future food security, genomic selection is emerging as a valuable tool for breeders to enhance genetic gains and introduce high-yielding varieties. However, predicting grain yield is challenging due to the genetic and physiological complexities involved and the effect of genetic-by-environment interactions on genomic prediction accuracy.

We utilized a chained model approach to address these challenges, breaking down the complex prediction task into simpler steps. A diversity panel with a narrow phenological range was phenotyped across three Mediterranean environments for various morpho-physiological and yield-related traits. In addition, canopy hyperspectral reflectance was captured by an unmanned aerial vehicle at heading.

The results indicated that a multi-environment model outperformed a single environment in prediction accuracy for most traits. However, no improvement in prediction accuracy was found for grain yield, ranging from 0.14 to 0.29. Spike number was assessed using machine learning from canopy spectra and exceed substances in a leave-one-environment-out validation.

The estimated spike number was utilized as a secondary trait in a multi-trait genomic selection and significantly improved grain yield prediction accuracy by 36% to 75% when only the calibration dataset includes the secondary trait.

This study emphasized the potential of hyperspectral-based high-throughput trait estimation as a secondary trait for improving genomic selection for wheat grain yield.

Workshop 5: Enhancing Wheat Productivity through Transformation and Genome Editing

Sunday, 22 September 2024
Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 7, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: A/Prof Alison Bentley, Australian National University, Australia** **Dr James Gaffney, United States Agency for International Development, USA**	
09:00 – 09:10	Introduction	
09:10 – 09:30	Enhancing wheat productivity through transformation and genome editing	**Dr James Gaffney** United States Agency for International Development, USA
09:30 – 09:50	Plant SynBio Australia – New opportunities for wheat improvement	**A/Prof Alison Bentley** Australian National University, Australia
09:50 – 10:10	HB4®: The World´s´ First GM wheat	**Mr Martin Mariani Ventura** Bioceres Crop Solutions, Argentina
10:10 – 10:30	Genome-editing to improve Australian wheat	**Mrs Tress Walmsley** Intergrain, Australia
10:30 – 10:50	**Tea/Coffee break**	
10:50 – 11:10	New technologies to accelerate wheat improvement	**Dr Melissa Garcia** Inari, USA

11:10 – 11:30	When will it be possible to trade gene-edited wheat?	**Prof Mike Jones** Murdoch University, Australia
11:30 – 11:50	Title to be announced	**Joanna Melonek** Australian National University, Australia
11:50 – 12:10	Growth regulators for improving wheat transformation	**Chaoqun Shen** University of Adelaide, Australia
12:10 – 12:30	Engineering adapted wheat for Australian growers	**Dr Jessica Hyles** CSIRO, Australia
12:30 – 12:50	From pathogen genomics to disease resistance engineering	**Dr Thorsten Langner** Max-Planck-Institute for Biology, Germany
12:50 – 13:10	Fine mapping and functional verification of adult-plant resistance gene yr54 to stripe rust in wheat	**Dr Shunda Li** Huazhong Agricultural University, China
13:10 – 13:30	CRISPR/CASΦ2-mediated gene editing and base editing in wheat and rye	**Mr Xiang Ji** Henan Agricultural University, China
13:30 – 13:35	**Closing remarks by** Co-Chairs	

Workshop 5 September 22, 2024, 09.10-9.30

Enhancing wheat productivity through transformation and genome editing

Dr Jim Gaffney[1]

[1]United States Agency for International Development, Alexandria, United States

Agriculture-led growth helps to lower food prices, creates greater prosperity and economic diversification, and is driven by technology and innovation. Wheat transformation has been validated in literature over several years to validate genetic effects and study phenotypic changes. Recent work has improved protocols for transformation, allowing increased scale and speed of development of transgenics.

The commercial release of HB4 wheat by Bioceres in Argentina paves the way for GM wheat to be grown around the world. Genome editing to improve crop production offers numerous benefits - predictability, precision, efficiency, and a more direct and shorter path to improved yield, quality, and resilience in varieties and hybrids.

Yet major challenges remain to fully capitalize fully on the promise including regulation of transgenics, and products from genome editing are a global policy patchwork, while intellectual property issues often mean that lengthy legal negotiations are needed for even simple research agreements. Finally, efficient and accessible plant transformation remains out of reach of many labs. Wheat offers its own unique challenges of having three genomes and is among the most traded of grains around the world which complicates acceptance of biotechnology products.

The workshop will bring together speakers to share their views and experiences on the importance of global agriculture-led growth and recent progress on transformation and genome editing in wheat, with recommendations for future research into application and downstream use.

This workshop will be co-hosted by USAID and the Australian National University who offer complementary global- and Australia-relevant perspectives on wheat transformation and genome editing.

The Center for Agriculture-led Growth at the United States Agency for International Development (USAID) focuses investments on research and development in plant and animal productivity. USAID supports current and past initiatives to improve wheat productivity, quality and resilience and is well aligned with international research to help deliver the next-generation of agricultural technologies into the hands of farmers.

Workshop 5 September 22, 2024,13.10-13.30

CRISPR/CasΦ2-mediated gene editing and base editing in wheat and rye

Sanzeng Zhao[1,†], Xueying Han[1,†], Yachen Zhu[1,†], Yuwei Han[1], Huiyun Liu[1], Zhen Chen[4], Huifang Li[1], Dan Wang[1], Chaofan Tian[1], Yuting Yuan[1], Yajie Guo[1], Xiaomin Si[2,*], Daowen Wang[1,3*] and **Xiang Ji**[1,*]

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The recent advancement in developing CRISPR/Cas9 system and various derivative tools such as base editors (BEs) has accelerated basic plant science research and crop improvement by creating multiple types of genetic variations. However, the use of Cas9 protein is frequently limited by the requirement of G/C-rich protospacer-adjacent motif (PAM) sequences, especially in triticeae plants, many of which are important food and forage crops carrying large and complex genomes.

In this study, we developed efficient gene and base editing tools by CRISPR/CasΦ2 system in wheat and rye. By sequentially testing crRNA expression, NLS incorporation and protein variants, we efficiently improved the efficiency of gene editing from near-background level up to 30% in transgenic wheat, with the mutations all being nucleotides deletions. Using the paired opposite crRNAs strategy in wheat and rye protoplasts, we have further improved the editing efficiency with ~1.5 fold higher than that produced with single crRNA. To develop CRISPR/CasΦ2-mediated base editors, we generated catalytically inactive dCasΦ2 by mutating the active sites of the RuvC domain (D394, E606, and D695), creating dCasΦ2-CBE and dCasΦ2-ABE.

Both base editors exhibited efficient C-to-T or A-to-G editing with very low levels of unwanted indels in wheat and rye. And the deamination window spanned from protospacer positions C2 to C17 or A9 to A11. Deep sequencing analysis indicated the high specificity in the editing activity of CRISPR/CasΦ2-mediated gene and base editing in transgenic wheat mutants.

With its unique properties, i.e., efficient use of TTN PAM and alternative base editing window, CRISPR/CasΦ2 provides a complementary genome engineering tool, which may find wide applications in future research on CRISPR/CasΦ2-mediated genome modifications, particularly for wheat and related triticeae crops.

Workshop 5 September 22, 2024,10.50-11.10

New technologies to advance wheat improvement

Dr Melissa Gracia, Inari, USA

Inari Agriculture is designing seeds to meet the challenges of feeding a growing global population while minimizing environmental impact. Our platform integrates Predictive Design and Advanced Multiplex Gene Editing tools to develop resilient seeds that require fewer natural resources and inputs, in a shorter time and lower costs than current approaches.

In Predictive Design, we harness the power of data, Artificial Intelligence, and cell-based assays to gain an understanding of the genes and pathways that underpin crop traits crucial for performance under varying environmental conditions.

Once the target sequences have been identified, we generate new allelic diversity using our Multiplex Editing toolbox to deliver multiple types of changes into elite parental lines. In 2021, we started a collaboration with InterGrain to work on wheat with a focus on increasing yield in Australia.
I will share some of what we have learned so far and my thoughts on how we can harness genome editing to enhance traits in wheat.

Workshop 5 September 22, 2024, 12.30-12.50

WS 8_Genomic Technologies for Ac

A. Cristina Barragan[1], Sergio M. Latorre[2], Aleksandra Białas[1], Angus Malmgren[1], Adeline Harant[1], Joe Win[1], Yu Sugihara[1,3], Hernán A. Burbano[2], Sophien Kamoun[1], Thorsten Langner[1,4]

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Genome structure and maintenance determine the evolvability of organisms. The genomes of fungal plant pathogens are often structured heterogeneously, harboring highly variable compartments and compartments of relative stability.

Often, rapidly evolving, virulence-related genes are associated with dynamic regions that are rich in repetitive elements, TEs and accessory genomic regions. An extreme case of such genomic structural variation are supernumerary mini-chromosomes (mChr), that are present in only some individuals of a species. We identified diverse mChr in the wheat and rice infecting blast fungus, *Magnaporthe* (syn. *Pyricularia*) *oryzae.*
These chromosomes are associated with intra- and inter-chromosomal rearrangements, copy number variation and horizontal transfer of genetic material and ultimately increase the genetic diversity and the adaptive potential of the blast fungus.

In addition, we have identified several virulence effector candidates on a mChr of a highly virulent pandemic wheat blast lineage. Here, I will discuss recent progress we made towards understanding mChr biology and potential strategies how we can use this information for pathogen-informed disease management via bioengineering.

Workshop 5 September 22, 2024,12.50-13.10

Fine mapping and functional verification of adult-plant resistance gene *yr54* to stripe rust in wheat

Shunda Li[1], Evans Lagudah[3], Ravi P. Singh [1,2], Caixia Lan [1,*]

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[3] CSIRO Agriculture and Food, Canberra ACT, Australia

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *Tritici*, is one of the major fungal diseases threatening wheat yield globally. Breeding resistant varieties is the most economical and effective way to manage this disease. *Yr54* derived from a CIMMYT spring wheat variety Quaiu#3 was located on the long arm of chromosome 2D (chr2DL) and expressed stable adult-plant resistance to stripe rust under many countries' rust environments, but its sequence is still unknown.

In the present study, the monogenic lines Aoc_*Yr54* only containing one major locus *Yr54* was crossed with the susceptible wheat line Apav#1 to construct an RIL mapping population. Combined the linkage analysis between phenotype and genotype and BSE-seq analysis, *Yr54* was limited to a 990 kb region on the end of chr2DL, with the aid of γ-ray fragment deletion susceptible mutants. In order to get the sequence information of target region, we sequenced and assembled a ~14.48 Gb Quaiu#3 genome using long-read sequencing and Hi-C.

We analyzed all of genes in the target region and verified the candidate genes by agrobacterium mediated genetic transformation. Finally, the candidate gene *G22* was identified as capable of enhancing stripe rust resistance in wheat, and its possible resistance mechanism may be involved in the accumulation of H_2O_2.
The related gene based molecular markers were developed and distributed to wheat breeders worldwide.

Workshop 5 September 22, 2024,12.10-12.30

Engineering adapted wheat for Australian growers

Jessica Hyles[1], Susan Hani[2], Richard Chambers[3], Steve Jobling[1], Ben Trevaskis[1], Donna Glassop[2], Dhara Bhatt[1], Jason Geijskes[2].

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While new genetic technologies such as gene editing provide potential to transform wheat breeding, there are current barriers to their adoption in Australian farming systems. The Office of the Gene Technology Regulator in Australia has classified organisms derived from SDN-1 editing and not containing a transgene as non-GM, but assurance of global market access will be required for security of export markets.

In addition, significant investment in commercial licensing of technologies alongside development of research capabilities are required. To re-coup these costs, the targeting of traits with demonstrated high value are attractive targets. It is important to note however, that this must not be at the expense of adaptation and yield which will be imperative to ensure grower adoption (and therefore return on investment).

A collaborative approach which involves multiple stakeholders will be necessary to deliver gene-edited crops to market. This presentation will discuss possible models and provide an example of engineering adapted wheat for Australian growers.

Workshop 5 September 22, 202411.10-11.30

When will it be possible to trade gene-edited wheat?

Michael G. K. Jones[1*], Muhammad Adeel[1,2], John Fosu-Nyarko[1] and Sadia Iqbal[1,3]

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More food is needed to feed the growing world population sustainably while preserving biodiversity for future generations. This requires the application of the best science and technology. Genome/Gene-Editing ('GEd') is providing exciting new opportunities for genetic crop improvement, and GEd is already contributing to a new understanding of gene function and to better produce.

The potential exists to break the nexus between Genetically Modified (GM) and GEd plants, and to avoid the issues that have prevented wider use of GM crops. Factors to consider related to commercial application include:

- Confirming the absence of 'external' nucleic acid sequence by sequencing the site of gene-editing, Q-PCR to show the absence of backbone T-DNA, and checking the sequence at the edited site to determine that there is no new ORF or new allergen.
- Complete genome sequencing is not necessary and would delay the application of GEd to wheat improvement.
- Possible off-target edits – the history of safe use of foods in which many mutations/edits are present shows that the vast majority off-target mutations/edits are of no biological significance to consumers
- Licensing of GEd technology
- Unintentional low-level presence of GEd seeds/produce in bulk trade

National and international policies/regulations on GEd produce are advancing rapidly. International harmonisation, or at least alignment, is a pre-requisite for achieving the full benefits and enabling international trade in GEd produce. Many countries in N & S America, the Asia-Pacific, and more recently in Africa and Europe, are reassessing their regulatory regimes.

The underlying principle is that if the GEd undertaken could have been achieved by conventional breeding, then there is no reason why produce developed using GEd, which achieves the same ends as conventional breeding but more rapidly and precisely, should be regulated any differently from conventionally bred varieties.

The international regulatory status of GEd crops will be presented, focusing on trade in the Asia-Pacific region.

Workshop 5 September 22, 2024, 9.50-10.10

HB4®: THE WORLD´S FIRST GM wheat

Martin Mariani Ventura[1,]

[1] Bioceres Crops Solutions, Rosario, Santa Fe, Argentina – Rosario National University,Rosario Santa Fe, Argentina

In 2003, Bioceres began a collaboration with the University of Litoral and the National Research Council to enhance the drought tolerance of key crops by the incorporation of new biotechnology discoveries, with particular focus on wheat, a crop abandon by biotechnology. This initiative was based on the research conducted by Dr. Raquel Chan on the genetic control of stress in plants and the discovery of the sunflower HaHB4 transcription factor.

The expression of the *HaHB4* gene in the model plant Arabidopsis resulted in a phenotype highly tolerant to drought. To address the deployment of this novel technology to commercial crops a series of regulatory validation steps were followed, including the full characterization of the GM crops and the final products obtained in field trials. During this process several notable scientific publications and patent families were published. After validating the results in wheat and soybean, Bioceres and its partners further developed the drought tolerance technology under the HB4® commercial brand.

In pursuing the global deployment of HB4® crops, initial efforts were directed towards obtaining regulatory approvals in countries that both produce and consume wheat and soybean. Wheat has been deregulated in Argentina, Brazil, and Paraguay for consumption and production, and it has been approved in other countries for consumption, including important markets such as United States, Australia and Indonesia.

In Argentina Bioceres has been commercializing the HB4® wheat technology in a first-to-market strategy, combining the HB4® varieties with biological seed treatments and digital components. By strategic alliances with breeding companies Bioceres is combining this novel drought tolerance trait with market-leading wheat germplasms to create unmatched varieties for the future seed markets. The out-license of the technology to seed companies will deliver the drought tolerance benefits to growers around the world.

Workshop 5 September 22, 2024, 9.30-9.50

Plant synbio Australia – new opportunities for wheat improvement

Alison R Bentley[1], Barry Pogson[1], Robert Furbank[1], Plant SynBio Australia partners[2], Darren Plett[2]

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In 2024, Bioplatforms Australia will launch Plant SynBio Australia, a new national capability for world-class synthetic biology applied to plant and crop species. It will address current bottlenecks in each step of the Design-Build-Test-Learn stages of the synthetic biology pipeline in plants. Within the facility initial work will focus on transformation and gene editing services across major crop species, integration of available 'omics technologies, and the development and deployment of new synthetic biology approaches that can be applied at scale through research to industry applications.

This introductory talk will provide an overview of this exciting new investment to accelerate Australia's capacity to deploy synthetic biology tools in crops, linking with and enhancing the existing portfolio of synthetic biology investments in Australia and beyond. It will also highlight specific opportunities for the Australian and international wheat community to access services and resources supporting future wheat improvement.

10:10 – 10:30	Genome-editing to improve Australian wheat	**Mrs Tress Walmsley** Intergrain, Australia

No abstract provided.

11:30 – 11:50	Title to be announced	**Joanna Melonek** Australian National University, Australia

No abstract provided.

Workshop 5 September 22, 2024, 11.50-12.10

Applications of developmental regulators in wheat elite cultivars

Chaoqun Shen[1,2], Matthew Tucker[1,2], Stuart Roy[1,2]

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Robust transformation technologies and efficient genome editing systems are essential for accelerated breeding and the production of enhanced wheat varieties. However, recalcitrance to genetic transformation in elite wheat cultivars significantly restricts the potential for wheat improvement through transgenic integration and genome editing. Recent studies have reported improvements in regeneration efficiency by manipulating the expression of developmental regulators such as WUSCHEL (WUS), BABY BOOM (BBM), GROWTH-REGULATING FACTOR 4 (GRF4) and its co-factor GRF-INTERACTING FACTOR 1 (GIF1), TaWOX5, and TaLAX1.

In this talk, I will summarize the application strategies of these developmental regulators in wheat and discuss the crosstalk between them. Additionally, I will present our latest results on testing the effects of these developmental regulators in different elite wheat cultivars. Finally, I will propose future aspects of developmental regulator application, including the potential use of protoplast regeneration systems to generate GMO-free gene-edited wheat cultivars.

3RD INTERNATIONAL WHEAT CONGRESS

Workshop 6: Agronomic Innovations

Sunday, 22 September 2024

Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 8, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Prof Richard Harper, Murdoch University, Australia** **Dr Kelly Pearce, WA Agricultural Research Collaboration｜DPIRD, Australia**	
09:00 – 09:10	Introduction	
09:10 – 09:30	Crop residue management for sustainable crop production	**Prof Kadambot Siddique** The University of Western Australia, Australia
09:30 – 09:50	Global wheat full semantic segmentation of complex canopies	**Dr Zijian Wang** The University of Queensland, Australia
09:50 – 10:10	The impact of nitrogen management on wheat protein composition in western Australian soils	**Mrs Samantha Harvie** The University of Western Australia, Australia
10:10 – 10:30	Enhancing winter wheat production systems in Flanders (Belgium) through reduced fungicide application and variety diversification	**Mr Bram Vervisch** Inagro, Belgium
10:30 – 10:50	**Tea/Coffee break**	

10:50 – 11:10	Tackling monocropping through crop rotation-based good agricultural practices in wheat cropping system in Ethiopia	**Dr Tadesse Dessalegn** GIZ/Green Innovation Center, Ethiopia
11:10 – 11:30	Breeding to improve the wheat phenotype while maintaining Epichloë endophyte compatibility	**Mrs Lisa Wood** Grasslanz Technology, University of Adelaide, Australia
11:30 – 11:50	Evaluating biostimulant efficacy for wheat performance as a sustainable fertilizer alternative in agriculture	**Dr Katharina Belt** The University of Western Australia, Australia
11:50 – 12:10	Wheatomics: A new journal for wheat community	**Dr Daolin Fu** Shandong Agricultural University, China
12:10 – 12:30	**Closing remarks by** Co-Chairs	

Workshop 6 September 22, 2024, 09.0-09.30

Crop residue management for sustainable crop production

Kadambot H.M. Siddique

The UWA Institute of Agriculture, The University of Western Australia, Perth WA 6001, Australia

Increasing crop production and enhancing soil carbon sequestration are essential for sustainable agriculture. Globally, a significant amount of crop straw is produced annually, and incorporating this straw into the soil is an effective agronomic practice with positive environmental and soil biological impacts. Straw incorporation can improve soil nutrient status, increasing crop growth and yield. However, improper straw management, such as discarding or burning it in fields after harvest, results in wasted resources and adverse effects.

Straw burning contributes to air pollution, deteriorates soil health, and poses serious health risks, such as respiratory infections and cardiac diseases due to increased particulate matter and greenhouse gases. Our field-based studies found that incorporating straw into the soil can significantly enhance soil organic carbon, total nitrogen, and nutrient availability.

Our recent studies have shown that ammoniated straw incorporation can increase crop yield by improving soil water retention and nutrient availability. Ammoniated crop straw has lower straw hemicellulose and lignin content, making it more easily decomposable by soil microbes and thus accelerating nutrient release.

Our synthesis of sequencing data from various published studies revealed significant changes in soil bacterial community diversity, composition, co-occurrence networks, and functional profiles following straw incorporation. In conclusion, our research provides a scientific basis for rationalising straw incorporation methods to enhance soil fertility, soil health, and crop yields.

Workshop 6 September 22, 2024, 10.10-10.30

Enhancing winter wheat production systems in Flanders (Belgium) through reduced fungicide application and variety diversification

Bram Vervisch[1], Jonas Claeys[1], Dorien Vanderveken[2], Veerle Derycke[3]

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Cereal production, especially winter wheat production, is an important economic crop in Europe, by extension worldwide. Furthermore, wheat is one of the most important sources of human food and animal feed. For this reason, its cultivation has become highly professionalized, including mechanization. But also by optimization of cultivation in terms of genetics and efficient use of crop protection products to reduce pressure from fungi, weeds, diseases and pests.

Also in Flanders, the yield of winter wheat was systematically further maximized in this way, but resulting in smaller financial margins. There is a fine line between the cost of inputs and economical gain from yield. The goal within this research is therefore to maximize the economic return of winter wheat cultivation, within the boundaries of IPM. These trials strive for an ‘optimal’ yield rather than a ‘maximum’. Which means lowering the cost of inputs, with an as high as possible yield output. This to economically assess low-input winter wheat production systems in Flanders.

During three consecutive growing seasons of winter wheat between 2019-2023, field trials were set up at three locations spread in Flanders. On the different locations the same varieties were sown. These were chosen based on disease tolerance or sensitivity. Which were combined with three fungicide schemes: (1) no treatment, (2) one treatment in final leaf stage (BBCH 39) and (3) two treatments, in second node (BBCH 32) and between ear formation until flowering (BBCH 55 -65).

After three seasons of trials on different locations the results show the underestimated importance of disease-tolerant varieties. The reduced usage of fungicides and a thoughtful choice of varieties can result in a lower yield (kg/ha), but also in lower costs of cultivation. Which leads to an equal or even higher economic return per ha.

It can be concluded that the added value of background knowledge of varieties, disease patterns and properties of crop protection products achieves an equivalent economic result with reduced inputs/costs in winter wheat in Flanders. Unnecessary treatments with fungicides puts pressure on the environment, but also on the authorization of active ingredients. Additionally this requests extra costs and time for the farmer.

Workshop 6 September 22, 2024, 09.30-09.50

Global wheat full semantic segmentation of complex canopies

Andreas Hund[1*], Jonas Anderegg[1], Alexis Carlier[2], Scott Chapman[3], Zhi Chen[3], Alexis Comar[4], Marie-Pia D'Argaignon[5], Sebastien Dandrifosse[6], Etienne David[7], Benoît De Solan[8], Benjamin Dumont[2], Joss Gillet[4], Evgeny Gladilin[9], Latifa Greche[10], Wei Guo[11], Malcolm J. Hawkesford[10], Kamran Irfan[4], Mehdi Khalaj[12], Norbert Kirchgessner[1], Shouyang Liu[13], Raul Lopez-Lozano[5], Afef Marzougui[1], Matthew Reynolds[14], Benoît Mercatoris[2], Ingo Mücke[9], Keyhan Najafian[12], Kerstin Neumann[9], Safaa Ouahid[15], Ian Stavness[12], Carlos Robles Zazueta[14], Nicolas Virlet[10], Andrea Visioni[15], Shuhei Nasuda[16], **Zijian Wang[3]**, Haozhou Wang[11], Marie Weiss[5], Radek Zenkl[1]

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Deep learning methods for image processing are rapidly advancing and imaging techniques have become a standard for classification and quantification in agriculture. Several studies already tackled crop segmentation of wheat under field conditions (Anderegg et al., 2023; Madec et al.,2023). However, most of the existing works focus on a two-classes segmentation problem, i.e., pixel-level classification of vegetation and background.
Global wheat (http://www.global-wheat.com/) aims to improve such efforts to train robust algorithms, which can segment leaves, stems, and inflorescences in complex wheat canopies. We collected ~40000 images from our phenotyping platforms spread across the globe.

The image information includes geographic location, developmental stage, genotype, or agricultural treatment. A diverse core set of 200 images is already labelled for all organs. Based on image tags and available meta-information, we currently select ~1000 images for further labelling.

This dataset will serve as a public benchmark for the training and validation of deep learning models. At IWC2024, we will i) present the dataset as a state-of-the-art benchmark for organ segmentation of wheat and ii) demonstrate the robustness of derived segmentation models under different testing environments.

Reference:

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Workshop 6 September 22, 2024, 11.10-11.30

Breeding to improve the wheat phenotype while maintaining *Epichloë* endophyte compatibility

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Epichloë endophytic fungi are used in commercial grass pasture throughout the world offering protection from insect herbivory and other biotic and abiotic stresses (these benefits are being discussed in Caradus et al. 2024 - BENEFITS OF AN OBLIGATE MUTUALIST ENDOPHYTE, *EPICHLOË*, IN WHEAT). *Epichloë* endophytes are mutualistic fungi that grow intercellularly within host grasses.

Visible growth is observed in sexual species where reproductive structures are formed. Asexual *Epichloë* show no visible external growth and it is these *Epichloë* that are commonly exploited in pastures. They are strictly vertically transmitted (maternally inherited), through seed to the next generation.

The success of this bio protection technology in temperate grasses, where in some cases *Epichloë* infection has increased dry matter production three times more than nil endophyte grasses, could have applicability to modern cereals, reducing the reliance on synthetic chemistry for controlling biotic stress.
However, modern wheat does not naturally host *Epichloë* endophytes and the genetic compatibility between host plant and endophyte has been a challenge. The most successful inoculation of *Epichloë* in wheat involved Chinese Spring with a *Leymus racemosus* H chromosome substitution (TACBOW0011).

Plants established a successful symbiosis with *Epichloë* and vertically transmitted the endophyte to the next generation. However, the initial *Epichloë* infected plants displayed undesirable phenotypes, a combination of the effect of the endophyte and the alien chromosome substitution from an ancient wheat species.

Endophyte positive plants show delayed maturity, have a stunted height and shrivelled seed. They also exhibit low germination and low endophyte transmission to the next generation. Here we describe the progress of improving the compatibility of *Epichloë* and modern wheat varieties to improve the plant phenotype.

Progress to date has resulted in maturity delay reducing to one week, a more acceptable plant height for commercial harvesting and increased seed size compared with the original inoculated TACBOW0011 material.
Germination and endophyte retention have also been improved. The future opportunities and challenges of using this endophyte technology in modern wheat is discussed.

Workshop 6 September 22, 2024, 11.30-11.50

Evaluating biostimulant efficacy for wheat performance as a sustainable fertilizer alternative in agriculture

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Enhancing plant production while reducing resource inputs is crucial for sustainable food security. My research is focused on refining low-input agricultural strategies, specifically tailored to Australian wheat varieties and soil conditions, particularly those found in Western Australia. These soils often present challenges due to their poor nutrient content, sandy texture, and arid nature.

Biostimulants are substances, either of microbial or non-microbial origin, that are applied to plants or soils to enhance nutrient uptake, stress tolerance, growth, and overall plant health. They differ from fertilizers in that they do not provide essential nutrients directly to plants but instead stimulate natural processes within the plant to improve its performance and resilience.

I investigate the effectiveness of both microbial and non-microbial biostimulants to optimize wheat plant production and development, aiming to decrease reliance on inorganic fertilizers. The implications of this research are profound for sustainable agriculture, providing insights into eco-friendly approaches that bolster plant performance, enhance stress responses, conserve resources, and mitigate the negative effects of synthetic fertilizers.

Understanding the mechanisms and positive impacts of biostimulants on plant development is crucial for transitioning towards more sustainable agricultural practices. Through wheat trials conducted in both controlled glasshouse and natural field conditions, we analyzed plant performance, development, yield, and grain protein outputs in plants treated with biostimulants or synthetic fertilizers. These trials serve as a foundation for further investigating the efficacy of biostimulant products and their efficient implementation in agriculture without compromising yield and profit.

In my presentation, I will share our current findings, comparing biostimulants to inorganic fertilizers, with the goal of optimizing farming practices, boosting plant production, and minimizing environmental impact to contribute to sustainable agriculture amidst global challenges such as population growth and climate change.

Workshop 6 September 22, 2024,10.50-11.10

Tackling monocropping through crop rotation-based good agricultural practices in wheat cropping system in Ethiopia

Tadesse Dessalegn[1*], Karl Kaizer[2], Bedada Begna[1], Meseret Shiferaw[1], Eshetu Lemma[1], Yitbarek Meles[1], Kassahun Mammo[1], Kebede Gutema[1], Tamiru Beyene[1], Samrawit Getachew[1], Magnus Schmid[3], Svenja Kirsch[1], Tanja Dorn[1]

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Wheat is among the major cereals and staple crops grown in Ethiopia. Wheat monocropping is a challenge contributing to lower productivity, declining soil fertility, recurring rust epidemics, and poorer grain quality. Diversifying with legume crops and improved wheat varieties will help to sustain wheat production. Hence, rotation based on-farm demonstrations (RoBOFDs) with good agricultural practices (GAP) was introduced by "GIZ/Green Innovation Centre – Ethiopia" project.

More than 399 *RoBOFDs* were established at eight districts in 110 villages at representative host-farmers' fields. The demonstrations were 1 ha based (2 wheat and 1 faba bean varieties) termed as main RoBOFD, and 0.5 ha based (1 improved wheat and 1 faba bean varieties) termed as satellite RoBOFD. The RoBOFDs were considered as core networking points to disseminate seeds of improved varieties and GAP knowledge to neighbouring non-host-farmers through participatory training.

The effectiveness of RoBOFD concept in disseminating new improved seeds of rotational crops, GAP knowledge, and ways to scale out was assessed through local social networks analysis. Each main and satellite RoBOFD host-farmer have shared GAP information with 10 and 6 other farmers, respectively, during 2018 and 2019. Those farmers who have received GAP information from main or satellite RoBOFDs have shared with 3 other farmers. Similarly, the improved seed exchange network analysis showed that each main and satellite RoBOFD host-farmers has shared with 10 and seven other farmers within two years.

Respondents showed that 60% of wheat and 58% of faba beans plots were covered by improved seed received from networks in 2018. One-fifth of wheat and 17 % of faba beans plots were covered with certified improved seed. "Ogolcho" followed by "Wane" and "Hidase" were improved wheat varieties. "Gabalcho" followed by "Gora" were faba bean varieties. Most of wheat and faba bean plots were planted with seeds exchanged through the social networks in both years.

Farmers (> 80%) believed wheat with faba beans rotation is important in restoring soil fertility, enhance soil biomass and improve land productivity. Two-third of farmers believed that rotation-based GAP was good practice in changing the mono-cropping of wheat. Others suggested faba beans serves as break crop for rusts, weeds control, save labour and to cope climate variability. Many farmers (70-75%) reported shortage of quality inputs and higher prices restricted applying rotation and GAP.

Availability of improved faba bean (75%) and wheat (67%) seeds was a major limiting factor. Hence, the RoBOFD concept can help to reach more smallholder farmers at shorter period with improved seed of rotational crops and GAP knowledge through their social networks.

Addressing availability of improved quality seeds of faba beans, biofertilizer, diversifying wheat varieties, and scaling out RoBOFDs to other wheat areas will help to harness monocropping and enhance sustainability of wheat production.

Workshop 6 September 22, 2024, 0950-10.10

The impact of nitrogen management on wheat protein composition in western australian soils

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Understanding the impact of nitrogen on wheat protein composition is crucial for enhancing the agronomic and commercial value of wheat. My research utilises a proteomic approach to analyse how different nitrogen management strategies effect the grain protein composition of wheat grown in the arid Western Australian soils.
The significance of my research stems from the need to optimise nitrogen utilisation while improving wheat grain quality without compromising yield.

To examine the influence of nitrogen on wheat protein composition, I performed a range of field and glasshouse trials and analysed plant development, yield and protein content in relation to the amount and timing of nitrogen applications. I identified specific changes in protein composition resulting from the different nitrogen application strategies. The findings of my research confirm that nitrogen application in terms of the amount and timing of nitrogen can significantly influence wheat grain protein composition, which could lead to downstream grain quality changes.

The contribution of my research provides valuable insights into the proteomic alterations associated with nitrogen management and suggests that protein composition can be altered through targeted nitrogen application strategies. Additionally, these findings will help develop protein targets for bioassays of harvested grain to guide future nitrogen management practices.

My research highlights the critical role of nitrogen management in shaping wheat protein composition and establishes a scientific foundation for advancing more sustainable and productive agricultural practices with potential lower nitrogen inputs. I will present the agronomic and proteomic outcomes of our trials on nitrogen application strategies, highlighting their implications for future wheat production.

Workshop 6 September 22, 2024, 11.50-12.10

Wheatomics: a new journal for wheat community

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Wheat is the largest cultivated cereal crop in the world and acts as a staple food for about 40% of humankind. A large scientific community is engaged in wheat research, aiming to enhance its production and to safe guard global food security. Thus, a dedicated journal is needed for improving wheat and its relative species (collectively *Triticeae*).

To fill this gap, we are leading an effort to establish a new journal – WheatOmics, which is hosted by the Shandong Agricultural University, China and will be published by the Springer Nature. We aim to publish high-quality, rigorously peer-reviewed articles, making WheatOmics a flagship international journal for *Triticeae* research. WheatOmics is an open-access international, peer-reviewed journal that publishes excellent, novel, rigorous research.

We are particularly interested in receiving manuscripts that offer significant insights, advancements, and innovations in agronomy, physiology, genetics, breeding, biotechnology, genome editing, microbiomes, artificial intelligence, and many more fields with full or partial integration of 'omics'. WheatOmics publishes original research, reviews, short communications and novel methods & techniques.

All submissions will go through rigorous peer review to ensure the high quality and novelty of published work. Our editorial board is committed to making fair and rapid publication decisions and aims to provide efficient services for readers and authors.

Please join us in advancing *Triticeae* research, submit your manuscripts to WheatOmics and be a part of this exciting journey.

3RD INTERNATIONAL WHEAT CONGRESS

Workshop 7: Biofortification of Wheat

Sunday, 22 September 2024
Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 9, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Dr Tona Sanchez-Palacios, Murdoch University, Australia** **Dr Velu Govindan, CIMMYT, Mexico** **Dr Roopali Bhoite, WADPIRD, Australia**	
09:00 – 09:10	Introduction	
09:10 – 09:30	Micronutrients in soils and plants: relationship to grain quality	**Prof Richard Bell** Murdoch University, Australia
09:30 – 09:50	Mainstreaming grain zinc in CIMMYT wheat breeding	**Dr Velu Govindan** CIMMYT, Mexico
09:50 – 10:10	Genetic strategies for iron biofortification of bread wheat	**Dr Alex Johnson** The University of Melbourne, Australia
10:10 – 10:30	Genetic biofortification to improve micronutrient efficiency in wheat – update for WA farming systems	**Dr Roopali Bhoite** Western Australia Department of Primary Industries and Regional Development, Australia
10:30 – 10:50	**Tea/Coffee break**	

10:50 – 11:10	Agronomic biofortification of Australian wheat through foliar zinc: improving the nutritional adequacy of food products	**Dr Tona Sanchez-Palacios** Murdoch University, Australia
11:10 – 11:30	Identification of quality-related ideotypes to improve common wheat end-use quality	**Dr Tatsuya Ikeda** National Agriculture and Food Research Organization (NARO), Japan
11:30 – 11:50	Genetic dissection of value-added quality traits for bread wheat through genome-wide association studies	**Dr Manish Kumar Vishwakarma** Borlaug Institute for South Asia (BISA), India
11:50 – 11:55	**Closing remarks by** Co-Chairs	

Workshop 7 September 22, 2024, 10.10-10.30

Genetic biofortification to improve micronutrient efficiency in wheat – update for wa farming systems

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Micronutrient trait/efficiency is a measure of the quantity of micronutrient present in harvested grains. Modern breeding practices have diluted micronutrient content in wheat grains significantly compared to historic varieties. In addition, a large proportion of Western Australian cropping soils are Zn deficient causing lesser Zn accumulation in grains. Improving Zn uptake and mobilization in grains through genetic biofortification present a sustainable solution.

The objective of the present study is to identify Zn-efficient lines both on +Zn and -Zn conditions in Western Australian soils. A selective subset of objective-specific international micronutrient panel containing introgressions of *Triticum* with wild relatives and landraces has been screened at Merredin in 2023 on a slightly alkaline (pH 7.4), Zn deficient (0.28 ppm) sandy loam paddock. A split-plot trial with +Zn and -Zn as main plots and varieties as subplots was used. Inductively coupled plasma mass spectrometry (ICP-MS) test was carried out on harvested grains, stems, and rachis to assess micronutrient content.

The genotypes were ranked in -Zn and +Zn plots based on grain Zn content. The zinc concentration ranged from 20.1 to 34.5 mg/kg and 32.7 to 58.5 mg/kg in -Zn and +Zn plots, respectively. HarvestPlus Zn target for healthy wheat is 33 mg/kg and recommended daily allowance is 45 mg/kg grains. In -Zn condition, six genotypes had higher grain Zn compared to WA check variety Scepter (28.8 mg/kg) with the highest one having 34.5 mg/kg Zn. In +Zn condition two genotypes were ranked above, and seven genotypes were in comparable range with Scepter (54.4 mg/kg). Manganese (Mn) and iron (Fe) were positively correlated with Zn. Zn mobilization from stem and rachis to grains was higher in high-Zn lines compared to low-Zn lines.

We have ongoing second round of screening using same set of lines in Merredin to validate the potential Zn-efficient lines. In conclusion, Zn-efficient lines will benefit wheat breeding programs to develop new genetically biofortified cultivars suitable for WA environment, build reputation of WA wheat in international market and boost exports and economic returns.

References:

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Workshop 7 September 22, 2024, 09.10-09.30

Micronutrients in soils and plants: relationship to grain quality

Richard W Bell and José Tonatiuh Sánchez-Palacios

SoilsWest, Centre for Sustainable Farming Systems, Food Futures Institute, Murdoch University

Humans require over 50 essential elements and molecules in their diet including 17 micronutrients, only 8 of which are also essential for plants (chlorine, iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu), molybdenum Mo), nickel (Ni)). Historically, micronutrients have received less attention in agriculture than N, P and K but their significance has gained prominence through increasing recognition that low levels in staple foods is a major factor in dietary deficiencies for billions of the world's population, particularly for Fe, Zn, iodine (I) and selenium (Se).

Studies on micronutrients in agriculture emphasise soil and foliar applications for maximising yield rather than their impacts on grain nutrient content. Differences among crop species and cultivars in their ability to mobilise micronutrients in soil explain their varied adaptation to low micronutrient soils. Variations in internal efficiency also exist but most micronutrients have variable phloem mobility which can be a constraint to loading into grain and foods.

For instance, micronutrients in a large collection of grain of an Australian high-yielding wheat cultivar ranged from 21.3 to 97.2 mg Fe/kg grain, 6.9 to 44.7 mg Zn/kg grain, 5 to 25 µg I/kg grain and 3.8 to 829 mg Se/kg grain. For Fe, Zn, Cu, Mo and B, phloem mobility is variable depending on supply, plant N status, plant part, and plant species. Advances in molecular biology are identifying transporters and channels that regulate uptake, distribution and redistribution of micronutrients within plants, especially to grain.

Among wheat genotypes, there are enormous variations in the capacity of plants to extract micronutrients from the soil so that plant breeding has been identified as a key strategy for biofortification of micronutrients in grain. However, efficiencies in uptake don't necessarily lead to increases in loading of micronutrients in grain. While foliar applications of micronutrients have been effective and accepted as an important strategy for boosting crop yield on low micronutrient soils, there is increasing evidence that efficiency of cellular uptake and retranslocation of common micronutrient salts and chelates is poor.

There is an opportunity to develop enhanced carriers and transporters of micronutrients. New products with enhanced mobility within the plants may also have a role in boosting root growth, particularly in subsoils that are low in Fe, Mn, Cu, Zn and B.

Another potential spin-off from biofortification of micronutrients in grain is increased seed vigour for crop establishment.

Workshop 7 September 22, 2024, 10.50-11.10

Agronomic biofortification of australian wheat through foliar zinc: improving the nutritional adequacy of food products

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Agronomic interventions such as foliar application of fertilisers can increase the mineral content of grains, consequently improving wheat flour for human consumption. We established a field trial at Wongan Hills in Western Australia's moderate rainfall zone (325-450 mm) during the winter season of 2021. Wongan Hills' soil is deficient in available Zn in subsoil layers containing 0.2 ± 0.1 mg Zn-DTPA kg^{-1}.

The topsoil layer, a pale-yellow sandy clay, is Zn adequate with 0.84 ± 0.2 mg Zn-DTPA kg^{-1}. First, we evaluated the efficiency of Zn foliar applications on wheat plants in producing Zn-enriched grains. Second, we used synchrotron radiation techniques to determine the localisation of Zn in grains. Third, we conducted grain quality studies to determine levels of mineral nutrition adequacy in food products. In the field, we tested two Zn forms including $ZnSO_4$ and Zn-EDTA, with or without soluble nitrogen (0.4% N) in the formulation.

Foliar treatments were applied four times from anthesis to grain-filling developmental stages. Foliar-control treatments with and without N produced wheat grains with 13.6 ± 0.4 mg Zn kg^{-1} and 12.9 ± 0.8 mg Zn kg^{-1}, respectively. Foliar $ZnSO_4$ with and without N resulted in a 2.1- and 1.7-fold increase in Zn concentration relative to controls.

Foliar Zn-EDTA with and without N resulted in a moderate 0.6- and 0.4-fold Zn increase relative to controls. Nitrogen in the formulation did not affect significantly grain yield or concentrations of Zn, iron and phosphorous in grains. X-ray fluorescence microscopy (XFM) studies revealed that Zn was accumulated in the embryo primarily, followed by aleurone layers and to a lesser extent in the crease region in control grains.

Grains of treated plants with Zn foliar fertilisers showed the same distribution pattern with a slight enrichment of Zn in the crease region. Flour milling and bread-making studies showed that Zn-enriched grains derived from $ZnSO_4$ treatment retained twice as much Zn in white bread products with 9.1 ± 0.1 mg Zn kg^{-1} Zn compared to the control white bread with 3.9 mg Zn kg^{-1}. A moderate increase of Zn relative to controls was observed in white bread produced from the Zn-EDTA foliar treatment at 5.7 ± 0.4 mg Zn kg^{-1}. Similarly, raw noodles from grains of the $ZnSO_4$ treatment retained 6.5 ± 0.1 mg Zn kg-^{1} which is higher than the control with 2.4 ± 0.05 mg Zn kg^{-1}.

Zinc concentrations in cooked noodles decreased compared to uncooked noodles but still the cooked noodles from flour of Zn enriched grains remained higher with 3.2 mg Zn kg^{-1} compared to the control at 1.5 mg Zn kg^{-1}.
In conclusion, applying Zn via foliar sprays on wheat resulted in enhanced grain products with dietary Zn advantage, making foliar biofortification a worthwhile agronomic method for agricultural systems with low Zn availability.

Workshop 7 September 22, 2024, 09.30-09.50

Mainstreaming grain Zn in CIMMYT Wheat Breeding

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Accelerating the rate of genetic gain for grain yield, grain Zn together with key agronomic traits is pivotal for delivering improved wheat varieties. Great progress has been made in the past decade in transferring alleles for high-zinc (Zn) and iron (Fe) from diverse genetic resources into elite wheat breeding lines. However, the major challenge is to maintain simultaneous and high rates of genetic gains for grain yield and grain Zn to meet the food and nutritional security demands through the continuous delivery of biofortified varieties that are competitive to replace non-biofortified varieties successfully.

Although a few intermediate effect QTL regions are identified for grain Zn, both yield and Zn content are quantitatively inherited. Increased breeding efforts and new approaches are therefore required to combine them in high frequency in CIMMYT's elite germplasm, ensuring that Zn levels are steadily increased to the required levels across the CIMMYT breeding pipelines. The addition of Zn as a core-trait will be achieved through significant acceleration in the breeding cycle, expanding population sizes, extensive Zn phenotyping, yield testing, phenotyping for biotic and abiotic stresses, molecular-assisted selection and genomic selection.

While continuing to increase agronomic performance, high Zn alleles has been added as a core-trait. Eventually Zn content will be increased in the elite lines annually along with the frequency of elite lines with high yield and other agronomic traits that have potential to be released by partners. A genomics assisted "rapid cycle recurrent selection" scheme achieved through rapid generation advancement approaches are being used in the CIMMYT wheat breeding program to mainstream grain Zn in the majority of elite lines.

Early-generation advancement using the speed breeding scheme that reduces operational costs and reduce time to recycle new parents; making advancement decisions for elite lines using data from intensive multi-trait, multi-year and multi-environment phenotyping; selection indices based parental selection and reycling and maintaining effective partnerships with the National Agricultural Research Systems for testing, releasing, and disseminating varieties to farmers.

Following activities implemented in the CIMMYT's spring wheat breeding program to accelerate rate of genetic gain.

- **Faster recycling approaches:** The 3-yr breeding cycle uses a rapid bulk generation advancement (RBGA) scheme fully optimized; The accelerated generation advancement coupled with field selections will shorten the breeding cycle from 6.25 to 3.65 years (saving 40% time).

- **Improved selection accuracy and increased selection intensity:** We optimized the selection strategy to identify parental lines combining high yield, disease resistance and high Zn by increasing selection intensity.
- **Optimized the testing strategy for stage 1 and stage 2 lines.** All stage 1 lines are phenotyped across 4 selection environments in Obregon, and the promising high yielding, high Zn lines are recycled after stage 1 while the best lines are advanced to stage 2 testing.
- **Centralized trait augmentation pipeline:** the centralized speed breeding trait-augmentation approach introgressed some key resistance genes for stem rust, yellow rust, wheat blast and spot blotch into elite backgrounds.
- **Zn mainstreaming:** Mainstreaming of grain Zn in elite lines was accelerated and about 60% of current crossing block parents possess high Zn; new crosses were made using parents with high breeding value for grain yield, high Zn and other agronomically important traits.

Workshop 7 September 22, 2024, 11.30-11.50

Genetic dissection of value-added quality traits for bread wheat through genome-wide association studies

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One of the grains that is most commonly consumed worldwide is bread wheat (*T. aestivum*). The following quality attributes were assessed for an association panel of 154 *T. aestivum* lines: protein percentage, SDS Sedimentation Value, Grain Appearance (GA) Score, Grain Hardness (GH), Phenol Reaction (PR) Score, and Test Weight (TWt).

Furthermore, the panel was also phenotyped at the BISA sites in Ludhiana and Jabalpur for grain production and associated characteristics like days to heading, days to maturity, plant height, and thousand kernel weight. Using 18,351 genotyping-by-sequencing markers, we conducted a genome-wide association analysis on this panel to identify marker-trait relationships for characteristics linked to grain yield and quality. On chromosomes 7B (10), 1A (9), 2A (8), 3B (6), 2B (5), 7A (4), 1B (3), 3A, 4A, and 6D, we found 55 SNP MTAs for quality-related attributes; the remaining chromosomes, 4B, 5A, 5B, and 1D, had one SNP MTA each.

Furthermore, on chromosomes 7D (4) and 4D (3), 20 SNP MTAs were found for yield-related variables in Ludhiana, while 44 SNP MTAs were identified for Jabalpur on chromosomes 2D (6), as well as 7A (5), 2A (4), and 4A (4).

The findings demonstrate the value of GWAS in determining the genetic underpinnings of quality traits in *T. aestivum*. More validation research on these loci will be beneficial for using them in marker-assisted selection to enhance hexaploid wheat for higher yield and improved grain quality.

Workshop 7 September 22, 2024, 11.10-11.30

Identification of quality-related ideotypes to improve common wheat end-use quality

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Various factors are involved in common wheat end-use quality. Besides protein contents, genotypes related to gluten strength, starch amylose content, grain hardness, and dough discoloration are critical attributes determining various end-use quality.

To clarify breeding targets to improve end-uses, eleven specific combinations of the genotypes (ideotypes) for each end-use are proposed based on the combination of these genotypes. Clarifying the ideotypes should accelerate wheat breeding for stable wheat supply while maintaining end-use quality.

Workshop 7 September 22, 2024, 09.50-10.10

Genetic strategies for iron biofortification of bread wheat

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Over two billion people worldwide are impacted by micronutrient deficiencies. The development of biofortified food crops is a sustainable way of increasing micronutrient intakes at no additional cost to growers and food manufacturers.

We have employed multiple genetic strategies to biofortify wheat with iron (Fe) including genetic engineering to overexpress genes and gene editing to mutate coding sequences involved in Fe uptake, transport and translocation.

Much of our research has centred on the *nicotianamine synthase* (NAS) and *hemerythrin motif-containing really interesting new gene (RING)- and zinc finger protein* (HRZ) gene families in bread wheat, however, we are also interested in upstream Open Reading Frames (uORFs) that are located within the 5' leader sequence of many eukaryotic genes.

This presentation will describe several aspects of our research and highlight results relevant to not only Fe biofortification but also improved plant tolerance to Fe deficiency.

3RD INTERNATIONAL WHEAT CONGRESS

Workshop 8: Genomic Technologies for Accelerating Wheat Research

Sunday, 22 September 2024
Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 10, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Dr David Chandler, Australian Genome Research Facility, Australia** **Prof Rudi Appels, University of Melbourne | La Trobe | Murdoch University, Australia** **Ms Anu Chitikineni, Murdoch University, Australia**	
09:00 – 09:10	Introduction	
09:10 – 09:30	Introduction - New and novel technologies at AGRF	**Dr Dhanya Sooraj** Australian Genome Research Facility, Australia
09:30 – 09:50	Introduction - Wheat Genome assembly and 3D-Genomics | Epigenetics	**Dr Brent Thomson** Dovetail Genomics, USA
09:50 – 10:10	High-throughput genotyping technologies	**Speaker to be announced**
10:10 – 10:30	Genomics technologies at Genomics WA	**Dr Alka Saxena** Genomics WA, Australia
10:30 – 10:50	**Tea/Coffee break**	

10:50 – 11:10	Optimized wheat genotyping arrays based on haplotype structure, marker quality, and candidate genes	**Dr Martin Ganal** TraitGenetics GmbH, Germany
11:10 – 11:30	Wheatpanomics- the next step in wheat breeding	**Prof Wolfram Weckwerth** University of Vienna, Austria
11:30 – 11:50	SNP-PORTRAIT: An innovative SNP data structure to improve genomic prediction accuracy using 2D-convolutional neural networks	**Dr Reem Joukhadar,** Qingdao Agricultural University, China & **Dr Abdulqadir Jighly,** AgriSustability, Australia
11:50 – 12:10	Integrating genomic databased with PRETZEL	**Dr Don Isdale** Agriculture Victoria Research, Australia
12:10 – 12:30	Gene mining for coordinated wheat improvement	**Dr Mao Long** Chinese Academy of Agricultural Sciences, China
12:30 – 12:35	**Closing remarks by** Co-Chairs	

Workshop 8 September 22, 2024, 10.50-11.10

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

Martin Ganal[1], Andreas Polley[1], Thomas Gross[1], Naser Poursarebani[1], Eva Grafarend-Belau[1], Joerg Plieske[1]

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Single nucleotide polymorphism (SNP) genotyping is nowadays widely used in wheat genetics and breeding. Examples for uses are the analysis of population structure and genetic relationships, the identification and location of qualitative and quantitative (QTLs) traits in appropriate populations, marker-assisted selection and backcrossing, and the identification marker-trait associations. Currently in most cases, SNP genotyping arrays are used which permit the simultaneous analysis of many thousands of SNP markers in a cost-efficient way with unparalleled precision. These relatively low-priced SNP arrays have also become a major tool in Genomic Selection within routine wheat breeding.

We describe the continuous development and optimization of our wheat genotyping arrays designed for wheat genome analyses and Genomic Selection based on important criteria: One is marker quality including the detection of only one specific locus in one of the wheat genomes and clear calling of the three allelic states.

Another emphasis is furthermore placed on the detection of as many haplotype blocks as possible based on a genotyping database including several thousand wheat varieties from the entire world. With this data set, we have selected 1-3 markers per identified haplotype block so that a maximum of information can be obtained with these arrays. A detailed analysis revealed that on the wheat genome sequence these haplotype-specific markers are highly clustered towards the end of the chromosomes where also most of the recombination events occur. To further increase the use of these arrays, we have added a set of several hundred publicly available markers that have been reported to be linked to specific traits in the literature.

Currently, we use two different genotyping arrays that include approximately 25,000 and 8,000 markers, respectively. The 25K SNP marker array is optimized for the detection of many haplotype blocks while the reduced 8K marker array can further decrease the costs per genotyped sample and is widely used for wheat breeding including marker imputation based on previous genotyping of parental lines with the 25K marker array or other available wheat genotyping arrays based on defined sets of common markers. These two arrays have been used for the genotyping of several hundred-thousands of lines from all over the world mainly in hexaploid wheat but also in tetraploid Durum wheat.

Workshop 8 September 22, 2024, 11.50-12.10

Integrating genomic databases with pretzel

Don Isdale[1], David Chisanga[1,*],Kenny Yu[1], Mahdi Rahimi[1], Kerrie Forrest[1], Debbie Wong[1], Matt May[2], Josquin Tibbits[1,3], Sally Norton[2], Gabriel Keeble-Gagnère[1], Matthew Hayden[1,3*]

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The exponential rate at which genomic data for plant genetic resources is being generated and made publicly available is increasing opportunities to accelerate research and breeding outcomes. A challenge for non-bioinformaticians is to integrate and visualize the different types of data distributed across multiple repositories.

To address this challenge, we developed the webtool *Pretzel,* which enables non-bioinformatics users to construct combined graphical visualizations of genetic maps, genome assemblies, alignments and QTLs using multiple data types from remote repositories, while avoiding the duplication and versioning of data and working with the existing permission structures of data repositories.

Through its graphical user interface, *Pretzel* allows users to select data sources, configure filter criteria, search for genomic locations of interest using different approaches, zoom to those locations to see highly resolved data, and arrange the graphical visualization to focus on their specific question.

Here, we illustrate the utility of *Pretzel* for linking research and breeding knowledge to plant genetic resources of wheat from the Australian Grains Genebank (AGG) that were genotypically characterized as part of a Strategic Partnership between Agriculture Victoria and the Grains Research and Development Corporation (GRDC). *Pretzel* forms part of a wider interoperable data infrastructure that will enable genomic data across genebanks to be integrated through standard interfaces such as the Breeding API (BrAPI), enabling connectivity with repositories such as Germinate, Gigwa and other BrAPI-compliant databases.

Workshop 8 September 22, 2024, 11.10-11.30

Wheatpanomics – the next step in wheat breeding

Wolfram WECKWERTH[1,2], Shuang Zhang[1], Arindam GHATAK[1,2], Palak CHATURVEDI[1]

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Integration of multiomics analysis using metabolomics, proteomics, RNAseq, metabolic modelling, AI, machine learning - in short PANOMICS technology - applied to natural variation of wheat germplasm collections goes beyond classical genomic techniques and offers valuable tools to characterize the system and understand evolutionary plasticity linking molecular regulation and phenotypes as well as plant- soil microbe interactions [1,2].

Wheatpanomics will exploit the natural variation of genomes, metabolomes, proteomes, transcriptomes in hundreds and thousands of different wheat genotypes and their causal link to phenotypic traits, such as yield, stress resistance, nutritional quality and many more.

The platform integrates also techniques of artificial intelligence and modelling to improve genomic predictions. The aim is to provide intimate molecular information for breeding programs addressing resilient and sustainable agricultural production systems [1, 2].

WheatOmics, a novel journal will provide an important podium for reporting the newest standards in wheatpanomics. WheatOmics encourages submissions that span Agronomy, Physiology, Genetics, Breeding, Biotechnology, Genome editing, Microbiome, Artificial Intelligence, and many more fields with full or partial integration of 'omics' (https://www.wheatomics.com/).

[1] Weckwerth W et al (2020) PANOMICS meets germplasm. Plant Biotechnol J 18: 1507-1525
[2] Ghatak, A., et al. (2023) PANOMICS at the interface of root-soil microbiome and BNI. Trends Plant Sci 28, 106-122

Workshop 8 September 22, 2024,11.30-11.50

SNP-Portrait: An innovative SNP data structure to improve genomic prediction accuracy using 2D convolutional neural networks

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Despite the promise of Deep Learning (DL) models in genomic prediction, their success in improving the prediction accuracy over standard linear models has been limited. Single nucleotide polymorphism (SNP) data is inherited in a 1D structure (along the chromosome) which typically fits the 1D convolutional neural networks (CNN) structure. Several attempts have been made to adapt SNP data to a 2D structure, suitable for 2D CNNs, which were originally designed to analyse graphic data.

These attempts have involved splitting allelic configurations into three channels (two homozygotes and one heterozygote), stacking multiple genomic relatedness matrices, or generating a 2D grayscale graph based on the Linkage Disequilibrium (LD) relations among SNPs.

However, these models have not consistently improved prediction accuracy. Here, we propose SNP-Portrait, a novel structure for SNP data that enhances the utilisation of loci interactions, thereby improving prediction accuracy. SNP-Portrait is designed to accommodate complex loci interaction scenarios and investigate epistatic interactions among two or more loci. SNP-Portrait was tested on a wheat dataset of 599 individuals phenotyped for grain yields in four environments.

We conducted 100 random replicates, each time using 20% of the population for validation. Our results showed that the model assuming 3-loci interaction resulted in the highest prediction accuracy with 4% improvement over the standard GBLUP model and 7% over the equivalent 1D CNN model. Unlike other DL models, SNP-Portrait consistently improved prediction accuracy across different tested scenarios.

Testing SNP-Portrait on more datasets is currently underway with promising results of achieving consistent improvements of prediction accuracy over standard models.

09:10 – 09:30	Introduction - Novel technologies in Genomics	**Dr Dhanya Sooraj** Australian Genome Research Facility, Australia

No Abstract Provided

09:30 – 09:50	Introduction - Wheat Genome assembly and 3D-Genomics | Epigenetics	**Dr Brent Thomson** Dovetail Genomics, USA

No Abstract Provided

09:50 – 10:10	Mechanisms for adaptation and increased virulence of the Australian *Parastagonospora nodorum* pathogen population of wheat	**Dr Huyen Phan** Centre for Crop Disease Management Curtin University

No Abstract Provided

10:10 – 10:30	Genomics technologies at Genomics WA	**Dr Alka Saxena** Genomics WA, Australia

No Abstract Provided

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State Agricultural Biotechnology Centre WESTERN AUSTRALIA
WHEAT INITIATIVE
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IWC 3RD INTERNATIONAL WHEAT CONGRESS

Workshop 9: Genebank Genomics

Sunday, 22 September 2024
Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 1, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs:** **Dr Matthew Hayden, Agriculture Victoria Research/La Trobe University Australia** **Prof Andreas Graner, Leibniz Institute of Plant Genetics and Crop Plant Research, Germany**	
13:00 – 13:10	Introduction	
13:10 – 13:30	Genomics-informed prebreeding unlocks the diversity in genebanks for wheat improvement	**Dr Martin Mascher** IPK Gatersleben, Germany
13:30 – 13:50	The Australian grains genebank enters the genomics era	**Mr Gabriel Keeble-Gagnere** Agriculture Victoria, Australia
13:50 – 14:10	Choosing and using genebank genomic resources to enable long-term analysis of CAIGE bread wheat trials	**Dr Nicholas Fradgley** Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia
14:10 – 14:30	Management and Utilization of Plant Genetic Resources in India	**Dr Gyanendra Pratap Singh** National Bureau of Plant Genetic Resources (NBPGR), India
14:30 – 14:50	**Tea/Coffee break**	

14:50 – 15:10	Chromosome-scale assembly of *Triticum timopheevii* enables identification of new introgressions in wheat	**Dr Surbhi Grewal** University of Nottingham, UK
15:10 – 15:30	Recreating adaptive traits in exotic germplasm to support pre-breeding in wheat	**Dr Sateesh Kagale** National Research Council Canada, Canada
15:30 – 15:50	Wheat's genetic diversity - maintenance and research	**Prof Andreas Börner** Leibniz Institute of Plant Genetics and Crop Plant Research, Germany
15:50 – 16:10	Wheat mutant resources enhance discovery of novel genes and functional genomics	**Dr Huijun Guo** Chinese Academy of Agricultural Sciences (CAAS), China
16:10 – 16:15	**Closing remarks by** Co-Chairs	

Workshop 9 September 22, 2024, 15.30-15.50

Wheat’s genetic diversity - maintenance and research

Andreas Börner[1*], Manuela Nagel[1], Mian Abdur Rehman Arif[1,2], David Riewe[1,3]

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Plant genetic resources play a major role for global food security. The most significant and widespread means of conserving plant genetic resources is *ex situ* conservation. Most conserved accessions are kept in specialized facilities known as genebanks. Worldwide, 7.4 million accessions are stored in about 1,750 *ex situ* genebanks. The largest collections by crop are wheat (860,000 accessions) and rice (780,000 accessions).

With a total inventory of 150,000 accessions, the ‘Federal *ex situ* Genebank of Germany’ in Gatersleben holds one of the most comprehensive collections worldwide. It comprises wild and primitive forms, landraces as well as old and more recent cultivars. Starting in the 1920’s material was accumulated systematically. Seed storage is managed in large cold chambers at -18°C. Seeds are kept in glass jars, covered with bags containing silica gel (active collection) and in aluminum bags under vacuum (base collection).

Since the majority of genebank accessions is stored in the form of seed, seed longevity is of particular importance for crop germplasm preservation. At the IPK research was initiated for crops stored in the genebank over decades. It was found that there is an intraspecific variation within genebank collections. It was concluded that the differences in germination after long term storage are genetically based. Therefore, genetic analyses of seed longevity were initiated. Here we provide results on wheat.

In addition, mass spectrometry based untargeted metabolite profiling experiments were performed in order to detect biochemical changes coinciding with loss in seed germination. The lipidomic composition of a wheat panel was investigated using high-resolution liquid chromatography-mass spectrometry (LC-MS). A high proportion of tentative oxidized lipids was detected, suggesting lipid oxidation as the causal trigger for membrane degradation.

Besides preservation of the germplasm, the evaluation of the collections is a very important task for further utilisation. It is the prerequisite for the identification of genes to be used in breeding programmes for crop improvement.

Therefore, a number of bi-parental mapping populations and association mapping panels have been established to allow for the genetic analysis of various traits. The current focus covers resistance/tolerance to a number of biotic and abiotic stresses, in particular drought and cold.

Workshop 9 September 22, 2024, 13.50-14.10

Choosing and using genebank genomic resources to enable long-term analysis of CAIGE bread wheat trials

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Several thousand bread wheat breeding lines have been tested in Australian field trials as part of the CIMMYT Australia ICARDA Germplasm Evaluation (CAIGE) project, but systematic genotyping of materials have only been conducted in recent years.

Here, I present my use case and experience of selecting and genotyping additional lines to enable a more complete analysis of a large multi-environment trial dataset with genetic information balanced across years. A diverse subset of lines was selected that minimises closely related individuals based on pedigree kinship.

Some degree of overlap between selected lines and those already genotyped with other platforms enabled linkage-based imputation across multiple genotyping platforms. Comparisons of genetic diversity of novel CAIGE lines from both CIMMYT and ICARDA compared to the Australian commercial controls highlighted the value of these materials for research and breeding.

Workshop 9 September 22, 2024, 14.50-15.10

Chromosome-scale assembly of Triticum timopheevii enables identification of new introgressions in wheat

Surbhi Grewal[1], Cai-yun Yang[1], Duncan Scholefield[1], Stephen Ashling[1], Sreya Ghosh[2], David Swarbreck[2], John Fellers[3], Ian P. King[1] and Julie King[1]

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Wheat (*Triticum aestivum*) is one of the most important food crops with an urgent need for increase in its production to feed the growing world. Wheat's wild relative species provide a hugely untapped reservoir of allelic and genetic diversity for wheat improvement. At the Nottingham Wheat Research Centre we are sequencing the genomes of wild relatives of wheat from its secondary and tertiary gene pools to speed up the identification of causal genes of beneficial traits and also to develop diagnostic markers for tracking in marker assisted backcross programmes.

Triticum timopheevii (2n = 4x = 28) is a tetraploid wheat wild relative species containing the A^t and G genomes that has been exploited in many wheat pre-breeding programmes over the last few decades. In this study, we report the generation of a chromosome-scale reference genome assembly of *T. timopheevii* accession PI 94760 based on PacBio HiFi reads and chromosome conformation capture (Hi-C).

The assembly comprised a total size of 9.35 Gb, featuring a contig N50 of 42.4 Mb, and 166,325 predicted gene models. Comparative genome analysis confirmed previously known chromosomal translocations and indicated new chromosome rearrangements. Analysis of the genomic distribution of DNA methylation showed that the G genome had on average more methylated bases than the A^t genome. The G genome was also more closely related to the S genome of *Aegilops speltoides* than to the B genome of hexaploid or tetraploid wheat.

The assembly enabled the characterisation of pre-known and identification of new *T. timopheevii* introgressions in the 10+ wheat genomes and 760 Genebank accessions. We show the utility of this resource in our pre-breeding programme for the characterisation of wheat-*T. timopheevii* introgression lines.

In addition, the genome assembly has been crucial in work being carried out with collaborators aimed at finding new disease resistance alleles/genes for Fusarium Head Blight and leaf rust found in the wheat-*T. timopheevii* introgression lines.

In summary, the *T. timopheevii* genome assembly provides a valuable resource for genome-informed discovery and cloning of agronomically important genes for future food security.

Workshop97 September 22, 2024, 13.10-13.30

Genomics-informed prebreeding unlocks the diversity in genebanks for wheat improvement

Martin Mascher[1], Albert W. Schulthess[1], Sandip M. Kale[1], Fang Liu[1], Yusheng Zhao[1], Norman Philipp[1], Maximilian Rembe[1], Yong Jiang[1], Ulrike Beukert[2], Albrecht Serfling[2], Axel Himmelbach[1], Jörg Fuchs[1], Markus Oppermann[1], Stephan Weise[1], Philipp H. G. Boeven[3], Johannes Schacht[3], C. Friedrich H. Longin[4], Sonja Kollers[5], Nina Pfeiffer[6], Viktor Korzun[5], Matthias Lange[1], Uwe Scholz[1], Nils Stein[1], Jochen C. Reif[1]

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The great effort spent in the maintenance of past diversity in genebanks is rationalized by the potential role of plant genetic resources in future crop improvement – a concept whose practical implementation some think has fallen short of expectations. Here, we implement a genomics-informed pre-breeding strategy for wheat improvement that does not discriminate against non-adapted germplasm.

We collect and analyse dense genetic profiles for a large winter wheat collection and evaluate grain yield and resistance to yellow rust in bespoke core sets. Breeders already profit from wild introgressions but plant genetic resources still offer useful, yet unused diversity. Potential donors of resistance sources not yet deployed in breeding were detected, while the pre-breeding contribution of plant genetic resources to yield was estimated through ‘Elite×Plant genetic resources’ F1 crosses.

Genomic prediction within and across genebanks identified the best parents to be used in crosses with elite cultivars whose advanced progenies can outyield current wheat varieties in multiple field trials.

Workshop 9 September 22, 2024, 15.10-15.30

Recreating adaptive traits in exotic germplasm to support pre-breeding in wheat

Xingliang Ma[1,2], Dhouha Kthiri[1,2], Krystalee Wiebe[2], Curtis Pozniak[2], Sateesh Kagale[1]

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Wild relatives of wheat are a valuable source of genetic variation for crop improvement. Determining the breeding value (ie., average downstream performance) of exotic germplasm is important in selecting which germplasm to integrate into a breeding program. Despite harboring desirable genetic traits, such as disease resistance and climate resilience, assessing the collective breeding value of these traits is challenging due to the influence of various factors like phenology, height, and flowering time, which mask their agronomic potential.

To address this challenge, we devised a CRISPR/Cas-based gene editing approach to recreate adaptive traits in exotic wheat germplasm, thereby unmasking beneficial traits to support pre-breeding. We employed a two-pronged approach to modify genes controlling adaptive and domestication traits in wheat: the first approach involves a combination of gene editing and hybrid wheat technologies, and the second approach involves direct modification of domestication related genes in wild emmer accessions.

We constructed a multiplex gene knockout construct targeting five key genes, *RhtB1*, *PpdA1*, *VRN2*, *Q*, and *Btr1* that regulate plant height, photoperiod response, vernalization requirement, seed threshability and brittle rachis in wheat, respectively. This construct was used to transform an elite durum wheat cultivar, CDC Fortitude, creating the Tetra^{OM} tester line.

Subsequent intercrossing of Tetra^{OM} and tetraploid emmer wheat accession (TG3487) produced F_1 plants, which were assessed for emergence date, plant height, heading date, time to flowering, maturity and spike characteristics. Although the F_1 plants exhibited delayed growth, reduced tiller numbers, decreased fertility rates, and considerably prolonged germination periods, we observed limited editing efficiency in the targeted genes, prompting us to explore alternative strategies.

Among these strategies, the VIGS-based gene editing system, particularly targeting the PDS and Q genes in TG3487, exhibited promising outcomes and is currently being tested on other domestication genes. Additionally, direct editing of domestication genes in the TG3487 background, utilizing the JD633 vector, showed successful transformation and regeneration, as evidenced by targeted disruption of the *Rht-B1* gene, resulting in phenotypically shorter plants compared to the wild type.

Further advancement involved a multi-guide approach targeting multiple genes (*BTR1*, *Q*, *PpdA1*, *VRN2*, and *Rht-B1*) simultaneously within a single construct. Transformation and sequencing analysis of T_0 plants demonstrated successful editing at all five target genes.

This breakthrough sets the stage for efficient simultaneous editing of multiple domestication genes in exotic germplasm within a relatively short timeframe, laying a solid foundation for pre-breeding endeavors involving wild relatives of wheat.

Workshop 9 September 22, 2024, 13.30-13.50

The Australian grains Genebank enters the genomics era

Gabriel Keeble-Gagnère[1], Don Isdale[1], David Chisanga[1], Kenny Yu[1], Mahdi Rahimi[1], Kerrie Forrest[1], Debbie Wong[1], Matt May[2], Josquin Tibbits[1,3], Sally Norton[2], Matthew Hayden[1,3]

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[2] Australian Grains Genebank, Agriculture Victoria, Horsham, VIC, Australia
[3] School of Applied Systems Biology, La Trobe University, Bundoora, VIC, Australia

The explosion of wheat genomic data over the last five years is expected to be dwarfed by data generation over the next five as the cost of assaying the genome continues to fall. Already costs permit the large-scale genotyping of entire genebanks and breeding programs to the generation of multiple pan-genomes. This has allowed genebanks to transition from physical seedbanks to data generators within the broader context of genomic data generation in research and breeding activities.

The Australian Grains Genebank (AGG) Strategic Partnership is a $30M joint investment between the Victorian Government and Grains Research and Development Corporation (GRDC) that aims to unlock the genetic potential of plant genetic resources for the benefit of Australian grain growers. The Partnership is transforming the AGG into a bio-digital resource centre through the genetic characterisation of its collection and implementation of digital and genomic tools to make the new resources highly accessible to researchers and breeders.

This talk will describe work underway to transform the AGG into a genebank for the future. It will detail how genotypic data from its collection is being made accessible through the development of industry-informed use cases; the development of digital tools and interfaces to provide routes to access for users of the AGG; the building of interoperable data infrastructure to support genebank-to-genebank data integration; and how the genebank's accessions are being connected to research and breeding knowledge to empower researchers and breeders to deliver the varieties of tomorrow.

Workshop 9 September 22, 2024, 14.10-14.30

Management and utilization of plant genetic resources in India

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Plant genetic resources (PGR) are the foundation of agriculture as well as food and nutritional security. The ICAR-NBPGR is the nodal institution at national level for management of PGR in India under the umbrella of Indian Council of Agricultural Research (ICAR), New Delhi. India being one of the gene-rich countries faces a unique challenge of protecting its natural heritage while evolving mutually beneficial strategies for germplasm exchange with other countries.

The Bureaus activities include PGR exploration, collection, exchange, characterization, evaluation, conservation and documentation. It also has the responsibility to carry out quarantine of all imported PGR including transgenics meant for research purposes. The multifarious activities are carried out from ICAR-NBPGR headquarters and its 10 regional stations located in different agro-climatic zones of India. It has linkages with international organizations of the Consultative Group on International Agricultural Research (CGIAR) and national crop-based institutes to accomplish its mandated activities. ICAR-NBPGR encompasses the National Genebank Network and at present, the National Genebank conserves more than 4.68 lakhs accessions.

ICAR-NBPGR collects and acquires germplasm from various sources, conserves it in the Genebank, characterizes and evaluates it for different traits and provides ready material for breeders to develop varieties for farmers. NBPGR works in service-mode for effective utilization of PGR in crop improvement programmes which depends mainly on its systematic characterization and evaluation, and identification of potentially useful germplasm. For effective utilization of germplasm, ICAR-NBPGR is actively engaged in organizing Germplasm Field Days in every crop season. Germplasm field days provide an opportunity to the users for on-the-spot assessment of variability and select the material of their interest.

The Bureau also develops genomic tools for enhanced utilization of crop germplasm; generates and conserves genomic resources; undertakes molecular profiling and DNA fingerprinting of crop varieties; and molecular diagnostics for GM detection. It is responsible for identifying trait-specific pre-adapted climate resilient genotypes, promising material with disease resistance and quality traits which the breeders use for various crop improvement programmes.

The system has contributed immensely towards safeguarding the indigenous and introducing useful exotic PGR for enhancing the agricultural production. Presently, our focus is on characterization of *ex situ* conserved germplasm and detailed evaluation of prioritized crops for enhanced utilization; assessment of impact of on-farm conservation practices on genetic diversity; genome-wide association mapping for identification of novel genes and alleles for enhanced utilization of PGR; identification and deployment of germplasm/ landraces using climate analog data; validation of trait-specific introduced germplasm for enhanced utilization.

Utilization of PGR stands at a crucial juncture and strategic decisions on the way forward are imperative. Traditional methods of PGR management are being challenged by changing needs, priorities, climate, technologies, and policies.

Envisaging the overwhelming pressure on the natural existence and evolution of PGR does not require prophecy. However, looking through more than four decades shows the promise of modern technologies in combating genetic erosion and enhancing utilization of PGR. It is in this context that adopting every upcoming technology is the best way forward.

Workshop 9 September 22, 2024, 15.50-16.10

Wheat mutant resources enhance discovery of novel genes and functional genomics

Huijun Guo*, Hongchun Xiong, Yongdun Xie, Linshu Zhao, Jiayu Gu, Huiyuan Li, Luxiang Liu*

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Mutation induction has been utilized in plant breeding for over 90 years, the heritable changes result in new traits and increase the genetic diversity of crops. With the development of functional genomics, mutants on variable traits have been applied to unveil functions and pathways of target genes in Arabidopsis, rice, maize and other crops.

Wheat functional genes have been uncovered rapidly in recent several years with the release of reference genome and low-cost sequencing technique, therefore it need more diversified mutant resources under multiple genetic background to accelerate gene function characterization and elaboration. We used a hexaploidy winter wheat variety, Jing411, as the wild type, and used gamma rays, carbon ion beams, spaceflight, ethyl methane sulphonate as mutagens, established a mutant resource with broad spectrum phenotypic variations and a mutant library with genotypic variations.

The phenotypic mutant resource was consist of advanced stable lines, which have been used to uncover novel QTLs, genes and/or alleles, such as the semi-dwarf gene *Rht8* and its alleles, flag leaf angel gene *SPL8* and its alleles, the *Q* gene and its alleles, mutants on starch and resistant starch etc..

The genotypic mutant resource (http://jing411.molbreeding.com/#/query) contained functional mutations, which covered 97.09% of high confidence genes and 71.12% of low confidence genes. Up-to-date, the mutants have been shared and utilized in wheat community, and over 600 samples have been provided to more than 50 research groups.

3RD INTERNATIONAL WHEAT CONGRESS

Workshop 10: Functional Genomics

Sunday, 22 September 2024

Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 2, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Prof Harvey Millar, The University of Western Australia, Australia** **Dr Phillippa Borrill, John Innes Centre, UK**	
13:00 – 13:10	Introduction	
13:10 – 13:30	The discovery of tandem kinase r-genes: origin, function, and potential in resistance breeding	**Prof Tzion Fahima** University of Haifa, Israel
13:30 – 13:50	Unveiling the micronutrient architecture of wheat grains through spatial transcriptomics at single-cell resolution	**Dr Chen Ji** John Innes Centre, UK
13:50 – 14:10	Deciphering the role of small RNAs in regulation of restorer-of-fertility gene expression in wheat	**Dao Anh Thien Tran** The University of Western Australia, Australia
14:10 – 14:30	Developing cisgenic resistance gene stacks for improved resistance to wheat stem rust disease	**Dr Ming Luo** Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia
14:30 – 14:50	**Tea/Coffee break**	

14:50 – 15:10	Nicotianamine synthase gene discovery, characterisation, and overexpression in bread wheat	**Dr Jesse Beasley** The University of Melbourne, Australia
15:10 – 15:30	Does-response assays and transcriptomic analyses evaluate novel fungicide-supplement combinations on elevating wheat rust disease resistance	**Dr Meng Li** University of British Columbia, Canada
15:30 – 15:50	Identification of wheat rDNA unit variants and changes in their expression following partial chromosomal deletions	**Mr Yohta Hyuga** Prefectural University, Kyoto, Japan
15:50 – 15:55	**Closing remarks by** Co-Chairs	

Workshop 10 September 22, 2024, 15.30-15.50

Identification of wheat RDNA unit variants and changes in their expression following partial chromosomal deletions

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Many crops are polyploid, and understanding the genetic and genomic changes during polyploidization is important for crop improvement.

rDNA (ribosomal DNAs) encodes rRNAs (ribosomal RNAs), components of the ribosome, and is located in chromosomal regions called NORs (Nucleolar Organizer Regions). The NORs contain hundreds to thousands of copies of rDNA units repeated in tandem. Although the sequences of each rDNA unit are highly similar, their expression is tightly regulated.

Nucleolar Dominance (ND) is the phenomenon that only one of the NORs inherited from both parents is expressed in hybrid offspring. Our project aims to reveal the genomic harmonization that occurred during the wheat’s allopolyploidization based on the analysis of ND.

In a previous report (Handa et al., 2018), we found sequence differences in the ETS region after the 26S rRNA gene on chromosomes 1B, 5D, and 6B, and identified four rDNA subtypes. Expression studies revealed that only B genome-derived subtypes were expressed in the normal hexaploid wheat. However, 5D chromosome-derived subtypes began to be expressed if the NORs on chromosome 1B or 6B were missing.

This time, we investigated the reference sequences of chromosomes 1A and 7D, which have yet to be analyzed in the five chromosomes with NORs in wheat, and we identified two new subtypes of rDNA unit. Together with the previous four subtypes, six were identified, providing a basis to investigate all NORs throughout the wheat genome. As with previous results, only B genome-derived subtypes were expressed in normal hexaploid wheat.

However, in strains lacking NORs on chromosomes 1B or 6B, subtypes derived from 5D and 7D were found to be expressed. In contrast, no expression of chromosome 1A-derived subtype was observed even when the B genome-specific NOR was deleted. These suggest a hierarchical structure in ND in wheat in the order B-genome>D-genome>A-genome. Further studies using strains with partial deletion of chromosome 1B revealed that as the degree of deletion of the short arm of chromosome 1B increased, the degree of recovery of expression of the D genome subtype also increased. This result contradicts the previous hypothesis that only the NOR on the B genome chromosome regulates ND and suggests there may be regulatory regions other than the NOR on the short arm.

Now, to clarify more details of ND in wheat, we are analyzing the changes in ND according to growth stage and wheat evolutionary processes.

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Workshop 10 September 22, 2024, 13.50-14.10

Deciphering the role of small RNAs in regulation of restorer-of-fertility gene expression in wheat

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Plant mitochondrial genomes may carry genes causing cytoplasmic male sterility (CMS), resulting in failed pollen development and seed set. Proteins encoded by nuclear Restorer-of-fertility (*Rf*) genes can block CMS gene expression, forming an effective breeding system (CMS/*Rf*) that controls self-pollination and restores fertility in F1 hybrids.

Although hybrid varieties have been developed successfully in various crops, wheat lacks an efficient breeding system, hindering hybrid seed production on a commercial scale. A promising system in wheat involves the mitochondrial *orf279* gene in *Triticum timopheevii* (T-CMS) and nuclear *Rf1* and *Rf3* genes. It has been shown that Rf1 and Rf3 proteins target the *orf279* transcript and induce cleavage to suppress the T-CMS phenotype.
However, seed-set experiments indicated neither of the two restorer genes can fully restore the fertility of F1 hybrids, a crucial requirement of a successful breeding system, contrasting with fully fertile *T. timopheevii* plants.

Using next-generation sequencing assays and PCR-based sequence analyses, we compared the expression and processing of *orf279* in *T. timopheevii* with transgenic wheat plants. We observed that *orf279* is more suppressed in *T. timopheevii* than transgenic wheat plants carrying T-CMS and single *Rf1* or *Rf3* or both genes. RNA-seq analysis indicated that the low expression levels of *Rf* genes may be insufficient to block the expression of *orf279* in transgenic lines.

Post-transcriptional gene silencing mechanisms have been reported to affect Rf gene expression in plants negatively. Our small RNA-seq and degradome-seq data analyses revealed several small RNAs targeting and cleaving *Rf* transcripts. To explore the extent to which the expression of *Rf* genes is regulated by those small RNAs, we investigated accessions with targeted changes on the small RNA binding sites.

Our comprehensive analysis sheds new light on the possible reasons for inconsistent fertility restoration using T-CMS/*Rf* systems, offering valuable insights for high-yielding hybrid wheat production.

Workshop 10 September 22, 2024, 15.10-15.30

Does-response assays and transcriptomic analyses evaluate novel fungicide-supplement combinations on elevating wheat rust disease resistance.

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Despite quarantine measures, new wheat rust fungal isolates enter Canada regularly, often from Mexico via the US by common airflows known as the Puccinia pathways. New incursions include mutated rust spores with changed virulence, aggressiveness, and sensitivities to fungicides. Evolved isolates can result in changed virulence towards current resistant wheat varieties and can be caused by mutated or lost avirulence effectors or nuclear haplotype exchange via hybridization.

Complementing rust disease control via genetic resistance occurs through fungicide application which has been estimated to cost on average $17.25 per acre annually in wheat fields on the Canadian Prairies. Even though fungicides are targeted to fungal pathogens, they have also been reported to trigger disease resistant effects on the host plant.

Therefore, our collaboration connecting industrial technology and wheat-rust research has developed an assay system combining plant genetics and fungicide plus formulation treatment on germinating urediniospores and detached or in-planta, infected leaves that allows for dramatic dose reductions of the synthetic fungicides Quadris and Proline often used for wheat leaf rust control.

To study the potential mode of actions (molecular mechanisms) of the commercial compounds, the effect of the supplement, and to identify potential biomarkers for diagnosing virulence-reducing and defense-enhancing responses in both fungus and wheat host, comparative transcriptomic analyses were carried out.
By comparing groups of leaf rust fungus and wheat transcripts, differentially expressing genes were identified as potential diagnostic biomarkers indicating resistance or susceptibility, and as potential regulators of defense responses as annotated by gene ontology searches.

This new combinational treatment will generate significant cost savings, increase options available to producers as well as reduce negative environmental impacts of synthetic fungicides while improving crop yield and quality.

Workshop 10 September 22, 2024, 13.30-13.50

Unveiling the micronutrient architecture of wheat grains through spatial transcriptomics at single-cell resolution

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Wheat (*Triticum aestivum*) grain filling is coordinated with cell expansion that significantly enlarges the grain size. This stage represents the peak period for nutrient and micronutrient transportation within the grains. Wheat grain has low levels of iron and zinc which contributes to micronutrient deficiencies in the billions of people who rely on wheat as a staple food. Due to technical limitations, many genes involved in micronutrient processes and their related mechanisms are yet to be discovered. Through understanding the transport processes, we aim to increase the levels of iron and zinc in wheat grains, to benefit human health.

In this study, we combined phenomics and transcriptomics to observe cell expansion, endosperm filling and micronutrient content in wheat cultivar Cadenza 12 days and 18 days after flowering. By leveraging XRF (X-ray Fluorescence Spectroscopy), ICP-OES (Inductively coupled plasma - optical emission spectrometry), Stereo-seq spatial transcriptomics and single-nucleus analysis, we delineated the regulatory networks and key transcription factors involved in the development of grains and the transport of Zn/Fe, with confirmation currently in progress through targeted in-situ analyses techniques.

We identified clusters specific to various tissue components and transporters that are specifically expressed in the embryo-adjacent endosperm and starchy endosperm, which we hypothesise are involved in the transport of micronutrients.

These findings offer a strategy for exploring gene regulatory networks at the single-cell level in wheat grains and provide insights into improving wheat grain micronutrient content.

Workshop 10 September 22, 2024, 13.10-13.30

The Discovery of Tandem Kinase R-GENES: Origin, Function, and Potential in Resistance Breeding

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Plants employ numerous innate immune receptors to perceive various immunogenic signals associated with pathogen infection and activate defense responses. These receptors include cell-surface receptor-like kinases (RLKs) and intracellular nucleotide-binding leucine-rich repeats (NLRs). Yet, pathogens continuously develop counter-defenses in an arms race with their hosts, destabilizing food production and sustainability and resulting in global food insecurity. Thus, breeders seek durable broad-spectrum resistance (BSR) that can last many years.

We previously cloned the yellow rust BSR resistance gene, *Yr15*, derived from wild emmer wheat (Nature Communications 2018) that encodes a protein with a kinase-pseudokinase domain architecture, designated as Wheat Tandem Kinase 1 (*WTK1*). The cloning of *WTK1* led us to discover a novel protein family with a unique tandem kinase protein (TKP) architecture distributed across the plant kingdom. Phylogenetic analysis indicated that TKP family members are associated with RLKs and originated from either gene duplication or gene fusion events, implying a polyphyletic origin of the TKPs by convergent molecular evolution.

We hypothesize that TKPs serve as decoys that counter-defend the suppression of RLKs by pathogen effectors. Whole transcriptome analysis of wheat near-isogenic lines revealed that WTK1 activates defense-associated transcriptional reprogramming upon pathogen infection, leading to disease resistance.

The decoy model can explain how TKPs regulate the activation of local programmed cell death immune responses and provide support for TKP's polyphyletic origin and evolution. Currently, nine functional wheat TKPs were described by different groups, conferring resistance against rusts

(*WTK1, WTK2*, *WTK5*, *WTK6-vWA*), powdery mildew (*WTK3*, *WTK4, WTK7-TM, Pm57*), and wheat blast (*Rwt4*) diseases. *Yr15* (*WTK1*) confers resistance to >2000 pathogen isolates from around the globe, and we developed functional molecular markers for marker-assisted breeding.

Thus, TKPs have a great potential for resistance breeding. Further studies are underway to elucidate the mechanism of resistance conferred by this extraordinary protein family.

Workshop 10 September 22, 2024, 14.50-15.10

Nicotianamine synthase gene discovery, characterisation, and overexpression in bread wheat

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Nicotianamine (NA) is a chelator of divalent metal cations that is synthesized by nicotianamine synthase enzymes (NAS) in all higher plants. We have previously described the identification of 21 *TaNAS* genes present in the bread wheat (*Triticum aestivum* L.) cultivar (cv.) Chinese Spring genome[1].

This study utilized up-to-date online wheat genomic resources to discover 33 *TaNAS* genes present in cv. Chinese Spring genome and an additional eight *TaNAS* genes throughout the genomes of ten other bread wheat cvs. The expression of most *TaNAS* genes was higher in wheat roots relative to other tissues, although some individual *TaNAS* genes displayed ubiquitous expression across tissues and developmental stages. The amino acid sequence of all TaNAS proteins contained two motifs for cytoplasmic vesicular localization, and extended N-terminal regions in TaNAS2-A1, TaNAS2-A2, TaNAS2-D3, TaNAS6-A1, TaNAS6-D1, TaNAS7-A1, TaNAS7-B1, and TaNAS8-D1 predicted subcellular localisation to mitochondria and/or chloroplasts.

To explore the effect of *TaNAS* overexpression on grain nutrition and plant growth we transformed bread wheat (cv. Fielder) with six independent constructs containing TaNAS coding sequences (*TaNAS1*-A1, *TaNAS3*-B1, *TaNAS4*-A1, *TaNAS6*-DL1, *TaNAS6*-DS1, and *TaNAS7*-A1) that represent a range of TaNAS protein structures and gene expression profiles. We isolated three single insert homozygous *TaNAS* events and a respective null segregant (NS) control for each construct and assessed plant growth and grain nutrition of T3 transgenic plants under both glasshouse and field conditions.

This study demonstrates the value of genomic resources for characterizing important gene families in bread wheat and provides further evidence of NA biofortification as a promising strategy for enhancing grain nutritional quality[2].

[1] Bonneau JP, Baumann U, Beasley JT, Li Y, Johnson AAT. Identification and molecular characterization of the nicotianamine synthase gene family in bread wheat. Plant Biotechnol J. 2016 Dec;14(12):2228-2239.

[2] Beasley JT, Bonneau JP, Moreno-Moyano LT, Callahan DL, Howell KS, Tako E, Taylor J, Glahn RP, Appels R, Johnson AAT. Multi-year field evaluation of nicotianamine biofortified bread wheat. Plant J. 2022 Mar;109(5):1168-1182.

Workshop 10 September 22, 2024, 14.10-14.30

Developing cisgenic resistance gene stacks for improved resistance to wheat stem rust disease

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Rust fungi cause severe diseases in plants and are a major threat to wheat production and global food security. Central to the success of rust infection is the secretion of effector proteins, which function to manipulate the host plant. Due to evolutionary pressures, pathogen effector proteins are often sequence diverse, but some are known to adopt conserved structural folds. To investigate conserved structural features of rust effectors that could be targeted for improved disease resistance we used AlphaFold2 to predict the structures of all secreted proteins from five rust species: wheat stem rust (*Puccinia graminis* f. sp. *tritici* (*Pgt*), wheat leaf rust (*P. triticina*), wheat tripe rust (*P. striiformis*), oat crown rust (*P. coronata* f. sp. *avenae*) secretomes, and flax rust (*Melampsora lini*).

As observed in flax rust, all six known *Pgt* avirulence effector proteins belong to different structural classes. This suggests that rusts employ a more structurally diverse set of effectors than oomycetes and powdery mildew pathogens, whose effectors predominantly belong to a single class (WY and RALPH, respectively). Recently, we experimentally determined the structure of the avirulence effector AvrSr27 from *Pgt*, which has a novel zinc-bound, duplicated domain structure.

We compared the AlphaFold2 predicted structure against our experimentally determined structure of AvrSr27 and demonstrated the prediction accuracy of AlphaFold2 for detecting zinc-binding sites. To determine if zinc binding is prevalent in rust secretomes we employed an established *in silico* screen, which revealed several other putative metal ion binding effector families, including those containing the recently cloned *Pgt* avirulence effectors, AvrSr13 and AvrSr22.

To validate our findings, we developed a workflow for the biochemical characterisation of metal-binding in rust effectors and showed that both AvrSr13 and AvrSr22 preferentially bind zinc ions. According to our predictions, zinc binding proteins account for about 13% of the secreted proteins of *Pgt*, and ~5-10% for the other studied rust species, suggesting that this may be a common feature in rust effectors.

3RD INTERNATIONAL WHEAT CONGRESS

Workshop 11: Predictive Breeding for Wheat Improvement

Sunday, 22 September 2024
Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 3, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Dr Dini Ganesalingam, InterGrain Pty Ltd, Australia** **Dr Scott Sydenham, LongReach Plant Breeders Management Pty Ltd, Australia**	
13:00 – 13:10	Introduction	
13:10 – 13:30	Wheat priority breeding traits for Europe: perspectives based on French genetic progress over 40 years	**Dr Jean-Pierre Cohan** Arvalis, France
13:30 – 13:50	Using deep learning to predict wheat spike volume to estimate fruiting efficiency	**Ms Olivia Zumsteg** ETH Zurich, Switzerland
13:50 – 14:10	Spatial analysis with UAS data in wheat breeding trials	**Dr Arron Carter** Washington State University, USA
14:10 – 14:30	Advancements in marker-assisted breeding for enhancing yellow color in durum wheat	**Dr Patricia Cabas-Lühmann** The Pontifical Catholic University of Chile, Chile
14:30 – 14:50	**Tea/Coffee break**	

14:50 – 15:10	Optimizing genomic selection for winter hardiness and Fusarium head blight resistance in winter durum wheat	**Mr Ritesh Yadav** University of Manitoba | Agriculture and Agri-Food, Canada
15:10 – 15:30	Genetics-inspired enviromic prediction to exploit impacts from changing climate to wheat performance	**Dr Xianran Li** US Department of Agriculture - Agricultural Research Service (USDA-ARS), USA
15:30 – 15:50	Optiwheat: genomic-enviromic modeling with bigdata enhances wheat grain yield predictions	**Mr Abhishek Gogna** Leibniz Institute of Plant Genetics and Crop Plant Research, Germany
15:50 – 16:10	**Closing remarks by** Co-Chairs	

Workshop 11 September 22, 2024, 15.30-15.50

Optiwheat: genomic-enviromic modeling with bigdata enhances wheat grain yield predictions

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Breeding programs in Europe often prioritize average genotype performance in target environments, potentially overlooking the late-stage selection of environment-optimized candidates. The latter translates into suboptimal cultivar performance at the farm level, especially under environment diversification exacerbated by climate change.

To address this challenge, we propose leveraging BigData and Artificial Intelligence (AI) for genomic predictions. In this study, we compiled data on winter wheat grain yield from 13,285 genotypes across 98,175 yield plots in diverse 117 central European environments from 2010 to 2022. With integrated genomic data we found that as the size of the training dataset increased, convolutional neural networks (CNN) outperformed traditional genomic best linear unbiased predictions (GBLUP) in predicting average genotype performance.

We then expanded our prediction models to account for genotype-environment interactions (GxE) by additionally including enviromic data. In doing so, we observed a notable improvement of up to 22% in predicting environment-specific performance of new hybrids within a network of testing environments. Lastly, to better understand environment factors driving GxE, we conducted analyses on a core set comprising 500 genetically diverse lines in the studied environments.

Using machine learning, we successfully identified pivotal markers for environment classification in Central Europe.

Our results suggest that BigData not only facilitates absorption of AI but also offers new avenues for widening the genetic bottleneck often encountered when progressing candidates from early limited-environment to late stage multi-environment evaluations.

Workshop 11 September 22, 2024, 15.10-15.30

Genetics-inspired enviromic prediction to exploit impacts from changing climate to wheat performance

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Environmental conditions play a significant role in shaping phenotypic variation observed in natural conditions, but it remains challenging to parse out and integrate environmental contributions into genetic analyses and breeding programs. Two primary obstacles are acquiring data from a large number of environments and implementing intuitive analytics.

Variety testing trial has been an essential component for modern breeding programs over decades, these publicly accessible performance records, coupling weather database, could be repurposed to overcome the data acquisition hurdle.

Newly developed machine learning and deep learning algorithms, inspired by well-established genetics approaches, can be seamlessly integrated into the classic Joint regression model pioneered by Australian Scientists Finlay and Wilkinson over 60 years ago.

Combining these innovative data acquisition and analytics strategies empowers exploit weather conditions to wheat performance.

Workshop 11 September 22, 2024, 13.10-13.30

Wheat priority breeding traits for Europe: perspectives based on French genetic progress over 40 years

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Wheat production in Europe is facing major challenges: improving its resilience against climate change, developing more efficient integrated diseases, pests and weeds management strategies relying less on pesticides and, adapting its quality criteria to new consumers demands. Innovative cultivars are needed to address all these issues. Consequently, wheat breeding programs must be managed carefully to integrate the priority traits to achieve these objectives.

As a starting point to define these traits, an unprecedented genetic progress study has been conducted in France using 40 years of data from the cultivars assessment trial networks and related research programs. Due to its geographical positioning, France presents contrasted cropping environments, allowing us to extrapolate some general conclusions to other European countries.

Yield genetic progress has been estimated at 0.054 and 0.042 $t.ha^{-1}.year^{-1}$ for bread and durum wheat respectively, stable since the early 1980s. This progress is significant even in constraint cropping environments and is mainly based on a higher number of $grain.m^{-2}$. Given the increasing occurrence of late heat and water stress, strengthen the grain filling capacity of wheat should now be a priority for breeding programs.

For 40 years, the average resistance of cultivars against the main wheat foliar diseases has improved. For brown and yellow rusts, this progress is particularly challenged by the rapid evolution of pathogen populations. In 2005, more than 70% of the bread wheat French cultivation area was cultivated with medium sensitive and highly sensitive cultivars for *Septoria*, while in 2022, more than 70% of the bread wheat French cultivation area was cultivated with medium resistant and highly resistant cultivars for this disease.

The main objectives of breeding programs should be to strengthen the durability of disease resistances by rapidly stacking different types of resistance genes against major pathogens.

Wheat quality traits often present a strong link with nitrogen use efficiency (NUE). For bread wheat, NUE has continuously been improved since the beginning of the 1980s, particularly its N utilization efficiency component.
Thanks to the enhancement of the Grain Protein Deviation, the decrease of grain protein concentration under the effect of yield progress has been limited.

For durum wheat, vitreosity index has been improved, despite the decrease of grain protein concentration due to the yield progress. High NUE should be kept as a target in breeding program to address both production and climate change mitigation challenges, as well as quality traits to respond to the diversified consumer demands.

Workshop 11 September 22, 2024, 14.50-15.10

Optimizing genomic selection for winter hardiness and fusarium head blight resistance in winter durum wheat

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Fusarium head blight (FHB) disease resistance and winter hardiness are the two priority traits in the Canadian winter durum wheat breeding program. These traits, influenced significantly by polygenic factors and environmental conditions, are crucial given the ongoing climate change challenges in Western Canada, such as rising temperatures and escalating drought risks.

There is only a single winter durum cultivar (OAC Amber) registered in Eastern Canada in 2010, which remains uncommercialized. At present, no winter durum wheat candidates are available for testing in Western Canada. Recently, joint Genome-Wide Association Study-Genomic Selection (GWAS-GS) and haploblock based GS approach have shown great potential for deciphering the genetic basis of complex traits, facilitating more accurate prediction of breeding values and enhancing genetic gains for difficult-to-phenotype and complex traits, such as FHB resistance and winter hardiness.

In our study, we assembled a diverse panel of 292 winter durum accessions from Canada, Europe, and the USA, including in-house winter hexaploid wheat x durum cross derivatives. The panel was phenotyped for FHB resistance and winter hardiness at multi-location trials in Winnipeg, Carman, and Ottawa during the 2021-23 seasons, with ongoing trials in 2023-24. Genotyping was performed using Genotyping-by-Sequencing (GBS), and SNPs were called against the durum cv Svevo.v1 and IWGSC CS RefSeq v2.1 reference genomes using an in-house GBS pipeline to enhance the robustness of the downstream genetic analyses.

Genetic structure analyses (phylogenetic, principal component and ancestry analyses) clustered the accessions into six sub-populations. FarmCPU GWAS detected loci associated with winter hardiness on chromosome 2A, 4B, 5A, 5B and 7B and FHB resistance on chromosome 3B and 7B.

Further, we aim to employ joint GWAS-GS approach using several parametric models such as GBLUP, EGBLUP, RRBLUP and Bayesian models (BRR, BLASSO, EBLASSO, Bayes B, Bayes C), non-parametric models such as Random Forest and RKHS, and haploblock based genomic prediction. We will utilize GWAS-tagged markers derived from multiple GWAS models such as, MLM+K+Q, FarmCPU, MLMM and BLINK in the GS models as fixed effects.

Significant markers derived from each GWAS models will be consolidated into a streamlined, non-redundant marker set for further use in genomic prediction. The haplotype blocks will be constructed using different fine-tuned LD thresholds, number of adjacent markers and algorithms available in HaploView and HaploBlocker (R package) and will be utilized for haploblock based genome prediction using suite of GS models.
Our ongoing study focuses on optimizing GS for Canadian winter durum development program.

Workshop 11 September 22, 2024, 13.30-13.50

Using deep learning to predict wheat spike volume to estimate fruiting

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Frequent drought and heatwaves due to climate change pose significant threats to global wheat yields. Number of grains per spike is an important yield component that shows potential for optimization. A way to increase the number of grains is to select for high fruiting efficiency (number of grains per gram of spike dry weight at anthesis). Currently, there is a lack of high-throughput phenotyping (HTP) methods that estimate fruiting efficiency. With our new sensor head, containing a rigid setup of 13 RGB cameras that captures multiple top-view images from hundreds of different genotypes, we are able to detect and to count spikes.

While spike dry weight is difficult to measure directly, the volume at flowering, the so-called fruiting capacity may be used as proxy for spike weight at anthesis and determines the capacity of the reproductive organ to be filled. Estimating fruiting capacity and efficiency and elaborating their relationship will allow to dissect genotype-specific differences in yield and in their underlying causes. Such insights are crucial to mitigate the impacts of heat and drought under projected climate conditions.

Developing deep learning models requires a solid data set for training and tests. For this work, approximately 1000 spikes from a diverse set of genotypes were labelled in the field, imaged both individually in the field with a smartphone, and at the plot level with the 13 cameras. After harvest, their volume was measured with a 3D scanner. As a proof of concept, volume estimation from RGB images was first tested on the close-up smartphone images using deep learning models and yielded an MAPE of 9.7%.

Results indicate that neural networks show a higher prediction accuracy compared to the baseline model, which fits smoothing splines along the axis and integrates over the splines to get the volume. Furthermore, augmenting the dataset with artificial images can both reduce the prediction error of the neural network compared to using real images only by 3.51 %, and reduce overfitting on the training set.

As an outlook, next steps will involve applying the developed models to predict in-field volumes directly with the multi-view sensor head. The extracted volume estimations over time will allow identifying genotypes with high fruiting capacity that can efficiently fill their potential volume even under stress conditions.

Ultimately, this will allow to achieve high yields despite heat and drought events throughout the season.

Workshop 11 September 22, 2024, 14.10-14.30

Advancements in marker-assisted breeding for enhancing yellow color in durum wheat

Patricia Cabas-Lühmann[1]*, Susanne Dreisigacker[2], Karim Ammar[2], Andrés Schwember[1], Maria Itria Ibba[2]*

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The yellow color in durum wheat (*Triticum turgidum* spp. *durum*) is an essential trait for breeders to meet consumer and pasta industry requirements. Given the complex inheritance of this trait, there is a continued need for new molecular markers targeting key genes, like the phytoene synthase (*Psy1*) gene.

This research aimed to (1) identify and characterize the allelic variants associated with *Psy1-A1* and *Psy1-B1* genes; (2) test previously published and newly developed molecular markers that target genes associated with the yellow index (YI); (3) perform a genome-wide association study (GWAS) analysis to validate the effectiveness of the tested markers and identify new genomic regions associated with grain yellowness.

A population of 269 durum genotypes from CIMMYT grown in three environments during 2020-2022 in Mexico was used. Thirteen molecular markers targeting specific alleles of *Psy1-A1* and *Psy1-B1* genes were employed. Five markers were developed at CIMMYT, while the remaining had already been published. Marker specificity was confirmed through sequence comparison and amplicon analysis. The population was genotyped with 26,349 SNPs and phenotyped for YI using the CIE b^* value in semolina and whole wheat flour. Statistical analyses included phylogenetic analysis, analysis of variance, heritability estimation, and GWAS. Phylogenetic analysis elucidated the genetic diversity of *Psy1-A1* and *Psy1-B1* alleles across different wheat species, providing insights into their evolutionary relationships.

The study also highlighted the importance of marker specificity and genetic diversity analysis in marker development and validation. GWAS results demonstrated the potential of developed markers in predicting yellow color, with markers from *Psy1-A1* and *Psy1-B1* genes showing significant associations ($P \leq 0.05$).
The most significant SNPs associated with semolina and whole wheat yellowness were identified on chromosomes 5B and 7A with percentages of variance up to 22% and 10%, respectively.

Results of this research contribute to the ongoing efforts in durum wheat breeding programs of enhancing the grain YI, thereby facilitating the development of high-quality durum wheat varieties capable of meeting consumer preferences and industry standards.

Workshop 11 September 22, 2024, 13.50-14.10

Spatial analysis with UAS data in wheat breeding trials

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An important aspect of reliable cultivar development is good field trial evaluations. In more extensive field experiments, trial design and modeling of spatial variability are critical to control field variability and minimize error in genotype evaluations. Unoccupied aircraft systems (UAS also known as drones or UAVs) are a popular high-throughput phenotyping tool that has been used to successfully evaluate plant stress and other canopy characteristics.

In precision agriculture applications, UAS imagery has been used to identify spatial variability in field settings. Here we use UAS spectral imagery to improve field trial spatial analysis, better control spatial variability and reduce error for more reliable selections. UAS imagery data was collected across 47 breeding trials planted in an augmented complete block design (ACBD) or alpha-lattice replicated designs from 2020 through 2023. Trials were evaluated using three spatial analysis strategies: linear models incorporating block effect, row-column effect, or 2D splines. UAS-derived spectral reflectance indices (SRI) were combined with each model as covariates.

Modeling strategies were used across all trials and evaluated for autocorrelation, model fitness, and coefficient of variation (CV). Akaike information criterion (AIC) was used to assess model fitness. For spatial analysis trials, SRIs lowered model AIC by an average of 38.4 for alpha-lattice trials and 69.1 for ACBD trials. CV scores were also lowered when SRIs were utilized, with average CV values being 2.6 lower for alpha-lattice and 2.1 for ACBD trials.

This study highlights the potential for SRIs to improve the analyses of field breeding trials, despite extreme environmental variables and climate conditions.

3RD INTERNATIONAL WHEAT CONGRESS

Workshop 12: Innovative Multi-Omics Technologies for Wheat and Beyond

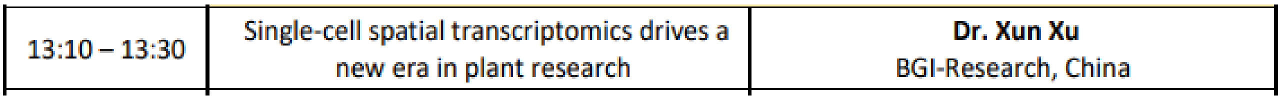
Sunday, 22 September 2024

Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 6, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Mr Richard Harrison, MGI Tech, Australia** **Dr Cen Tong, BGI Research/Murdoch University, China/Australia**	
13:00 – 13:10	Introduction	
13:10 – 13:30	Single-cell spatial transcriptomics drives a new era in plant research	**Dr. Xun Xu** BGI-Research, China
13:30 – 13:50	Going against the grain: Investigating C4 Wheat Theory Through STOmics Spatial Transcriptomics Platform	**Tori Millsteed** The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Australia
13:50 – 14:10	Dynamic Gene Regulation in Developing Wheat Spike at Single-Cell Spatiotemporal Resolution	**Prof Jun Xiao** Institute of Genetics and Developmental Biology, Chinese Academy of Science, China
14:10 – 14:30	Single-cell Transcription Atlas of Seed Germination in Barley	**Dr. Cen Tong** BGI-Research; Murdoch University, China/Australia
14:30 – 14:50	**Tea/Coffee break**	

14;50 – 15:10	Spatial Transcriptomic Study of Early Developing Wheat Inflorescence Using STOmics Stereo-seq	**Dr. Yue Qu (Julian)** The University of Adelaide, Australia
15:10 – 15:30	Optimising Plant Breeding Research with Low-pass Whole Genome Sequencing	**Dr. Dou Hu** MGI Tech, China
15:30 – 15:50	CropGS-Hub: A Comprehensive Database of Genotype and Phenotype Resources for Genomic Prediction in Major Crops	**Dr. Cong Tan** BGI-Research; BGI-Bioverse, China
15:50 – 16:10	A Bumper Crop: Latest Multi-Omics tools for Agriculture	**Dr. Paul Donohoe** MGI Tech, Australia and New Zealand
16:10 – 16:15	**Closing remarks by** Co-Chairs	



13:10 – 13:30	Single-cell spatial transcriptomics drives a new era in plant research	**Dr. Xun Xu** BGI-Research, China

No Abstract Provided

13:30 – 13:50	Going against the grain: Investigating C4 Wheat Theory Through STOmics Spatial Transcriptomics Platform	**Tori Millsteed** The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Australia

No Abstract Provided

13:50 – 14:10	Dynamic Gene Regulation in Developing Wheat Spike at Single-Cell Spatiotemporal Resolution	**Prof Jun Xiao** Institute of Genetics and Developmental Biology, Chinese Academy of Science, China

No Abstract Provided

14:10 – 14:30	Single-cell Transcription Atlas of Seed Germination in Barley	**Dr. Cen Tong** BGI-Research; Murdoch University, China/Australia

No Abstract Provided

14;50 – 15:10	Spatial Transcriptomic Study of Early Developing Wheat Inflorescence Using STOmics Stereo-seq	**Dr. Yue Qu (Julian)** The University of Adelaide, Australia

No Abstract Provided

15:10 – 15:30	Optimising Plant Breeding Research with Low-pass Whole Genome Sequencing	**Dr. Dou Hu** MGI Tech, China

No Abstract Provided

15:30 – 15:50	CropGS-Hub: A Comprehensive Database of Genotype and Phenotype Resources for Genomic Prediction in Major Crops	**Dr. Cong Tan** BGI-Research; BGI-Bioverse, China

No Abstract Provided

15:50 – 16:10	A Bumper Crop: Latest Multi-Omics tools for Agriculture	**Dr. Paul Donohoe** MGI Tech, Australia and New Zealand

No Abstract Provided

Workshop 13: Floral Biology Traits for Hybrid Wheat

Sunday, 22 September 2024

Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 11, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: A/Prof Ryan Whitford, Murdoch University, Australia Dr Antje Rohde, BASF, Belgium**	
13:00 – 13:10	Introduction	
13:10 – 13:30	Reflections on pollination control options for hybrid crops	**Dr Marc Albertsen** Albertsen Crop Genetics for Humanity, Llc, USA
13:30 – 13:50	Cytoplasmic male sterility and restorer-of-fertility genes in wheat and its relatives	**Prof Ian Small** University of Western Australia, Australia
13:50 – 14:10	An efficient and effective system for hybrid wheat production	**A/Prof Peng Zhang** University of Sydney, Australia
14:10 – 14:30	**Tea/Coffee break**	
14:30 – 14:50	Introducing male sterility genes into plant mitochondria by gene editing	**Dr Hajime Sakai** Napigen, USA
14:50 – 15:10	Unlocking the secrets of female fertility in wheat for improved hybrid seed production	**Dr Marina Millan** John Innes Centre, UK

15:10 – 15:30	Transcriptional signatures associated with female receptivity and longevity in a genetically male-sterile wheat cultivar Chris	**A/Prof Ryan Whitford** Murdoch University, Australia
15:30 – 15:50	Insights into floral biology to enhance hybrid seed setting	**Dr Samira El Hanafi** Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany
15:50 – 16:10	The breeder's perspective on desirable floral trait for hybrid wheat	**Dr Jacob Lage** KWS, France
16:10 – 16:15	**Closing remarks by** Co-Chairs	

Workshop 13 September 22, 2024, 15.10-15.30

Transcriptional signatures associated with female receptivity and longevity in a genetically male-sterile wheat cultivar chris

Ryan Whitford[1,2*], Ute Baumann[2], Xiujuan Yang[2], Matthew R. Tucker[2], Faezeh Mahdavi[3], Indeewari Dissanayake[3], Kewin Ogink[3], Mark Davey[3], Antje Rohde[3]

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[2] Waite Research Institute, School of Agriculture, Food and Wine, University of Adelaide, Urrbrae, SA 5064, Australia
[3] BASF Belgium Coordination Center – Innovation Center Gent, Technologiepark 101, 9052 Ghent, Belgium

Wheat hybrids have potential to capture the yield benefits arising from heterosis, but their commercial deployment is restricted due to high seed production costs, itself a result of wheat's floral architecture and poor outcrossing characteristics. These can, in part, be attributed to poor female receptivity to airborne pollen conditioned by wheat's floral morphology.

This study presents a comprehensive transcriptome dataset spanning the life cycle of pistils and stigmas of individual emasculated flowers from wild-type and genetically male-sterile wheat cv. 'Chris'. By comparing gene expression levels to hand pollinated seed-set at successive time points post gaping, we identified gene networks implicated in stigma presentation, receptivity, and longevity.

These include networks linked to GA homeostasis likely associated with styloid and stigma hair elongation, and with increased expression of peroxidases and boron transporters correlating with peak receptivity. *KIL-B1* and several *ZmNAC76* orthologues were also identified to be upregulated at the onset of stigma senescence. By profiling female reproductive tissue specific marker genes, we further determined that physical emasculation (*Ms1*) advances female development by approximately 2 to 3 days relative to genic male sterility (*ms1d*).

The dataset represents a novel genomics resource for identifying and/or engineering improved gene variants for use in breeding female wheat inbreds with improved seed set, and hence, a more cost-effective hybrid seed production.

Workshop 13 September 22, 2024, 14.50-15.10

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

Marina Millán Blánquez[1-2], Matthew Hartley[1-3], Nicholas Bird[4], Yann Manès[5], Cristóbal Uauy[1], Scott Boden[6]

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[2]Laboratoire Reproduction et D’eveloppement des Plantes, ENS de Lyon (INRAE), Lyon, France
[3]European Bioinformatics Institute (EMBL-EBI), Cambridge, UK
[4]KWS UK Ltd, Hertfordshire, UK
[5]Syngenta, Chartres, France
[6]University of Adelaide, Adelaide, Australia

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.

Workshop 13 September 22, 2024, 15.30-15.50

Insights into floral biology to enhance hybrid seed setting

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Unlike inbred crops, hybrids deliver more stable yields and benefit from the phenomenon of heterosis. Efficient hybrid seed production relies on the optimization of both female and male ideotypes with high cross pollination capability. However, due to wheat's domestication as a self-pollinating species and centuries of breeding for cleistogamy, its cross-pollination ability may have decreased in elite varieties.

Therefore, understanding the key aspects that enhance pollen receptivity and boost female hybrid seed set will help in developing and selecting optimal plant ideotypes. To fulfill this aim, we tested hybrid seed set of diverse elite material and a double haploid population, emasculated using a chemical hybridization agent (CHA), across several environments.

Large-scale field screenings demonstrated that high anther extrusion and maximum pollen shedding significantly enhanced the likelihood of successful pollination. Although high levels of these indirect traits already offer a good starting point, a more targeted approach was used to investigate whether factors beyond pollen traits may affect female receptivity. We focused on male lines with sufficient-to-high anther extrusion levels and we investigated the interactions of various traits to better understand their impact on seed set.

Flowering begin and duration showed the largest proportion of the variance in seed set. Therefore, employing a multivariate approach to examine the genetic covariances among these traits could elucidate how their combined expression influenced the overall seed set performance, providing then a strategic pathway for enhanced hybrid wheat breeding.

While genomic assisted breeding of favourable parental ideotypes identified several QTLs with minor to large effect, seed set showed a single major QTL on chromosome 1B, which accounted for 21% of the total phenotypic variation, emphasizing the complex polygenic architecture underlying this particular trait.

Workshop 13 September 22, 2024, 15.50-16.10

The breeder’s perspective on desirable floral trait for hybrid wheat

Jacob Lage

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Hybrid wheat has been the elusive target for industry and academia for over 50 years. Several major attempts to commercialize non-chemically based hybrid wheat have been launched, most terminated at the end of the funding period, or when economic milestones became unachievable. One common thread in the boom-and-bust cycle of hybrid wheat research and development is linked to the costs of hybrid seed.

At KWS, we re-started our focus on hybrid wheat over ten years ago, convinced that heterotic pools either exists, or can be created, and that many wheat growers will adopt hybrid wheat if they see a clear benefit. Like others, we identified two key criteria for success: an efficient hybridization system and cost-effective hybrid seed production. Both criteria are closely linked to wheat floral biology.

As a seed company, KWS’ experience is in breeding and seed production, and less on fundamental research. Consequently, we partnered with the University of Sydney to develop an efficient nuclear male sterility hybrid wheat system. Today we are close to achieving this goal and have begun the transition from discovery to implementation and validation.

Of similar importance for the future success of hybrid wheat are the ‘Cost of Goods Sold’ (COGS). Wheat being a self-pollinated crop, the challenge is to improve the cross-pollination abilities of wheat flowers. To achieve this objective, we teamed up with trusted partners in academia and industry, focusing on pollination duration, stigma morphology and receptiveness, floral gaping, anther size and filament length, pollen count etc.

The progress has been impressive, but more is needed. Our understanding of wheat floral traits has advanced, but so far, the focus has predominantly been on exploiting natural variation in wheat. Moving forward, we need to think bigger and look further away for inspiration and genetics. Our goal must be to obtain near-perfect seed set on male-sterile wheat with limited land used for male pollinators, thereby lowering the COGS of commercial hybrid wheat seed.

The level of COGS will inevitable be the driver, or failure, of commercial large-scale hybrid wheat. At KWS we feel optimistic about deploying a new and efficient hybridization system, a hugely important step achieved via a public-private partnership. Similar, we are looking at the wider research community to continue and even expand the focus on floral biology. We believe that only through partnerships can we redesign wheat floral biology to make it a commercially viable hybrid crop globally.

Workshop 13 September 22, 2024, 13.30-13.50

Cytoplasmic male sterility and restorer-of-fertility genes in wheat and its relatives

Ian Small[1], Joanna Melonek[2], Thien Tran[1], Gilang Bintang Fajar Suhono[1], Laurent Beuf[3], Jean-Philippe Pichon[3], Jacques Rouster[3], Sang Huynh[1], Catherine Colas des Francs-Small[1]

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[2] Research School of Biology, The Australian National University, Canberra, ACT 2600, Australia
[3] Centre de Recherches de Chappes, Limagrain Field Seeds, Route d'Ennezat, 63720 Chappes, France

Cytoplasmic male sterility (CMS) is a widespread trait in flowering plants that has been exploited by breeders for producing hybrid seed for a century or more. Unlike many other 'domestication' traits, CMS is commonly found in the wild. CMS is a maternally inherited trait caused by expression of aberrant mitochondrial genes in anthers leading to a failure to produce viable pollen.

In natural populations, *cms* genes are balanced by nuclear *Restorer-of-fertility* (*Rf*) genes. Most *Rf* genes encode members of the pentatricopeptide repeat (PPR) family — sequence-specific RNA binding proteins that act in mitochondria or chloroplasts. Rf proteins suppress expression of mitochondrial *cms* genes by specifically binding to and inducing cleavage of *cms* transcripts. *Rf* and *cms* genes are widely used in three-line hybrid seed production systems in many vegetable and field crops, such as maize, rice, sorghum and canola.

There has been much interest in exploring their use for hybrid seed production in wheat and other *Triticeae* crops, but development of effective fertility control has proved difficult, in part because of the particularly complex genetics surrounding CMS traits and fertility restoration in *Triticeae*.

The advent of high-quality chromosome-scale genome assemblies for *Triticeae* species has revealed unprecedented numbers of *Rf-like* PPR genes (e.g. ~200 for hexaploid wheat versus ~20–30 for rice and barley; Melonek & Small, 2022). The complexity is compounded by an even more massive and abrupt expansion in genes encoding a second family of organellar RNA-binding proteins known as mTERFs (mitochondrial transcription termination factors) that may also play a role in fertility restoration in cereals.

It seems certain, therefore, that within the *Triticeae* there are multiple different causes of CMS and multiple *Rf* alleles active against them and that disentangling them may be difficult. Nevertheless, progress has been made with the identification of *Rf1* and *Rf3*, encoding PPR proteins that bind to and induce cleavage of the CMS-inducing mitochondrial gene *orf279* (Melonek *et al.* 2021).

This CMS gene is from *Triticum timopheevii*, where its expression is naturally suppressed far more effectively than has been achieved so far in *T. aestivum*, even by combining *Rf1* and *Rf3* in the same genotype. It seems therefore probable that one or more additional *Rf* genes remain to be discovered in *T. timopheevii*.

Recently we have discovered a characteristic C-terminal sequence in Rf-like proteins that correlates with their ability to induce RNA cleavage (Huynh et al. 2023) that should make it easier and faster to identify *Rf* candidates from genome or transcriptome data.

Melonek, J., Duarte, J., Martin, J., Beuf, L., Murigneux, A., Varenne, P., Comadran, J., Specel, S., Levadoux, S., Bernath-Levin, K., Torney, F., Pichon, J.-P., Perez, P., & Small, I. (2021). The genetic basis of cytoplasmic male sterility and fertility restoration in wheat. *Nature Communications*, *12*(1), 1036.

Melonek, J., & Small, I. (2022). *Triticeae* genome sequences reveal huge expansions of gene families implicated in fertility restoration. *Current Opinion in Plant Biology*, *66*, 102166.

Huynh, S. D., Melonek, J., Colas des Francs-Small, C., Bond, C. S., & Small, I. (2023). A unique C-terminal domain contributes to the molecular function of Restorer-of-fertility proteins in plant mitochondria. *The New Phytologist*, *240*(2), 830–845.

Workshop 13 September 22, 2024, 14.30-14.50

Introducing male sterility genes into plant mitochondria by gene editing

Hajime Sakai, Dilbag Multani, Cheryl Caster, Sachie Kimura, Colleen McMichael, Zoe Bara, Mina Hamidi, Dawn Eigner, Debora Wilkinson, Emil Orozco, Jr.

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Cytoplasmic male sterility (CMS) has become the genetic foundation of creating F1 hybrid seed to unlock unprecedented yield potentials in many crop plants. Wheat is a prime example of a crop that can benefit from hybridization. It has been reported that yield gains of 15% or more could be achieved in F1 hybrids of various wheat lines. Encouraged by this promise, private and public organizations have invested significant efforts over past decades to turn the biological gains of hybrid vigor into agricultural advantages.

While the efforts continue, two challenges remain recalcitrant. One is the male sterility of female and the other is the pollen shedding of male plants. The former is perhaps most critical for large-scale hybrid seed productions. So far, the source of male sterility in industrial operations has been limited to a few CMS lines that were originated from crosses of modern wheat plants with less cultivated, often wild, wheat species as cytoplasmic donors.

These alloplasmic plants tended to have unstable mitochondrial genomes, producing novel DNA molecules through complex recombination. A few of the rearranged mitochondrial DNAs resulted in CMS genes. However, most of CMS plants exhibit conditional male sterility. Additionally, due to incompatibility between the nuclear and cytoplasmic genomes, many of the alloplasmic plants are agronomically inferior over cultivated wheat, making their industrial use challenging.

To create better CMS plants, it is highly desirable to have molecular tools to engineer mitochondrial genomes to precisely introduce the best CMS genes into the wheat germplasm pool. Motivated by the potential benefits to our society, we founded NAPIGEN to develop such tools for mitochondrial gene editing.

Our success in single cell organisms, yeast and alga, has now been replicated with plant mitochondrial genomes. We have demonstrated the integration of exogenous genes into specific sites of the rice mitochondrial genome, leading to proper gene expression. We are introducing CMS genes into rice and wheat mitochondrial genomes to enable the creation of customized CMS plants in elite crop backgrounds.

We expect this will help circumvent the alloplasmic and phenotypic expression problems of existing CMS plants. Our technology will improve current hybrid wheat production systems and facilitate robust yield gains.

Workshop 13 September 22, 2024, 1310-13.30

Reflections on pollination control options for hybrid crops.

Dr Marc Albertsen[1]

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Approaches to pollination control, and their impact on facilitating hybrid crops, most often reflect the technology of the time and the specifics of the crop to which they are being applied. Each approach to pollination control has led to a different hybridization platform, whether it be a genetic manipulation, a mechanical intervention, or a chemical intervention. Perfect or imperfect floral structure also impacts the type of pollination control that can be achieved.

The imperfect, monoecious floral structure of maize, for example, greatly facilitated early hybrid development that demonstrated the role that hybrid vigor can play in improving crop productivity. Although perfect flowers presented a challenge to enabling hybrid development in other crops, the recognized value of hybridity spurred the development of crop relevant, cost-effective hybridization platforms.

Whether pollination control was purely mechanical, or whether it was under chemical or genetic control, the desired outcome was a platform that offered the lowest cost of goods (COGs) possible in the production of that hybrid. Cytoplasmic and nuclear male sterility were described decades ago. Recent technology developments have enhanced the likelihood of incorporation of these long-established platforms both into existing hybrid crops and into crops in which hybrids are not yet economically feasible.

In addition to the hybridization platform itself, technologies have been developed that enable the collection and redistribution of pollen, potentially altering the deployment of some hybridization platforms. Still other technologies are under development that could alter how hybrids are produced and maintained.

These technologies, along with development of cost-effective hybrid production practices, have potential in facilitating delivery of affordable hybrid wheat

Workshop 13 September 22, 2024, 13.50-14.10

An efficient and effective system for hybrid wheat production

Peng Zhang[1], Chongmei Dong[1], Jianbo Li[1], Nicholas Bird[2], Nizam Ahmed[1], Amit Singh[1], Jacob Lage[3], Richard Trethowan[1]

[1] Plant Breeding Institute, University of Sydney, Cobbitty, Australia
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Wheat is the world's second largest crop and is also Australia's major crop, which contributes more than $6 billion to the Australian economy. There has been an urgent need to increase the productivity of wheat in an increasingly hostile farming environment, mainly due to climate change. In addition, wheat yields have plateaued making it imperative that new avenues for improving the productivity are found. The benefits of hybrid vigour or heterosis in crops such as maize and rice is well known.

However, like rice, wheat is self-fertilizing and therefore not amenable to simple hybrid seed production. Various strategies for overcoming the self-fertilization barrier have been attempted. However, none provided complete sterility and/or fertility restoration besides other shortcomings, making them less attractive for commercialization.
We have implemented the previously reported genetic blue aleurone (BLA) hybrid wheat system in diverse germplasm. The blue seeded materials have an alien chromosome addition with a blue colour gene on the long arm and a fertility restorer on the short arm, and carry a deletion of the male fertility gene Ms1 on chromosome 4BS. When self-fertilized, these materials produce white and blue seed in a 1:1 ratio. The blue seed have 43 chromosomes and maintain the system, whereas the white seed carry a normal complement of 42 wheat chromosomes and are male sterile as they also carry the homozygous Ms1 deletion.

These white seed are used as the female parent in generating F1 hybrid seed, with any wild-type wheat genotype restoring the fertility in the F1 progeny. The monosomic alien chromosome occasionally mis-divides during meiosis and white seed with the fertility restorer are produced.

The overall consequence is that small proportion of the F1 seeds are not hybrid. We have been using different approaches to rearrange the alien chromosome to eliminate/greatly reduce the impact of mis-division.
The BLA chromosome has been sequenced, which allowed us to design oligo-FISH probes to determine the structure of the original BLA chromosome and any rearranged chromosomes. Updates on the progress will be presented.

Once confirmed, this new hybrid wheat system will provide a pathway to more productive, sustainable, and profitable wheat production globally.

3RD INTERNATIONAL WHEAT CONGRESS

Workshop 14: Physiological insights informing breeding and agronomy

Sunday, 22 September 2024
Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 8, PCEC

Tentative Program

Sunday, 22 September 2024

Time	**Co-Chairs: Dr Dion Nicol, DPIRD, Western Australia, Australia** **Dr Cathrine Ingvordson, Australian Grains Technologies, Australia**	
13:00 – 13:05	Introduction	
13:05 – 13:25	Wheat yield relationships in Western Australia	**Dr Dion Nicol** Department of Primary Industries and Regional Development (DPIRD), Australia
13:25 – 13:45	Yield and resilience of wheat in southern Chile	**Dr Daniel Calderini** Universidad Austral de Chile, Chile
13:45 – 14:05	The relevance of sink strength in determining wheat yield	**Prof Gustavo Slafer** University of Lleida – ICREA, Spain
14:05 – 14:25	N uptake dynamics of high—and low-protein wheat genotypes	**Dr Amanda de Oliveira Silva** Oklahoma State University, USA
14:25 – 14:45	**Tea/Coffee break**	
14:45 – 15:05	Wheat phenotypes for frugal agriculture	**Dr Victor Sadras,** South Australian R&D Institute, Australia

15:05 – 15:25	Accelerated integration of physiology-based wheat traits within a commercial breeding programme – learnings from the project	**Dr Cathrine Ingvordsen** Australian Grain Technologies, Australia
15:25 – 15:40	Physiological approaches to breeding and use of Plant Genetic Resources	**Dr Matthew Reynolds** CIMMYT, Mexico
15:40 – 16:10	How do we make physiology have impact?	**Everyone**
16:10 – 16:15	**Closing remarks by** Co-Chairs	

Workshop 14 September 22, 2024, 14.45-15.05

Phenotypic integration in wheat

Victor Sadras

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From a developmental perspective, connectance is defined as the level of linkage between organs or traits (Biosystems 56, 1-11). Development is not a continuous process but alternates stabilisation during discrete stages (*i.e.*, phenophases) and dismantling of relational networks between organs in the intervening, shorter critical periods (Theory in Biosciences 123, 17).

In this context, the critical period is a transient phase of isolation of the system that enables its evolution towards equilibrium; the transition from dissipative to isolated system is the source of newly emerging dissipative structures, *i.e.*, new phenophases, in which environmental or developmental disturbances are adaptively integrated. We calculated the connectance of 17 traits in a historic collection of 13 wheat cultivars grown in four field environments.

The frequency distribution of connectance, which varied from 0.09 to 4.2, conformed to a power law with slope = -1.67. Several traits, including time to anthesis and seed weight, clustered below 0.3. Traits related to growth during the pre-anthesis period including shoot biomass at anthesis, nitrogen uptake at anthesis, evapotranspiration from sowing to anthesis, heads per m^2 and grains per head, clustered in the range from 0.3 to 0.5.

Shoot biomass at maturity had the largest connectance (2.1) and related traits including grain yield, nitrogen uptake at maturity, biomass per unit evapotranspiration at maturity, all had connectance above 1.

Genotype-dependent median connectance varied 2.4-fold, from 0.28 in Condor, the first semi-dwarf cultivar in the sampled series, to 0.67 in Gladius. Connectance of yield increased at 0.028 per year over the period 1958 to 2007.

15:25 – 15:40	Physiological approaches to breeding and use of Plant Genetic Resources	**Dr Matthew Reynolds** CIMMYT, Mexico

No Abstract Provided

15:40 – 16:10	How do we make physiology have impact?	**Everyone**

No Abstract Provided

15:05 – 15:25	Accelerated integration of physiology-based wheat traits within a commercial breeding programme – learnings from the project	**Dr Cathrine Ingvordsen** Australian Grain Technologies, Australia

No Abstract Provided

14:05 – 14:25	N uptake dynamics of high—and low-protein wheat genotypes	**Dr Amanda de Oliveira Silva** Oklahoma State University, USA

No Abstract Provided

13:45 – 14:05	The relevance of sink strength in determining wheat yield	**Prof Gustavo Slafer** University of Lleida – ICREA, Spain

No Abstract Provided

13:25 – 13:45	Yield and resilience of wheat in southern Chile	**Dr Daniel Calderini** Universidad Austral de Chile, Chile

No Abstract Provided

13:05 – 13:25	Wheat yield relationships in Western Australia	**Dr Dion Nicol** Department of Primary Industries and Regional Development (DPIRD), Australia

No Abstract Provided

iWC 3RD INTERNATIONAL WHEAT CONGRESS

Workshop 15: Balancing Wheat-based Product Quality and Nutritional Quality Requirements

Sunday, 22 September 2024

Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 9, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: A/Prof Vicky Solah, Murdoch University, Australia** **Mr. Sean Masterson, Managing Director, Asia Pacific ARYZTA**	
13:00 – 13:10	Introduction	
13:10 – 13:30	Balancing nutrition and quality	**Dr Haelee Fenton** InterGrain, Australia
13:30 – 13:50	A new collaborative approach for sourdough bread	**David Doepel** Murdoch University, Australia
13:50 – 14:10	How does protein content affect bread wheat dough properties and gluten composition?	**Dr Bin Xiao Fu** Grain Research Laboratory, Canadian Grain Commission, Canada
14:10 – 14:30	From lab to farm: a success story of breeding specialty wheats to ensure nutritional security	**Dr Achla Sharma** Punjab Agricultural University, India
14:30 – 14:50	**Tea/Coffee break**	
14:50 – 15:10	Upcycling spent grains for food applications	**Dr Suwimol Chockchaisawasdee** Murdoch University, Australia

15:10 – 15:30	Australian wheat – quality ingredient for SEA Noodles	**Dr Larisa Cato** Australian Export Grains Innovation Centre (AEGIC), Australia
15:30 – 15:50	Impact and mechanism of sulphur-deficiency on modern wheat end-use and nutritional properties	**Prof Wujun Ma** Murdoch University, Australia
15:50 – 15:55	**Closing remarks by** Co-Chairs	

Workshop 15 September 22, 2024, 13.50-14.10

How does protein content affect bread wheat dough properties and gluten composition?

Bin Xiao Fu*, Carly Isaak, and Kun Wang

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Seven composites of AAC Brandon, the dominant variety in Canada Western Red Spring wheat class, were prepared by blending 2021 harvest samples based on their protein content in bands with 1% increments, spanning <11% to >17% (13.5%mb). Farinograph dough development time and stability increased with the increase of protein from 3.25 to 14.5 min and 8.0 to 20.0 min, respectively.

Dough strength, as measured with extensograph R_{max} increased from 465 BU to 732 BU, and dough extensibility also increased with protein content, resulting in well balanced dough properties for all composites.

Further analysis of the samples with gluten index and GlutoPeak showed little change in gluten strength parameters. Parameters generated with farinograph and extensograph reflect both the density and strength of gluten network, while gluten index and GlutoPeak measure the strength of the hydrated and aggregated polymers largely independent of protein quantity.

Flour proteins were fractionated into monomeric proteins, soluble glutenin, and insoluble glutenin. The proportion of 0.6% SDS insoluble glutenin remained little changed among the seven aggregates at 17.7-19.0%. With the increase of protein, the proportion of monomeric proteins increased gradually from 55.9% to 58.9% %, and soluble glutenin decreased from 26.4% to 22.2%.

Further analysis of 45% propanol insoluble glutenin with RP-UPLC found little change in the proportions of insoluble glutenins across the samples, and confirmed that the total amounts of insoluble glutenin were exclusively a function of wheat protein content.

The variation was also small among the seven aggregates in the proportions of individual HMW glutenin subunits and the ratio of HMW-GS/LMW-GS.

Results generated from the unique sample set used in this study provided commercially applicable information on the relationship between wheat protein content and processing quality, and the biochemical basis of the changes in functionality with protein content for a given variety.

Workshop 15 September 22, 2024, 14.10-14.30

From lab to farm: a success story of breeding speciality wheats to ensure nutritional security

Achla Sharma[1], Satindee Singh[1], Lenika Kashyap[1], G S Mavi[1], Puja Srivastava[1], Satinder Kaur[1], Jaspal Kaur[1], Ritu Bala[1], Parveen Chhuneja[1] and V S Sohu[1]

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In the post green-revolution decades, Indian Punjab's agriculture and its position as the pre-eminent contributor of food grains to the country has been sustained by well-tuned varietal input by the Punjab Agricultural University (PAU). The future viability of the program, however, is challenged by changes not only in the technological and policy arena, but also the consumer preferences and awareness.

The breeding programmes operating initially in a food deficit context could pay scant attention to quality parameters so that even the farmer became hesitant to grow the limited quality wheat germplasm available indigenously, for example *sphaerococcums* which were native to Punjab, were totally phased off due to lesser yields and hence marginal profits. However, the transition has been initiated at PAU by shifting the focus towards enhancing quality & end product specificity which has let to success stories by development of specialty wheat varieties. **PBW 1 Zinc** (2017) and PBW Zinc 2 (2023) has almost 25% more zinc in the grain. **PBW Zinc 2** has been developed through wide hybridization and MAS using *Triticum boeoticum* as source of high zinc content and PBW 703 as a source of high yield, stripe and leaf rust resistance.

PBW RS1, developed by PAU (2023) is a first wheat variety of India combining five novel *SBEII* alleles along with stripe rust resistance and high protein genes having enhanced amylose content which is known to have nutraceutical value owing to the decreased digestibility of starch and lower glycaemic index. It has 3 – 4 times higher resistant starch than the check varieties.

Wheat breeding program at PAU has responded to changing expectations regarding processing traits which has resulted in the development of '**PBW 1 Chapati**' and '**PBW 1 Biscuit**'. PBW 1 *Chapati* cooks soft, whitish and sweet premium *chapati* with desirable palatability and texture, comparable to '*desi wheats'*. PBW 1 biscuit has been developed using winter wheat as softness donor and has medium soft grains with spread factor of 9.8/10, suitable for making biscuit having desirable colour, texture and cracks.

The development of wheat varieties combining two or more novel nutritional components in future is a prized target of the team.

Workshop 9 September 22, 2024,15.10-15.30

Australian wheat - quality ingredient for SEA noodles

Larisa Cato[1], Kishor Sharma[1], Matthew Watkinson[1]

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Wheat based products are major part of dietary requirements around the world. Asian noodles have been important for Asian cuisine for many centuries and continue to play a central role in many dishes in Asia but are also becoming very popular and widely consumed all over the world.

Often, the quality of Asian noodles is described by noodle appearance (noodle colour) and eating quality (noodle texture). The suitability of wheat for noodles is assessed in terms of product quality but also processing properties.

This research identified the key wheat and flour quality preferences and ideal target levels and attributes for major types of noodle products in Southeast Asian markets. Benchmark samples from Vietnam, Philippines, Taiwan and Indonesia were used as quality targets and benchmarked to Australian milling grades.
Yellow alkaline noodles (YAN) were made using small scale noodle making process. Colour of raw and boiled noodles was measured using Minolta Chroma Meter CR310. Noodle colour and appearance of Australian samples was superior to any and benchmark sampels used from various markets. Enzymes alpha-malt and pasta-zyme were used to assess colour and appearance improvement potential.

Their impact on noodle appearance was measured and discussed as noodle brightness (L*), yellowness (b*) and redness (a*). Colour stability and discolouration over a period of time measured and will be discussed in this paper.

Workshop 15 September 22, 2024,13.10-13.30

Balancing nutrition and quality

Haelee Fenton

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Innovation in reformulations of grain foods to provide more nutritious options, while satisfying consumer demand for simpler ingredients and for cleaner labels is a challenge. The opportunity however to address key nutrition and health concerns, including increased dietary fibre, lower cholesterol, and improved glucose control, through the inclusion of additional grains is of interest.

Recently, breeding of cereal varieties for specific nutritional properties, developments and expanding knowledge in processing technologies, changes in legislation, and consumer interest are driving the development of composite grain products.

The inclusion of oat and barley grains in food products has real promise in addressing both nutrition and health concerns, as well as potential for promotion as a sustainable prospect in the long term.

While nutritional profiles of value to consumers can be created, meeting the expectations of quality, and providing appealing sensory experiences are harder to deliver. In this workshop session, the benefits of including oat and barley in baked products will be discussed.

Workshop 15 September 22, 2024, 15.30-15.50

Impact and mechanism of sulphur-deficiency on modern wheat end-use and nutritional properties

Zitong Yu[1], Maoyun She[1], Ting Zheng[1], Dean Diepeveen[3], Shahidul Islam[1], Yun Zhao[1,2], Yingquan Zhang[1], Guixiang Tang[1], Yujuan Zhang[1], Jingjuan Zhang[1], Christopher L. Blanchard[4], **Wujun Ma**[1,2]

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Two challenges that the global wheat industry is facing are a lowering nitrogen-use efficiency (NUE) and an increase in the reporting of wheat-protein related health issues. Sulphur deficiencies in soil have also been reported as a global issue.

The current study used largescale field and glasshouse experiments to investigate the sulphur fertilization impacts on sulphur deficient soil. Here we show that sulphur addition increased NUE by more than 20% through regulating glutamine synthetase. Alleviating the soil sulphur deficiency highly significantly reduced the amount of gliadin proteins up to 82%, indicating that soil sulphur levels is related to the biosynthesis of proteins involved in wheat-induced human pathologies.

The sulphur-dependent wheat gluten biosynthesis network was studied using transcriptome analysis and amino acid metabolomic pathway studies. Results revealed that S-adenosylmethionine plays the key role in modulating the biosynthesis of gluten components.

The study concluded that sulphur deficiency in modern farming systems is not only having a profound negative impact on wheat productivity but is also impacting on wheat consumers' health.

Workshop 15 September 22, 2024, 13.30-13.50

A new collaborative approach for sourdough bread

David Doepel, Research Manager, Murdoch University

The opportunities for pre-breeding programs to access previously unavailable genetic data will be of critical importance in the face of a changing climate. The genetic bonanza can also be harnessed to assist breeders to work closely with artisan bakers to create desirable flavour profiles from grains that are more suited to long-fermentation breads, particularly sourdoughs.

This presentation maps a methodology for collaboration and development that prioritises flavour and possible nutrition components that are aligned and the specific physicochemical attributes of flours best suited for sourdough production.

14:50 – 15:10	Upcycling spent grains for food applications	**Dr Suwimol Chockchaisawasdee** Murdoch University, Australia

No Abstract Provided

iWC 3RD INTERNATIONAL WHEAT CONGRESS

Workshop 16: Biological Nitrification Inhibition (BNI)

Sunday, 22 September 2024
Perth Conference & Exhibition Centre (PCEC), Perth, Western Australia

Meeting Room 10, PCEC

Tentative Program		
Sunday, 22 September 2024		
Time	**Co-Chairs: Prof Wolfram Weckwerth, University of Vienna, Austria** **Dr Susanne Dreisigacker, CIMMYT, Mexico** **Dr Carolina Saint Pierre, CIMMYT, Mexico** **Moderators: Viktor Kommerell, BNI-Mission Director, CIMMYT** **Dr Tony Fischer, Honorary Visiting Scientist, Australian National University**	
13:00 – 13:10	Introduction	
13:10 – 13:30	BNI-Wheats – A new category of wheats that are highly nitrogen-efficient, requires less nitrogen-inputs, highly productive and leak less nitrogen	**Dr Guntur Subbarao** Japan International Research Center for Agricultural Sciences, Japan
13:30 – 13:50	CropSustaiN: The ‘Low-N footprint’ WHEAT MISSION	**Dr Carolina Saint-Pierre** CIMMYT, Mexico
13:50 – 14:10	An overview of BNI wheat research in India	**Dr Pradeep Kumar Bhati** CIMMYT, Mexico
14:10 – 14:30	Exploring the genetic frontiers of BNI	**Dr Julio Huerta** CIMMYT, Mexico
14:30 – 14:50	**Tea/Coffee break**	

14:50 – 15:10	Exploring BNI in Winter Wheat	**Dr Guillermo Gerard** CIMMYT, Mexico
15:10 – 15:30	Natural variation of BNI and root exudate metabolomes in wheat germplasm collections	**Dr Wolfram Weckwerth** University of Vienna, Vienna
15:30 – 15:50	Modelling plausible impacts of biological nitrification inhibition in Australian farming systems – outline of current research	**Dr Kirsten Verburg** Commonwealth Scientific and Industrial Research Organisation (CSIRO) Agriculture and Food, Australia
15:50 – 15:55	**Closing remarks by** Co-Chairs	

Workshop 16 September 22, 2024, 13.30-13.50

CropSustaiN: The 'Low-N footprint' WHEAT MISSION

Carolina Saint Pierre

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The four-year CropSustaiN mission aims to establish the potential of the biological nitrification inhibition (BNI) trait in spring and winter wheat across the Global South and North. This initiative seeks to reduce agriculture's nitrogen footprint through seed-based and agronomic solutions, complementary to other mitigation measures.
The Novo Nordisk Foundation asked CIMMYT to lead this multidisciplinary initiative, in collaboration with nearly 50 partners. The vision is for farmers to reduce nitrogen fertilizer.

Deploying BNI-wheat could reduce GHG emissions by 0.19 gigatonnes CO2-equivalent emissions per year, and up to 6% of total global N2O emissions annually, as well as lowering nitrate pollution - whilst maintaining crop productivity and soil health. This is critical to satisfy growing demand and meet food and nutrition security SDG targets.

CropSustaiN will demonstrate the economic, environmental, and societal value of the BNI trait at farm, value chain, and policy-making levels. The initiative is structured around three integrated research areas.

First, it develops and validates standardized protocols for measuring and documenting BNI-wheat cropping management, soil sampling, and nitrogen cycling parameters to produce accurate, reliable, and comparable data across the diverse global partners' sites and over time. This will ensure accessibility and interoperability of the extensive dataset adhering to FAIR principles. Second, it focuses on breeding, agronomy, and field trials to incorporate the BNI trait into elite spring and winter wheat lines.

Third, it conducts discovery research in genomics, microbial ecology, and agricultural innovation systems to enhance understanding of BNI processes and support technology adoption. Academic institutions, industry, farmers, and policymakers are all integral to the innovation systems that will enable BNI-wheat scaling across geographies.

The CropSustaiN initiative aims to engage stakeholders along this impact pathway.

Workshop 16 September 22, 2024,15.10-15.30

Natural variation of biological nitrification inhibition and root exudate metabolomes in wheat germplasm collections

Wolfram WECKWERTH[1,2]

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All plant roots secrete various metabolites into the root-soil system making up to 40% of their photosynthetic productivity [1]. These enormously complex root exudates have various functions for nutrient acquisition, and most obviously plant – soil microbiome interactions. Plants control the soil microbiome with these root exudates adjusting the species composition of the rhizosphere microbiota involved in nutrient transformations, decomposition and mineralization of organic substances which eventually determines soil quality.

One remarkable process is the secretion of metabolites from the root system which are able to inhibit nitrification processes of bacteria and archaea called biological nitrification inhibition [1]. In effect BNI can lead to less nitrate loss from the soil, especially in conjunction with nitrogen fertilization, and eventually improve nitrogen use efficiency (NUE) of the plant [1]. We are investigating these processes in various cereals such as millets and wheat. Intriguingly, BNI activity is also depending on drought-stress conditions, offering a potential route to improve NUE and drought resistance [2].

Recently, we have introduced the concept of Panomics applied to large germplasm collections [3]. Panomics applies integrative analysis of metabolomics, proteomics, RNAseq, metabolic modelling, AI, machine learning and genomic prediction to natural genetic variation of large crop germplasm collections [3]. The aim is to provide intimate molecular information for breeding programs addressing resilient and sustainable agricultural production systems [1, 2].

Using this platform we investigated various wheat genotypes with respect to their root exudate metabolomes and corresponding effects on the soil microbiota, as well as nitrifiers and BNI activity.

[1] Ghatak, A., et al. (2023) PANOMICS at the interface of root-soil microbiome and BNI. Trends Plant Sci 28, 106-122
[2] Ghatak, A. et al. (2022) Root exudation of contrasting drought-stressed pearl millet genotypes conveys varying biological nitrification inhibition (BNI) activity. Biol Fertil Soils 58, 291-306,
[3] Weckwerth W et al (2020) PANOMICS meets germplasm. Plant Biotechnol J 18: 1507-1525

Workshop 16 September 22, 2024, 14.10-14.30

Exploring the genetic frontiers of BNI in CROPSUSTAIN

Julio Huerta[1,2], Susanne Dreisigacker[1], Guillermo Gerard[1], Velu Govindan[1], Leonardo Crespo-Herrera[1], Carolina Saint-Pierre[1]

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Translocations from *Leymus racemosus* have been introgressed into sets of CIMMYT differential genetic backgrounds since the first phases of BNI research activities. Several T3BL.3NsbS translocation lines have been initially tested and are being distributed to partners globally for broader evaluation.

The translocation is now transferred into recent high-performance CIMMYT spring wheat, aiming to provide BNI as a new trait in conjunction with climate resilience traits, broad-spectrum disease resistance, and ensuring nutritional and end-use quality. Molecular markers specific for T3BL.3NsbS have been developed through genotyping-by-sequencing deployed in marker-assisted backcrossing. To achieve a greater range of BNI translocation sources, additional chromosome regions are explored that contribute to BNI activity.

Translocations in different chromosomes other than chromosome 3B, translocations from *Leymus mollis*, and translocations shorter than the current T3BL.3NsbS are investigated. BNI varieties with a shorter translocation are expected to have better agronomic performance than those with a larger chromosome translocation, while BNI activity will need to be confirmed.

Workshop 16 September 22, 2024, 14.50-15.10

Exploring BNI in Winter Wheat

Guillermo Sebastian Gerard[1]

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Biological Nitrification Inhibition (BNI) describes the ability of certain plants to specifically inhibit the growth and activity of nitrifying microorganisms in rhizosphere soil. This trait is a promising plant breeding target, allowing for increased N fertilizer use efficiency while mitigating the release of the potent greenhouse gas N_2O in the farmer's field.

The International Maize and Wheat Improvement Center has been able to transfer the BNI capacity from *Leymus racemosus* into spring wheat. Preliminary studies using a set of different elite spring wheat lines carrying the translocation have shown no negative effects on agronomically important traits while showing promise in reducing agriculture's nitrogen footprint. One goal within the CropSustaiN 4-year mission is to expand and explore the potential use of the BNI trait in winter wheat.

The transfer of the BNI translocation from spring to different winter wheat backgrounds is being carried out in collaboration with different public and private partners globally. Field studies using winter wheat BNI isogenic lines are expected to be carried out in the short-medium term to assess potential effects on agronomically important traits, BNI activity, and Nitrogen losses aiming to determine the technology's potential in winter wheat backgrounds/environments.

Workshop 16 September 22, 2024, 13.50-14.10

Investigation of BNI expression in wheat field systems

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The introduction of the Lr#N short arm to elite spring wheat lines resulted in the release of BNI root exudates confirmed with a micro-bioassay. The inhibition potential on nitrifying microbes under laboratory conditions is promising, but it remains unclear if the BNI trait introduced to various wheat lines can significantly reduce nitrification under field conditions.

Measuring the expression of BNI in the field is challenging and there is no methodology that allows BNI quantification in soil-root systems. This study aims to test the yield potential of BNI translocation spring wheat lines and simultaneously confirm BNI expression under field conditions. Six pairs (parental/control vs. translocation line) were planted under four N fertilizer application rates (0, 75, 150 and 225 kg N ha^{-1}) in three locations in Mexico. Stability for yield quantity and quality was observed after the first crop seasons.

Grain protein was higher for some BNI wheat lines. Expected reduced nitrate formation in the root zone and lower potential nitrification rates in post-harvest sampled soil could not be confirmed after one planting season. It is assumed that a significant expression of the BNI effect requires more than one planting season since both, accumulation of actively released BNIs and passively released BNIs during root turnover after harvest are responsible for significant reduction of nitrate formation.

Therefore, CIMMYT will test within the BNI mission ‘CropSustaiN’ a set of BNI translocation lines in Latin America, Africa and South Asia under different N fertilizer and soil pH conditions in the next years to verify the global potential of the BNI trait.

Workshop 16 September 22, 2024, 15.30-15.50

Modelling plausible impacts of biological nitrification inhibition in australian farming systems, outline of current research

Kirsten Verburg[1], Alison Bentley[2], Ismail Garba[3], Wolfram Buss[2], Enli Wang[1], Cathryn O'Sullivan[3], Gupta Vadakattu[4]

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Retaining nitrogen (N) in soils in the form of ammonia by inhibiting nitrification processes has been long hypothesised as a solution to N loss from denitrification and leaching of nitrate below the root zone. However, the findings of field experiments testing synthetic nitrification inhibitors have shown variable results.

More recently, nitrification reactions have been found to be inhibited by root exudates from some crops (e.g., bracharia, wheat, rice, sorghum, maize, canola). Research into the role of these biological nitrification inhibitors (BNIs) in reducing N loss and improving N use efficiency is currently actively being progressed. Agricultural systems modelling has demonstrated that the variable benefits of synthetic nitrification inhibitors and of controlled-release fertilisers relate to system interactions affecting the longevity of the protection of the fertiliser N, the timing of N loss events and the crop's ability to respond to retained fertiliser N.

In this presentation we outline new research that is modelling the plausible impacts from BNI for wheat, canola and sorghum crops in Australian farming systems. Model conceptualisations will be based on a review of available experimental evidence on BNI. Systems analyses using the APSIM model will then be used to explore impacts on both productivity and emissions under different scenarios.

This work complements the ongoing international experimental research by outlining the systems aspects that may impact on benefits that can be achieved with BNI in the field. As such it can be seen as pre-experimentation modelling that informs further research and can be improved over time as more knowledge and field trial data becomes available.

Workshop 16 September 22, 2024, 13.10-13.30

BNI-Wheats: A new category of elite-wheats that are highly nitrogen-efficient and highly productive

Guntur V Subbarao, Kishii Masahiro and Tadashi Yoshihashi

Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba, Ibaraki, Japan.

Wheat, a major staple food crop, grown over 220 million ha globally, consumes a third of nitrogen fertilizer produced worldwide. Nearly 70% of this nitrogen fertilizer is lost from wheat fields either through nitrate-leaching or gaseous emissions of nitrous oxides, causing ecological and environmental concerns.

Nitrogen fertilizers are mostly in ammonium form or converted into ammonium (from urea) when applied to wheat fields, bound to the soils, and not harmful to the environment. However, nitrifying soil bacteria has become aggressive in modern production systems and using most of fertilizer-N as energy source and turning it into harmful byproducts, NO_3^-, NO_2, and NO, which then leaks out of farmlands, causing inefficiency, polluting waterbodies and harming environment.

Biological nitrification inhibition (BNI) is a natural plant function where nitrification inhibitors are released from roots to suppress nitrifier activity. Cultivated wheat does not have sufficient BNI-capacity in root systems. Wild-wheat (*Leymus racemosus*) has BNI-ability several-fold higher than in cultivated wheats; BNI-trait is in the short-arm of ‘n’ chromosome (Lr#n-SA), which is successfully introduced into and expressed in several elite-wheat varieties. Elite-wheat carrying Lr#n-SA are ‘BNI-wheats’, considered as a special category. BNI-wheats produce 3-5-fold higher BNI-activity from root systems, suppress soil-nitrate formation (30-50%), emission of nitrous oxides (about 30%) and require 30-50% less nitrogen fertilizers to produce similar grain yields.

The yield potential of BNI-wheats is like or better (10-20% of non-BNI controls) than present day wheat varieties. By transferring Lr#n-SA carrying BNI-trait, present-day wheat varieties can be converted into BNI-wheat varieties.

As BNI-wheat technology is seed-based, scaling up is easier; as this involves no-additional cost, farmer adoption of BNI-wheat varieties will be faster.