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Poster Presentations
Abstract Book

Editors:
Rajeev K Varshney and Anu Chitikineni

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3RD INTERNATIONAL
WHEAT CONGRESS

22 – 27 September 2024 • Perth, Western Australia

Hosted By
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Preface

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

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

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

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

Thanking you.

Editors
Rajeev K Varshney, &
Anu Chitikineni

Table of Authors

Author	Poster No.	Author	Poster No.
Abbai, R	217	Al-Sham'aa, K	222
Abbas, A	411, 267, 359	Al-sham'aa, K	603
Abbasi, M	287	Alsheikh, M	53, 242
Abbasnejad, E	404	Amamou, A	525
Aboukhaddour, R	464	Amaral, A	374
Abrouk, M	219	Ambrózy, Z	176
Acarapi, J	166	Ampatzidis, Y	273
Acharya, J	273	Anderson, K	48
Achilli, A	548	Andersson, B	227
Acosta, A	374	Angirekula, U	119
Adewale, S	273	Aouini, L	219
Aggarwal, H	518	Arief, V	493
Ahunchain , R	275	Arif, M	498
Ai, G	607	Arora, S	361
Akhunov, E	203	Arranz, E	236
AL ABDALLAT, A	616	Arriagada, O	235
Alabdullah, A	151	Asefa, A	413
Alamrani, M	257, 53, 227	ASHFAQ, W	553
Alamrani, M	342	Ashling, S	417, 94
Alam Khan, M	638	Asseng, S	633
Alarcon Reverte, R	521	Asuke, S	115

Author	**Poster No.**	**Author**	**Poster No.**
Alarcon-Reverte, R	131, 130	Atienza, S	60, 59, 57
Alasti, O	553	Atienza, S	56
Albiston, R	401	Atkinson, J	417
Albrecht, J	529	Awais, M	638
Alfaro, C	204, 182, 185, 237	Awasi, M	471
Alfaro, C	235	Ayele, B	617
Ali, C	184	Ayliffe, M	594
Ali, S	498	AZMAN HALIMI, R	553

B

Author	Poster No.	Author	Poster No.
B. Ferreira, T	633	Bhatt, D	381
Babar, A	273	Bhattarai, R	507
Bai, G	420, 372, 203	Bhatti, P	578
Bai, G	564	Bhavani, S	454
Baidani, A	525	Bhavani, S	475
Baig, I	184	Bhosale, R	651
Baillie, J	259	Bhosale, R	404
Baizerman, M	330	Bi, H	272
Baker, J	203	Biddulph, B	345, 373
Baker, S	203	Biligetu, B	581
Bakkeren, G	287	Bird, N	187
Bakonyi, J	156	Bizerman, M	329

Ed-Daoudy, L	603	Ellwood, S	346, 61
Edin, E	227	Elsheikh, O	196
Edwards, C	651	Ennian, Y	242
Edwards, K	125	Ens, J	83
Edwards, S	417	Erenstein, O	633
Edwards, T	20	Esposito, S	367, 462
Author	**Poster No.**	**Author**	**Poster No.**
El Hanafi, S	254	Esposito, S	504
Elbashier, E	196	Éva, C	176
Elbashir, A	196	Evers, B	266

F

Author	Poster No.	Author	Poster No.
Faci, I	0	Finkbeiner, A	146
Fadida-Myers, A	329	Fisher, B	118
Fahima, T	218	Fisk, I	404
Fan, K	200	Foiada, F	189
Fanelli Carvalho, H	451	Földi, M	230
Fang, Q	482	Forestan, C	451
Fang, Q	637	Forestan, C	504
Fang, S	655	Formby, S	287
Fania, F	462	Forrest, K	499
Farhad, M	578	Förter, L	146
Farhad, M	215	Fossati, D	189

Gerardi, C	367
Germán, S	260
Germinara, G	367
Geyer, M	124
Ghannoum, O	359
Ghannoum, O	411
Gho, C	493
Gibberd, M	610, 317
Gilbert, D	361
Gill, H	643
Gill, R	531
Gill, U	643
Giongo, V	527, 374
Giovinazzo, G	367
Giraldo, P	348
Giulini, A	451
Giunta, R	178
Glassop, D	598
Gnocato, F	626, 625
Gogniashvili, M	190
Golan, G	217
Gulyas, Z	252
Gunjača, J	249
Guo, H	390
Guo, L	23, 35
Guo, W	641
Guo, W	652, 580
Gupta, A	332, 119
Gupta, O	344
Gupta, P	464
Gupta, P	387
Gupta, R	643
Gupta, V	344
GUPTA, D	553
Gutiérrez, L	476
Gutiérrez, L	342, 260
Guwela, V	408
Guzman, C	348
Guzmán López, M	121
Guzmán López, M	236
Gyorgy, M	334

H

Author	Poster No.	Author	Poster No.

Qiao, Y	172	Quiroz-Chavez, J	577
Qiaofeng, Z	642	Qureshi, N	454

R

Author	Poster No.	Author	Poster No.
Rader, R	88	Ren, Y	575, 288, 65
Rahimi, M	432	Renis, M	367
Rahman, A	557	Requena-Ramírez, M	57
Rahman, M	486	Requena-Ramírez, M	60
Rajković, B	253, 249	Requena-Ramírez, M	59
Ral, J	582, 296	Requena-Ramírez, M	56
Ramakers, J	493	Reveco, S	235
Ramasundaram, P	344	Reynolds, M	167
Rameez Khan, M	498	Reynolds, M	475, 464
Ramírez González, R	577	Rheault, M	464
Rana, A	274	Riche, A	404
Rana, V	332, 274	Riella, V	260
Rana Shahi, P	88	Rivera, J	548
Rasheed, A	84	Robertson, R	633
Rathjen, T	373	Rodrigo, J	548
Rathore, M	555	Rodríguez-Suárez, C	60, 59, 57, 56


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WHEAT CONGRESS
22 – 27 September 2024 • Perth, Western Australia

Shewry, P	651	Sun, X	288
Shi, C	288	Sun, X	655
Shi, X	576	Sun, Y	615
Shuangjun, G	642	Sun, Z	575
Sibisi, P	566	Sura, S	300
Siddique, A	634	Sutton, R	203
Siddique, K	507	Sydenham, S	407
		Szabados, F	252

T

Author	Poster No.	Author	Poster No.
Tabuchi, M	133	Thiyagarajan, K	565
Tadesse, W	603	Thiyagarajan, K	294, 299
Tahir, I	196	Thomas, S	521
Tahir, I	225, 488	Thompson, I	543
Takahashi, D	117	Tian, H	575
Takumi, S	170, 133, 120	Tian, R	172
Tamagno, S	251	Tian, R	171
Author	**Poster No.**	**Author**	**Poster No.**
Tamm, I	449	Tian, W	84
Tan, K	61, 51, 136	Tibbits, J	499
Tan, K	137	Tidiane sall, A	222
Tang, J	288	Tikhonova, M	250
Tang, L	212	Till, B	512

W

Author	Poster No.	Author	Poster No.
Walker, J	417	Wang, Z	652, 631
Walkowiak, S	393, 287	Warnasooriya, A	144
Walter, J	316	Warren, F	651
Walter, N	94	Watanabe, S	89
Wang, A	381	Weckwerth, W	624
Wang, A	594	Wei, G	642
Wang, D	272, 288, 268, 337	Wei, Y	575
Wang, D	326	Wei, Y	582
Wang, D	167	Wells, D	417
Wang, D	220	Wenling, Z	642
Wang, E	373, 206	Whalley, R	125
Wang, F	371	WheatNet Consortium	234
Wang, H	326	Whish, J	373
Wang, J	272	Whitworth, M	404
Wang, K	111	Wiebe, K	83
Wang, P	35	Wijeweera, S	604
Wang, W	305, 300	Wilkins, R	331
Wang, W	631	Wilson, L	408
Wang, W	652	Wilson, M	94
Wang, X	591, 615	Windju, S	53
Wang, X	577, 576	Winn, Z	195

Author	Poster No.	Author	Poster No.
Yadav, M	119	Yang, Z	652
Yadav, S	329	Yang, Z	271
Yamamori, K	270	Yao, Y	580
Yan, G	507	Yates, R	20
Yan, J	63	Yates, S	189
Yan, M	200	Yin, G	288
Yan, Q	564, 606	Yin, H	655
Yan, W	607	Ying, L	642
Yang, C	417, 408	Yoden, Y	120
Yang, C	94	Yoon, M	505
Yang, D	23, 35	Yoon, M	473
Yang, Q	582	Yoshida, K	170, 209, 133, 120
Yang, X	575	Yoshikawa, T	270
Yang, X	615	Yoshioka, M	115
Yang, X	45	Yu, G	216
Yang, X	272	Yu, H	632
Yang, Y	607	Yu, K	467
Yang, Y	288, 337	Yu, R	296

Z

Author	Poster No.	Author	Poster No.
Zaim, M	616	Zhao, J	326
Zambounis, A	346	Zhao, L	68

Paper #257

Pre-breeding for climate resilience and pathogen resistance in nordic spring wheat

Marwan Alamrani[1], Laurène Mailhan[1], Ronja Wonneberger[1], Therése Bengtsson[1], and the CResWheat Consortium[1-9]

E-mail: marwan.alamrani@slu.se

Spring wheat (*Triticum aestivum L*.) is one of the most important crops in northern Europe, particularly in regions where winter wheat cultivation is challenging. Climate change is expected to exacerbate the occurrence of pests and diseases, including the emergence of new pathogen races. To address this challenge, the CResWheat project has been established as a collaborative pre-breeding initiative involving Nordic spring wheat breeders and researchers.

The primary goal is to enhance the climate resilience of Nordic spring wheat by improving grain yield and its stability across different environments (sites and years), and self-sufficiency. CResWheat project evaluated more than 20 characteristics of 184 breeding lines and cultivars in 24 field trials at seven locations across four Nordic countries from 2021 to 2023. Most of the material was of European origin except for a few cultivars from the USDA and CIMMYT.

The focus was on agronomic traits, host plant resistance to various pathogens, and pre-harvest sprouting resistance. Yellow rust, caused by (*Puccinia striiformis f.sp. tritici)*, a disease that has a severe effect on the yield of spring wheat, is one of the traits evaluated.. During 2022 and 2023 the screening was done at four different sites: Svalöv in Sweden, Holeby and Horsens in Denmark, and Vollebekk in Norway. Results from a genome wide association study (GWAS) across locations revealed quantitative trait loci (QTL) on wheat chromosomes 4A, 5B and 6A for resistance to yellow rust. Excluding the breeding lines from CIMMYT, two additional QTL were found on chromosomes 3B and 7A. Additionally, four and eight QTL were found in separate GWAS for Vollebekk and Holeby, respectively. The QTL on chromosome 6A has previously been identified incultivars and breeding lines with Nordic origin.

Based on the results of their phenotypic performance and further genetic analyses, genotypes were selected as parents for the development of segregating populations. These populations are currently under development and will be evaluated in multi-environment trials in the Nordic Region and Baltic States in the next years.

The project’s main outcomes will be DNA markers associated with important characteristics, populations and lines with combinations of beneficial characteristics as well as exchange of knowledge and material between the project partners, thus greatly benefiting breeding for climate-resilient spring wheat in northern Europe.

Affiliations

1. Marwan Alamrani, Ronja Wonneberger, Laurène Mailhan, Therése Bengtsson, Rodomiro Ortiz, Swedish University of Agricultural Sciences, Department of Plant Breeding, Box 190, 234 22 Lomma, SWEDEN
2. Mogens Støvring Hovmøller & Mehran Patpour, Aarhus University, Department of Agroecology, Forsøgsvej 1, 4200 Slagelse, DENMARK
3. Merja Veteläinen, Outi Manninen & Tarja Niemelä, Boreal Plant Breeding Ltd, Myllytie 10, 31600 Jokioinen, FINLAND
4. Muath Alsheikh, Jon Arne Dieseth & Susanne Windju, Graminor AS, Hommelstadvegen 60, 2322 Ridabu, NORWAY
5. Pernilla Vallenback & Firuz Odilbekov, Lantmännen ek. för., Udda Lundqvists väg 11, 268 31 Svalöv, SWEDEN
6. Marja Jalli & Annika Johansson, Natural Resources Institute Finland (Luke), Tietotie 4, 31600 Jokioinen, FINLAND
7. Morten Lillemo & Min Lin, Norwegian University of Life Sciences, Department of Plant Sciences, Kirkeveien 12, 1433 Ås, NORWAY
8. Ahmed Jahoor, Jeppe Reitan Andersen, Pernille Bjarup, Janni Hedensvang Jørgensen & Jihad Orabi, Nordic Seed A/S, Kornmarken 1, 8464 Galten, DENMARK
9. Rasmus Lund Hjortshøj, Linda Kærgaard Nielsen & Charlotte Damsgård, Sejet Plant Breeding, Nørremarksvej 67, 8700 Horsens, DENMARK
10. Jan Svensson, NordGen- The Nordic Genetic Resource Center, Box 162, 234 23 Lomma, SWEDEN
11. Liina Jakobson, Reine Koppel & Anne Ingver, METK- The Centre of Estonian Rural Research and Knowledge, J. Aamisepa 1, Jõgeva, 48309 Jõgeva maakond, ESTONIA

#60

Carotenoid esterification as a strategy to increase carotenoid retention during storage of durum wheat grains

María Dolores Requena Ramírez[1], Cristina Rodríguez Suárez[1], Dámaso Hornero Méndez[2], Sergio G. Atienza[1]

[1] Instituto de Agricultura Sostenible, CSIC, Alameda del Obispo, s/n, E-14004 Córdoba, Spain.
[2] Department of Food Phytochemistry, Instituto de la Grasa, CSIC, Campus Universidad Pablo de Olavide, Edificio 46, Ctra de Utrera, Km 1, E-41013 Sevilla, Spain.

Carotenoid pigments are considered essential nutrients for humans and animals because they cannot synthesize them and must therefore be incorporated from the food. They are responsible for the yellow pigment content (YPC), an important quality trait of durum wheat for making pasta and couscous. However, the storage and industrial processing of wheat grain can reduce the carotenoid content and consequently the YPC. Carotenoid esterification is a general mechanism for carotenoid accumulation, sequestration, and storage in plants, and can be a good strategy to increase carotenoid retention in the durum wheat food chain.

In this work, we investigated the effect of carotenoid esterification with fatty acids on pigment retention during grain storage in durum wheat. For this purpose, we used two consecutive storage experiments: 1) In a preliminary work, two landraces with esterification ability (BGE047520 and BGE047535) conserved at the National Plant Genetic Resources Centre (INIA-CSIC) and two durum wheat varieties (‘Amílcar’ and ‘Olivadur’) without lutein esters were compared for the carotenoid content and profile; 2) Second, F_2 segregating individuals derived from the BGE047535 × ‘Athoris’ cross were analyzed contrasting for esterifying ability to validate our results.

The results show that carotenoid esterification leads to a higher carotenoid retention during storage in durum wheat. Thus, the use of the carotenoid esterification would be useful as an additional strategy to ongoing efforts to improve carotenoid retention in the durum wheat food chain as a step forward in breeding to improve the commercial and nutritional quality during grain storage.

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#59

Impact of post-anthesis short heat waves on quality traits in durum wheat

Requena-Ramírez MD[1], Rodríguez-Suárez C[1], Hornero-Méndez D[2]; Atienza SG[1]

[1] Institute for Sustainable Agriculture, CSIC, Avda, Menéndez Pidal s/n, E-14004, Córdoba, Spain
[2] Department of Food Phytochemistry, Instituto de la Grasa, CSIC. Campus Universidad Pablo de Olavide, Edificio 46. Ctra. de Utrera, Km 1, E-41013, Sevilla, Spain

Global warming is a serious concern for agriculture. The Intergovernmental Panel on Climate Change has projected a global warming trend of 0.3-1.7ºC by 2100. As temperatures increase, heat waves (a phenomenon when temperatures are exceptionally high for a continuous period of several days) will become more frequent, longer and severe.

Pre-anthesis heat stress affects yield mainly by increasing floret mortality, either by direct or indirect effects, and thus reducing the number of grains. Post-harvest heat stress also affects yield by impairing grain development, resulting in lower grain weight, although it may also cause kernel abortion. In addition to this, heat stress during grain development also affects the deposition pattern of phytochemical compounds, thus impacting on grain quality.

The yellowness of pasta and couscous is the first attribute that consumers evaluate when determining the quality of a cereal-derived food product, which determines its acceptability and consumption. Carotenoids are responsible for the yellow colour of durum wheat grains, making it an important quality target for durum wheat breeding worldwide.

In this work, we investigated the impact of Short Heat Waves (SHW) during grain development on grain carotenoid content and profile in durum wheat. Four durum wheat varieties, 'Don Ricardo', 'Don Ortega', 'Athoris' and 'Amilcar' were used. Together, these varieties represented almost 50% of the market share in Spain during the 2022-2023 season.

For the experimental trials, heat stress conditions were imposed by installing structures (1.5 m height, 1.5 m width, 1.5 m long) over the field plots. These structures were covered with 125 µm transparent polyethylene film, but leaving the bottom 30 cm of each side open to facilitate gas exchange.

SHW impacted total grain carotenoid content and thousand kernel weight with reductions around 25% and 8% respectively, on the contrary no changes in grain protein were observed. Thus, heat stress is not only a concern regarding yield but also grain quality in Mediterranean environments.

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#637

Controlling sodium accumulation within chloroplasts underpins leaf tissue tolerance to salinity in wheat

Nivethitha Baluchamy[1], Nishanth Baluvanahalli Manjunath[1], Qui Fang[1], Niharika Sharma[2], Yogendra Kalenahalli[3], Michelle Mak[1], Jayakumar Bose[1,4]*

[1]School of Science, Western Sydney University, Penrith, NSW 2751, Australia
[2]NSW Department of Primary Industries, Orange, NSW 2800, Australia
[3]International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India
[4]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia

Contact email- 22114944@student.westernsydney.edu.au, J.Bose@westernsydney.edu.au

Wheat is the major cereal crop providing 20% of global dietary energy and protein intake. Rapidly increasing human population demands a doubling of wheat production by 2050. However, arable lands are becoming increasingly saline and posing a major threat to wheat production by causing up to 60 per cent yield reduction. Hence, improving wheat salt tolerance is critical to ensure global food security.

Salt exclusion from the photosynthetic tissue has been considered a common salt-tolerant mechanism in wheat, however, some genotypes showed higher salt accumulation while maintaining comparable photosynthetic performance. This observation led us to test how salt loads are managed within the mesophyll cells, in particular vacuole and chloroplasts of contrasting wheat genotypes. We treated nine genotypes of *Triticum aestivum* with AABBDD genome, and its relatives *T. durum* with AABB, *T. monococum* with AA, *Aegilos tauschii* with DD genomes to 150mM NaCl stress and observed their leaf tissue tolerance.

We measured chlorophyll content, photosynthetic rate, stomatal conductance and chlorophyll fluorescence parameters such as *Fv/Fm* ratio, *PhiNO* and PhiPSII up to three weeks of salt treatment from the fourth leaf. Sodium and potassium accumulation in leaves after three weeks of salt treatment was quantified using ICP-OES. Vacuolar and chloroplast Na accumulation within leaf mesophyll cells were quantified using the Na-selective fluorescent dye CoroNa green.

We also measured the gene expression of key transporters implicated in vacuolar sequestration (NHX), and chloroplast ion homeostasis (MSL2/3, BASS2, KEA1) to understand the molecular mechanism involved in leaf tissue tolerance. Our results suggested that controlling Na accumulation within the mesophyll chloroplasts through regulated expression of transport proteins is a key determinant to maintaining photosynthetic function during salt stress.

#156

UAV based high-throughput phenotyping system in the Hungarian cereal breeding at Martonvásár

Judit Bányai[1], György Balassa[2], József Bakonyi[3], Mónika Cséplő[1], Klára Mészáros[1], Ottó Veisz[1], Péter Mikó[1]

[1] Agricultural Institute, HUN-REN Centre for Agricultural Research, Martonvásár 2462, Hungary
[2] AGRON Analytics Ltd., Budaörs 2040, Hungary
[3] Plant Protection Institute, HUN-REN Centre for Agricultural Research, Budapest 1022, Hungary

E-mail: banyai.judit@atk.hun-ren.hu

Over the last two decades various high-throughput phenotyping techniques have been developed, which can be used in wheat breeding, genetic analysis and model compilation for the study of disease resistance, drought and heat tolerance. The long-range spectroscopic measurements are a huge help in examining the field areas observed by breeders in minutes, enabling the recording of coordinate-location data in seconds.

Because drones, thanks to their flexibility, under the right conditions, are able to fly and hover above the given area, they are suitable for carrying out plot data recordings. With this high-throughput phenotyping system, changes occurring in experiments involving up to thousands of plots can be easily monitored, and by analyzing the huge amount of data that can be collected in one growing season, species-specific plant models can also be created from germination to ripening.

By using drones, the breeder's selection process can be simplified, accelerated and made more objective, and the use of multispectral imaging sensor systems allows access to information that is not visible to the naked eye. So multi or hyperspectral imaging of a plant is more than 'photography'. The goal is none other than the quantitative measurement of the examined phenotypic properties through the interactions between light (reflected, absorbed, transmitted photons) and the plant.
Drone flights have been carried out at the Agricultural Institute, Cereal Breeding Department, Martonvásár, Hungary, since 2019. In addition, in autumn 2021 and 2022, an expanded reference variety trial was established, consisting of 11 winter wheat, barley, durum wheat and triticale genotypes, which are susceptible and/or tolerant to disease, drought and heat stress.

The trial was artificially inoculated with leaf rust and additionally with *Pyrenophora teres* f. *maculata* in case of barley. Four out of eight randomly arranged replications were treated with fungicide. In each growing season the drone flew from spring onwards weekly over the plots at a height of 100 meters.

Altogether, 17 phenotypic parameters were recorded, 47 spectral data were measured by the drone on 12 occasions, and all plots were harvested in the summer. The orthophotos show whether there is soil patches in the experimental area, how the field is covered with vegetation, the development of the plant population, whether there is weeding or wildlife damage.The emergence of individual plant diseases can be tracked, and infection hotspots can be detected linked to the corresponding GPS and vegetation index data. Our goal is to identify the spectral bands and indices that offer the greatest differences between genotypes, and to show, already at an early stage of development, that a change has occurred in the physiological processes of the examined line.

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#234

Wheatnet, the csic initiative to boost scientific and technical research on wheat in spain

Francisco Barro [1] (fbarro@ias.csic.es), The WheatNet Consortium [2]

[1] Institute for Sustainable Agriculture-Spanish National Research Council (IAS-CSIC), Córdoba 14004, Spain
[2] https://conexion-trigo.csic.es/integrantes-3/

Wheat is the most important source of protein and provides 20% of the calories consumed daily by humans. Wheat consumption is increasing, being estimated that the global demand for wheat production will increase by 60% in 2050 to meet the requirements of a growing population. Increasing wheat production and quality in a changing scenario requires joint efforts to reach a holistic approach encompassing and connecting different disciplines to ensure food production, make more sustainable agriculture, reduce greenhouse gas emissions, and promote alternative production systems with a lower carbon footprint.

'WheatNet' is an initiative funded by the Spanish National Research Council (CSIC) to boost scientific and technical research in wheat. WheatNet is comprised of researchers from CSIC, universities, and other public research centers, and focuses on project coordination and joint activities to promote the collaboration, internationalization, and specialization of its members. Specific objectives are:
To create a knowledge base for wheat yield improvement for present and future environmental conditions.

1. To propose suitable approaches to improve the quality and added value of food products demanded by the final consumer.
2. Establish a network of field trials and technologies to understand environmental responses and enhance sustainable production in changing conditions.
3. To recruit young talent to boost early scientific vocations by offering a complete training program on wheat research to ensure a high qualification carrier in this field.
4. To promote interdisciplinary research initiatives that require collaboration between research groups from different fields to come together and address complex wheat research questions collectively.
5. To raise awareness of the importance of continuous investment in wheat research to enhance food security.

'WheatNet' is organized into 5 Work Packages (WP). The WP1 aims to promote and optimize omics technologies, breeding techniques, scientific infrastructures, and plant genetic resources that are used in the other WPs. The WP2 seeks to improve the water and nutrient use efficiency in different agroclimatic conditions to reduce environmental impact and promote sustainable agriculture.

The WP3 will face challenges related to biotic and abiotic stresses, for the development of more resilient wheat varieties. The WP4 integrates strategies to improve the end-use quality of wheat, and how to use wheat to fight against allergies and intolerances. The WP5 addresses the wheat crop from a social perspective and the circular economy.

The project was funded by the WheatNet ("Conexión TRIGO") of the Spanish National Research Council (CSIC)

#294

Exploring biological nitrification inhibition (BNI) in wheat

Pradeep Kumar Bhati[1,2], Manish Kumar Vishwakarma[2], Narain Dhar[2], Uttam Kumar[3] ,Karthikeyan Thiyagarajan[1] and Arun Kumar Joshi[1,2]

[1]International Maize and Wheat Improvement Center (CIMMYT) New Delhi India
[2]Borlaug Institute for South Asia (BISA) New Delhi India
[3]Astralyn Agro Pvt. Ltd, Shamli Uttar Pradesh India

Email: pk.bhati@cgiar.org

A huge amount of nitrogen-based fertilizers is used in modern agriculture. Wheat is one of the top three crops whose production largely depends on the application of nitrogen fertilizer. However, around 70% of fertilizer applied in the field is not absorbed by the crops. Unabsorbed nitrogen leads to water pollution or adds to the existing greenhouse effect. To improve NUE, a new JICA-CIMMYT-BISA-ICAR partnership was initiated in 2022 through a JICA funded project for the Establishment of Nitrogen-Efficient

Wheat Production Systems in Indo-Gangetic Plains by the Development of BNI Technology. It aims to enhance nitrogen retention and reduce nitrous oxide emissions in the wheat-growing zone, particularly in the Indo-Gangetic plains of India. The focus is on developing new wheat varieties with the BNI trait. For this, field trials were conducted using BNI and non-BNI lines at the three BISA research farms – Ludhiana (Punjab), Jabalpur (Madhya Pradesh), Samastipur (Bihar), and three ICAR institutions (IIWBR Karnal, IARI New Delhi and CSSRI Karnal).

Evaluation trials included 3 BNI lines, 3 BNI parents, and 3 local checks. Simple lattice design with 2 replications, 4 nitrogen treatments (0% N_2, 50% N_2, 75% N_2, and 100% N_2 of recommended doses), standard package of practices, and propiconazole 25EC fungicide spray to avoid infection of spot blotch and stripe rust were used in these trials. More than 20 traits were recorded. It was found that BNI Vorobey showed 7-8% grain yield superiority under 75% N_2 compared to normal Vorobey. A strong breeding pipeline involving BNI lines and population wheat varieties of India is being developed for releasing BNI wheat varieties in near future.

In addition to this, the research consortium successfully identified molecular markers from the de-Novo sequence of the *Leymus recemosus* that enable a clear-cut identification of translocated segments conferring BNI and non-translocated segments of non-BNI-wheat varieties. The newly identified molecular markers will enhance the efficiency of the breeding process.

The development of BNI technology and its introduction in wheat varieties demonstrates a way forward towards sustainability and innovation that could fundamentally alter the course of agricultural development.

#368

Development of heat tolerance lines for south Asia

Pradeep Kumar Bhati[1,2], Manish Kumar Vishwakarma[2], Narain Dhar[2], Uttam Kumar[3], Sundeep Kumar[4], Gyanendra Pratap Singh[4] and Arun Kumar Joshi[1,2]

[1]International Maize and Wheat Improvement Center (CIMMYT) New Delhi India
[2]Borlaug Institute for South Asia (BISA) New Delhi India
[3]Astralyn Agro Pvt Ltd Shamli Uttar Pradesh India
[4]ICAR-National Bureau of Plant Genetic Resources Pusa Campus New Delhi India

Email: pk.bhati@cgiar.org

Terminal heat stress which occurs during the reproductive phase causes significant loss of crop yield in wheat round the world. It has been well documented in wheat, causing morphophysiological alterations, biochemical disruptions, and reduction of genetic potential. Combined with the consequences of climate change, we must ensure that farmers across the world are equipped to overcome the negative impact of heat stress. To address the issue of terminal heat stress, a new partnership between BISA-ICAR-NBPGR was initiated in 2020 for germplasm characterization and trait discovery in wheat using precision phenotyping and genotyping approaches for improving climate resilience, productivity and nutritional quality.

The main goal of this partnership was to develop, evaluate and disseminate wheat germplasm in which grain yield is sustained not compromised too much under heat stress. A set of 3200 diverse lines including newly developed CIMMYT advanced lines, landraces, primitive and traditional cultivar, wild and Indian varieties were evaluated to extreme heat under field conditions. These lines were evaluated for grain yield under optimum and heat stress condition for two years (2020-21 and 2021-22) with two replications at three different locations in BISA India: Ludhiana, Jabalpur and Pusa. Genomic data was also used to discard nonperforming lines.

A set of 560 heat tolerant entries were selected based on two years data of 3200 lines. These selected 560 entries were again planted at the three sites of BISA. The planting was done in replicated standard plots ($3.02m^2$) for yield and yield-related parameters under optimum (early November) and heat stressed (Late December) conditions. About 20 traits were recorded at each BISA site and more than 80,000 data points were recorded digitally in one year of testing. The yield of the optimum sown trial at Ludhiana ranged from 3.41 to 7.78 t/ha while under heat stress it was 2.26 to 7.34 t/ha.

Similarly, the optimum and heat-sown trials yield at Jabalpur ranged from 4.44 to 8.18 and 3.65 to 6.21 t/ha respectively. The plot yield at Samastipur, Bihar under normal sown conditions ranged from 2.29 to 7.58 t/ha while it was 1.09 to 4.69 t/ha under heat stress. The effect of heat stress on the mean grain yield of genotypes was significant in each of the three zones but was highest (2.4 t/ha) in the northeast plain zone (Samastipur) and lowest (1.0 t/ha) in the northwest plain zone (Ludhiana). In the central zone (Jabalpur) the average yield decline was 1.8 t/ha.

Based on heat susceptibility index (HSI), 162 heat tolerant lines were identified. Most (128) of them were from Ludhiana, while about 32 were from Jabalpur. At Samastipur, only 2 lines were able to express heat tolerance. These lines have been provided to national partners for either direct release or use in their breeding program.

#272

TaXTH13 is required for efficient water stress tolerance through promoting ABA biosynthesis and signaling

Huihui Bi[1], Zeliang Liu[1], Shanshan Liu[1], Jiayi Wang[1], Xuewei Yang[1], Minghui Zhao[2], Wenchen Qiao[2], Kunpu Zhang[1], Daowen Wang[1]

[1] National Key Laboratory of Wheat and Maize Crop Science, College of Agronomy, Henan Agricultural University, Zhengzhou 450002, China
[2] Key Laboratory of Crop Drought Resistance Research of Hebei Province, Dry Farming Institute, Hebei Academy of Agriculture and Forestry Sciences, Hengshui 053000, China

Correspondence: dwwang@henau.edu.cn (D. W.)

Drought stress severely limits worldwide wheat productivity. Consequently, studying and improving wheat drought tolerance and water use efficiency (WUE) are an urgent need for securing sustainable wheat production. However, drought tolerance and WUE are complex quantitative traits, which are difficult to dissect especially in polyploid wheat. In previous studies, we found that the wheat gene *TaXTH13*, encoding xyloglucan endotransglucosylase/ hydrolase, was induced by water stress, and could enhance drought tolerance when overexpressed in wheat.

Further research revealed that *TaXTH13* improves the drought tolerance of wheat by promoting ABA synthesis, reducing stomatal opening and enhancing reactive oxygen species (ROS) scavenging. Interestingly, we found that *TaXTH13-B* has two haplotypes, namely $\mathrm{Hap1}^{\mathrm{TaXTH13\text{-}B}}$ and $\mathrm{Hap2}^{\mathrm{TaXTH13\text{-}B}}$. The promoter activity of $\mathrm{Hap2}^{\mathrm{TaXTH13\text{-}B}}$ was significantly stronger than that of $\mathrm{Hap1}^{\mathrm{TaXTH13\text{-}B}}$, which correlated with higher drought tolerance index values exhibited by the cultivars carrying $\mathrm{Hap2}^{\mathrm{TaXTH13\text{-}B}}$.

Altogether, our results suggest that *TaXTH13* plays an important role in shaping wheat drought tolerance via its impact on ABA biosynthesis and signaling and that marker-assisted selection of $\mathrm{Hap2}^{\mathrm{TaXTH13\text{-}B}}$ is valuable for breeding more drought tolerant wheat cultivars in the future.

#380

Identification of QTL involved in tolerance to phosphorus deficiency in bread wheat

Alexia Crézé[1], Francesca Degan[2], Yann Brandt[3], Regis Helias[3], Jean-Pierre Cohan[4], Matthieu Bogard[1]

1 Arvalis Institut du végétal, 110 Chemin de la côte vieille, Baziège, France
2 Arvalis Institut du végétal, Station expérimentale, ZA des Graviers, 91190 Villiers-le-Bâcle, France
3 Arvalis Institut du végétal, 3 chemin de Bellevue, 81600 Montans, France
4 Arvalis Institut du végétal, La Chapelle-Saint-Sauveur, 579 route de la Jaillière 44370 Loireauxence, France

Email: m.bogard@arvalis.fr

Phosphorus (P) is a macronutrient involved in key metabolic activities and structures in plants (photosynthesis, cell membrane, energy, and nucleic acid synthesis). The world phosphorus resources, contained in phosphate rocks, are being exhausted. Therefore, breeding for tolerant varieties to phosphorus deficiency is becoming a target to maintain sustainable cropping systems.

The objective of this study was to identify genetic factors determining tolerance to P deficiency in bread wheat using a genome-wide association study (GWAS) approach. A panel of 199 winter bread wheat (Triticum aestivum) varieties released between 1988 and 2022 mainly in France and in the United-Kingdom was used. The panel was genotyped using SNP markers from the TaBW280K, the 90K iSelect and the 35K breeder arrays.

This panel was tested in three trials in France in 2021 and 2022 under optimal and low soil P concentrations. Different traits were measured such as grain yield (GY) and its components, earliness, plant height, grain P concentration and P use efficiency (PUE) and tolerance indices were calculated. The heritability of PUE ranged from 0.23 to 0.59 and interactions between genotypes and environments were observed. Out of the 201,557 SNPs used for the GWAS, 120 were found significantly associated to PUE related traits above the LOD threshold of 6.05. Based on linkage disequilibrium, we identified 18 quantitative trait loci (QTL) on 10 chromosomes (2A, 2B, 2D, 3A, 4B, 4D, 5A, 5B, 6B and 7B) for traits related to PUE.

Some of the QTL found in this study colocalized with known genes such as Ppd-D1 and Phosphate starvation response. A genomic prediction model for PUE was calibrated and showed moderate predictive ability after cross-validation (r = 0.47). Markers and genomic prediction models obtained in this study could be useful in breeding for PUE in the early stage of a breeding program.

#302

Investigating the influence of circadian variation in wheat on leaf senescence and heat tolerance

Joshua Boyte[1], Chris Buckley[1], Mike Haydon[1]

[1]University of Melbourne, Australia

Wheat yield and grain quality are heavily influenced by environmental and genetic factors. Understanding the genetic basis of adaptability to environmental variations, particularly temperature fluctuations, is crucial for enhancing agricultural productivity. The circadian clock, a conserved gene regulatory network orchestrating rhythmic biological processes, is a pivotal player in plant adaptation to environmental cues, including temperature.

However, its functional and regulatory mechanisms in wheat remain largely unexplored. In my PhD, I will investigate genetic variation in circadian rhythms in wheat and the impact on two traits known to be influenced the circadian clock: adaptation to warm temperature and timing of senescence. I aim to comprehensively assess circadian trait variation within Australian wheat cultivars and identify associated loci using genome-wide association studies (GWAS). By measuring circadian rhythms of delayed fluorescence (DF), I will measure circadian phenotypes in addition to key agronomic traits, including timing of senescence, grain protein content (GPC), and nitrogen use efficiency (NUE), in a panel of Australian wheat cultivars.

This investigation will shed light on the genetic architecture underlying circadian rhythms and its implications for grain quality. Furthermore, I will explore the influence of the circadian clock on temperature responsiveness by performing quantitative polymerase chain reaction (qPCR) analysis of clock gene transcripts under varying temperature conditions. Specifically, I will examine the role of key clock components, such as ELF3, in mediating temperature compensation and its influence on response to heat stress.

Additionally, I will investigate the involvement of the circadian clock in regulating leaf senescence, a critical developmental process influencing grain quality and yield. My PhD will integrate molecular genetics, phenomics, and field trials to unravel the intricate relationship between the circadian clock, temperature adaptation, and senescence control in wheat.

By elucidating the underlying mechanisms, I aim to identify novel genetic targets for breeding resilient wheat varieties capable of thriving in changing environmental conditions, thereby contributing to global food security.

#610

Meta-analysis of quantitative trait loci for Septoria nodorum blotch and Tan spot in wheat

Cabral AL, Gibberd MR and Phan HTT

Curtin University, Centre for Crop and Disease Management, Kent Street, Bentley WA 6102

Necrotrophic fungal diseases *Septoria nodorum* blotch (SNB) and Tan or Yellow Spot cause marked reductions in wheat quality and yield. Quantitative trait loci (QTL) associated with both diseases have been identified in bi- and multiparent populations, association mapping panels (AMPs) and global collections challenged with pathogen isolates and genotyped with molecular markers [Single Nucleotide Polymorphisms (SNP), Diversity Arrays Technology (DArT), Simple Sequence Repeats (SSR) or Expressed Sequence Tags (EST)].

Hence, across studies, a given QTL might have varied centiMorgan (cM) map locations on the same chromosome. A meta-analysis of SNB and Tan spot QTL was carried out at the Centre for Crop and Disease Management (CCDM) located at Curtin University in Western Australia. A total of 153 published SNB and Tan spot (TS) QTL were projected onto a consensus map and helped identify RefSeq v2.0 assembly-based physical locations of 10 meta-QTL (MQTL) regions on chromosomes 1B, 2D, 4B, 4D, 5A and 5B in addition to potential candidate genes for SNB and TS.

The current study provides a consensus on genetic and physical locations of MQTL identified for SNB and Tan Spot, besides their associated candidate genes, and should serve as a resource for fine-mapping studies of both diseases.

#529

Impact of sowing location and nitrogen fertilizer on tropical wheat quality for cookie production in brazil

Martha Zavariz de Miranda[1], Eduardo Caierão[1], Joaquim Soares Sobrinho[1], Ricardo Lima de Castro[1], Vanoli Fronza[1], Pedro Luiz Scheeren[1], Júlio César Albrecht[2], Jorge Henrique Chagas[1]

[1] Brazilian Agricultural Research Corporation (Embrapa) – Embrapa Wheat, Passo Fundo, RS 99050-970, Brazil
[2] Brazilian Agricultural Research Corporation (Embrapa) – Embrapa Cerrados, Planaltina, DF 73310-970, Brazil

Brazil ranks as the fourth-largest cookie producer globally, with a significant portion of its production being exported. In 2022, the cookie industry received 10% of the total wheat flour produced in the Brazilian trade. Cookies generally have a long shelf life, and crisp texture, being flour an important ingredient for product quality. During the 2021 crop season, different wheat lines were evaluated for their suitability in cookie production across three environments (Uberaba, MG, Luiz Eduardo Magalhães, BA, and Planaltina, DF).

Following the evaluation, the most promising line was identified, and subsequent agronomic adjustments were made for the 2022 crop season, in the same three locations. Wheat treated with different nitrogen doses (N = 0, 20, 80, and 120 kg/ha) was evaluated for technological quality (physicochemical and rheological analyses) and also through cookie testing. Results indicated that wheat flour from Luiz Eduardo Magalhães exhibited higher protein and gluten contents, gluten strength, and farinograph stability. Meanwhile, the greatest flour yield across all samples was observed in Planaltina.

Both Uberaba and Planaltina samples showed suitable gluten strength, water absorption, and flour stability for sweet cookie production across all nitrogen doses. Furthermore, regardless of location and dose, all samples exhibited desirable light flour color and extensible dough, which are suitable for sweet cookies. In particular, samples from Planaltina yielded the best-performance in the cookie test, as evidenced by the smaller thickness and larger expansion factors.

As conclusion, the choice of sowing location exerted a more pronounced influence on the physical-chemical and rheological characteristics of wheat flour compared to nitrogen doses, which was corroborated by the cookie testing. However, further data will be obtained in the next crop season. Nevertheless, the selected wheat line confirmed its great potential for cookie production.

#527

Impact of sowing location and nitrogen fertilizer on tropical wheat quality for cookie production in brazil

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#258

Contrasting nitrogen use efficiency of wheat in a high-yielding environment

Fabián Carrasco[1,2] and Daniel F. Calderini[2*]

[1]Graduate School, Faculty of Agricultural Sciences, Campus Isla Teja, Universidad Austral de Chile, Valdivia, Chile
[2]Institute of Plant Production and Protection, Faculty of Agricultural Sciences, Campus Isla Teja, Universidad Austral de Chile, Valdivia, Chile

Nitrogen use efficiency (NUE) has not improved over the last 20 years, making it crucial to identify wheat genotypes with high NUE and the traits that contribute to higher NUE. In this study, we conducted two field experiments in the high-yielding environment of Valdivia, Chile. In Experiment 1, we evaluated 20 wheat genotypes: 6 from Chilean breeding programs plus 14 from CIMMYT's Wheat Association Mapping Initiative (WAMI) population under two contrasting nitrogen (N) availability levels: a control without N fertilization and a fertilized treatment supplying 280 kg N ha^{-1}.

Wheat genotypes were sown in a split-plot block design, where the main plot was assigned to the nitrogen availability level and the subplots to genotypes in three blocks. Genotypes showed different grain yield ranking between both nitrogen treatments. Two contrasting genotypes were selected: WBLL1*2/KUKUNA (WBLL1), which achieved high yield in both fertilization treatments, and ATTILA/3*BCN*2///BAV92 (ATTILA), which showed contrasting yields depending on the N treatment.

In Experiment 2, these two contrasting genotypes were evaluated under two nitrogen availabilities as in Experiment 1. As expected, WBLL and ATTILA demonstrated contrasting responses between the nitrogen treatments. In control treatments, grain yield of WBLL was 13% lower than in the high nitrogen treatment, while this difference reached 36% in ATTILA (WBLL1: 1294 and 1122 g m^{-2}; ATTILA: 1126 and 725 g m^{-2}). WBLL and ATTILA showed similar ($p > 0.05$) N uptake (28.6 and 31.1 g m^{-2}, respectively) under the fertilized treatment. However, in the control treatment N uptake was higher in WBLL than in ATTILA, because of a higher nitrogen uptake efficiency (NUpE).

This difference allowed higher grain yield by WBLL in low N availability. In addition, WBLL1 exhibited a lower depletion in grain N concentration than ATTILA in the control treatment, regarding his lower grain N concentration (WBLL1: 1.81 and 1.73 %; ATTILA: 2.36 and 2.07 %), which could be attributed to a greater ability to translocate N under lower N availability and its higher N utilization efficiency (NUtE). Therefore, WBLL demonstrated higher NUE (54% higher) than ATTILA.

Wheat breeding programs focused on enhancing NUE could benefit from these differences allowing higher N uptake, especially in restricted N conditions.

#245

Outbreak of yellow rust in Chile: a new race?

Castillo, D. [1]; Sagredo, B. [2]; Matus, I.[1]; Correa, F.[2]; Muñoz, V.[2]

[1] Instituto de Investigaciones Agropecuarias. INIA-Quilamapu. Av. Vicente Méndez 515. Chillán. Chile.
[2] Instituto de Investigaciones Agropecuarias. INIA-Rayentué. Avda. Salamanca s/n, Km 105 ruta 5 sur, sector, Los Choapinos. Rengo. Chile

dalma.castillo@inia.cl, bsagredo@inia.cl, imatus@inia.cl, fcorreas23@gmail.com, v.munozquiroz.inia@gmail.com

Wheat is one of the most important crops in South America. In 2023 the production rise 24.10 MT, and the main producers' countries were Argentina, Brazil, Chile, Paraguay, Uruguay, and Peru. Since 2017, the production has been under threat because of the presence of yellow rust (*Puccinia striiformis*) in a significant area of production.

If no fungicides are employed, this disease might result in a 40% or a total loss of production. In some cases, the farmers require at least three applications per season, realizing that *P. striiformis* is one of the limiting factors for the sustainable production of this cereal.
Many studies show that *P. striiformis* populations have been constantly changing with high capacity for adaptation to new environments, features that are concomitant with more aggressiveness of races producing high quantity of urediospores in shorter period of time.

These characteristics added long-distance dispersal of yellow rust populations, and the rapid evolution of new races, have led to the rapid erosion of effective resistance genes. This has significantly reduced the number of sources of resistance for the development of new varieties. In Chile the situation became very complex, the epidemics are frequent, and there is a high genetic diversity for the *P. striiformis* populations collected.

Further, the wheat breeding program has reported that the area affected by this pathogen has increased, with higher number of wheat fields infected for *P. striiformis* each season, as well as the species affected, such as bread wheat, durum wheat, triticale, and rye, causing total losses in some cases. In this scenario we need to identify the races of *P. striiformis* that explain this new pattern of the disease in Chile. We collected yellow rust samples in different seasons, from diverse sites of the cereal production of the country from bread wheat, durum wheat, winter triticale and spring triticale.

An isolate obtained from pustule was used to extract total DNA to be sequenced. Its genome will be compared with known genome sequenced available at database. Both known reference genes and SSR markers will be used to infer about genetic diversity and races exist in Chile.

#204

Anken-Inia, a new Chilean bread wheat variety resilient to drought stress

Castillo, D[1].; Matus, I.[1]; Jobet, C.[2]; Zúñiga, J.[2]; Alfaro, C.[3]; Del Pozo A.[4]

[1] Instituto de Investigaciones Agropecuarias. INIA Quilamapu. Av. Vicente Méndez 515. Chillán. Chile.
[2] Instituto de Investigaciones Agropecuarias. INIA Carillanca. Km 10 Camino Cajón Vilcún. Temuco. Chile
[3] Instituto de Investigaciones Agropecuarias. INIA Rayentué. Avda. Salamanca s/n, Km 105 ruta 5 sur, sector Los Choapinos. Rengo. Chile
[4] Universidad de Talca. Av. Lircay s/n. Talca. Facultad Ciencias Agropecuarias. Chile.

In Chile, bread wheat accounts for 92% of the total wheat area and consumption is 96 kg per capita, which represents 2.4 million tons per year. Spring wheat varieties are sown in the Mediterranean and temperate regions of the country (between 33°40' and 40°53' S). This large environmental diversity challenges the wheat breeding program, whose principal aim is to create new cultivars with increased resilience to drought stress. Anken-INIA is a spring wheat that originated from a crossing made by the wheat breeding program of INIA, Chile, in 2000. Some characteristics of this variety are: height at adult stage 85–105 cm, white spike, with long awns along it.

The grain is red, vitreous, with an average thousand kernel weight between 46–54 gr. Anken-INIA exhibits alleles 1, 17+18 and 2+12 at the homoeologous GluA1, GluB1 and Glu1D loci. The variety does not carry the 1BL.1RS rye translocation, and thus its Corrected Glutenin Score reaches a maximum value of 8. In early sowing dates (beginning of August), flowering occurs after 98-100 days. Up to the 2023-2024 season, Anken-INIA has shown resistance to stripe rust (*Puccinia striiformis* West. f. sp. *tritici*), leaf rust (*Puccinia triticina* Erikss.), powdery mildew (*Blumeria graminis* DC. f. sp. *tritici* Marchal), and and tolerant to Zymoseptoria *tritici* (*Mycosphaerella graminícola* (Fuckel) J. Schröt.)

The cross and pedigree is: PASTOR//SRMA/TUIC 8176-4C-1C-0C-1C-0C. The parentals are from CIMMYT nurseries and were selected in trials distributed in central-south of Chile. The parental SMRA/TUI comes from the 8th SAWYT (semi-arid wheat yield trial), and PASTOR is a CIMMYT genotype evaluated in drought and high-temperature conditions in northern Mexico.

Ankén-INIA was evaluated in field experiments between 2009 and 2022, in rainfed and irrigated environments: Cauquenes (rainfed; 35°58'S, 72°17'W), Santa Rosa (irrigated, 36°31'S, 71°54'W), Yungay (rainfed and irrigated, 37°14'S, 72°01'W), Humán (irrigated, 37º43'S, 72°41'W) and Carillanca (rainfed, 38°69'S, 72°41'W).

In rainfed environments, grain yield of Ankén-INIA was 6.11% higher than control varieties Pantera-INIA and 5.33% higher than Pandora-INIA from 2009/2010 to 2011/2012, and 27,73% and 10,99% higher than Pantera-INIA and Pandora-INIA, respectively, between 2020/2021 to 2022/2023. Under irrigation conditions, Ankén-INIA presented a high yield potential between 8-11 t ha^{-1}.

All these productive and quality characteristics make Ankén-INIA a new alternative for farmers who produce under drought and temperature stress conditions.

#561

Glutopeak as a tool to differentiate baking potential of Australian wheat

Larisa Cato[1], Junhong Ma[1], Cassandra Kok[1]

[1]Australian Export Grains Innovation Centre (AEGIC), South Perth, WA 6151, Australia

larisa.cato@aegic.org.au

Southeast Asia (SEA) is the largest and fastest growing market for Australian wheat. The volume of the total bread segment in SEA is approximately 4.5mmt. Achieving the ideal loaf volume is the single most important valued attribute for flour millers when selecting wheat for bread. Other bread attributes, such as crumb softness, whiteness and structure and dough rheological properties of flour are secondary to loaf volume.

The most important flour and dough rheology characteristics were: water absorption, dough stability time, development time and strength, specifically, dough and fermentation tolerance along with wet gluten content. Many of these characteristics are associated with water holding capacity and retaining bread volume and shape.

A rapid shear-based test using a GlutoPeak instrument was compared with commonly used tests to assess the potential of this instrument to discriminate between wheat varieties and grades varying in protein content and strength. Fifty wheat flour samples derived from Australian varieties grown in different seasons and environments were analysed by Glutomatic, Farinograph, Extesnograp and GlutoPeak.

A subset was also tested for bread baking potential. Five different salts, NaCl, KCl, Mg$_2$Cl, CaCl and NH$_4$Cl at different concentrations were investigated. At lower concentrations all salts appeared to have similar impact. Type of salt and concentration will be discussed here and correlated to ability of GlutoPeak to discriminate between different wheat types and their suitability for bread baking in SEA.

#23

Genomic-wide analysis reveals Seven in absentia genes regulating grain development in wheat (*Triticum aestivum* L.)

Tao Chen[1, 2, *], Delong Yang[1, 2, *], Weidong Gao[2], Yanyan Zhang[2], Peipei Zhang[1], Lijian Guo[1]

[1] State Key Laboratory of Aridland Crop Science (Gansu Agricultural University), Lanzhou 730070, China
[2] College of Life Science and Technology, Gansu Agricultural University, Lanzhou 730070, China

* Correspondence: Delong Yang, yangdl@gsau.edu.cn; Tao Chen, chent@gsau.edu.cn

Seven in absentia (SINA) proteins, which contain a conserved SINA domain, are involved in regulating various aspects of wheat growth and development, especially in response to environmental stresses. However, it is unclear whether TaSINA family members are involved in regulating grain development until now. In this study, the expression pattern, genomic polymorphism, and relationship with grain-related traits were analyzed for all TaSINA members.

Most of the *TaSINA* genes identified showed high expression levels in young wheat spikes or grains. The genomic polymorphism analysis revealed that at least 62 *TaSINA* genes had different haplotypes, and the haplotypes of five genes were significantly correlated with grain-related traits. Kompetitive allele-specific PCR (KASP) markers were developed to confirm the single nucleotide polymorphisms in *TaSINA101* and *TaSINA109* among the five selected genes in a set of 292 wheat accessions.

The *TaSINA101-Hap II* and *TaSINA109-Hap II* haplotypes had higher grain weight and width compared to *TaSINA101-Hap I* and *TaSINA109-Hap I* in at least three environments, respectively. The qRT-PCR assay revealed that *TaSINA101* was highly expressed in the palea shell, seed coat, and embryo in young wheat grains.

The TaSINA101 protein was unevenly distributed in the nucleus when transiently expressed in the protoplast of wheat.
Three homozygous *TaSINA101* transgenic lines in rice showed higher grain weight and size compared to the wild type. These findings provide valuable insight into the biological function and elite haplotype of *TaSINA* family genes in wheat grain development at a genomic-wide level.

#575

A TaSRT1-TaHSP18.6 module confers resistance to Fusarium crown rot by mediating auxin content in wheat

Xia Yang, Leilei Zhang, Jiajie Wei, Xiangning Yan, Minjie Yuan, Lingran Zhang, Ning Zhang, Yan Ren, Feng Chen*

National Key Laboratory of Wheat and Maize Crop Science / CIMMYT-China Wheat and Maize Joint Research Center /Agronomy College, Henan Agricultural University, Zhengzhou 450046, China

Corresponding author: fengchen@henau.edu.cn (86-0371-56990337)

Fusarium crown rot (FCR) is one of the most serious underlying diseases to threaten wheat yield and quality recently. Here, we identified an antioxidant enzyme gene catalase (*TaCAT*) through genome wide association study (GWAS) and whole-exome sequencing (WES) in two nested bi-parental populations.

We verified the function of *TaCAT* regulating wheat FCR resistance by virus-induced gene silencing (VIGS), EMS mutants and overexpression lines.
Moreover, we screened a TaSnRK1α that interacted with TaCAT through phosphorylation *in vitro* and *in vivo*. Subsequently, we identified an FCR-resistance haplotype TaCAT$^{214\mathrm{Ser}/418\mathrm{Lys}}$, and confirmed that Ser214Thr of TaCAT was a key phosphorylation site by TaSnRK1α.

We also found that TaSnRK1α improve more protein accumulation in TaCAT-R$^{214\mathrm{Ser}}$ than TaCAT-S$^{214\mathrm{Thr}}$, possibly resulting in an enhanced FCR resistance in TaCAT-R wheat plants.
Furthermore, the function of TaSnRK1α regulating FCR resistance was verified in wheat EMS mutants and overexpression lines. Taken together, we proposed a TaSnRK1α-TaCAT model to mediate FCR resistance by scavenging the ROS in wheat plants.

#50

Three near-complete genome assemblies reveal substantial centromere dynamics from diploid to tetraploid in Brachypodium genus

Chuanye Chen[1†], Siying Wu[1†], Yishuang Sun[2,3†], Jingwei Zhou[1], Yiqian Chen[1], Jing Zhang[2], James A. Birchler[4], Fangpu Han[2,3], Ning Yang[1] and Handong Su[1,5*]

[1] National Key Laboratory of Crop Genetic Improvement, Hubei Hongshan Laboratory, Shenzhen Institute of Nutrition and Health, Huazhong Agricultural University, Wuhan 430070, China
[2] State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Innovation Academy for Seed Design, Chinese Academy of Sciences, Beijing 100101, China
[3] University of the Chinese Academy of Sciences, Beijing 100049, China
[4] Division of Biological Sciences, University of Missouri, Columbia, MO 65211, USA
[5] Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China

Centromeres are critical for maintaining genomic stability in eukaryotes, and their turnover shapes genome architectures and drives karyotype evolution. However, the co-evolution of centromeres from different species in allopolyploids over millions of years remains largely unknown. Here, we generate three near-complete genome assemblies, a tetraploid Brachypodium hybridum and its two diploid ancestors, Brachypodium distachyon and Brachypodium stacei.

We detect high degrees of sequence, structural, and epigenetic variations of centromeres at base-pair resolution between closely related Brachypodium genomes, indicating the appearance and accumulation of species-specific centromere repeats from a common origin during evolution. We also find that centromere homogenization is accompanied by local satellite repeats bursting and retrotransposon purging, and the frequency of retrotransposon invasions drives the degree of interspecies centromere diversification.

We further investigate the dynamics of centromeres during alloploidization process, and find that dramatic genetics and epigenetics architecture variations are associated with the turnover of centromeres between homologous chromosomal pairs from diploid to tetraploid. Additionally, our pangenomes analysis reveals the ongoing variations of satellite repeats and stable evolutionary homeostasis within centromeres among individuals of each Brachypodium genome with different polyploidy levels.

Our results provide unprecedented information on the genomic, epigenomic, and functional diversity of highly repetitive DNA between closely related species and their allopolyploid genomes at both coarse and fine scale.

#323

Identification of genes increasing the number of grains per spikelet in a pyramidale wheat

Tiantian Chen1, Kazusa Nishimura2, Kazuki Murata1, Kyoka Nagasaka1, Yu Iwahashi1, Takahiro Maki1 , Yu Kinoshita1, Hiromo Inoue1, Ryohei Nakano1, and Tetsuya Nakazaki1

1 Graduate School of Agriculture, Kyoto University, 4-2-1, Shiroyamadai, Kizugawa city, Kyoto, 619-0218, Japan. 2Graduate School of Environmental, Life, Natural Science and Technology, Okayama University, 1-1-1 Tsushima-naka, Kita-ku, Okayama City, 700-8530, Japan

Grain yield components, such as grain number per spikelet (GPSt) and spikelet number per spike, are crucial factors determining wheat yield. Sakuma et al. (2019) demonstrated that the less functional GNI-A1 allele contributed to higher GPSt, resulting in an increase in final grain yield by 10-30%. TN28, a tetraploid pyramidale wheat line, was found to harbor even more GPSt, as many as five grains in a spikelet with an average of 2.61, than ‘Langdon’ (LDN) which carry the less functional GNI-A1 allele with an average of 2.02. This suggests that TN28 harbors novel genes that increases GPSt with effects surpassing GNI-A1.

To identify the genes, we performed QTL analysis using 144 F2 individuals derived from the cross between two tetraploid wheat TN28 and LDN in a previous study in year 2020. We detected a major QTL for grain number and grain weight in a spikelet located on chromosome 7AL, which was named as qGNI-7A. Moreover, a major QTL for GPSt was detected on chromosome 7B named as qGNI-7B and a significant digenic interaction was observed between qGNI-7A and qGNI-7B for GPSt where qGNI-7A can only increase GPSt when they are TN28 alleles at both qGNI-7A and qGNI-7B.

To validate these QTLs of TN28, we performed QTL analysis in year 2022 under two environments with different fertilizer conditions (designated as 22AC and 22AF) using other F2 populations from the same cross. The genetic linkage map was constructed using all three populations.

To evaluate grain number distribution within the spike, we also evaluated grain number along the spike by dividing the spike into several parts (n = 2 to 10; e.g. n= 3 means three parts within the spike: apical, central and basal parts of the spike). qGNI-7A was detected for grain number at the apical 10-20% portion of the spike in 22AC, although the two previously detected QTLs were not detected in either condition.
Also, the interactions between qGNI-7A and qGNI-7B were not reproduced in 22AC and 22AF. All in all, a specific-genotype and specific-environment effects of QTLs on chromosome 7A and 7B may contribute to a higher GPSt in TN28. Better understand their effects and interactions in isogenic backgrounds in multiple environments is needed in the future work.

#505

Gwas to identify the genomic loci associated with heading trait under fall-sowing conditions

Changhyun Choi, Yurim Kim, Myoung-Goo Choi, Kyeong-Min Kim, Han-Yong Jeong, Chon-Sik kang, Myoung Hui Lee, Ki-Chang Jang, Mira Yoon

National Institute of Crop Science, Rural Development Administration, Wanju 55365, Korea

The current study aimed to analyze the allelic variations in major heading-date regulating genes and conducted a genome-wide association study (GWAS) using a wheat core collection to explore the genetic loci related to heading date under fall sowing conditions in East Asia.

Significant differences in heading dates were observed based on the genotype of the Ppd-D1 gene, whereas no significant difference was seen based on the genotypes of Vrn-1 and Ppd-A1. Furthermore, despite Korean varieties exhibiting extremely early heading worldwide, the allelic diversity in Vrn-1 and Ppd-1 is limited. Through GWAS, using the entire wheat core collection (n = 518), two significant SNPs (AX-95222044 and AX-94685526) were selected as the major loci associated with the heading date.

Two more SNPs (AX-94550996 and AX-94970315) were chosen from the GWAS using the accessions (n = 231) carrying the Vrn-1 and Ppd-1 genotypes that were identical to 40 Korean wheat varieties. Among them, genes harboring AX-95222044 and AX-94970315 had previously been reported to be associated with wheat heading dates, and the gene carrying AX-94550996 was reported to be related to flowering time in the orthologous gene of Arabidopsis.

Therefore, resources possessing early heading alleles for the four SNPs identified through GWAS, along with Ppd-D1, were considered potential candidates for early heading breeding materials. Five Korean resources (IT nos. 14303, 15864, 116118, 175533, and 237807) met this criterion, with an average DTH of 179 days. Utilization of the four SNPs selected from GWAS in this study could help develop molecular markers for breeding early maturing varieties in East Asia.

#252

Bread wheat carrying *RHT-B1b* and *RHT-D1b* dwarf alleles in the mirror of climate change

Andras Cseh[1], Andrea Lenyko-Thegze[1],Diana Makai[1], Fanni Szabados[1],Zsolt Gulyas[1], Tibor Kiss[1,2], Ildiko Karsai[1], and Adel Sepsi[1]

[1] HUN-REN, Centre for Agricultural Research, Martonvasar, 2462, Hungary
[2] Food and Wine Research Institute, Eszterhazy Karoly Catholic University, Eger, 3300, Hungary

The development and worldwide spread of wheat varieties carrying the *Rht-B1b* and *Rht-D1b* dwarfism alleles, starting from the 1950s 'Green Revolution', resulted in an unprecedented increase in wheat yields. Height-reducing (*Rht*) and yield-increasing alleles are being widely used during the breeding of high-yielding wheat varieties. Their beneficial effects are essential for future breeders to maintain food security for the growing population.

It is therefore essential to understand how dwarfing alleles affect wheat fertility under the climatic conditions caused by global warming. While a temperature of around 20°C is optimal for the early generative development of wheat, the daily maximum temperature corresponding to the season may exceed the threshold value of 30°C by 2050.
The aim of the present research was to investigate the effect of short-term (24h) high temperature stress (30°C) on the early generative stage and fertility of wheat lines carrying the *Rht-B1b* or *Rht-D1b* alleles, with a focus on meiotic cell division, the process that leads to gamete formation.

Our results showed that the increased temperature caused a significant yield loss in the main ears of *Rht-B1b* and *Rht-D1b* wheats, greater than the tall wheats carrying the wild type alleles. Our results showed that fertility losses correlated with abnormal forms of meiotic cell division, including defective synaptonemal complex structure, a reduced frequency of homologous recombination and more frequent chromosome mis-segregation.

Additionally, *Rht-B1b* and *Rht-D1b* mutants showed meiotic defects at optimal temperature and were more sensitive to high temperature than their high counterparts. Our results show that the identification and introduction of alternative dwarfism alleles into modern breeding programs is essential for the production of high-yielding wheat varieties resistant to high temperatures.

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#79

Abiotic stress responses in local varieties of Triticum turgidum subsp. Durum

Pasquale L. Curci[1,*], Giacomo Mangini[1], Emanuela Blanco[1], Diana L. Zuluaga[1], Francesco Punzi[1,2], Gaetano Laghetti[1], Gabriella Sonnante[1,*]

[1]Institute for Biosciences and BioResources, National Research Council (CNR), Via G. Amendola 165/A, 70126 Bari, Italy
[2]Department of Biosciences, Biotechnologies and Environment, Botanic Garden Museum, University of Bari, Via E. Orabona 4, 70125 Bari, Italy

Under the urgent threat of climate change, the agricultural stability of the Mediterranean basin faces increasing challenges due to a combination of rising temperatures and reduced rainfall. These environmental changes cause abiotic stresses, including drought, salinity, heat, and nutrient deficiencies, which seriously affect the sustainability and yield of many crops, such as durum wheat (*Triticum turgidum* subsp. *durum*).

In this context, durum landraces represent a valuable resource to explore and leverage for their effective application in crop breeding. Within the project PSR Puglia 2014-2020; Op. 10.2.1 “Biodiversità dei cereali antichi pugliesi per la sostenibilità e la qualità - SAVEGRAIN-CER”, we aim at a molecular characterization of durum landraces subjected to abiotic stresses. By employing RNA sequencing, this research seeks to investigate the global transcriptional responses of different plant organs (including flag leaf and developing grain) of durum landraces, to highly impacting abiotic stresses.

This study aims at identifying both shared and unique stress response mechanisms, enhancing our understanding of the biological processes involved in the stress tolerance. Such insights will be critical for developing effective strategies to improve crop resilience and maintain agricultural productivity in changing climatic conditions.

#393

Unveiling the genetic architecture of ergot resistance in durum wheat

Tinku Gautam[1], Samia Berraies[1], Yuefeng Ruan[1], Ron Knox[1], Amir Souissi[1], Brad Meyer[1], Christopher Sehn[1], Malina Poppy[1], Curtis Pozniak[2], Jim Menzies[3], Sean Walkowiak[4], Richard Cuthbert[1]

[1]Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, Swift Current, SK, Canada
[2]Crop Development Centre and Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada
[3]Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden, MB, Canada
[4]Grain Research Laboratory, Canadian Grain Commission, Winnipeg, MB, Canada

Correspondence: samia.berraies@agr.gc.ca; yuefeng.ruan@agr.gc.ca

Ergot, caused by the fungus *Claviceps purpurea* (Fr.) Tul. (Cp), affects plants during anthesis, leading to the formation of black sclerotia. While the sclerotia themselves do not cause significant yield losses, the toxic alkaloids within them result in grain downgrading and economic losses during market grading.

Commercial durum wheat cultivars in Canada are susceptible to ergot disease. Although host resistance is rare, a few resistance loci have been identified. This study aimed to confirm existing and identify additional genetic determinants of ergot resistance in two doubled haploid durum wheat populations (A1560& and A1561&). The common parent, A0709-BX05, is the most ergot-resistant line developed at the Swift Current Research and Development Centre (SCRDC), Agriculture and Agri-Food Canada (AAFC).

The populations were grown in the SCRDC greenhouse from 2021 to 2023 and inoculated with three ergot isolates. Visual assessments of honeydew and sclerotia production were conducted. Furthermore, the A1561& population, along with parents and checks, were assessed for six post-harvest sclerotial traits using Vibe QM3i image analyzer. Both populations and parents were genotyped using the 25K Wheat Array, following the Trait Genetics manufacturer’s protocol.

Linkage maps were independently generated and quantitative trait locus (QTL) mapping was conducted. The QTL mapping revealed significant loci associated with honeydew resistance and sclerotia rating. For the A1560& population, a QTL was associated with honeydew resistance on each of chromosome 2A and 5B. Notably, the QTL on chromosome 2A emerged as the major contributor to honeydew resistance, explaining up to 54% of the phenotypic variance.

The resistant parent A0709-BX05 contributed low honeydew production alleles for both QTL. In the case of A1561&, six QTL associated with eight different traits (honeydew production, sclerotia production, number of sclerotia, sclerotia length, sclerotia width, sclerotia area average, sclerotia total weight, and sclerotia total area) were identified on chromosomes 1A, 1B, 2A, and 5B. Notably, the QTL on chromosomes 2A and 5B were major contributors to resistance, explaining from 25 to 75% of the phenotypic variance for honeydew resistance and sclerotia rating.

All resistance loci were contributed by the resistant parental line A0709-BX05. These findings will aid in the development and enhancement of markers for ergot resistance in durum wheat.

#636

Molecular identification of the wheat stripe rust resistance gene *yr10*

Katherine Dibley[1], Robert McIntosh[2], Evans Lagudah[1] & Peng Zhang[2]

[1]CSIRO Agriculture and Food, GPO Box 1700, Canberra, ACT 2601, Australia.
[2]The University of Sydney, School of Life and Environmental Sciences, Plant Breeding Institute, Cobbitty, NSW 2570, Australia.

Wheat stripe rust disease, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a threat to global wheat production. The molecular identification of resistance genes is essential for enabling efficient deployment to combat yield losses to disease. The wheat stripe rust resistance gene, *Yr10,* was originally identified in Turkish wheat landrace PI 178383 and deployed into US germplasm in the 1960s.

Although defined by a unique array of responses to multiple *Pst* isolates, the molecular identification of the causative gene of *Yr10* has been elusive and controversial. Candidate genes previously proposed include a canonical resistance gene of the CC-NBS-LRR gene family, and more recently a NAM-ZnF-BED domain gene. Here, we present sequence analysis and disease resistance phenotyping in mutants generated in a universal reference *Yr10* stock, Avocet S + *Yr10*, to confirm that *Yr10* is encoded by the NAM-ZnF-BED domain gene *YrNAM.*

An agreed simplification of the separate gene identities, *YrNAM* and *Yr10*, conferring a common specificity to a single *Yr10* designation will benefit the wider cereal research community. We also draw parallels between *YrNAM* and the recently described, structurally-similar barley leaf rust resistance gene *Rph7*, and propose this new *R* gene class be designated as Triticeae NAC-BED domain (TNB) proteins.

#70

Tiller fertility is critical for improving grain yield, photosynthesis, and nitrogen efficiency in wheat

DING Jin-feng[1, 2, 3,*], DING Yong-gang[1], ZHANG Xin-bo[1], MA Quan[1], LI Fu-jian[1], TAO Rong-rong[1], ZHU Min[1], 2, LI Chun-yan[1, 2], ZHU Xin-kai[1, 2], GUO Wen-shan[1, 2]

[1] Jiangsu Key Laboratory of Crop Genetics and Physiology/Jiangsu Key Laboratory of Crop Cultivation and Physiology/Agricultural College, Yangzhou University, Yangzhou 225009, P.R. China
[2] Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, P.R. China
[3] Jiangsu Ruihua Agricultural Technology Co., Ltd., Suqian 223800, P.R. China

Genetic improvement has promoted wheat’s grain yield and nitrogen use efficiency (NUE) during the past decades. In China, the Yangtze River Basin is an important wheat production region, constituting 16% of the total wheat production area and accounting for 25% of total production. The current wheat cultivars exhibit higher grain yield and NUE than previous cultivars in this region since the 2000s.

However, the agronomic traits of the high-grain yield and high-NUE cultivars that were released in recent years have not been characterized, and the mechanisms by which higher grain yield and NUE levels have been achieved are not known. In the present study, 21 weak-winter wheat cultivars (*Triticum aestivum* L.) that were released in the Yangtze River Basin after 2000 were cultivated for three seasons from 2016 to 2019.

Significantly positive correlations were observed between grain yield and NUE in the three years. The cultivars were grouped into high (HH), medium (MM), and low (LL) grain yield and NUE groups. The HH group exhibited significantly high grain yield and NUE.

High grain yield was attributed to more effective ears by high tiller fertility and greater single-spike yield by increasing post-anthesis single-stem biomass. Compared to other groups, the HH group demonstrated a longer leaf stay-green ability and a greater flag leaf photosynthetic rate after anthesis. It also showed higher N accumulation at pre-anthesis, which contributed to increasing N accumulation per stem, including stem and leaf sheath, leaf blade, and unit leaf area at pre-anthesis, and promoting N uptake efficiency, the main contribution of high NUE.

Moreover, tiller fertility was positively related to N accumulation per stem, N accumulation per unit leaf area, leaf stay-green ability, and flag leaf photosynthetic rate, which indicates that improving tiller fertility promoted N uptake, leaf N accumulation, and photosynthetic ability, thereby achieving synchronous improvements in grain yield and NUE.

Therefore, tiller fertility is proposed as an important kernel indicator that can be used in the breeding and management of cultivars to improve agricultural efficiency and sustainability.

#595

Leveraging X-ray Micro CT and image segmentation to explore wheat grain anatomy

Kateryna Dugina1, Craig Sturrock2, Ian Fisk3,4

1 School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, United Kingdom. 2 Hounsfield Facility, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, United Kingdom. 3 International Flavour Research Centre, Division of Food, Nutrition and Dietetics, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom. 4 International Flavour Research Centre (Adelaide), School of Agriculture, Food and Wine and Waite Research Institute, The University of Adelaide, PMB 1, Glen Osmond, South Australia 5064, Australia.

kateryna.dugina@nottingham.ac.uk, craig.sturrock@nottingham.ac.uk, ian.fisk@nottingham.ac.uk

Wheat, a fundamental cereal grain sustaining 36% of the global population, stands as a cornerstone in worldwide nutrition. As milling processes demand precise separation of wheat grain tissues, understanding its intricate anatomical structure becomes paramount. This paper delves into the application of X-ray micro-computed tomography (X-ray µCT) coupled with image segmentation techniques to analyze the wheat grain's internal composition.

The anatomy of wheat grain comprises the starchy endosperm, germ, and outer seed coat, each delineating unique nutritional and functional properties. Conventional methods for anatomical assessment, while informative, often lack precision and involve intrusive sample preparations. Advanced imaging techniques like Fourier Transform Infrared (FT-IR) microspectroscopy and Scanning Transmission X-ray Microscopy (STXM) provide detailed chemical insights but suffer from low throughput.

To address these limitations, the study evaluated X-ray µCT as a novel non-invasive imaging method to understand wheat grain anatomy. Whilst previous studies have highlighted its utility as an imaging technique, our study significantly advanced this field. We demonstrated that X-ray µCT serves as a rapid, high-throughput analysis tool capable of visualizing individual grain anatomy. This was achieved by implementing specialized in-house structural segmentation methods, namely Histogram-based segmentation and Trainable Weka Segmentation (TWS).

These methods proved to be robust and reliable in delineating internal structures such as the endosperm and germ, offering automatic micron-scale insights into the grain's structure.
Ultimately, this research aims to assess the feasibility and accuracy of these segmentation methods in delineating wheat grain tissues, offering potential advancements in understanding and optimizing milling processes for enhanced grain tissue separation and nutritional value extraction.

#603

The effect of R*hizobium* in improving drought tolerance in spring bread

Ed-daoudy Lamyae [1,2], Khaled Al-sham'aa [3], Laila Sbabou [2], Wuletaw Tadesse [1]

[1] International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco.
[2] Laboratory of Microbiology and Molecular Biology, Faculty of Sciences, Mohammed V University, Rabat, Morocco.
[3] International Center for Agriculture Research in the Dry Areas (ICARDA), Giza, Egypt.

edda.lamyae@gmail.com

Bread wheat (Triticum aestivum L., 2n = 6x = 42, AABBDD) is critical for food security, especially in Central and South Asia, North Africa, and Sub-Saharan Africa. Drought is having an increasing detrimental impact on bread wheat output as a result of climate change.
In Morocco, a country primarily reliant on wheat production, drought has resulted in significant output losses over the last five years.

Over a century, researchers have investigated the use of plant growth-promoting rhizobacteria as an alternate technique for improving plant drought tolerance. Despite this understanding, the interaction of rhizobia with non-legumes was generally overlooked as a research topic until the last few decades.

Here we show that the application of *Rhizobium* as plant growth promoter under abiotic stress can have a positive effect on wheat yield components. Two elite wheat genotypes, one capable of withstanding drought and the other vulnerable to it, were primed with Three *Rhizobium leguminosarum* strains, and cultivated in plastic tunnels in the main growing season in Morocco under drought and well-watered conditions.

The strains were evaluated for their ability of phosphate solubilization (PSA) and phytohormones production under drought conditions. The application of the *Rhizobium* strains B1 and B2 had a positive effect on either wheat genotype. Under drought conditions, priming with these two *Rhizobium* strains led to an increase in seed number and number of tillers by 38% and 32%, respectively, compared to the uninoculated retrospective genotype. Interestingly, wheat genotypes act differently with the strains used for the inoculation.

The third *Rhizobium* strain (B3) had no effect on plant performance in the majority of the conditions, and multi-strain inoculation resulted in lower yield than when these micro-organisms were used alone allowing us to explore the genetic factors that control *Rhizobium* interactions with wheat in the future.

#578

Exploring early planting strategies for spring wheat in Dinajpur, Bangladesh vs. Ludhiana, India

Md. Farhad[1]*, Md. Abdul Hakim[1], Md. Monwar Hossain[1], Uttam Kumar[2], Pradeep Bhatti[3], Arun Kumar Joshi[3,4] and Golam Faruq[1].

[1.] Bangladesh Wheat and Maize Research Institute, Dinajpur, Bangladesh
[2.] Astralyn Agro (OPC) Pvt. Ltd., Ludhiana, Punjab-141001, India
[3.] Borlaug Institute for South Asia (BISA), New Delhi, India
[4.] International Maize and Wheat Improvement Centre (CIMMYT), New Delhi, India.

*Presenting author Md. Farhad; email ID: md.farhad@bwmri.gov.bd

The Indo Gangetic Plain, often referred to as the South Asian breadbasket, plays a critical role in feeding a substantial portion of the world's population. However, climate change poses significant challenges to wheat production in this region. Early planting has been found to be beneficial for boosting wheat yield, especially in the face of climate change. While it has been proven beneficial in most regions of the Indo Gangetic Plain, it has not been extensively studied in short-wintered areas like Bangladesh.

A study assessed the feasibility of early planting strategies for spring wheat in Dinajpur, Bangladesh, by comparing the results with Ludhiana, Punjab, India. Specifically, we investigated the impact of early planting on yield, heading days, grain filling duration, and other relevant parameters. Field trials were conducted in the BWMRI research field in Dinajpur and compared with previously established results from trials in the BISA research field at Ludhiana.

The research compared early planting (before the typical sowing window) with timely planting (within the recommended period). Bangladeshi wheat genotypes were used in Dinajpur, while CIMMYT developed advanced lines were utilized in Ludhiana, Punjab, India. Increased heading days were exhibited and extended grain filling duration with early planting in Ludhiana. This ensured better source-to-sink dynamics and ultimately higher yields.

In Dinajpur, early planting led to fewer days to heading, but the grain filling duration remained similar across planting times. Early planting significantly boosted yield in Ludhiana. Some wheat varieties were already released to support farmers' demand for early planting for India.
In contrast, early planting in Dinajpur resulted in lower average yields compared to timely planting. Heat stress during the early season affected flowering time, potentially forcing early heading in Dinajpur. Prolonged vegetative periods, known to enhance yield, were not observed in the Bangladeshi environment.

The search for heat-tolerant genes and germplasms remains crucial to support successful early wheat establishment in Bangladesh. Developing varieties specifically adapted to short winter conditions is essential. Early planting strategies have varying impacts on spring wheat yield in different regions. While Ludhiana benefits from extended grain filling duration, Dinajpur faces challenges related to heat stress.
This study underscores the need for tailored approaches to wheat cultivation based on local conditions and genetic resources.

#154

QTLs for seedling and adult plant resistance to leaf rust derived from cultivar popo detected in south africa and argentina environents

Sandiswa Figlan1,2*, Tsepiso Hlongoane1, Carlos Bainotti3, Pablo Campos4, Leonardo Vanzetti3,5, Gabriela Edith Tranquilli6, Toi John Tsilor7

1 Agricultural Research Council - Small Grain Institute, Bethlehem 9700, South Africa. 2 Department of Agriculture and Animal Health, University of South Africa, Florida, Johannesburg 1710, South Africa. 3 EEA INTA Marcos Juárez., Marcos Juárez (CP 2580), Córdoba, Argentina. 4 INTA Bordenave, CC 44, Bordenave (CP 8187), Buenos Aires, Argentina. 5 Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. fInstituto de Recursos Biológicos, INTA Castelar, Hurlingham, Argentina. 6 Instituto de Recursos Biológicos, INTA Castelar, Hurlingham, Argentina. 7 Department of Life and Consumer Sciences, University of South Africa, Florida, Johannesburg 1710, South Africa

Leaf rust, caused by the fungus Puccinia triticina Eriks (Pt), is a destructive disease affecting wheat (Triticum aestivum L.) production in many countries, and a serious threat to food security. As a result, several breeding programs have included leaf rust resistance as an important trait.

The discovery and identification of new resistance genes that could aid in incorporating durable or long-lasting leaf rust resistance into wheat is fundamental in these breeding programs. The present study aimed to identify quantitative trait loci (QTLs) for leaf rust resistance in 127 recombinant inbred lines (RIL) developed from the cross between the resistant cultivar Popo and the susceptible cultivar Kariega. The RIL population and parental lines were phenotyped for leaf rust infection type and severity at seedling and adult plant stage, respectively.

The former in the greenhouse (in Argentina) and the latter in multiple field test environments comprising 3 locations in South Africa (in Tygerhoek in the Western Cape Province during the 2014, 2015, 2017 and 2018 cropping seasons; Clarens during 2014, 2016 and 2017 cropping seasons and in Bethlehem in the Free State Province during 2017 cropping season) and in 1 location in Argentina (during the 2017 and 2018 cropping seasons in Marcos Juárez, Córdoba Province). The population was genotyped using genotyping-by-sequencing.

A total of 12,080 silicoDArT and 2,669 SNP markers were used for QTL analysis. In total, 25 putative QTLs for resistance to leaf rust at seedling and adult plant stages were identified, including 5 QTLs for seedling and 20 QTLs for adult plant resistance (APR). Interestingly, both Popo and Kariega contributed with alleles for resistance. Significant loci for reducing leaf rust infection at seedling stage were designated QLr.arc-1A, QLr.arc-2B, QLr.arc-5B, QLr.arc-6A and QLr.arc-6D.

Three minor QTLs derived from Popo designated as QLr.arc-1B, QLr.arc-2D and QLr.arc-3D were also detected from the field tests, explaining 5-10%, 10-16% and 5-7% of the phenotypic variance, respectively.

The identified QTLs and their closely linked silicoDArT and SNP-based markers can be used for fine mapping and candidate gene discovery in wheat breeding programs targeting durable leaf rust resistance.

#508

Enhanced activity of photosystem I as a result of temperature acclimation improves thermostability of wheat

Andrej Filaček[1], Marek Živčák[1], Lorenzo Ferroni[2], Marek Kovár[1], Mária Barboričová[1], Marián Brestič[1]

[1]Department of Plant Physiology, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture, Trieda A. Hlinku 2, 949 76 Nitra, Slovakia
[2]Laboratory of Plant Cytophysiology, Department of Environmental and Prevention Sciences, University of Ferrara, Corso Ercole I d'Este 32, 44100 Ferrara, Italy

High temperatures affect practically all physiological and metabolic processes in plants. Therefore, plants are forced to change their metabolism in order to minimize damage caused by heat because they are stationary organisms that cannot escape the heat in true sense of word. This process is known as acclimation.

The main goal of the study was to assess how long-term plant acclimation to high temperatures affected the ability of photosynthetic apparatus to withstand heat stress. In experiment focusing on the effects of high temperature, three genotypes of wheat differing in leaf and photosynthetic traits were analyzed: Thesee (*Triticum aestivum* L.), Roter Samtiger Kolbenweizen (*Triticum compactum*) and ANK 32A *(Triticum aestivum* L.). The pot experiment was carried out in outdoor conditions (non-acclimated variant). Acclimation to high temperature was induced by transferring half of the plants to a foil tunnel with elevated temperature for 12 days as soon as all plants had fully developed flag leaves (acclimated variant).

Subsequently, exposition of the both groups of plants (acclimated and non-acclimated) in a growth chamber with artificial light and air temperature up to 45 °C for approximately 12 hours prior to the measurements induced a severe heat stress. The realized measurements of gas-exchange and rapid kinetics of chlorophyll *a* fluorescence demonstrated that the ability of the genotypes to respond to acute heat stress varies. The effects of high temperature were manifested by non-stomatal inhibition of the photosynthetic process and the rapid kinetics of chlorophyll fluorescence confirmed the mostly reversible effects due to a moderate decrease of parameters associated with PSII activity.

All genotypes exhibited a decrease in the total activity of PSII (PIabs) ,the number of active PS II reaction centers (RC/ABS) and maximum quantum yield of PSII (Fv/Fm) due to a effect of acute heat stress. On the other hand, severe heat stress adversely affected the PS I (parameter ψREo) in the non-acclimated variants only, while a significant increase was observed in the acclimated variants, which was the most pronounced in heat sensitive and chlorophyll b-deficient genotype ANK-32.

This finding supports the hypothesis that increased PSI activity may play a key role in protection of plants against adverse effects of acute temperature stress. Research activities were realized under the projects: APVV-22-0392, VEGA 1-0664-22, VEGA 1-0425-23.

#146

Breeding progress for stem water-soluble carbohydrates in European winter wheat

Lukas Förter[1], Andris Finkbeiner[1], Andreas Stahl[2], Tsu-Wei Chen[3], Eva Herzog[4], Benjamin Wittkop[1], Rod J. Snowdon[1]

[1] Department of Plant Breeding, Justus-Liebig University, Giessen, Germany
[2] Julius Kühn Institute, Institute for Resistance Research and Abiotic Stress Tolerance, Quedlinburg, Germany
[3] Groupe of Intensive Plant Food Systems, Humboldt University Berlin, Germany
[4] Biometry and Population Genetics, Justus-Liebig University, Giessen, Germany

Contact: Lukas.Foerter@ag.uni-giessen.de

Plant breeding has contributed to a steady yield progress in European winter wheat varieties released since the 1960s. This progress was particularly driven by improving sink-related traits like grain number per spike. However, since yield formation relies on complex source-sink interactions throughout the entire vegetation period, an additional investigation of source-related traits is essential for understanding and further improving yield progress in wheat breeding. Accumulation of stem water-soluble carbohydrates (SWSC) is one determinant for source capacity after the shift to generative growth and is known to be a key factor for yield formation.

To assess the SWSC concentration in the context of genotype-environment-interactions, 50 well-characterized, elite European winter wheat varieties representing the last five decades of breeding progress were sown in a field trial with two irrigation treatments (rainfed and additional irrigation) at our field station in Gross Gerau.

Plant samples were taken at four timepoints (flag leave, anthesis, milk/dough stage, maturity) to investigate temporal dynamics of SWSC under abiotic stress conditions. Besides classical chemical analysis of SWSC (anthrone method), NIR spectra of the plant samples were recorded in order to develop calibration equations for establishment of a high-throughput analysis method for samples from field trials in multiple years, locations and treatments.

As expected, preliminary results from the 2023 field trial confirmed the superior yield performance of modern varieties. Moreover, the SWSC concentration showed a similar linear improvement over time in relation to the year of cultivar registration and was correlated to grain yield under both irrigation treatments. In summary, modern varieties tend to have higher yields as well as a higher source capacity than older varieties.

We thus assume that SWSC, as a source-related trait, has been a key contributing factor to historical yield progress in winter wheat breeding. The findings are being validated with further sample analysis and multi-location field trials in 2024.

High-throughput phenotyping for SWSC via NIRS will enable collection of large datasets that provide valuable insight into the complex genotype-environment-interactions affecting SWSC and yield performance, especially under abiotic stress conditions. Ultimate, this will facilitate a more direct approach to selection for yield improvement in future wheat breeding programs.

#189

Genomic selection for low don content in winter wheat

Dario Fossati[1], Flavio Foiada[2], Patrick Krähenbuhl[2], Steven Yates[3], Bruno Studer[3], Andreas Hund[3], Chalhoub Boulos[1], Karl-Heinz Camp[2],

[1] Agroscope, Field-Crop Breeding and Genetic Resources, Nyon, Switzerland
[2] Delley Seeds and Plants, Delley, Switzerland
[3] ETH Zurich, Institute of Agricultural Sciences, Zürich, Switzerland

dario.fossati@agroscope.admin.ch

Wheat contamination with mycotoxins caused by Fusarium is of great economic importance. The current routine method of disease evaluation (single-location trial based on visual symptoms) does not yield the comprehensive data needed to accelerate breeding for resistance to Fusarium. We intend to lay the foundation for more efficient resistance breeding against Fusarium, by targeting deoxynivalenol (DON), the most important mycotoxin, and improve resistance to DON accumulation by genomic selection (GS).

A reference set (RS) of 300 cultivars and breeding lines was phenotyped in a three-location, artificially inoculated trial over two years. We evaluated resistance to Fusarium by visual scoring of symptoms, expressed as Area Under the Disease Progress Curve (AUDPC), and by measuring DON content of harvested grain by HPLC. We additionally scored plant height, heading date and anther extrusion. In the third year, a validation set (VS) of 225 new lines was integrated in the same experimental setting.

The purpose of the VS was, on the one hand, to evaluate the potential of GS by comparing the observed phenotypes with corresponding genomic predictions obtained from models trained using the RS. On the other hand, the new lines would ultimately extend the number of genotypes available for GS model training to a total of 525.

We obtained satisfactory phenotypic results for all years and most locations, with generally high and homogeneous Fusarium pressure and subsequent DON accumulation, resulting in high heritability for both AUDPC and DON. The correlation between Fusarium symptoms and DON was moderate (r = 0.54), confirming that measurement of DON is a valuable asset for resistance breeding. Correlations with other traits were low to moderate, but significant, and confirmed that both height and earliness influence Fusarium infection and should be considered into account when implementing GS.

For both Fusarium resistance traits, the first independent validation of GS through the VS yielded moderate prediction abilities (0.3 - 0.4), while prediction abilities from cross-validation schemes with the full set of available data (525 genotypes, 3 years) reached values of 0.5 to 0.6. Realistic estimates of prediction abilities for future GS-use should lie in the 0.4 – 0.5 range, and are sufficient for significant breeding progress through GS.

By the end of the project, we are thus able to employ predictions for 5’000 already genotyped breeding lines, which will help selection across all stages of the breeding program, from the choice of crossing parents up to selection of advanced lines.

#642

Fine mapping of *PmHHM*, a broad-spectrum gene conferring both seedling and adult powdery mildew resistance in a wheat landrace Honghuamai

Bisheng Fu[1], Zhixin Lin[2], Lijuan Yan[3], Qiaofeng Zhang[1], Caiyun Liu[1], Jin Cai[1], Wei Guo[1], Ying Liu[1], Wenling Zhai[1], Feng Xu[2], Shuangjun Gong[4], Jizhong Wu[1,*]

[1]Institute of Germplasm Resources and Biotechnology/Jiangsu Provincial Key Laboratory of Agrobiology, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, Jiangsu, China
[2]College of Agriculture, Anhui Science and Technology University, Fengyang 233100, Anhui, China
[3]School of Life Sciences, Henan University, Kaifeng 475004, Henan, China
[4]Institute of Plant Protection and Soil Science, Hubei Academy of Agricultural Sciences, Wuhan 430064, Hubei, China

* Corresponding author: wujz@jaas.ac.cn

Common wheat is a crucial global food crop with a direct impact on food security. Wheat powdery mildew (PM), caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), poses a significant threat to wheat yield and flour quality. The identification and utilization of broad-spectrum resistance genes against PM are essential for effective disease control. The wheat landrace Honghuamai (HHM) has demonstrated remarkable resistance in adult plants in the field and high resistance to all 25 tested *Bgt* isolates during the seedling stage, making it a valuable source of powdery mildew resistance.

This study employed five genetic segregation populations to investigate the inheritance of PM resistance in HHM at both the seedling and adult stages. Genetic analysis showed that PM resistance in HHM is determined by a single dominant gene, temporarily named *PmHHM*.

By utilizing BSA separation analysis and molecular marker mapping, the gene was fine-mapped to a genetic distance of 0.0024 cM, corresponding to a physical interval of 242.5 kb based on the IWGSC v2.1. Seven high-confidence genes were predicted, with five of them annotated as RPP13-like proteins 1 clustering in the target region.

Comparative genomic analysis revealed the presence of presence/absence variations (PAVs) in the *PmHHM* region among genomes of different ploidy wheat and common wheat varieties, leading to varying recombination rates between populations. These closely linked molecular markers will not only benefit the cloning of *PmHHM* but also facilitate the efficient utilization of the gene in breeding.

#435

Production of synthetic octaploid wheat by wild hybridization between bread wheat and aegilops tauschii

Yan Gao[1], Yoshihiro Matsuoka[2], Hisashi Tsujimoto[3], Masahiro Kishii[4], Shun Sakuma[5], Takayoshi Ishii[3,6]

[1] United Graduate School of Agricultural Sciences, Tottori University, Tottori 680-8553, Japan. [2]Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan. [3] Arid Land Research Center (ALRC), Tottori University, Tottori 680-0001, Japan. [4] Japan International Research Center for Agricultural Sciences (JIRCAS), Tsukuba 305-8686, Japan. [5] Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan. [6] International Platform for Dryland Research and Education (IPDRE), Tottori University, 680-0001, Japan

E-mail: d23a3003b@edu.tottori-u.ac.jp

Wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD) is vulnerable to high temperatures and is severely affected by global warming. There is a need to increase the genetic diversity of bread wheat to adapt to climate change. The use of wild species in bread wheat is one of the most important means of improving wheat. *Ae. tauschii* (*Aegilops tauschii* Coss., 2n=2x=14, D^tD^t) is the D genome doner of wheat.

By crossing wheat directly with *Ae. tauschii*, synthetic octaploid wheat (2n=8x=56, $AABBDDD^tD^t$) can be produced without altering the genetic composition of the wheat AABB genome. This approach is a good strategy to expand the genetic diversity of wheat D genome. In the present study, a Japanese wheat cultivar Norin 61 (N61) and CIMMYT wheat varieties Borlaug 100 (B100) were used as female parents, and 210 lines of *Ae. tauschii* were used as male for synthetic octaploid wheat production.

The synthetic octaploid wheat will be backcrossed with wheat (N61 or B100) to create lines that possess various *Ae. tauschii* D^t genome fragments within the wheat N61 or B100 genomic background. So far, forty-five lines of synthetic octaploid wheat with N61 and one line with B100 have been produced. The seed setting rates in the hybridization crosses (i.e., cross ability) varied depending on the crossing combination N61 (0%~56.3%) and B100 (0%~6.3%).

We measured the ploidy levels of 103 plants derived from 40 lines of octaploid wheat by Flow-cytometry. Out of these, 85 plants were octaploid, 16 plants were hexaploid, and 2 plants had the fewer chromosomes than 42 (hexaploidy). These ploidy levels were confirmed by the chromosome counting. These findings suggest that N61 was relatively easy to cross with *Ae. tauschii*. However, this cross ability was dependent on the genotypes of the male parents of *Ae. tauschii*.

About 82.5% of octaploid wheat maintained the ploidy and 17.5% of octaploid wheat lost the chromosomes and experienced reduction in their ploidy levels during propagation. We are in the process of producing the backcrossing population to exploit the genetic diversity of *Ae. tauschii* into bread wheat.

#104

A high throughput pipeline for phenotyping, object detection and quantification of trichomes

Catherine Gardener[1], Andrea González-Muñoz[1], Dai-Jie Wu[2], Ana Belén Perera-Rodríguez[1], Harryson Avila[1,4], Guotai Yu[1], Oadi Matny[3], Brian J. Steffenson[3], Konstanze Laquai[1], Mohamed Rekik[2], Justine Braguy[2], Silvio Giancola[2], Brande B. H. Wulff[1]

[1]King Abdullah University of Science and Technology (KAUST), Biological and Environmental Science and Engineering Division, Thuwal, Saudi Arabia. [2]Thya Technology, KAUST, Thuwal, Saudi Arabia
[3]University of Minnesota, Minneapolis, MN 55455, USA. [4]Present address: Purdue University, 610 Purdue Mall, West Lafayette, IN 47907, USA

Access to increasing amounts of high-quality DNA sequence data for many plant species is allowing for faster, more accurate gene identification. To maximize the use of this sequence data for gene identification and validation, it must be coupled to phenotype data.
However, phenotype acquisition can present a bottleneck in studies requiring many datapoints, such as diversity panels for genome-wide association studies.

Here we developed a handheld device—the Tricocam—and method for image capture and semi-automatic quantification of leaf edge trichomes in grass species of the Poaceae.
Trichomes have been implicated in abiotic and biotic stress tolerance in grasses. We also refined and implemented the AI detection processes underpinning the web-based image quantification platform from Thya Technology, to rapidly quantify leaf edge trichomes in Poaceae diversity panels.

In making the Tricocam 3D print design and AI visual detection model public, we hope to deliver useful resources for the plant science community to use or adapt for other large-scale phenotyping projects.

#374

Water ecotoxicity footprint of rainfed wheat in Brazil

Vanderlise Giongo[1], Adão da Silva Acosta[1], Álvaro Augusto Dossa[1], Anderson Santi[1], André Júlio do Amaral[1], Eduardo Caierão[1], José Eloir Denardin[1], Osvaldo Vasconcellos Vieira[1], Maria Cléa Brito de Figueirêdo[2], Marília Ieda da Silveira Folegatti Matsuura[3], José Paulo Pereira das Dores Savioli[3], Tatiane Battistelli4, Bruno Ricardo Silva4, Bruno Estefano Pires4, Mônica da Silva Santana1,5

[1]Brazilian Agriculture Research Corporation (Embrapa), Embrapa Wheat, Passo Fundo, RS, Brazil. [2]Brazilian Agriculture Research Corporation (Embrapa), Embrapa Tropical Agroindustry, Fortaleza, CE, Brazil. [3]Brazilian Agriculture Research Corporation (Embrapa), Embrapa Environment, Jaguariúna, SP, Brazil. [4]Moageira Irati Wheat Mill, Irati, PR, Brazil. [5]Edmundo Gastal Agricultural Research and Development Support Foundation, Pelotas, RS, Brazil.

In order to address the global food crisis and to promote sustainable food production through efficient use of natural resources and pollution mitigation, collaboration among researchers, policymakers, farmers, and stakeholders is essential to identify hotspots for improvement in cultivation practices. Water plays a crucial role in this process. Wheat ranks as the fourth most-produced staple food worldwide, with 783 million metric tons harvested in 2023.
Despite being the 14th largest global wheat producer, Brazil is actively working on enhancing food security by developing a sustainable wheat production system.

This initiative involves assessing the environmental impact of wheat production through life cycle assessment, focusing on a water ecotoxicity footprint (WEF), encompassing the impact categories of terrestrial, freshwater, and marine ecotoxicities. Impact results are related to 1 kg of grain, with data from 12 rainfed wheat farms in southern Brazil, adopting the scope from cradle to farm-gate.

Emissions of pesticides, heavy metals and nutrients from farm use of inputs were accounted applying the BR-Calc methods. The ecotoxicity impacts were assessed using the ReCiPe 2016 Midpoint (H) V1.08 / World (2010) H method in SimaPRO 9.5.0.2.
Terrestrial ecotoxicity values ranged from 0.43 to 1.96 kg 1,4-DCB eq kg-1, while freshwater and marine ecotoxicities ranged from 0.002 to 0.014 kg 1,4-DCB eq kg-1 and 0.006 to 0.021 kg 1,4-DCB eq kg-1, respectively.

The use of phosphate fertilizers, key input in wheat cultivation, generated emissions that significantly contributed to the WEF (38%, 56%, and 45% for terrestrial, freshwater, and marine, respectively) followed by the wheat cultivation process (37%, 20%, and 37%).
Fertilizer application lead to ecotoxicity due to depositing heavy metals in soil, transported to water resources. Productivity had a direct effect on the WEF, as evidenced by the farm producing 4.5 ton ha-1 of wheat resulting in the lowest WEF values, while the farm yielding 2.5 ton ha-1, the highest.

Thus, increasing the use efficiency of phosphate fertilizers and ensuring higher productivity are the best alternatives to reduce the WEF of Brazilian wheat.

#190

Haplotype analysis of the wknox1 gene of hexapoid wheats and their d genome progenitor

Mari Gogniashvili [1,2], Yoshihiro Matsuoka [3], Natia Tephnadze [1], Nana Kunelauri [1], Mirian Chokheli [2], Tengiz Beridze [1]

1 Institute of Molecular Genetics, Agricultural University of Georgia, Tbilisi, Georgia. 2 Scientific Research Center of Agriculture, Tbilisi, Georgia. 3 Graduate School of Agricultural Science, Kobe University, Kobe, Japan

The aim of the presented study is a genetic characterization of the hexaploid wheat (Triticum aestivum L.) by PCR-based haplotype analysis of the fourth intron of Wknox1d and of the fifth-to-sixth-exon region of Wknox1b of 20 hexaploid wheat samples. PCR-based haplotype analysis of the fourth intron of Wknox1d and of the fifth-to-sixth exon region of Wknox1b of all 20 hexaploid wheat samples was carried out.

Fifth-to-Sixth Exon Region of Wknox1b PCR-based haplotype analysis of the fifth-to-sixth exon region of Wknox1b showed that the 157-bp MITE inserted band (588 bp) is present in T. turgidum subsp. durum cv. ‘Langdon’, CS, ‘Red Doly’. This band is absent in tetraploid T. turgidum subsp. carthlicum, T. aestivum L. subsp. carthlicoides, T. aestivum subsp. macha (M1–M13). In the fourth intron of Wknox1d in common wheat, a 122-bp MITE insertion has been reported.

The MITE containing band (411 bp) was missing in all tetraploid wheat accessions and was observed in all subspecies of common wheat. In the case of subsp. macha (11 samples) the 453 bp band was observed. PCR-based haplotype analysis of the fourth intron of Wknox1d and the fifth-to-sixth-exon region of Wknox1b provides an opportunity to make an assumption that hexaploid wheats T. aestivum subsp. macha var. palaeocolchicum and var. letshckumicum differ from other macha samples by the absence of a 42 bp insertion in the fourth intron of Wknox1d.

One possible explanation for this observation would be that two Aegilops tauschii Coss. (A) and (B) participated in the formation of hexaploids through the D genome: Ae. tauschii (A) - macha (1–5, 7, 8, 10–12), and Ae. tauschii (B) - macha M6, M9, T. aestivum subsp. aestivum cv. ‘Chinese Spring’ and cv. ‘Red Doly’.

#541

Impact of reduced irrigation and heat stress on bread wheat industrial quality

H. Gonzalez-Santoyo[1], María Itria Ibba[1], L. Crespo Herrera[1], C. Velazquez-Luna[1], N. Hernandez-Espinosa[1], Huizar L. Díaz-Cisneros[2]

[1]International Maize and Wheat Improvement Center (CIMMYT), El Batan, Texcoco 56237, México. [2]Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Norman E. Borlaug, Cd. Obregón, Sonora, México

h.gonzalez@cgiar.org, m.ibba@cgiar.org, l.crespo@cgiar.org, c.velazquez@cgiar.org, n.h.espinosa@cgiar.org, diaz.huizar@inifap.gob.mx

Wheat cultivation and trade form a significant part of the global economy. As one of the most widely cultivated cereal grains worldwide, its production and trade contribute to the livelihoods of millions of people involved in farming, processing, and distribution. Wheat plays a crucial role in ensuring food security for many nations. Therefore, ensuring adequate wheat grain and processing quality under the current climate change scenario is of utmost importance and a key target for wheat improvement.

In the present study, the end-use quality of a set of 271 spring bread wheat elite lines grown under optimal conditions, reduced irrigation, and late heat stress during the 2021-2022 cycle was analyzed.

The parameters most influenced by the abiotic stresses were thousand kernel weight (TKW) (r^2=0.703), followed by test weight (TESWT) (r^2=0.61), grain protein content (GRNPRO) (r^2=0.54), and bread loaf volume (LOFVOL) (r^2=0.50). In the detailed analysis by environment, it was found that for the full irrigation environment, both test weight (81.5 kg/hl) and thousand kernel weight (53.0 g) yielded the highest values, while the lines grown under reduced irrigation exhibited the lowest grain quality, with average TESTWT values of 77.6 kg/hl and TKW values of 42.1 g.

Regarding grain and flour protein content (FLRPRO), the lines grown under late heat stress presented the highest values (GRNPRO = 14.4%; FLRPRO = 12.0%), followed by those harvested under reduced irrigation (GRNPRO = 14.0%; FLRPRO = 11.1%).
Similarly to protein content, the overall gluten strength and elasticity of the analyzed lines increased under reduced irrigation (Alveograph W = 443 x 10^{-4} J; Alveograph Ie = 62.1%) and late heat stress (Alveograph W = 394 x 10^{-4} J; Alveograph Ie = 62.5%) compared to the values obtained under optimal conditions (Alveograph W = 253 x 10^{-4} J; Alveograph Ie = 45.8%).

An average increase of 80 cm^3 was also observed in the loaf volumes of the lines grown under abiotic stress compared to those grown under optimal conditions.
Based on these results, it is clear that the grain physical quality of wheat grown under stress is reduced. However, it is evident that these conditions are generally associated with an increase in bread-making quality. Further studies across different years and using different germplasm will be required to confirm this trend and to support the development of high-quality and climate-resilient wheat.

#543

Gene expression between compatible and resistance responses of wheat

Cassidy R. Million[1], Charles F. Crane[1], Ian Thompson[1] and Stephen B. Goodwin[1]

[1]USDA-Agricultural Research Service, Crop Production and Pest Control Research Unit, 915 Mitch Daniels Blvd., West Lafayette, Indiana 47907-2054, USA

Septoria leaf blotch disease is caused by closely related but different species on cereal crops: *Zymoseptoria tritici* affects wheat but not barley, while *Z. passerinii* infects barley but not wheat. To test for differences between susceptible, R-gene incompatible and non-host resistance responses, wheat and barley cultivars were inoculated with isolates of *Z. tritici* or *Z. passerinii* and analyzed for gene expression at 1, 3, 6, 10 and 17 days after inoculation (DAI).

The highest numbers of differentially expressed genes for the R-gene interaction on barley occurred at 1 and 10 DAI, corresponding to the times of initial penetration by the pathogen and its transition from biotrophic to necrotrophic growth, while for the non-host interaction it occurred only at 1 DAI. Very few differentially expressed genes relative to the water controls were conserved across all five time points indicating large changes in gene expression over time. The highest numbers of differentially expressed genes across all time points was with the R-gene interactions.

The two R genes tested in wheat, *Stb2* and *Stb3*, each had very different patterns of expression in response to pathogen inoculation, but how much of this is due to resistance genes versus other differences between the cultivars is not known. GO and KEGG analyses were performed to try to identify possible biological functions of the differentially expressed genes. One gene for a possible SCP-like extracellular protein showed differential expression between the susceptible and non-host responses at all time points except for 1 DAI and could be a possible candidate gene for resistance.

Large differences in expression between the two resistance genes in wheat plus the wheat and barley non-host responses compared to susceptible interactions likely indicate that the different types of resistance response occur by disparate mechanisms. Lack of similarity among homologous genes in wheat and barley for the same type of response indicate that resistance mechanisms likely are species specific.

Ultimately, we hope that analyses between responses of closely related pathosystems to compatible and non-host pathogens could identify broad-spectrum resistance genes that could function across species to improve resistance in the future.

#225

Can eastern wheat break the yield-barrier: adaptability of Japanese wheat cultivars to hot and dry environments

Yasir S. A. Gorafi[1,2], Izzat S. A. Tahir[2,3] and Hisashi Tsujimoto[3]

[1] Graduate School of Agriculture, Kyoto University, 606-8502, Japan
[2] Agricultural Research Corporation, P. O. Box 126, Wad Medani, Sudan
[3] Arid Land Research Center, Tottori University, Tottori 680-0001, Japan.

Email: seragalnor.yasir.5x@kyoto-u.ac.jp

The *Rht*, the semidwarf gene of the green revolution, was introduced from the Japanese wheat ‘Norin 10’. However, recent studies highlighted that despite their significant role in the green revolution, Japanese wheat and other traditional Asian bread wheat cultivars are still underexploited for breeding.

In the light of the imminant threat of climate change and the observed temperature rise, which is already being felt, and the urgency to exploit several germplasm sources for better adaption to heat stress, we evaluated a set of 100 Japanese wheat core collections in Sudan to explore their potentiality under the hot, dry- environment of Sudan.

First, we planted the 100 lines in an observation nursery at the optimum sowing date. Out of the 100 lines, 40 flowered and matured during the Sudanese short-growing season (mid-November to mid-March). Then, these 40 lines were further evaluated in replicated trials executed in an alpha lattice design with 10 Sudanese heat-tolerant wheat cultivars in six environments (a combination of seasons, sowing dates and locations; Wad Medani and New Halfa). Mean grain yield ranged from 1778 kg/ha in Wad Medani late sowing to 2917 kg/ha in Wad Medani optimum sowing, averaging 2370 kg/ha across the six environments.

The Japanese wheat cultivars Kantou 107 and Norin 61 had better grain yield than three Sudanese heat-adapted wheat cultivars. In heading time, Naka Soushuu and Kounosu 25 were one week earlier than the earliest Sudanese cultivar ‘Bohaine.’ In grain number per spike, ‘Fujimi Komugi’ and ‘Kanto 107’ had more grains than six Sudanese cultivars while maintaining reasonable seed weight. Interestingly, ‘Norin 75’ had the highest number of spikes/m^{2,} exceeding some Sudanese heat-adapted cultivars.

Considering the big difference between the Japanese and Sudanese environments, it is interesting that some Japanese wheat cultivars performed comparably to the Sudanese heat-adapted high-yielding cultivars.

These findings could encourage a more in-depth exploration of these 40 lines to utilize their unique traits. For example, Sudan is expected to be affected by intensive heat stress; therefore, early maturing varieties would be one of the options to sustain wheat production in Sudan.

In this regard, the earliness observed in Naka Soushuu and Kounosu 25 could be a favorable trait in wheat breeding in Sudan.

#346

Australia-wide pan-genomics of multiple crop pathogen species to study virulence gene evolution

Naomi Gray[1], Mohitul Hossain[1], Pavel Misiun[1], Elzette Palmiero[2], Antonios Zambounis[3], Simon Ellwood[1], Pao Theen See[1], James Hane[1]

[1]Curtin University, [2]Centre for Crop and Disease Management, [3]Hellenic Agricultural Organization DEMETER, Institute of Plant Breeding and Genetic Resources

Globally, fungal plant pathogens are a significant threat to food security as they rapidly adapt to disease management strategies. One aspect of improving fungal disease outcomes is developing a better understanding of effector proteins. Effectors are secreted by plant pathogens to facilitate infection and are the primary determinants of fungal crop disease outcomes.

Therefore, accurate effector profiling can be used for improving crop disease resistance. However, low sequence homology between effector proteins and their location in highly plastic regions of the genome makes this challenging. Fungal pathogens often possess genomes compartmentalised into core conserved regions and variable accessory regions. Accessory regions are subjected to high mutation rates and may contain several effector genes.

The variation observed in the accessory genome is driven by a variety of mutagenesis mechanisms such as repeat-induced point (RIP) mutation, mesosyntenic rearrangements, and lateral gene transfers (LGT).

The number of available fungal genomes has increased exponentially over the last 20 years, improving the feasibility of using genomics to better understand the molecular mechanisms that drive fungal adaptation.

Currently, we have a wide range of datasets available - that include closely-related species with differing host ranges, and pathogenic lifestyles – allowing broad comparative/pan-genomics to be performed across a wide range of pathosystems spanning multiple hosts (wheat, barley, grasses, peas, chickpea, beans, lentil, lupin, pistachio, pomegranate, pear) and fungal pathogen genera (Parastagonospora, Pyrenophora, Fusarium, Blumeria, Ascochyta, Septoria, Botrytis, Sclerotinia and Rhizoctonia).

This will enable the discovery and further study of sets of genes associated with host specificity. In particular, pan-genomic datasets of Pyrenophora spp. (tritici-repentis (n=163), teres f. sp. teres (n=307) and teres f. sp. maculata (n=59)) and Parastagonospora nodorum (n=650), both infecting wheat and barley hosts, will provide new insight into wheat-, barley- and cereal-specific pathogenicity gene sets.

These comparisons will also improve our understanding of the mechanisms, taxonomic ranges and frequencies of pathogenicity-associated genome features including accessory compartmentalisation, LGT, RIP mutation, and mesosynteny. New effector prediction methods will be applied - which rank candidates based on physicochemical properties and disease phenotype association - and functionally tested using synthetic effector constructs.

#617

Detection of genetic loci for lodging related traits in spring wheat through genome-wide association study

Ginelle Grenier[1], Muhammad Iqbal[2], Curt McCartney[1], Gavin Humphreys[3], Dean Spaner[2], Belay T. Ayele[1]

[1] Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada. [2] Department of Agricultural, Food and Nutritional Sciences, Edmonton, Alberta, Canada. [3] Ottawa Research and Development Center, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada

Correspondence to: grenie19@myumanitoba.ca

Lodging, which refers to the permanent displacement of plants from their upright positions, is one of the major constraints to spring wheat (Triticum aestivum L.) production since it negatively impacts harvest efficiency, and grain yield and quality. Lodging is a quantitative trait regulated by many genes that interact with environmental factors. The main genetic approach deployed to mitigate the negative effects of lodging involves the use of semi-dwarfing genes, which may limit the yield potential of modern cultivars through decreasing photosynthetic areas.

Therefore, there is a need to identify alternate genetic components which contribute to lodging resistance without affecting photosynthesis potential of the plant. To this end, this study conducted a genome-wide association study of a diverse association mapping panel consisting of 181 spring wheat genotypes in multiple environments.

The mapping panel was phenotyped for lodging-related culm traits, including the breaking strength of the second basal internode, bending moment, and lodging index. The panel was also genotyped using the 90K iSelect BeadChip Array, resulting in a filtered set of 18,611 single nucleotide polymorphism markers used to determine their association with the lodging-related traits examined in this study.

Our analysis identified multiple quantitative trait loci associated with breaking strength, bending moment, and lodging index traits across all trial environments. The findings of this study may have potential use in marker-assisted selection for lodging resistance in spring wheat.

#652

Charting wheat germplasm resources with innovative computational tools and databases

Zihao Wang1, Yongming Chen1, Zhengzhao Yang1, Wenxi Wang1, Huiru Peng1, Zhongfu Ni1, Qixin Sun1, Weilong Guo1,*

1 Frontiers Science Center for Molecular Design Breeding, Key Laboratory of Crop Heterosis and Utilization (MOE), Beijing Key Laboratory of Crop Genetic Improvement, China Agricultural University, Beijing 100193, China

Email: guoweilong@cau.edu.cn

Wheat provides about one-fifth of the calories consumed by humans worldwide. However, with a huge genome size of ~16G bp and complex structural variations, progress in studying on wheat genomics is substantially trailed behind those of the other two major crops, rice and maize, for at least a decade.

With rapid advances in genome assembling and reduced cost of high-throughput sequencing, emerging de novo genome assemblies of wheat and whole-genome sequencing data are leading a paradigm shift in wheat research. In the past several years, we have invented a panel of novel methods for decoding the complex genomes and evolutionary histories of wheat.

Based on an observed trimodal distribution of the genetic variant densities across the genomic windows, we developed two algorithms, IntroBlocker and ggComp, for dissecting the wheat germplasm resources with haplotypes at two levels, ancestral haplotype group (AHG) and the germplasm-based haplotypes (gHap), respectively. We constructed a pan-ancestry haploblock map across tetraploid and hexaploid wheats, and revealed the ancestral mosaics foundations of A&B genomes that emerged from the admixture of six founder wild emmer lineages.

We further build an evolution model of polyploid wheat highlighting the key roles of wild-to-crop and interploidy introgressions in shaping the reticulate evolutionary trajectory of wheat. We also build a multiscale germplasm network of wheat, that is efficient in clarifying pedigree relationships, demonstrating genetic flow during breeding, identifying key founder lines, and also provide a framework for mining beneficial gene alleles.
We also build a wheat integrative gene regulatory network, wGRN, by integrating gene expression, chromatin accessibility, TF binding, and TF motif information to guide trait-associated gene discovery for wheat improvement.

In summary, the utilization of efficient tools and multi-omics data will drive a transformation in wheat breeding technologies, thereby enhancing the development of superior wheat varieties to meet the increasing food demand of the growing population under ever-changing climatic conditions.

#643

Elucidating genetic determinants for salt tolerance in spring wheat (*Triticum aestivum* L.)

Santosh Gudi[1,4], Harsimardeep S Gill[2], Serena Collins[3], Jatinder Singh[1], Devinder Sandhu[3], Sunish K Sehgal[2], Upinder Gill[1] and Rajeev Gupta[4]*

[1]Department of Plant Pathology, North Dakota State University, Fargo, ND, USA
[2]Department of Agronomy, Horticulture, and Plant Science, South Dakota State University, Brookings, SD, USA
[3]US Salinity Laboratory (USDA-ARS), Riverside, CA, USA
[4]Edward T. Schafer Agricultural Research Center, USDA-ARS, Fargo, ND, USA

*Correspondence Rajeev.Gupta@usda.gov

High concentrations of soluble salts in soil or irrigation water cause salinity stress in various crops, including wheat (*Triticum aestivum* L.). Salinity stress affects the physiological, biochemical, and agronomic traits at various developmental stages of wheat, which significantly reduce the production potential of popular cultivars in major wheat-growing areas worldwide.

Identifying and introducing salt tolerant genes into cultivars is crucial for maintaining sustainable yields under salt-affected conditions. In this study, we evaluated 228 diverse spring wheat accessions, the subset of exome sequenced panel, in greenhouse lysimeters at the US Salinity Laboratory, Riverside, CA.

Evaluation was carried out under control (with electrical conductivity of irrigation water (EC_{iw} = 1.46 dS m^{-1})) and salt stress (EC_{iw} = 14 dS m^{-1}). Salt stress significantly reduced shoot height (17.45%), root length (15.51%), tiller number (43.83%), shoot weight (44.61%), and root weight (35.82%) compared to control. However, there was a significant increase in root length by shoot height ratio (3.75%) and root weight by shoot weight ratio (28.02%) under salt stress.

Based on seedling traits and their stress tolerance indexes, four highly salt-tolerant and four highly salt-sensitive lines were identified. Multi-locus genome-wide association studies (GWAS) using 297,104 SNPs identified 487 significant SNPs associated with seedling traits under control and salt stress. Among 487 SNPs, 14 were highly-significant (LOD > 6.77), 18 were significant (LOD = 5.9-6.67), and 455 were suggestive (LOD = 4.2-5.9).

Furthermore, linkage disequilibrium (LD) based grouping consolidated 487 SNPs into 219 QTLs. Future studies must focus on developing molecular markers from these QTL regions to facilitate the marker-assisted transfer of candidate genomic regions from salt-tolerant lines into cultivar background. This will accelerate the development of salt-resilient wheat varieties, ensuring sustainable production in salt-affected areas.

Keywords: Wheat, Salinity, Stress tolerance index, SNPs, GWAS, QTLs

#236

Low-gluten and low-immunogenic RNAi wheat lines: A promising alternative for celiac and wheat allergy patients

Francisco Barro [1] (fbarro@ias.csic.es), The WheatNet Consortium [2]

Naomi Gray[1], Mohitul Hossain[1], Pavel Misiun1, Elzette Palmiero[2], Antonios Zambounis[3], Simon Ellwood[1], Pao Theen See[1], James Hane[1]

[1]Curtin University, [2]Centre for Crop and Disease Management, [3]Hellenic Agricultural Organization DEMETER, Institute of Plant Breeding and Genetic Resources

Globally, fungal plant pathogens are a significant threat to food security as they rapidly adapt to disease management strategies. One aspect of improving fungal disease outcomes is developing a better understanding of effector proteins. Effectors are secreted by plant pathogens to facilitate infection and are the primary determinants of fungal crop disease outcomes.

Therefore, accurate effector profiling can be used for improving crop disease resistance. However, low sequence homology between effector proteins and their location in highly plastic regions of the genome makes this challenging. Fungal pathogens often possess genomes compartmentalised into core conserved regions and variable accessory regions.

Accessory regions are subjected to high mutation rates and may contain several effector genes. The variation observed in the accessory genome is driven by a variety of mutagenesis mechanisms such as repeat-induced point (RIP) mutation, mesosyntenic rearrangements, and lateral gene transfers (LGT).

The number of available fungal genomes has increased exponentially over the last 20 years, improving the feasibility of using genomics to better understand the molecular mechanisms that drive fungal adaptation.

Currently, we have a wide range of datasets available - that include closely-related species with differing host ranges, and pathogenic lifestyles – allowing broad comparative/pan-genomics to be performed across a wide range of pathosystems spanning multiple hosts (wheat, barley, grasses, peas, chickpea, beans, lentil, lupin, pistachio, pomegranate, pear) and fungal pathogen genera (Parastagonospora, Pyrenophora, Fusarium, Blumeria, Ascochyta, Septoria, Botrytis, Sclerotinia and Rhizoctonia).

This will enable the discovery and further study of sets of genes associated with host specificity. In particular, pan-genomic datasets of Pyrenophora spp. (tritici-repentis (n=163), teres f. sp. teres (n=307) and teres f. sp. maculata (n=59)) and Parastagonospora nodorum (n=650), both infecting wheat and barley hosts, will provide new insight into wheat-, barley- and cereal-specific pathogenicity gene sets.

These comparisons will also improve our understanding of the mechanisms, taxonomic ranges and frequencies of pathogenicity-associated genome features including accessory compartmentalisation, LGT, RIP mutation, and mesosynteny.
New effector prediction methods will be applied - which rank candidates based on physicochemical properties and disease phenotype association - and functionally tested using synthetic effector constructs.

#334

The effect of drought stress on the root development of different winter wheat varieties

Márton György, András Farkas, Zsuzsanna Farkas, István Molnár, and Balázs Varga

HUN-REN Centre for Agricultural Research - Agricultural Institute (MGI), Martonvásár, Hungary

Climate change resulting in increasingly frequent and severe drought periods highlights the importance of research and breeding which aims to identify and produce better drought stress-tolerant wheat lines, thus increasing crop security. One of the most important components of surviving the drought period are the dynamics of root development, and the shape of root structure, which helps the plant through more efficient water absorption and also as an emergency reserve for "hard times" until the end of the generative phase.

In our experiments, we investigated the early root development of 18 winter wheat seedlings in a hydroponic system and the root structure of the same 18 full-grown (BBCH 85) wheat variates in greenhouse cultured in classified KH 30 sand in PVC tubes with 75 cm height and 11 cm diameter.

The seedlings were watered with Hoagland's solution in a half-strength dose. In order to osmotically inhibit the water absorption of the treated group, polyethylene glycol (PEG-6000) was applied at a concentration of 18%. After four days of treatment, the root parameters were measured using the WinRHIZO root scanner and software (Regent Instruments Ltd, Canada). As a result of PEG treatment, it is declarable, that the total length, surface and volume of the root in all varieties decreased, but the rate of the reduction showed significant differences among the varieties.

The greatest reduction was measured in the Babuna and Bayraktar varieties, where the reduction in length exceeded 90% (91.8%, 93.5%), while the decrease in root length of four varieties (Disponent, Aura, Salamouni and KWS Scirocco) didn't even reach 50%. All four varieties can be classified into middle and late ripening groups.

The decrease in root length (and volume) caused by PEG treatment during germination can be paralleled with the degree of drought tolerance simulated during field and rain shelter experiments. However, some cultivars, showing good drought tolerance under field conditions, did not tolerate water shortages well during the seedling stage.

Those wheats which grew in sand tubes and examined at BBCH 85 stage got complete water withdrawal from BBCH 30 stadium. As a result, total root length got significantly shorter in most of the varieties, but the average diameter got significantly thicker. Therefore, it was concluded that the water shortage resulted in a decrease in the total root length while the root volume increased under drought stress condition. The root surface area shoved high variability.

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#640

Soil ph management boosts nematode diversity and wheat productivity in Western Australia

Ramesha H. Jayaramaiah[1], Gaus Azam[2], Reza Ghaderi[3] Francesca L. Brailsford[1], Wei Xu[1], Fran C. Hoyle[1], Chris Gazey[2], Ji-Zheng He[3], and Daniel V. Murphy[1]

[1] SoilsWest, Centre for Sustainable Farming Systems, Food Futures Institute, Murdoch University, 90 South Street, Murdoch WA 6150, Australia
[2] Department of Primary Industries and Regional Development, 75 York Rd, Northam, WA 6415, Australia
[3] School of Agriculture, Food and Ecosystem Sciences, Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, VIC 3010, Australia

Soil acidity is a significant constraint to wheat production in the Western Australian (WA) wheat belt, affecting over 70% of topsoils and 50% of subsurface soils. This study investigates the complex relationships between soil acidity, nutrient cycling, and soil fauna dynamics, with a specific focus on implications for wheat cultivation. Through a combination of field sampling across the WA wheat belt and controlled glasshouse experiments, we examined how varying soil pH levels influence soil fauna communities, nutrient availability, and wheat growth parameters.

Our research revealed critical pH thresholds for optimal soil fauna activity and nutrient cycling processes essential for wheat production. The study demonstrated significant correlations between soil pH, fauna diversity, and nutrient availability, with direct implications for wheat root development, nutrient uptake, and overall crop performance.

Results showed that in WA dryland agricultural soils, approximately 0.6 million DNA copies/g soil of free-living nematodes were measured, indicating their potentially significant role in nutrient transformations. Lime application (4 t/ha) effectively managed soil acidity, leading to reduced metal toxicity and increased nutrient availability.

Notably, nematode population and diversity increased with lime application. Wheat productivity improved by 0.3 t/ha and 0.6 t/ha in the 2023 season (1 decile) under surface application and incorporation of lime, respectively. Long-term monitoring suggests these yield improvements are sustained over at least three growing seasons.

These findings provide valuable insights for developing targeted soil management strategies in WA wheat production systems and potentially other acidic agricultural regions globally. Our results suggest that optimizing soil pH through appropriate liming practices could significantly enhance wheat productivity by fostering beneficial soil faunal communities and improving nutrient cycling efficiency.
This research contributes to the development of sustainable agricultural practices that balance wheat yield optimization with long-term soil health maintenance in acidic agricultural landscapes, potentially increasing regional wheat production while promoting soil biodiversity.

Keywords: Soil acidity management, wheat production, soil fauna, nutrient cycling efficiency, lime application, free-living nematodes

#195

Identifying novel resistance to wheat stem sawfly (cephus cinctus norton) using multivariate genome-wide association studies

Mik Hammers[1*], Zachary J. Winn[2], Punya Nachappa[1], R. Esten Mason[1]

[1] Colorado State University, Fort Collins, Colorado, USA. [2] United States Department of Agriculture Agricultural Research Service, Raleigh, North Carolina, USA.

hammers@colostate.edu, zjwinn@ncsu.edu, punya.nachappa@colostat.edu, esten.mason@colostate.edu

Wheat stem sawfly (Cephus cinctus Norton) is grass feeding insect native to North America. Damage from the pest predisposes the wheat to lodging, or cutting, which disrupts agricultural practices and is responsible for an estimated $350 million USD loss annually.

Since most of the lifecycle of the wheat stem sawfly occurs within the stem, traditional pest management measures such as pesticides are not as usable. Therefore, the use of other management practices such as biocontrols and genetically resistant wheat cultivars are widely studied. Currently, the only characterized genetic resistance is the solid stem trait (SSt1) on chromosome 3B.

While the solid stem trait helps mitigate damage by limiting movement of the sawfly within the stem, it is an environmentally variable trait. Additional genetic resistance would allow for improved defenses against wheat stem sawfly and limit damage to grower fields.

To identify potential genomic regions of interest for non-solid stem resistance, we observed a historic panel of 1110 Colorado State University wheat breeding program lines from 2014 to 2023 that had cutting, heading date, and solid stem rating data.

Multivariate genome wide association studies using one, two, and all three traits were utilized. This research study is ongoing, so results are not yet finalized. We expect final results within the next couple months.

#115

Exploring wheat blast resistance genes in Japanese wheat accessions

Kei-ichi Sota[1], Soichiro Asuke[2], Motohiro Yoshioka[2], Muhammad Rezaul Kabir[3], Pawan Kumar Singh[4], Yukio Tosa[2], Hirokazu Handa[1]

[1] Laboratory of Plant Breeding, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto 606-8522, Japan
[2] Laboratory of Plant Pathology, Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan
[3] Wheat Breeding Division, Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur, 5200, Bangladesh
[4] International Maize and Wheat Improvement Center (CIMMYT), El Batan, Texcoco, Mexico

email: hirokazu@kpu.ac.jp

Wheat blast caused by *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) pathotype *Triticum* (MoT) was first observed in Brazil in 1985 and has recently spread to South Asia and Southern Africa, potentially becoming a pandemic disease. Several resistance genes/genomic regions have been identified in wheat against wheat blast, including the 2NS/2AS translocation from *Ae. ventricosa*, *Rmg7*, and *Rmg8,* but the number of reported resistance genes is still limited. Therefore, it is desirable to identify new resistance genes and to breed durable resistant varieties by pyramiding diverse resistance genes.

We have screened a total of 192 Japanese wheat accessions in the wheat blast precision phenotyping platform (PPP) at Jashore, Bangladesh. From this, we identified four accessions (R1, R2, R3, and R4) that exhibited relatively strong resistance in the field. These four accessions were then subjected to indoor inoculation tests with MoT isolate Br48 carrying *AVR-Rmg8* (the avirulence gene corresponding to *Rmg8*) and Br48ΔA8 strain/isolate lacking *AVR-Rmg8*. Both R3 and R4 lines showed resistance to both the isolates, indicating that they possess a novel resistance gene distinct from *Rmg8*.

Further segregation analysis of resistance in the F_2 population obtained by crossing R3 or R4 with susceptible accessions revealed that resistance and susceptibility segregated at nearly 3:1. These results suggest that a single dominant gene controls the resistance in these two accessions.
However, the degree of resistance was relatively stronger in R3 than in R4. In addition, these two accessions responded differently to inoculation with MoT isolates from Bangladesh, indicating the presence of different resistance genes in the two accessions.

A genome-wide association study (GWAS) using the field trial data from 192 accessions (three-year average of resistance in each line) detected MTAs above $-\log_{10}(p)=3.5$ on chromosomes 2B, 4B, and 7A. Currently, we are mapping the resistance gene regions using $F_{2:3}$ populations derived from R3 and R4.

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#470

Unlocking the genetic potential of plant genetic resources to accelerate wheat improvement

Matthew Hayden[1,3], Gabriel Keeble-Gagnere[1], Josquin Tibbits[1,3], Kerrie Forrest[1], Sally Norton[2]

[1] Agriculture Victoria, AgriBio, Centre for AgriBioscience, Bundoora, VIC, Australia. [2] Australian Grains Genebank, Agriculture Victoria, Horsham, VIC, Australia. [3] School of Applied Systems Biology, La Trobe University, Bundoora, VIC, Australia

Wheat grain makes an important contribution to the Australian economy in terms of gross agricultural production value and export income. This success is predicated on the availability of improved wheat varieties, whose development is supported from the outset by access to plant genetic resources (PGRs) imported and conserved through the Australian Grains Genebank (AGG) and subsequent underpinning research, development and extension. In the context of global food security and climate change, PGRs are important for the development of climate-change resilient, high-yielding and high-value wheat varieties into the future.

Here, we describe a 5 year $30M strategic investment by the Grains Research and Development Corporation and Agriculture Victoria that is transforming the AGG from a traditional seedbank into a bio-digital resource center. The investment aims to unlock the genetic potential of PGRs, which includes about 50 000 wheat accessions, through digital and genomic innovation to enable the AGG to acquire, curate and distribute PGRs more sustainably and efficiently, whilst ensuring that the Australian grains industry has facilitated and informed access to the plant biodiversity it needs to sustain national productivity and resilience targets into the future.

#639

Implementation and evaluation of genomic selection in a recurrent selection wheat pre-breeding scheme

Lezaan Hess[1], Willem Botes[2], Aletta Ellis[3], Rayganah Rhoda[4]

Department of Genetics, University of Stellenbosch, Stellenbosch, South Africa

The wheat production areas of South Africa are affected by several diseases. Among them, the three rusts, namely stem rust, leaf rust and stripe rust caused by *Puccinia graminis* f. sp. *tritici* Eriks. & E. Henn., *Puccinia triticina* Eriks and *Puccinia striiformis* West. f. sp. *tritici* Eriks. & E. Henn. respectively, are the most prominent. Their severity and importance in a specific area is largely influenced by climatic factors and they can lead to yield losses of more than 50% (Terefe et al. 2024).

Wheat rusts can effectively be controlled by the deployment of multiple resistance genes that confer durable resistance. The utility and durability of resistance genes can be extended considerably if multiple genes are combined in new varieties, thus creating more complex genetic barriers that will less likely be overcome by the pathogen (Mapuranga et al. 2022). One of the most effective strategies to incorporate resistance genes is by a structured process of pre-breeding based on biotechnology tools such as molecular marker-assisted selection (MAS), the production of doubled haploids (DH) and genomic selection (GS).

With the rapid decline in genotyping costs and the development of statistical methods to accurately predict marker effects, GS has been accepted as a molecular breeding tool for the improvement of complex traits where many loci of small effects control the trait (Crossa et al. 2014; Crossa et al. 2017). It has the potential to reduce the number of cycles in a breeding process and decrease phenotyping costs. Moreover, it facilitates the use of recurrent selection in wheat, a well-documented breeding method that increases favourable alleles in a population (Rutkoski et al. 2015).

Therefore, this study aims to implement genomic selection in a recurrent selection pre-breeding program and evaluating its ability to rapidly and accurately predict rust resistant genotypes to be selected as crossing parents for crop improvement and potential release as cultivars in breeding programs.

#536

Pyramiding stem rust resistance genes into South African cultivars

Tsepiso Hlongoane[1*], Timmy Baloyi[1], Toi Tsilo[1], Hussein Shimelis[2]

1Agricultural Research Council, Small Grain, P Bag X29, Bethlehem, South Africa, 9700
2University of KwaZulu Natal, South Africa, College of Agriculture, Engineering and Science, Pietermaritzburg campus, Scottsville, 3209

Correspondence: HlongoaneT@arc.agric.za

As the demand for wheat grows rapidly across the globe, Ug99 responsible for tremendous economic losses continuously evolve and spread threatening food security. Developing wheat varieties with moderately effective slow rusting genes and race specific genes is useful in developing more stable and long lasting resistance to curtail the evolution of Ug99 and newly aggressive stem rust races.

This study aimed to pyramid diverse sources of resistance, *Sr25*, *Sr26* and a slow rusting resistance gene *Sr2*, in the background of three locally adapted bread wheat cultivars because of their effectiveness against Ug99 and other stem rust races. Specific markers for each gene; *csSr2*, *BF145935* and *Sr26#43* and *BE518379* for *Sr2*, *Sr25* and *Sr26* respectively were used to confirm the presence of all the three genes that help to expedite breeding technologies through marker assisted selection (MAS). F$_1$ lines carrying the pyramided genes were selected and crossed to the recurrent parents.

These lines were checked for homozygosity for the three genes up to the BC$_3$F$_2$ generation.
A total of 64 lines carrying all the three stem rust resistant genes were obtained and screened at both seedling and adult plant stage for their infection response and disease severity to the currently prevalent *Pgt* races in South Africa. Marker assisted background selection accelerated recovery of the recurrent parents.

The lines developed in the present study provide improved versions of the three recurrent parents with good disease resistance of stem, leaf and stripe rust and desirable agronomic performance. The balance between desirable agronomic traits and excellent disease resistance would promote the use of these lines and to provide immediate useful germplasm for disease resistance.

#424

Identification and validation of qtls for heading date in Korean doubled haploid lines

Sumin Hong[1], Sun-Hwa Kwak[1], Youngjun Mo[1], Chul Soo Park[1,*]

[1]Department of Crop Science and Biotechnology, Jeonbuk National University, Jeonju 54896, Republic of Korea
Sumin Hong: qkqh7695@jbnu.ac.kr

*Corresponding Author C.S. Park: pcs89@jbnu.ac.kr

Understanding the genetic mechanisms underlying heading date is essential in wheat breeding to maximize productivity in different environments. As Korean wheat breeding programs have focused on developing early heading cultivars since the 1970s, most recent commercial cultivars already carry the alleles for early heading at the major genes such as VRN1 and PPD1.

This study aimed to identify new genetic factors influencing heading date by dissecting Quantitative Trait Loci (QTL) associated with days to heading date (DHD) in Korean Doubled Haploid lines. Ninety-four lines were evaluated for eight yield-related traits, including DHD, over three growing seasons (2017, 2018, and 2019).

A linkage map was constructed using 641 SNPs from 35K SNP Array, leading to the identification of three stable quantitative trait loci (QTLs) associated with days to heading date (QDhd.jbnu-3B, QDhd.jbnu-6B, and QDhd.jbnu-7D). Compared to the lines carrying the allele for late heading, those carrying the allele for early heading at QDhd.jbnu-3B, QDhd.jbnu-6B, and QDhd.jbnu-7D headed 3.1, 2.0, and 1.7 days earlier, respectively.

As the alleles for early heading at all three QTLs were associated with decreased kernels per spike, caution is required when deploying these alleles to minimize the negative impacts on yield. Nonetheless, the accumulation of alleles for early heading at the three QTLs resulted in an acceleration of heading date by approximately one week, indicating that these QTLs provide useful genetic resources for fine-tuning heading date.

We also developed a KASP marker for QDhd.jbnu-3B, the most impactful QTL for accelerating heading, and validated using 49 Korean cultivars. Cultivars with the early-heading allele headed 5.5 days earlier than those with the late-heading allele, suggesting that this KASP marker will be useful for further marker-assisted selection in Korea. This study provides valuable information for developing wheat cultivars with optimal heading dates.

#425

Correlation between root system architecture, coleoptile length, and grain characteristics in korean wheat cultivars

Sumin Hong[1], Sun-Hwa Kwak[1], Youngjun Mo[1], Chul Soo Park[1,*]

[1]Department of Crop Science and Biotechnology, Jeonbuk National University, Jeonju 54896, Republic of Korea
Sumin Hong: qkqh7695@jbnu.ac.kr

*Corresponding Author C.S. Park: pcs89@jbnu.ac.kr

Root characteristics are important to increase water utilization rate in water-scarce environmental conditions due to climate change, but few studies have been conducted in Korean wheat. This study aimed to evaluate the root characteristics of 37 Korean wheat cultivars at early seedling stage and analyze the relationship between seedling growth and grain characteristics.

A total of six root traits and coleoptile length were evaluated from images captured seven days after seeding on germination paper, and grain characteristics, including embryo size, thousand kernel weight (TKW), and test weight (TW), were measured from dried seeds harvested over two years (2021 and 2022). Seminal root length showed a positive correlation with maximum depth and total root length, but a negative correlation with root number and seminal root angle.

Embryo length exhibited a significant positive correlation with the number of roots and coleoptile length, while TKW and TW have no correlation with root characteristics. We also conducted genotypic analysis of semi-dwarfing genes (RhtB1, Rht-D1) and grain size related genes (TaCWI-4A, TaCWI-5D, TaGW2-6A, TaSus2-2B). Rht-D1b was associated with decreased maximum depth, increased root angle, and reduced coleoptile length, indicating their negative impact on early seedling growth.

The alleles for large grain size (TaCwi-A1a, Hap-5D-C, Hap-L, and Hap-6A-G) at all four genes were associated with increased number of roots and maximum depth, indicating that cultivars with large grain size have favorable seedling growth. Korean wheat cultivars were clustered into three groups: Cluster I with fifteen cultivars, Cluster II with nine cultivars, and Cluster III with thirteen cultivars, based on root, grain, and coleoptile characteristics. Cluster II exhibited the largest grain size and TKW, as well as the longest coleoptile length and seminal root length, suggesting favorable early seedling conditions.

However, caution is required when developing cultivars with superior root characteristics to minimize the negative impact of large grain size on yield and quality, as it increases the risk of pre-harvest sprouting and insufficient grain filling in Korea due to the short maturation period. Our study provides valuable insights for selecting wheat cultivars with optimal root growth and grain characteristics to ensure stable wheat production.

#328

Molecular genetic examination of a main circadian clock gene (*CCA1*) in hexaploid wheat

Ádám d. Horváth [1, *], Tibor Kiss[1,2], , Zita Berki[1], Ádám Horváth[1], Balázs Kalapos[1], Krisztina Balla[1] , András Cseh[1], Ildikó Karsai[1]

[1] Agricultural Institute, Centre of Agriculture, ELKH, Martonvásár, Hungary
[2] Food and Wine Research Institute, Eszterházy Károly Catholic University, Eger, Hungary

The circadian rhythm is an endogenous regulatory mechanism (autonomous oscillator) that enables the plants to synchronize their internal biological processes with the changes in daily temperature and light conditions of the external environment. It also participates in the regulation of photosynthesis, carbohydrate biosynthesis, biotic and abiotic stress responses. The regulatory mechanism of the plant circadian rhythm has already been significantly explored in *Arabidopsis* and many homologous genes have been described in wheat, but only a few of these have been studied more intensively.

Therefore, (1) we determined the daily expression patterns of a major circadian clock gene (*CCA1*). Three winter wheat cultivars with different plant development and genetic diversities were examined (‘Mv Toborzó’ /AT1, early heading/, from Hungary, ‘Tommi’ /AT3, late heading/, from Germany and ‘Charger’ /AT20, late heading/, from Great Britain) under four controlled environmental conditions (18 °C vernalized/unvernalized, 25 °C vernalized in the phytotron and 14-18 °C vernalized in the greenhouse) applying long photoperiod (16h). The leaf sampling started at 6:00 a.m. (1 h after the start of the light period - ASLP), and it was carried out every three hours, for two consecutive days (48h) using two-week old plants.

The relative gene expression was normalized with three housekeeping genes. In parallel to the gene transcription studies (2) we also carried out *in silico* researches using an online database (https://plants.ensembl.org). The sequenced region of *CCA1* was analyzed and compared with CLC Genomics Workbench 3.6.5. program to determine the possible mutations. In addition, the phylogenetic tree of *CCA1* was also constructed with MEGA 11 software to examine the possible evolutionary diversities between the homologue genes of *Arabidopsis* and two cereal species (*Hordeum vulgare* and *Triticum aestivum*).

The expression of *CCA1* showed a definite diurnal activity irrespective of genotypes. This finding was in a good agreement with the expression of homologue gene in *Arabidopsis*. In the case of the early cultivar (‘Mv Toborzó’) the peak expression of this gene nearly doubled at 3h ASLP under 18 °C (vernalized). According to the phylogenetic analysis of *CCA1*, the genotypes were positioned in five distinctly separated subgroups.

A stronger association between the subgroups and the genomes of different species (7A, 7B, 7D of hexaploidy wheat, 7H of barley and 2G of *Arabidopsis*) was observed, that was due to the length variation in intron 3. A larger insert section of intron 3 region was identified in barley (1913 bp), which was even longer in hexaploid wheat (3300 – 3600 bp) compared to the relevant region of the homologue gene in *Arabidopsis* (479 bp). In addition, a few single nucleotide polymorphisms (SNPs) were also identified in the exon 5 of hexaploid wheat.

One non-functional mutation was found both in chromosome 7A in ′Landmark’ (4591 bp) and in chromosome 7B in ′Lancer’ varieties (5240 bp). A synonymous mutation was found both in ′Chinese Spring’ (GGG (Gly)-GAG (Glu)) and in ′Lancer’ (GTG (Val)-GCG (Ala)) in chromosome 7B. Moreover, both ′Kariega’ and ′Lancer’ have a same functional mutation (GAC (Asp)-AAC (Asn) in chromosome 7D.

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#585

Characterising disease tolerance in wheat

Robabeh Hosseini[1], Dion Bennett[2], Ayalsew Zerihun[1] Mark R. Gibberd[1], Fatima Naim[1]

[1] Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Bentley, Western Australia 6102, Australia. [2] Australian Grain Technologies, WA

robabeh.hosseini@postgrad.curtin.edu.au, dion.bennett@agtbreeding.com.au, fatima.naim@curtin.edu.au, m.gibberd@exchange.curtin.edu.au, a.zerihun@curtin.edu.au

Towards future food security, it is necessary to improve the durability of crop plants against devastating diseases that result in large yield losses. Plants have two defence mechanisms to cope with disease pressure: resistance and tolerance. Although much research is dedicated to characterising disease resistance and susceptibility genes, very little is known about cellular mechanisms that enable cereal crops to tolerate foliar diseases in the Australian context.

This study aims to generate foundation knowledge to assess Australian wheat cultivars' ability to tolerate foliar fungal diseases of concern. For this, a field trial was established in York, Western Australia, and a set of 24 wheat genotypes were exposed to natural infection with predominant fungal diseases.

We applied a combination of high-resolution micro and macro phenotyping and molecular characterisation techniques to associate macro phenotypes to the amount of pathogen present within the leaf. Understanding the phenotypic and genotypic mechanisms of tolerance will, in turn, enable breeders to select from a wider pool of traits to breed for more durable crop plants. I will present our preliminary results on the progress we have made with assessing tolerance.

#466

Identifying genomic regions controlling resistance to foliar diseases in a anadian spring wheat population

Muhammad Iqbal[1,*], Kassa Semagn[1], Klaus Strenzke[1], Izabela Ciechanowska[1], Colin Hiebert[2], Brent McCallum[2], Dean Spaner[1], Gurcharn S. Brar[1]

[1] Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada.
* Email: mi1@ualberta.ca
[2] Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden, MB, Canada
[3] Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Morden, MB, Canada.

This study aimed to identify genomic regions controlling leaf rust, stripe rust, and leaf spotting resistance in a recombinant inbred line (RIL) population derived from two Canadian spring wheat cultivars, AAC Ryley and AAC Tenacious. The population, consisting of 279 RILs and the two parents, was genotyped using the 90K wheat Infinium iSelect SNP array.

Evaluation for disease resistance was conducted over multiple years and locations: leaf rust in Edmonton, Alberta (four years), Morden, Manitoba (two years); stripe rust in Edmonton, Alberta (three years), Creston, British Columbia (two years); and leaf spotting diseases in Edmonton, Alberta (three years). Preliminary QTL analysis utilizing 9015 polymorphic SNP markers and mean disease scores revealed 32 significant regions associated with resistance.

Thirteen regions were identified for leaf rust on chromosomes 1A, 3A, 3B, 4A, 4B, 4D, 5A, 5D, 7A, and 7D. Twelve regions were associated with stripe rust resistance on chromosomes 2B, 2D, 3A, 3D, 4A, 4D, 5A, and 7D, while five regions were linked to leaf spotting resistance on chromosomes 4A, 4D, 5A, 6D, and 7A.

The leaf rust QTLs explained 3-19% of the phenotypic variation, stripe rust QTLs explained 0.4-18%, and leaf spotting QTLs explained 4-12%. Notably, the QTL for leaf rust resistance on chromosome 7D is hypothesized to be the adult plant leaf rust resistance gene *Lr34*, and the QTL on 7A is likely the leaf rust resistance gene *LrCen*. Ongoing analysis seeks to determine the novelty of the other identified QTLs.

#89

Effective accumulation of target genes with consecutive crossing and speed breeding

Goro Ishikawa[1, *], Fuminori Kobayashi[1], Takeyuki Okada[1], Makoto Tougou[1, 3], Koichi Hatta[1, 4], Keiko Kizawa[2], Shota Watanabe[2], Katsuyuki Hayakawa[2]

[1] Institute of Crop Science, National Agriculture and Food Research Organization, Tsukuba, Ibaraki 305-8518, Japan. [2] Cereal Science Research Center of Tsukuba, Nisshin Flour Milling Inc., Tsukuba, Ibaraki 300-2611, Japan

[*] goro@affrc.go.jp

Present address:
[3] Agriculture, Forestry and Fisheries Research Council, Ministry of Agriculture, Forestry and Fisheries, Chiyoda-ku, Tokyo 100-8950, Japan. [4] Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, Kasai, Hokkaido 082-0081, Japan

For developing cereal cultivars with improved yield, quality, and other complex traits, it is essential to establish a robust technology that can accumulate many useful genes in a short period of time. Here, we accumulated three to five genes within 1 year by combining consecutive crossing, high-throughput genotyping, and a speed breeding method (accelerating generation advancement).

First, we selected one allele of each of 11 genes in wheat genetic resources as targets for accumulation. Using the spring wheat ‘Yumeshiho’ as a recurrent parent, we conducted five consecutive crosses to produce near isogenic lines (NILs) for each target gene by selecting heterozygous individuals using Kompetitive Allele Specific PCR (KASP) markers.

This step took 18 months with speed breeding. Next, to accumulate the target gene alleles, we crossed the NILs with each other and repeated crossing for three generations. During these steps, the frequency of the target alleles was gradually increased, and individuals homozygous for the target alleles in three genes were obtained within 1 year.

By three consecutive crosses, a population in which the frequency of target alleles was around 19% was developed, and individuals homozygous or heterozygous for the target allele in up to five genes were obtained. In general, a large population size is required to accumulate genes in a short period of time. Here, however, the population size per generation was kept at 384 individuals for the convenience of KASP genotyping, which was performed in 384-well PCR plates.

To investigate whether the technology can be applied to other genetic backgrounds, we examined a method called “seed-green vernalization” using cultivars with different vernalization requirements. Cultivars with winter alleles in three Vrn-1 genes usually require about two months for vernalization.
However, using this method, about 30 days of vernalization was sufficient to fully vernalized and the time from sowing to heading was 60 days. The result suggests that this technology can be applied to cultivars with different backgrounds.

#250

Effectiveness of powdery mildew resistance genes in European wheat

Bulat Islamov[1], Marina Tikhonova[2]

[1]Leibniz Institute of Plant Genetics and Crop Plant Research, Corrensstraße 3, Seeland OT Gatersleben 06466, Germany
[2]Centre of Estonian Rural Research and Knowledge, J. Aamisepa 1, Jõgeva vald 48309, Estonia

islamov@ipk-gatersleben.de
marina.tikhonova@metk.agri.ee

Wheat is the most widely grown crop in the Baltic Sea Region. Although wheat production has seen a significant increase in recent years, thanks to the availability of locally adapted varieties and enhanced agricultural practices, it still faces challenges from adverse weather conditions and disease pressure. Powdery mildew is a major biotrophic plant pathogen that significantly impacts wheat production in the region.

However, the deployment of resistance gene combinations effective at different developmental stages using marker selection can help control the disease. In this study we evaluated seedling and adult plant resistance of wheat germplasm and tested the applicability of marker selection. We used 55 spring and winter wheat varieties widely grown in the region as well as wheat lines with known resistance genes as controls to be challenged by a diverse collection of European powdery mildew isolates. We hypothesised that seedling resistance to a wide spectrum of isolates could predict resistance observed in the field.

We postulated the presence of common resistance genes by comparing seedling resistance patterns of wheat varieties with those of control lines, for which resistance genes are already described. The existence of shared resistance genes was further confirmed by using specific DNA markers to Pm1a, Pm3 and Pm6. In addition, we screened the material for adult plant resistance genes and visually assessed disease severity in the field.

According to seedling resistance data, Pm1a, Pm3d and Pm6 are only partially effective against the local powdery mildew population and the protective effect can be increased by combination of two or more Pm genes. Powdery mildew severity data on adult plants in the field experiment confirmed our hypothesis that broad spectrum seedling resistance is also effective in adult plants.

Even though wheat varieties with a broader spectrum of seedling resistance exhibited lower disease severity in the field, a small portion of varieties lacking seedling resistance also showed lower disease severity compared to the control lines. This can be explained by the presence of yet undescribed adult plant resistance genes in these lines.
Identification of seedling resistance to multiple isolates combined with disease severity data obtained from the field trial and DNA marker analysis can aid selection for powdery mildew resistance.

#590

Harnessing barley’s non-host resistance for wheat improvement: A microphenomics approach

Bulat Islamov[1]*, Stefanie Lück[1], István Molnár[2], Simon Krattinger[3], Philipp Schulz[4], Dimitar Douchkov[1]**

[1]Leibniz Institute of Plant Genetics and Crop Plant Research, Corrensstraße 3, Seeland OT Gatersleben 06466, Germany. [2] Agricultural Institute, Centre for Agricultural Research Biology Resources, Martonvásár, Brunszvik u. 2, 2462, Hungary. [3] King Abdullah University of Science and Technology, Cereal Genetics and Genomics Lab, Thuwai, Jeddah 23955-6900, Kingdom of Saudi Arabia. [4] Julius Kühn-Institute (JKI) - Federal Research Institute for Cultivated Plants, Institute for Field Crops and Grassland Protection, Messeweg 11/12, 38104 Braunschweig

* Presenting author. E-mail: islamov@ipk-gatersleben.de
** Corresponding author. E-mail: douchkov@ipk-gatersleben.de

Wheat production faces significant challenges from fungal plant pathogens capable of adapting to commonly used resistance genes. Consequently, there is an increasing need for new, durable, disease-resistant genes to prevent pathogen-induced yield losses without relying on chemical plant protection products.

Although the barley gene pool has been identified as a potential source of resistance genes effective against wheat pathogens, barley genetic resources have not yet been extensively utilized for wheat improvement. This study aims to reveal the genetic landscape of barley resistance against non-adapted wheat pathogens, specifically powdery mildew and stripe rust, within a genetically diverse barley panel.

By employing a novel micro- and macro-phenotyping approach and leveraging a high-throughput phenotyping platform alongside available genotypic data, we aim to uncover the genetic basis of non-host resistance that could be harnessed to develop durable disease resistance in wheat.

#582

Genome-editing of five starch synthesis genes produces highly resistant starch and dietary fiber in barley

Qiang Yang[1,2,3], Jean-Philippe Ral[3], Yuming Wei[1], Youliang Zheng[1], Zhongyi Li[3*], Qiantao Jiang[1*]

[1] State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Triticeae Research Institute, Sichuan Agricultural University, Chengdu, Sichuan, 611130, China
[2] Institute of Quality Standard and Testing Technology Research, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China
[3] CSIRO Agriculture and Food, Black Mountain, Canberra, ACT 2601, Australia

A barley starch synthase IIa (ssIIa) null mutant has been commercialized as a healthy grain of its benefits for human health; however, it has a shrunken grain and reduced yield. Here, we used a multiplex genome-editing system to produce single, double, and triple null mutations of five barley starch synthesis enzyme genes for either increasing the content of beneficial grain ingredients or increasing the grain yield compared to ssIIa mutant.

The grain ingredients of these mutants were analyzed, and the ssIIa null mutant exhibited the highest content of β-glucan. The sbeIIasbeIIb and ssIIasbeIIasbeIIb mutants produced amylose and resistant starch over 86% and 12%, respectively, which were extremely higher than that in the ssIIa mutant. Furthermore, these mutants have greater 1000-grain weight, and less fructan and dietary fiber contents than those of the ssIIa mutant.

The ssIIassIVa mutant produced similar resistant starch content and 1000-grain weight, higher levels of fructan compared to the ssIIa mutant. The ssIIassIIIassIVa mutant had a similar 1000-grain weight, and less resistant starch but more fructan and dietary fiber than the ssIIa mutant. The ssIIassIIIa mutant had a greater 1000-grain weight, similar resistant starch content, and lower levels of fructan, and dietary fiber compared to the ssIIa mutant.

Three mutants, ssIIassIVa, sbeIIasbeIIb and ssIIasbeIIasbeIIb, exhibited improved levels of amylose and dietary fibre and/or a higher grain weight compared with the single ssIIa mutant. These were determined to be the best choices among the five polygenic mutants for future applications in the production of healthy food products.

Our study demonstrates that Cas9-mediated multiplex gene editing is feasible for modifying starch to generate grain with higher RS contents than targeted editing of single genes and provides a theoretical basis and genetic resource for breeding barley with improved health benefits.

#608

Genetic basis analysis of tillering angle dynamics in common wheat

Liujie Jin[1], Yunzhen Li[1], Wenhao Yan[1, *]

[1]National Key Laboratory of Crop Genetic Improvement, Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan, 430070, China

*Correspondence: Wenhao Yan (yanwenhao@mail.hzau.edu.cn)

Tillering is a special branch formed during the growth and development of gramineous crops such as wheat, and during overwintering, when the angle between wheat tillering and the main stem and the ground (tillering angle) is reduced to the extreme, wheat creeping grows. Creeping growth affects agronomic traits such as wheat yield, but there are relatively few genetic studies on the dynamic changes of wheat tillering angle, especially tillering angle.

The QTL mapping of this trait and the discovery of related candidate genes are of great significance for the improvement of wheat plant type and yield. In this study, the association analysis of Yangmai 16/Zhongmai 895 DH population and the natural population was used to investigate the phenotypic data of wheat tillering angles at the overwintering stage, and two significant QTLs were identified through the association analysis of GWAS populations, which were mainly distributed on chromosomes 2B and 5A, among which QTa-5A was mainly concentrated in about 589Mb, which happened to be the interval of vernalization gene VRN1A.

The SNPs on chromosome 5D are concentrated at about 469 Mb, which is the interval of the vernalization gene VRN1D. The QTL mapping results of DH population showed that the QTL controlling creeping was located on chromosome 5D, which happened to co-localize with the natural population.

Combined with the results of GWAS and DH population mapping, it was speculated that VRN1 may be a candidate gene and the functional verification was carried out, and the transgenic results showed that the mutant vrn1 could significantly increase the tillering angle, indicating that VRN1 negatively regulated the tillering angle of wheat.

In the later period, the tillering angle dynamics of the whole growth period of the natural population were studied, and the R2 measured by machine measurement and manual measurement was 0.832, and it was found that QTa-5A did not drive before low temperature and at the beginning of low temperature, and when it lasted for a period of low temperature, QTa-5A gradually exercised its function and continued for a period of time to control the creeping change of wheat, and the QTL function was gradually inhibited when the temperature increased.

The dynamic change of "erect-prostrate-semi-prostrate-erect" plant type provides a reference.

#182

Monarka inia wheat: potential productive for southern Chile

Claudio Jobet[1]*, Iván Matus[1], Dalma Castillo[1], Javier Zuñiga[1], Rafael López[1], Sebastián Meier[1] and Christian Alfaro[1]

[1] Instituto de Investigaciones Agropecuarias (INIA). Chile

Email: cjobet@inia.cl

MONARKA INIA is a new winter wheat variety (Triticum aestivum L.) developed by the Wheat Breeding Program of INIA Carillanca (38°50'S and 72°25'W) at the Araucanía Region. This variety was selected from a nursery received from the NordSaat of Germany, which arrived in 2015.
Due to its performance and agronomic characteristics, was selected among many other lines and was evaluated in a preliminary winter yield trial during 2016/17. Subsequently was included in the elite winter wheat yield trial since 2017/18, which was sown in different locations in southern Chile, from Chillán (36°31'S/71°54'W) to Purranque (40°51'S/73°09'W).

Monarka-INIA, in average outperformed, in all locations, compared with the INIA´s control varieties Maxwell, Rocky-INIA and Kiron-INIA. It was similar to Chevignon, a European cultivar belongs to ASUR Plant Breeding, which is currently the most sown variety in France. The yield of Monarka-INIA in average of all locations and for all evaluated years was 12.7% higher than Maxwell; 14.5% higher than Rocky-INIA and 13.6% higher than Kiron-INIA.

Compared with Chevignon, Monarka-INIA yielded in average 2.7% higher than Chevignon, with not significant statistically differences. Monarka-INIA has a good hectoliter weight (over 82.5 kg/hL-1) and a hard grain texture. It has a protein percentage over 9,0%, a sedimentation volume over 38 ml, and a wet gluten content of 26%, classifying it in the category of medium type wheat (NCh 1237-2000).
In average its alveographic characteristics (rheological parameters) are good, with a work value W of 222.5, adequate resistance to stretching (120.2), a dough extensibility (L) equal to 49.0. Its mass toughness (P/L ratio) is slightly above 2.0, which may require the use of ingredients that improve extensibility.

The other outstanding characteristic of this variety is the color of its flour, which is white, and therefore, more accepted by the milling industry where this characteristic is increasingly demanded.

#185

Monarka inia wheat: production circuit for the Southern Chile

Claudio Jobet1*, Iván Matus1, Dalma Castillo1, Christian Alfaro1, Juan Lobos1, Jaime Santander2 and Juan Carlos García3

1Instituto de Investigaciones Agropecuarias (INIA), Chile. 2SOFO, Temuco, Chile. 3Universidad de la Frontera, Escuela de Agronomía, Temuco, Chile.

*email: cjobet@inia.cl

As a way to evaluate the yield potential of Monarka-INIA under farm or commercial conditions, a ***productive circuit*** was conducted in an area of approximately 10 hectares with four farmers in four locations, Collipulli, Freire, Radal and La Unión, of the southern area of Chile.

The crop was conducted under the farmer agronomic management. The objective was to break their own production records. An agreement was established between INIA and each of participating farmers in order to regulate the final destination of the production as well as the control of the weighing and surface area by an external Minister of Faith.

As a result, Monarka-INIA reached 16.33 tons per hectare with a high quality grain in Collipulli. The farmers of Freire and Radal managed to exceed 15.60 and 14.60 tons per hectare, respectively, being for all of them a production record and also superior quality. In the case of La Unión, yield was affected by winter conditions, soil and rotation problems; however, despite this, Monarka-INIA managed to exceed 13.80 tons per hectare.

In overall, the average of the production circuit exceeded 15.05 tons per hectare with an intermediate to strong grain quality, in a condition of good agronomic management and with a relatively wet spring that in critical periods allowed a good grain filling. It is worth noting that Collipulli was the only locality where irrigation was carried out during wheat development.

The farmers participating in this circuit were Christofer Jequier (Collipulli), Elena Fitzner (Freire), José Quintas (Radal) and Patricio Avilez (La Unión).

#627

The genotype x environment interaction and stability under the influence of heat of grain yield and selected in bread wheat in the southern regions of Uzbekistan

D. T. Juraev[1*], O. A. Amanov[1], Sh. D. Dilmurodov[1],N. Boysunov[1], A.Buronov[2]
S. M. Turaeva[3]

[1]Southern Research Institute of Agriculture, Kashkadarya Region, Uzbekistan
[2] Chirchik pedagogical university
[3]S.Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of Uzbekistan

*Emails: jurayevdiyor9@gmail.com, di.yor@mail.ru,

Wheat (*Triticum aestivum* L.) is considered one of the world's most important cereal crop. In recent years, climate changes issues have a negative impact on the morphological and physiological traits of wheat including grain productivity. Therefore, it is important to develop bread varieties resistant to abiotic factors varieties. Effect of high temperature (more than 30°C), during grain filling period, on grain quality and productivity has been studied thoroughly for southern parts of Uzbekistan.

At the study, temperatures during the growth period were calculated and daily temperatures during the grain filling period were determined. The effect of air temperature on growth and development, plant height (cm), spike length (cm), number of spikelets per spike (unit), spike grain weight in a spike(g), TKW (g), test weight (g/l), protein content (%), gluten content (%) were studied.

Results shows that heading period of later sowing varieties and accessions began 9-10 later compared to the optimal sowing and the yield decreased by 1.53– 2.76 t/ha, TKW decreased by 0,25-9,85 gr due to the temperature increase during "heading-maturity" stage. Several varieties and accessions had the highest resistance to heat under optimum planting date while varieties and samples under late sowing period had lowest heat resistance.

#222

Implementation of an accelerated genomic selection scheme in the icarda durum wheat breeding program.

Hafssa Kabbaj[1], Zakaria Kehel[1], Khaled Al-Sham'aa[2], Amadou Tidiane SALL[3], Rodomiro Ortiz 4and Filippo M[1].

[1] International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco. [2] International Center for Agricultural Research in the Dry Areas (ICARDA), Cairo, Egypt. [3] Senegalese institute for agronomical research (ISRA), saint louis, Senegal. [4] Swedish university of agriculture (SLU), Alnarp, Sweden

Hafssa Kabbaj : h.kabbaj@cgiar.org, Zakaria kehel : z.kehel@cgiar.org, Khaled Al shamaa: k.el-shamaa@cgiar.org. Amadou Tidiane Sall : tidianeassall11@gmail.com, Rodomiro Ortiz: rodomiro.ortiz@slu.se, Filippo bassi : f.bassi@cgiar.org

Global wheat production needs to increase by approximately 1.5% yearly. Combining genomic selection and speed breeding appears the successful strategy to deliver this level of genetic gain, thereby ensuring the rapid recycling and the pyramiding of multiple target traits.

An accelerated recurrent genomic selection scheme by full sib mating has been identified as the new breeding strategy for the ICARDA durum program to develop super lines to fit the needs of the six different mega product profiles from the Central and West Asia, North Africa, and West Africa.

100 crosses are conducted yearly to initiate cycle-0 for the six MPPs. Where speed breeding is used for the generation advancement. At F4 generation, genomic selection is applied using 89 KASP SNPs, the selected lines are field tested as stage 0. In parallel, full-sib mating-cycle1 is begin. While stage 1 yield trials are tested at the targeted population of environments. Similarly cycle-2 is processed.

#215

Exploiting 2NS and non-2NS based resistance in wheat breeding for blast resistance

Muhammad Rezaul Kabir[1], Krishna Kanta Roy[1], Md. Abdul Hakim[1], Golam Faruq[1], Abul Awlad Khan[1], Md. Farhad[1], Md. Monoar Hossain[1], Xinyao He[2], Pawan Kumar Singh[2]

[1] Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur, Bangladesh
[2] International Maize and Wheat Improvement Center (CIMMYT), Mexico

Email: Muhammad Rezaul kabir* (rezaul.kabir@bwmri.gov.bd), Krishna Kanta Roy (kkroy@bwmri.gov.bd), Md. Abdul Hakim (mahakim@bwmri.gov.bd), Golam Faruq (dg.bwmri@bwmri.gov.bd), Abul Awlad Khan (aa.khan@bwmri.gov.bd), Md. Farhad (md.farhad@bwmri.gov.bd), Md Monoar Hossain (monoar.hossain@bwmri.gov.bd), Pawan Kumar Singh(pk.singh@cgiar.org)

Wheat blast has become an economically important disease in Bangladesh. Intercontinental jump of the disease caused by pathogen *Magnaporthe oryzae* pathotype *Triticum* (MoT) from South America to Asia and Africa and possibility of further spread to other vulnerable areas across the globe poses a serious threat to global wheat production. Development of wheat blast resistant varieties is the most effective and environmentally friendly approach to manage the disease. Available information suggests that field resistance for wheat blast is largely influenced by 2AS/2NS translocation from *Ae. ventricosa.* However, the effectiveness of 2NS translocation often influenced by the background genetic makeup of a particular wheat line.

Virulent strains of MoT on this translocation have been reported across South America over time which demands to find out new sources of resistance (especially non-2NS based) . In South Asia, several varieties have been released having 2NS translocation. Bangladesh Wheat and Maize Research Institute (BWMRI) has released 3 wheat blast resistant wheat varieties so far. Among them, “BARI Gom33,” a zinc bio-fortified blast-resistant wheat variety released in Bangladesh in 2017 is containing 2AS/2NS translocation.

This variety is covering approximately 40% of the total wheat growing area in Bangladesh. Dependency on single gene-based resistance (2NS translocation) over a large acreage may make this variety vulnerable to new MoT isolates. BWMRI has been working to identify both 2NS and non-2NS based resistance sources since 2017. Precision Phenotyping Platform (PPP) has been established with the aim of evaluating wheat germplasm with improved precision and identification of sources of resistance against the disease.

During 2023-23 growing season, fifty-one advance breeding lines was evaluated under artificial inoculated condition in PPP, Jashore. Out of this, twenty-four lines found 2NS positive where 8 lines showed less than 10% disease index. In contrast, among other 27 non-2NS lines, only 2 advanced lines showed resistant reaction.

Beside this, forty-five advance breeding lines have been screened under greenhouse conditions and found some 2NS/ non-2NS based promising lines (BAW 1272, BAW 1286, BAW 1328, BAW 1408, BAW 1422, and BAW 1424) against the disease. This non-2NS lines could be crossed with other non-2NS resistant lines (identified before at BWMRI) together with 2NS genotypes to accumulate resistance genes to achieve high and durable WB resistance.

Thematic area: Enhancing wheat resistance against biotic stresses

#573

Targeting novel traits for wheat quality improvement

Lenika Kashyap[1], Achla Sharma[1], Satinder Singh[1], Arshvir Kaur[1], Shivani Upadhyay[1], G S Mavi, Puja Srivastava[1], Satinder Kaur[1], V S Sohu[1]

[1]Punjab Agricultural University, Ludhiana-141004, Punjab, India

Wheat (Triticum aestivum L.), provides 20% of the total calories to humans across the globe and is a rich source of starch, dietary fiber, minerals, proteins and is consumed in varied forms such as chapati (Indian flat bread), bread, pasta, noodles, biscuits etc. Being a staple cereal, development of nutritionally enhanced wheat holds promising prospects of being sustainable solutions to combat the malnutrition.

Though various factors play a key role in causing hidden hunger, however confining daily calorie intake to limited range of food is one of its key determinants. To meet the challenges of feeding a hungry planet, our commitment to innovation has led to development of diverse range of wheat varieties, having not only high yields but also to the discerning tastes and specific requirements of targeted populations.

In addition to other long-term objectives, the objective of development of product-oriented wheat varieties by reorienting the research focus is the newest one. Plant breeding and molecular biology techniques have been used to develop nutritionally enriched staple grains. Enormous potential of grain protein content gene (GpcB1) in wheat has been targeted and strengthened breeding for nutritious wheat grain for providing nutrition to humans by utilizing wild progenitors.
The consumption of whole grain of wheat has been associated with health promoting roles owing to presence of spectrum of beneficial phytonutrients in it. Recently, concept of colored grains or pigmented grains has grabbed the attention of both researchers and end-users owing to their immense nutraceutical values.

Their genetics, biochemistry, quality, product development and consumer impact are being investigated so as to tailor this germplasm for biofortification of elite wheat cultivars and transforming the colored grain into commercial products in order to provide sustainable, targeted and cost effective nutritional and health benefits.

These pigments possess numerous health promoting roles such as owing to their anti-oxidative potential as they reduce the oxidative damage to biological membranes, aging and prevent several chronic diseases.

Further, phytic acid in cereals acts as a chelator of major micronutrients such as iron and zinc, thus lowering their bioavailability. Various promising genotypes having low phytic acid have been identified and characterized for use in wheat breeding programme to breed biofortifed wheat cultivars with higher micronutrient and reduced phytic acid concentration combined with enchanted abiotic stress tolerance which can potentially help in alleviating the hidden hunger under changing climatic conditions.

#581

Unocovering the genetic diversity of bluebunch wheatgrass a progenitor of hybrid wheatgrass

Nadeem Khan[1], Yuanyuan Ji[1,2], Sampath Perumal[1], Pierre Hucl[3], Bill Biligetu[3], Lingling Jin[2,*] and Andrew Sharpe[1*]

[1]Global Institute for Food Security, Saskatoon, SK, Canada
[2]Department of Computer Science, University of Saskatchewan
[3]Crop Development Centre and Department of Plant Sciences, University of Saskatchewan

Emails: Andrew.sharpe@gifs.ca; lingling.jin@cs.usask.ca

Hybrid Wheatgrass (HWG) is an important, palatable, perennial forage crop cultivated in Saskatchewan and other Western provinces. It results from crosses between quackgrass (QG; *Elymus repens*, 2n = 6x = 42 SSSSHH) and North American and Eurasian bluebunch wheatgrasses (BBG; *Pseudoroegneria* spp., 2n = 1x = 14 SS, and 2n = 2x = 28 SSSS). QG is a hardy species with a weedy and invasive nature, but also valuable for wheat improvement programs, capable of crossing with wheat to produce introgression lines with enhanced resistance to *Fusarium* head blight and stripe rust. Similarly, BBG plays a crucial role in ecosystem stability, soil conservation, and providing forage, but its genetic diversity remains underexplored.

In this study, we conducted a comprehensive analysis of genetic diversity in BBG using single nucleotide polymorphisms (SNPs) across a collection of 145 accessions. First, single nucleotide polymorphisms (SNPs) were mapped using Bowtie, and subsequently identified with GATK, and after filtering, a total of 61,101 (61K) high-quality SNPs were retained for further analysis. SNP density analysis revealed significant variation on chromosomes 2 and 7.

Population structure analysis indicated three major genetic groups, reflecting significant differentiation among populations. Principal component analysis (PCA) highlighted significant axes of variation, with PC1 and PC2 explaining 14.35% and 6.88% of the variation, respectively. Phylogenetic analysis showed well-supported clades, with *Pseudoroegneria spicata* forming major clades, suggesting high conservation.

Genetic differentiation (FST) and neutrality tests (Tajima's D) indicated substantial genetic divergence between populations. Our results provide new insights into the genetic diversity and structure of BBG, with significant implications for conservation and management efforts.

While this study focuses on the genetic analysis of BBG we are also currently developing reference genomes for HWG, BBG, QG and sequencing selected wheat introgression lines, serving as a valuable resource for advancing wheat pre-breeding programs and contributing to sustainable agriculture.

#118

Targeting wheat senescence to improve grain protein content

Emilie Knight[1], Becca Testa[1], Sophie Harrington[1], Benjamin Fisher[2], Sadiye Hayta[1], Mark Smedley[1], Philippa Borrill[1].

[1] John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom. [2] University of Birmingham, Edgbaston, Birmingham, B15 2TT, United Kingdom.

emilie.knight@jic.ac.uk

Over the last few decades, bread wheat (Triticum aestivum) has been selected for high yield. This has come at a cost because of the trade-off between yield and protein content. We are interested in improving grain protein content without hindering yield. To this purpose, we study the processes involved in wheat senescence (when nutrients get remobilised from the vegetative tissues to the maturing spike); the timing of which directly impacts yield and protein content.

Here we investigate the potential role in senescence of three transcription factors (TF), which stood out in a gene regulatory model of senescence: NAC3 and two RWP-RK genes; using TILLING mutants, gene editing and a protoplast-based method to identify the downstream target genes of these TF.

NAC3 was found to interact with NAM-B1, a known senescence regulator, through a yeast two-hybrid experiment. Double mutants of NAC3 obtained from the crossbreeding of two tetraploid TILLING lines with a deleterious mutation for each copy of the gene showed a delay in senescence. To confirm the role of NAC3 in regulating senescence, we have generated gene edited wheat plants cv. Cadenza, using sgRNAs targeting all three copies of NAC3. We have obtained several T0 plants with edits in all 3 copies of NAC3. Senescence phenotyping of homozygous knock-out T1 plants will be carried out in the next few months.

RWP-RK genes are regulators of nitrogen metabolism and are potential new candidate genes in the senescence process. Indeed, one of these genes has predicted shared targets with NAM-A1, a TF known to have a key role in senescence. We are generating double and triple knock-out mutants in tetraploid (cv. Kronos) and hexaploid (cv. Cadenza) wheat plants, using TILLING lines. Senescence phenotyping of these mutants is underway and shows a trend towards an involvement in the senescence process.

Finally, we are adapting the TARGET method from Arabidopsis to accurately identify targets of TF of interest within wheat cells. Protoplasts are transformed with a construct carrying a TF known to be involved in senescence.

Transformed cells are selected and treated with chemicals causing the TF to move to the nucleus or not, thereby allowing for RNA sequencing to determine true primary targets of the TF and eliminating secondary targets and background noise.
These experiments will identify new genes to manipulate senescence and provide a step towards improving yield and grain protein content simultaneously.

#170

Production and agronomic traits evaluation of synthetic polyploid wheats having diverse genomes

Fuminori Kobayashi[1*], Nobuyuki Mizuno[1], Shigeo Takumi[2], Yoshihiro Matsuoka[2], Kentaro Yoshida[3].

[1] Institute of Crop Science, National Agriculture and Food Research Organization (NARO), Tsukuba, Ibaraki 305-8518, Japan. [2] Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan. [3] Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan.

* kobafumi@affrc.go.jp

Wild wheat relatives carry abundant natural variations and have been used for wheat breeding through synthetic hexaploids and alien chromosome introgression lines. We have newly generated about 200 synthetic polyploids through interspecific crossing between a tetraploid wheat accession Triticum turgidum L. ssp. durum cv.

'Langdon' [AABB genome] as the female parent and various wild relatives as the pollen parents. The synthetic polyploids include hexaploids, octoploids and decaploids, and their pollen parents are diploids (Ae. tauschii Cosson [DD genome], Ae. umbellulata Zhuk. [UU genome], Ae. uniaristata Vis. [NN genome], Ae. commosa Sm. In Sibth. & Sm. [MM genome], T. monococcum ssp. aegilopoides (Link) Thell. [AmAm genome] and T. urartu Tumanian ex Gandilyan [AA genome]), tetraploids (Ae. biuncialis Vis. [UUMM genome], Ae. columnaris Zhuk. [UUMM genome], Ae. kotschyi Boiss. [SSUU genome], Ae. geniculate Roth [UUMM genome] and Ae. peregrina (Hackel in J. Fraser) Maire & Weille [UUSS genome]) and hexaploids (Ae. juvenalis (Thell.) Eig [DDMMUU genome] and Ae. vavilovii (Zhuk.) Chenn. [DDMMSS genome]), respectively.

In general, Ae. tauschii is the most frequent parent of the synthetic polyploids, but we have succeeded in generating novel synthetic polyploids using other wild relatives. In this study, we investigated the characteristics of these lines and analyzed the effects of genomes derived from wild relatives on tested traits. We evaluated several agronomic traits of synthetic polyploids under field conditions. In the synthetic hexaploids, alterations in traits occurred depending on the genome of the wild relatives.

For example, the AABBDD and AABBAmAm lines had longer culm and spike lengths and more spikelets and grain number per spike than the other lines. The AABBUU, AABBNN and AABBMM lines had higher numbers of effective tillers than the others. On the other hand, no clear differences in traits were observed depending on the genome of the wild relatives in the synthetic octoploids.
We have succeeded in screening salt-tolerant lines through salt stress test during germination.

In the future studies, we plan to evaluate the agronomic traits of synthetic polyploids under dry conditions. It is thus expected that the resources we have generated can contribute to improving wheat in response to recent and future climate changes. This study was supported by Cabinet Office, Government of Japan, Moonshot R&D Program for Agriculture, Forestry and Fisheries (funding agency: Bio-oriented Technology Research Advancement Institution).

#209

Duplication of the distal region of chromosome 5A reduced the culm length in γ-irradiated mutant

Shoya Komura[1*], Makiko Chono[2], Hitoshi Matsunaka[4, 5], Kazuhiro Nakamura[2, 4], Goro Ishikawa[3], Fuminori Kobayashi[3], Kentaro Yoshida[1]

[1] Graduate School of Agriculture, Kyoto University, Kyoto, Japan. [2] Headquarters, National Agriculture and Food Research Organization, Ibaraki, Japan. [3] Institute of Crop Science, National Agriculture and Food Research Organization, Ibaraki, Japan. [4] Kyushu Okinawa Agricultural Research Center, National Agriculture and Food Research Organization, Fukuoka, Japan. [5] Hokkaido Agricultural Research Center, National Agriculture and Food Research Organization, Hokkaido, Japan

Email: komura.shouya.48p@st.kyoto-u.ac.jp

Reduction of culm length is one of the most essential traits in wheat breeding to avoid lodging, which causes severe damage to the yield and quality. In this study, we irradiated the Japanese wheat variety 'Chikugoizumi' with gamma-rays and obtained a mutant with a shorter culm length than the wild type. To determine the genomic regions responsible for the mutant phenotype, we applied whole genome sequencing to the mutant and wild type.

Since gamma radiation often causes structural variants such as deletions, the depth of coverage across the genome was estimated to detect deletions. By comparing the depth-of-coverage between wild-type and mutants, we detected a deletion of 0–36 Mbp region of chromosome 2B and a duplication of 636–713 Mbp region of chromosome 5A. In addition, we confirmed that the duplication region of chromosome 5A was translocated to the end of the deleted chromosome 2B by fluorescence in situ hybridization (FISH).

Segregation analysis using the F2 population generated from a cross between the mutant and the wild-type showed that the chromosome 2B-5A translocation was significantly associated with the reduction of culm length. The translocation region contained two previously reported culm length-related genes, Q (TraesCS5A03G1116700) and Rht12 (TraesCS5A03G1269600). Since Rht12 encodes the gibberellic acid (GA) 2-oxidase protein, to determine whether the reduction in culm length was caused by a gibberellin-related response, we treated the mutant and the wild type with exogenous GA3.

The GA3 treatment increased the culm length in both the wild-type and the mutant. In particular, the increase in the short culm mutant was remarkable. The results suggested that exogenous GA3 compensated for endogenous GAs inactivated by Rht12 and that the amount of inactivated GA is higher in the mutants.

Furthermore, even after elongation by exogenous GA3, the wild type exhibited longer culm length than the mutant indicating that there is a factor causing culm length reduction independent of the GA pathway. Q has been reported to reduce culm length in a GA-insensitive manner. Therefore, it was inferred that both Q and Rht12 caused the reduction of culm length through increased copy number resulting from the chromosome 5A translocation.

Our results would provide useful resources for improving plant height. This study was supported by MAFF Commissioned project study on "Smart-breeding System for Innovative Agriculture [DIT1002]".

#399

Mapping of dwarf gene Rht5 and candidate gene mining

Xianglan Kong[1], Chunge Cui[1], Liang Chen[1*], Yin-Gang Hu[1, 2*]

[1]State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, College of Agronomy, Northwest A&F University, Yangling, Shaanxi, China. [2]Institute of Water Saving Agriculture in Arid Regions of China, Northwest A&F University, Yangling, Shaanxi, China.

Email: 2021060047@nwafu.edu.cn; huyingang@nwafu.edu.cn

Wheat is an important grain crop in the world, and the height of wheat plant is one of the important phenotypes to determine the yield, in which dwarf genes play a key role. The gibberellin-sensitive (GAR) dwarf gene Rht5 can reduce plant height and improve lodging resistance, but does not affect the coleoptile length and seedling viability, and can significantly increase the effective panicle number, panicle number and harvest coefficient, therefore, Rht5 mapping and gene mining are of great significance for wheat dwarf breeding.

Then two F2 populations derived from the crosses of Jinmai47 and Ningchun45 with Marfed M (Rht5) were employed to identify the genetic region of Rht5, and their derived lines were used to evaluate its efects on plant height and main agronomic traits. Rht5 was fine-mapped between markers Kasp-25 and Kasp-23, in approximately 1 Mb region on chromosome 3BS, which harbored 17 high-confidence annotated genes based on the reference genome of Chinese Spring (IWGSC RefSeq v1.1).
Combined with the recombinant inbred lines derived from the hybridization of Jinmai 47 and Mafed M, homozygant tall and short lines from different F3:4 isolated lines were selected for RNA-Seq sequencing, so as to mine genes related to stem development.

Among all possible genes, TraesCS3B02G025600 was suggested as a potential candidate gene of Rht5. There is a 30 bp deletion in the frist exon of its coding region was found in Marfed M, as compared to Jinmai47, Nicngchun45 and Chinese spring, which leaded to variations in amino acid sequence. It encodes a CYP450 protein which is a massive family contains plenty of members and is vital in the biosynthetic of phytohormones.

GA and BR were vital to the growth of plant, and a considerable number of genes involved in the biosynthesis and metabolism of GA and BR encoded CYP450 proteins. TraesCS3B02G025200 encoding fructosebisphosphate aldolase.

Fructose 1, 6-diphosphate aldolase is involved in glycolysis, glucose production and the Calvin cycle, providing energy and substances for plant growth and development.That two genes may be Rht5 candidate genes.

Screening Rht5 candidate genes and studying the mechanism of Rht5 dwarfism are of great significance for wheat dwarf breeding.

#98

Evaluation of potential minor wheat species for nordic-baltic future climate

Reine Koppel[1,*], Anne Ingver [1]

[1] Centre of Estonian Rural Research and Knowledge (METK), J. Aamisepa 1, Jõgeva alevik, 48309, Estonia

Reine.Koppel@metk.agri.ee, Anne.Ingver@metk.agri.ee

Wheat is the largest cereal crop in most of the Nordic-Baltic countries, including Estonia. New productive varieties provide income to the growers, as wheat is also an important export item. At the same time, there is increasing interest among producers and consumers in lesser-known types of wheat in the region, which are more resilient, suitable for specific products (durum wheat) or offer health benefits (einkorn, emmer).

The Centre of Estonian Rural Research and Knowledge (METK) has started research of less common diploid and tetraploid wheat species, both, spring and winter types of these species are studied.

Winter types. We started in 2020 with 2 einkorn and 5 emmer varieties. All the genotypes survived the winter conditions well. Winter hardiness is the most critical feature for winter types. The average yield was 3.8 for einkorn and 3.6 t/ha for emmer. Since 2022 the number of einkorn and emmer genotypes has increased.

There were 33 einkorn genotypes in the trial, winterkill depended on the trial location – einkorn and emmer had lower winter damage levels in warmer and drier soils. Older genotypes had problem with lodging resistance, less lodging was estimated for newer varieties. Disease infection rates were low.
Fewer varieties of winter durum wheat have been studied so far. Winterhardiness has been in average level, depending on the variety. Winter durum also had good lodging resistance and length of growing period.

Spring types. In the case of spring types, one of the most important characteristics in northern conditions is the suitable length of the growing period. Three durum and one emmer variety have been tested since 2021 in our trials.

The both types had more than 30% lower yield compared to the standard common wheat variety. Spring emmer had longer growing period, taller plants, smaller kernels, better disease resistance, and higher gluten content but softer gluten. Durum varieties had bigger kernels and higher protein content.

This study was financed by the Estonian Rural Development Plan 2014–2020: METK 7-2.1/36 project "Growing single grain wheat in Estonia and diversifying the use of wheat and rye" and the Center of Excellence "Agroecology and new crops in future climates" funded by the Ministry of Education and Research of Estonia.

#0

Physical map of QTLs for six spike related traits detected in multiple environments with emphasis on heat tolerance in wheat

Sourabh Kumar[1*], Sachin Kumar[1], Hemant Sharma[1], Vivudh Pratap Singh[1], Aanchal Kumari[1], Kanwardeep Singh Rawale[2,5], Sunil Kumar Bhatt[3], Ramanathan Vairamani[4], Kulvinder Singh Gill[2], Harindra Singh Balyan[1]

[1]Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India. [2]Department of Crop and Soil Sciences, Washington State University, Pullman, Washington, USA. [3]Research and Development Division, JK Agri-Genetics Limited, Hyderabad, Telangana, India. [4]Rallis India Limited, Mumbai, Maharashtra, India. [5]Geneshifters, LLC, Pullman, Washington, USA

[*]Presenting authors (sourabhkumar7669@gmail.com)

Wheat is a vital staple food world-wide, but its grain production is adversely impacted by rising global temperatures, posing a serious threat to food security. To unravel the genetic basis of key spike traits under heat stress conditions, a study was conducted using a doubled-haploid (DH) mapping population of 177 lines, developed from a cross between a heat-sensitive cultivar (PBW343) and a heat-tolerant genotype (KSG1203).

This population was phenotyped for six spike-related traits in 15 different environments, including timely, late, and very late sowing conditions, over three years and two locations within Indo-Gangetic plains. Composite interval mapping was performed using the best linear unbiased estimates for each trait and a sequencing-based genotyping (SBG) SNP physical map containing 5,710 markers, spanning a genomic distance of 14,263.4 Mb. In total, 52 QTLs (17 for timely, 11 for late, 18 for very late sown conditions, and six common) with PVE (%) ranging from 7.1% to 23.6%, were identified and integrated into the wheat physical map.

These included 12 stable QTLs displaying high PVE (%). These QTLs were recommended for marker-assisted recurrent selection (MARS) for wheat improvement in both optimal and heat stress conditions. These QTLs were validated in high yielding DH lines. A number of QTLs overlapped the known genes (TaHsfC2a-B, TaCol-B5, WAPO1, starch synthase I, TaGW2-B1, TaGW2-B1 and PI1-1B/WPI-1-1B0).

These genes are involved in heat shock response, starch synthesis, spikelet number per spike, spike architecture (including spikelets and floret development) grain weight, grain yield, and other aspects of spike and grain development. Moreover, 74 candidate genes (CGs) linked to heat tolerance, encoding 37 distinct proteins, were also identified.

The important QTLs recommended for MARS and the identified CGs constitute important genomic resources which may prove useful in breeding for heat-tolerant wheat varieties/germplasm enhancing the crop's resilience under adverse climatic conditions.

Keywords: Wheat, doubled haploid population, heat stress tolerance, SBG-SNPs, quantitative trait loci, candidate genes

#572

Introgression of 2 and 7u chromosomes from aegilops kotschyi for enhancement of β-glucan in wheat

Upendra Kumar[1], Sourav Panigrahi[2], Rita Goswami[2], Yogita Singh[3], Priyanka Balyan[4], Sundip Kumar[5], Reyazul Rouf Mir[6]

[1] Department of Plant Science, Mahatma Jyotiba Phule Rohilkhand University, Bareilly-243006, India. [2]Department of Molecular Biology & Biotechnology, College of Biotechnology, CCS Haryana Agricultural University, Hisar,125004, India. [3]Stockbridge School of Agriculture, University of Massachusetts, Amherst, USA. [4]Department of Botany, Deva Nagri P.G. College, CCS University Meerut-245206, India. [5]Molecular Cytogenetics Laboratory, Department of Molecular Biology & Genetic Engineering, College of Basic Sciences & Humanities, GB Pant University of Agriculture & Technology, Pantnagar, 263145, India. [6]Division of Genetics and Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), Srinagar (J& K), India

Addressing the urgent demand for biofortified wheat enriched with health-beneficial dietary fibres such as β-glucan, this study employed a meticulous crossbreeding process between established wheat cultivars and the β-glucan-rich wild relative Aegilops kotschyi accession “AK-3790”.
Within this context, a derivative line encompassing a pair of 7U chromosomes from Ae. kotschyi, denoted as 63-2-13 (Tiwari et al., 2010), was identified. The presence of the 7U chromosome in this line was confirmed through a comprehensive molecular marker and genomic in situ hybridization (GISH) analysis. To augment β-glucan content in hexaploid wheat, two distinct backcross populations were developed utilizing the 63-2-13 line as the donor parent and two separate recurrent parents (WH1105 and HD3086).

These populations underwent an exacting selection regimen, encompassing parent-like phenotypes, heightened yield, and robust resistance to yellow rust, meticulously tracked across successive generations until the BC2F2 stage. Among the outcomes, selected BC2F2 progenies exhibited remarkable enhancements in β-glucan levels, with a notable contender (BC2F2 23-5) showcasing an impressive 1.76% grain β-glucan content.

Despite a discernible reduction in yield compared to their high-yielding counterparts, BC2F223-5 demonstrated a harmonious trait profile, encompassing heightened β-glucan content and moderate yellow rust resistance, thus positioning it as a compelling candidate for subsequent refinement endeavours.

This research notably underscores the substantial potential of precise introgression strategies for amplifying β-glucan content in wheat, thereby underscoring the imperative of adept trait optimization to ensure both yield stability and nutritional enhancement.

#458

Exploring underutilized common wheat germplasm for diversifying breeding materials in korea: a phenotypic study

Sun-Hwa Kwak[1], Ho-Sun Cheon[1], Sukyeung Lee[2], Young-ah Jeon[2], Sieun Choi[1], Chul Soo Park[1], Youngjun Mo[1]*

[1]Department of Crop Science and Biotechnology, Jeonbuk National University, Jeonju 54896, Republic of Korea. [2]National Agrobiodiversity Center, National Institute of Agricultural Sciences, Rural Development Administration, Jeonju 54874, Republic of Korea

*Corresponding author: Youngjun Mo (yjmo@jbnu.edu)

Wheat, a staple crop globally, ranks as the second most consumed food crop in Korea following rice. However, the self-sufficiency rate of wheat is low (1.1%, as of 2021) in Korea, leading to a high dependency on imports. Therefore, developing improved wheat varieties for Korean environments is vital to improve food security. As wheat breeding in Korea has focused on developing early-maturing varieties suitable for the rice-wheat double cropping system, genetic diversity is highly limited.

Hence, it is crucial to establish a genetically diverse germplasm pool to discover and utilize valuable breeding materials for the development of competitive varieties. This study aimed to assess the genetic diversity among 359 underutilized wheat accessions collected from 44 countries based on 13 major agronomic traits evaluated under field conditions and select accessions suitable as foundational materials for developing a breeding population. The agricultural traits of 359 accessions were compared with those of Geumgang, one of the major wheat varieties cultivated in Korea.

Among the agricultural traits investigated, days to heading from sowing (DTH) averaged 195 days (range 168–217 days, Geumgang 171 days). Spikelet number per spike (SNS) and thousand grain weight (TGW) averaged 20.8 (range 12.8–30.2, Geumgang 14.4) and 37.3 g (range 21.8–64.6 g, Geumgang 45.3 g), respectively.

Using k-means cluster analysis, the 359 resources were classified into three groups, and characteristics among these groups were compared to understand the genetic diversity within the population. Based on the results, promising accessions with high SNS and TGW with suitable maturity under Korean environment were selected.

The average SNS of the selected accessions was 24.3, 69% higher than that of Geumgang, while the average TGW of the selected accessions was 55.1 g, 22% higher than that of Geumgang.
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#134

Quality and noodle-making performance of flour with varying gluten strengths treated with different xylanases

Eunbin Ha[1], Jeongeon Kim[2], Yujin Moon[2], Tingting Zhang[2], Meera Kweon[2]

[1] Department of Nutrition Education, Pusan National University, Busan 46241, Korea, ot4321@pusan.ac.kr
[2] Department of Food Science and Nutrition, Pusan National University, Busan 46241, Korea,
wjddjs8@pusan.ac.kr; amoebacul@pusan.ac.kr; xixidog@pusan.ac.kr; meera.kweon@pusan.ac.kr

Arabinoxylans (AXs) have long been recognized as a major fiber constituent in wheat grain cell walls, offering numerous health benefits. Based on their solubility in water, AX can be classified as either water-extractable or water-unextractable arabinoxylans (WEAX and WUAX). However, AXs can have adverse effects on processing and product quality.

When WUAX is enzymatically converted into WEAX using xylanase, it improves dough development, resulting in enhanced bakery product quality. This study compared the quality characteristics and noodle-making performance of flour with varying gluten strengths (S, M, and W) treated with two xylanases (Pentopan 500BG and Shearzyme 500L), which selectively act on WUAX and WEAX, respectively, at different concentrations.

The addition of both xylanases led to a significant decrease in solvent retention capacity (SRC) in water, with a slightly different pattern by increasing the xylanase concentration. SDS sedimentation volume of flour significantly increased with varied extents by adding both xylanases, suggesting the production of hydrolyzed AXs that contributed to varied swelling capacities in SDS-lactic acid solution.
Additionally, flour with weak gluten strength (W) showed a greater increase in SDS sedimentation volume with added xylanases than flour with strong gluten strength (S) and flour with medium gluten strength (M), indicating the impact of gluten strength.

Dough mixing patterns with adjustment of water based on water SRC also showed changes with xylanase addition. Textural properties of fresh noodle with the addition of xylanases showed a slight change in S and M, but a significant change in W, with different impacts from different xylanases and water adjustment. Shearzyme showed a greater impact than Pentopan.
The firmness and chewiness of cooked noodles also decreased significantly with increasing xylanase concentration, with a slightly greater impact from Shearzyme than Pentopan, highlighting the role of both WEAX and WUAX in noodle-making.

When water reduction was based on SRC values, the textural properties of both fresh and cooked noodles were less affected by xylanase treatment, confirming the reduced competition of WUAX or WEAX for water during noodle dough development. Additionally, the gluten strength of flour exhibited potential variations in impacts from different xylanases.

#367

Biological, chemical and genetic bases of purple wheat resistance to the granary weevil

Barbara Laddomada[1,*], Carmela Gerardi[1], Giovanna Giovinazzo[1], Maria Vittoria Renis[1], Ilaria D'Isita[2], Antonella Marta Di Palma[2], Giacinto Salvatore Germinara[2], Salvatore Esposito[3], Pasquale De Vita[3]

[1] Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Lecce 73100, Italy
[2] Department of the Sciences of Agriculture, Food and Environment (DAFNE), University of Foggia, 71122 Foggia, Italy
[3] Research Centre for Cereal and Industrial Crops (CREA), 71122 Foggia, Italy

*barbara.laddomada@ispa.cnr.it

Enhancing wheat resistance against the granary weevil, *Sitophilus granarius* (L.), is urgent to counteract the severe quantitative and qualitative wheat grain losses caused by this pest during post-harvest. The actual control strategy of the granary weevil relies on the use of fumigants and broad-spectrum contact insecticides, but due to their impacts on health, food safety and environment, there is an urgent need for a more sustainable and low-impact control approach.

The development of resistant wheat varieties is one of the most promising alternatives to insecticides in the integrated management of stored grain pests. Here we present a project recently funded by the Italian Ministry of University and Research (MUR) aimed at investigating on the sources and mechanisms of host-plant resistance to *S. granarius* in wheat. Previous studies showed that granary weevil adults perceive and behaviorally respond to a range of wheat grain volatile organic compounds (VOCs) [1,2].

In fact, differently from yellow wheats, some Ethiopian purple wheats besides being unattractive to *S. granarius* adults have antifeedant and toxic effects associated to anthocyanins accumulation [3]. Our project has the ambition to gain a deeper insight into the defense mechanisms of purple wheat against *S. granarius.* To this aim we will evaluate the biological, chemical, and genetic factors involved in the host finding and host acceptance by adult weevils.

A set of durum and bread wheat Near-Isogenic Lines will be used, each derived from heterogeneous inbred families contrasting for the color of the pericarp due to the diverse anthocyanin composition and content. Next generation technologies will be used to search for molecular markers associated to the key variants and target genes that significantly change their expression level within each NIL pair.

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#178

The project savegrain-cer for the collection, characterization, and safeguard of local diversity of triticum spp.

Gaetano Laghetti*, Pasquale L. Curci, Emanuela Blanco, Marcella Urbano, Giacomo Mangini, Diana L. Zuluaga, Paolo Direnzo, Rosella Giunta, Francesco Losavio, Mariano Zonna, Gabriella Sonnante

Institute of Biosciences and BioResources, National Research Council (CNR), Via Giovanni Amendola 165/A,
70126 Bari – ITALY

* Corresponding author: gaetano.laghetti@ibbr.cnr.it

Cereal landraces, selected over the time by local farmers and well adapted to the climatic conditions of the Apulia region (southern Italy), are threatened by genetic erosion/extinction, as a result from the gradual replacement with modern high-yielding cereal cultivars.

For these reasons, within the program PSR Puglia 2014-2020, Op. 10.2.1, the Apulian region is funding the project “Biodiversità dei cereali antichi pugliesi per la sostenibilità e la qualità (SAVEGRAIN-CER)”. This project is coordinated by the Institute of Biosciences and Bio-Resources of the National Research Council (IBBR-CNR) and is carried out in partnership with public research Institution, Universities, and private companies.

The main goals of SAVEGRAIN-CER are: i) to collect and to store the Apulian cereal landraces; ii) to perform morphological, genetic and bio-agronomic evaluation; iii) to exploit the typical end-product obtained with this local germplasm. The activities also include the collection of historical documents validating the relationship between the recovered landraces and their areas of cultivation.

Over 100 cereals, including common and durum wheat, landraces were collected in different habitat and areas outside and inside protected areas, where traditional agricultural practices still survive. The collected germplasm was stored ex situ in the Mediterranean Germplasm Seed Bank of IBBR-CNR and safeguarded on farm by custodian farmers.

The preliminary results of the project show the potential use of landraces to increase the development of sustainable agriculture.

#609

Hypoallergenic wheat

Debbie Laudencia-Chingcuanco*, Jeanie Lin, Henri Jenoudet, Albert Wong, Naxin Huo, and Yong Gu

Crop Improvement and Genetics Research Unit, United States Department of Agriculture-Agricultural Research Service, Western Regional Research Center, 800 Buchanan St. Albany, CA 94710

*debbie.laudencia@usda.gov

The gluten protein composition is a major determinant of the technological and nutritional properties of wheat-derived food products. The same proteins in gluten, however, can trigger several human health-related risks like wheat-dependent exercise induced anaphylaxis (WDEIA) and celiac disease. WDEIA is an IgE-mediated food allergy that is triggered by ingestion of wheat combined with certain cofactors like exercise, alcohol or non-steroidal anti-inflammatory drugs.

Allergic response can range from hives, difficulty in breathing, low blood pressure, angioedema, to anaphylaxis. The omega-5 gliadin, one of the proteins in gluten, is a major allergen responsible for WDEIA. To develop wheat lines with reduced allergenicity we generated and screened ethyl-methyl sulfonate and fast neutron radiation (FNR) mutagenized populations of a commercial variety of bread wheat (cv Summit) for the presence or absence of gluten proteins.

We identified several independent mutations for each of the 12 loci that encode gluten genes (Glu-1, high-molecular weight glutenin subunits; Glu-3, low-molecular weight glutenin subunits; Gli-1, omega, and gamma gliadins; and Gli-2, alpha gliadins). Exome capture assays using the ProEnSeq platform identified 143 glutenin and gliadin genes in the Summit genome, more than half of which are pseudogenes.

The in silico derived protein sequences of glutenin and gliadin genes surveyed for the presence of the 11 epitopes reported for WDIEA showed that 96 epitopes are in seven of the active gluten genes, 92 of which were on the three omega gliadin genes encoded by Gli-1B locus.

Mapping of the FNR induced mutation in Glu-3B-S225E line identified a 2 Mb deletion of Gli-1B/Glu-3B loci that removed 92 of the of the 96 WDEIA epitopes identified. Grain protein content, kernel weight, gluten-index and bread volume of Glu-3B-S225E was not significantly different from wild type. Hypoallergenic elite bread wheat lines with suitable agronomic traits can be used for healthier wheat products including plant-based meat alternatives.

#473

Development of pcr-based markers for identification of wheat hmw glutenin glu-1bx and glu-1by alleles

Myoung Hui Lee, Kyeong-Min Kim, Chon-Sik Kang, Mira Yoon, Ki-Chang Jang, and Changhyun Choi

National Institute of Crop Science, Rural Development Administration, Wanju, 55365, Korea

In common wheat (*Triticum aestivum* L.), allelic variations in the *Glu-B1* locus have important effects on grain end-use quality. The *Glu-B1* locus consists of two tightly linked genes encoding x- and y-type subunits that exhibit highly variable frequencies. However, studies on the discriminating markers of the alleles that have been reported are limited.

Here, we developed 12 agarose gel-based PCR markers for detecting Glu-1Bx and Glu-1By alleles. By integrating the newly developed markers with previously published PCR markers, nine Glu-1Bx locus alleles (Glu-1Bx6, Glu-1Bx7, Glu-1Bx7*, Glu-1Bx7^{OE}, Glu-1Bx13, Glu-1Bx14$^{(-)}$, Glu-1Bx14$^{(+)}$/Bx20, and Glu-1Bx17) and seven Glu-1By locus alleles (Glu-1By8, Glu-1By8*, Glu-1By9, Glu-1By15/By20, Glu-1By16, and Glu-1By18) were distinguished in 25 wheat cultivars.

Glu-1Bx6, Glu-1Bx13, Glu-1Bx14$^{(+)}$/Bx20, Glu-1By16, and Glu-1By18 were distinguished using the newly developed PCR markers. Additionally, the Glu-1Bx13 and Glu-1Bx14$^{(+)}$/Bx20 were distinguished by indels in their promoter regions. The Glu-1Bx6, Glu-1Bx7, Glu-1By9, Glu-1Bx14$^{(-)}$, and Glu-1By15/By20 alleles were distinguished by using indels in the gene-coding region. Glu-1By13, Glu-1By16, and Glu-1By18 were dominantly identified in the gene-coding region. We also developed a marker to distinguish between the two Glu-1Bx14 alleles.

However, the Glu-1Bx14$^{(+)}$ + Glu-1By15 and Glu-1Bx20 + Glu-1By20 allele combinations could not be distinguished using PCR markers. The high-molecular-weight glutenin subunits of wheat varieties were analyzed by ultra-performance liquid chromatography and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and the findings were compared with the results of PCR analysis.
These newly developed PCR markers could facilitate more effective analysis of the molecular variations of the *Glu-B1* alleles to improve the end-use quality of common wheat.

#288

Pyramiding 4 wheat durable resistant genes for rusts and powdery mildew control

Wei Li, Guoqi Song, Yulian Li, Shujuan Zhang, Rongzhi Zhang, Jihu Li, Jie Gao, Genying Li*
Crop research institute, Shandong Academy of Agricultural Sciences, Jinan, Shandong 250100, China

Email: lgy111@126.com

Lr34/Yr18/Pm38/Sr57, *Lr27/Yr30/Sr2*, *Lr46/Yr29/Pm39/Sr58* and *Lr67/Yr46/Pm46/Sr55* are four durable resistant genes in wheat. Each one provides partial resistance without race-specificity to leaf rust, stripe rust, stem rust and powdery mildew in adult plant stage. When deployed with each other, resistance will be enhanced, but the effect of four genes together is unclear.

In this study, we pyramided these four genes into recurrent parent Jimai229 through marker assisted selection. Four BC_6F_4 lines, namely 2L819, 2L857, 2L859 and 2L861, contain all four genes, one BC_6F_4 line namely 2L863 contains none of the four genes were developed to evaluate disease resistance and yield. All lines contain four genes are highly resistant to leaf rust, stripe rust and powdery mildew at adult plant stage in the field, but the leaf tip necrosis become more and more severe from heading stage to grain filling stage. At late Grain Filling stage, half of the flag leaf are necrotic.

We also observed the pseudo-black chaff, symptoms of *Sr2*, at flowering stage. Yield was evaluated with interval contrast design with three duplication in Jinan, Shandong Province. The production of 2L863 is 8.06 t ha^{-1}, while the production of 2L819, 2L857, 2L859 and 2L861 are 7.96, 7.07, 7.41 and 7.90 t ha^{-1}. Compared with 2L863, production reduction are 1.23%, 12.30%, 8.10%, 1.95%,.

There is no significant differences for Plant height, grain number per spike and Spike number per acreage. The thousand grain weight of 2L863 is 36.1g, while that of 2L819, 2L857, 2L859 and 2L861 are 33.87g, 33.10g, 32.04g and 34.38g respectively. In conclusion, pyramiding 4 wheat durable resistant genes improve resistance to rusts and powdery mildew effectively, but leaf tip necrosis are too severe to maintain production.

How many genes and what combination will provide adequate resistance with less production penalty need further investment.

#587

Allele distribution of 55 genes in wheat lines participating in 2019-2020 Shandong trial

Wei Li[1], Min Zhang[2], Guoqi Song[1], Yulian Li[1], Jie Gao[1], Dungong Cheng[1], Haosheng Li[1*], Genying Li[1*]

[1] Crop research institute, Shandong Academy of Agricultural Sciences, Jinan, Shandong 250100, China. [2] College of Agronomy, Qingdao Agricultural University, Qingdao, Shandong 266109, China

Email: lihaosheng810@163.com; lgy111@126.com

To analyze gene distribution in wheat lines involved in 2019-2020 Shandong regional trial, one hundred and twenty-six wheat lines were tested with 64 Kompetitive Allele Specific polymerase chain reaction (KASP) markers which were specific to 55 genetic loci. The markers are related to yield, quality, resistance, flower time and etc. Over 90% KASP markers give acceptable genotyping, which provides an efficient technology for gene identification.

Favorable allele frequencies of 20 genes are above 80%, including Vrn-A1, Vrn-B1, Vrn-D1, Ppd-A1, Ppd-B1, Ppd-D1, Psy-D1, Glu-A3g, Rht-D1, Pinb-D1, TaCwi-A1-1, TaGW2-6B, TaSus1-7B, TaGASR7-A1, 1-feh-w3, TaDreb-B1, PRR-A1, PRR-B1, TaFT3-B1 and TaMOT1-D1. Favorable allele frequencies of 26 genes are below 30%, including Vp1-B1, Ppo-D1, TaPds-B1, Zds-A1, TEF-7A, Lr46, Glu-B3g, TaGS5-A1, TaCwi-4A, 1B/1R, Rht-B1, Lr68, TaELF3-B1, Pina-D1, Sbwm1, TaPHS1, Pm21, COMT-3B, TaCKX-D1, TaSdr-B1, Pch1, Lr34, Yr15, Fhb1, TaMoc-7A and TaGS-D1, notably, favorable alleles of TaMoc-7A, Lr34, Fhb1, Pch1, Yr15, and TaSdr-B1 are not detected.

Favorable allele frequencies of 9 genes are between 30% and 80%, including Pinb2-V, TaPod-A1, Lox-B1, Glu-A1, Glu-D1, TaGS2-B1, TaGW2-6A, TaSus1-7A, and Lr14a. Favorable allele frequencies of 55 genes show polarization trend. Among all tested lines, JinongCH03, CG086, and JinongCH01 are the only one possessing favorable alleles of TaCKX-D1, Pm21, and COMT-3B, which could be used as gene donors for kernel weight, powdery mildew resistance, and stem lignin content improvement and breeding.

In this study, genotypes of 55 genes in 126 wheat lines are clarified, allelic distribution of important agronomic genes in wheat lines attending the Shandong regional trial was basically clarified, which will serve as the foundation for marker assistant selection breeding.

#588

Pyramiding 4 wheat durable resistant genes for rusts and powdery mildew control

Wei Li, Guoqi Song, Yulian Li, Shujuan Zhang, Rongzhi Zhang, Jihu Li, Jie Gao, Genying Li*

Crop research institute, Shandong Academy of Agricultural Sciences, Jinan, Shandong 250100, China

Email: lgy111@126.com

Lr34/Yr18/Pm38/Sr57, *Lr27/Yr30/Sr2*, *Lr46/Yr29/Pm39/Sr58* and *Lr67/Yr46/Pm46/Sr55* are four durable resistant genes in wheat. Each one provides partial resistance without race-specificity to leaf rust, stripe rust, stem rust and powdery mildew in adult plant stage. When deployed with each other, resistance will be enhanced, but the effect of four genes together is unclear.

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How many genes and what combination will provide adequate resistance with less production penalty need further investment.

#87

Difference in starch structure and physicochemical properties between waxy wheat and non-waxy wheat subjected to temporary heat stress during grain filling

Xin Liu [1, 2, †], Dongdong Zhou [1, 3, †], Cunhu Dai [1, 2], Yangyang Zhu [1, 2], Min Zhu [1, 2], Jinfeng Ding [1, 2], Xinkai Zhu [1, 2], Guisheng Zhou [1, 4], Wenshan Guo [1, 2], Chunyan Li [1, 2, *]

[1]Jiangsu Key Laboratory of Crop Genetics and Physiology, and Jiangsu Key Laboratory of Crop Cultivation and Physiology, Agricultural College of Yangzhou University, Yangzhou 225009, China;

19515797879@163.com (X.L.); 18762550988@163.com (D.Z.); dai457600@163.com (C.D.); 19851384855@163.com (Y.Z.); minzhu@yzu.edu.cn (M.Z.); jfdin@yzu.edu.cn (J.D.); xkzhu@yzu.edu.cn (X.Z.); gszhou@yzu.eud.cn (G.Z.); guows@yzu.edu.cn (W.G.); licy@yzu.edu.cn (C.L.)
[2]Jiangsu Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China
[3]Huai' an Agricultural Technology Promotion Center, Huai'an 223001, China
[4]Joint International Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou University, Yangzhou 225009, China
† These authors contributed equally to this work.

*****Correspondence: licy@yzu.edu.cn (C.L.)

The formation process of starch in the wheat grain is influenced by both genetic characteristics and environmental factors, which can affect starch quality. Waxy wheat Yangnuo1 (YN1) and non-waxy wheat Yangmai15 (YM15) were subjected to heat stress of day (35 °C) / night (25 °C) at early, medium, and late grain filling stages using artificial intelligence temperature control. Heat stress increased the short chain content of amylopectin in both cultivars and decreased their amylose contents. The effect of heat stress on wheat amylopectin structure was most pronounced in 16–20 days after anthesis (DAA).

The crystallinity and enthalpy of starch decreased, as did the swelling potential, solubility and transmittance, but the retrogradation degree showed an opposite trend after heat stress. Compared with YM15, YN1 exhibited superior physical and chemical properties as well as anti-aging properties of starch and consequently had greater thermal stability under heat stress due to its higher degree of branching.

The most sensitive stage to heat stress for yield was 6–10 DAA, resulted in significant decreases in grain number and 1000-grain-weight, followed by 16–20 DAA, resulted a significance only in 1000-grain-weight.

Our study indicated that heat stress during the early stage of grain filling resulted in a decrease in both grain weight and yield, whereas during the middle stage of grain filling, which led to a decline in starch quality, especially in non-waxy wheat.

#325

Exploration of elite alleles of dwarf genes in wheat and analysis of their action mechanisms

Huiyuan Li[1, †], Linlin Wang[1, 2, †], Kangnan Jia[1, 2, †], Hongchun Xiong[1], Huijun Guo[1], Yongdun Xie[1], Chunyun Zhou[1, 3], Meiyu Fu[1], Yumei Zhang[1, 2], Meiai Zhao[1, 2], and Luxiang Liu[1*]

[1] Institute of Crop Sciences, Chinese Academy of Agricultural Sciences/National Engineering Laboratory for Crop Molecular Breeding, National Center of Space Mutagenesis for Crop Improvement, Beijing 100081, China. [2] College of Life Science, Qingdao Agricultural University, Qingdao 266109, China. [3] State Key Laboratory of Crop Stress Biology in Arid Areas and College of Agronomy, Northwest A&F University, Yangling, Shaanxi 712100, China
[†] These authors contributed equally to this work.

[*] Corresponding author. E-mail address: liuluxiang@caas.cn.

The Green Revolution, based on dwarf breeding, has skyrocketed wheat grain yields mainly through the regulation of phytohormone gibberellin (GA) metabolism and signal transduction. Currently, alleles of available dwarf genes are very poor in breeding, and nitrogen use efficiency has been negatively affected.

Novel allelic variations are therefore essential for expanding dwarf gene resources and cultivating green and efficient wheat dwarf varieties. In this project, we will take the GA-sensitive *Rht8* cloned by the project team and the GA-insensitive *Rht-1* (Green Revolution gene) as research objects.
By using the wheat mutant library and surrogate prime editors developed previously, we will create innovative dwarf mutants and obtain target mutations in the promoter region, UTR, and important functional domains.

Moreover, we will further analyze the effects of various allelic variations on plant height, nitrogen use efficiency, and yield under low nitrogen and normal nitrogen conditions and screen for variant types that are superior to the Green Revolution dwarfing alleles.

Finally, we will analyze the mechanism by which different allelic variations affect gene function, examine the expression changes of *GRF4*/*NGR5* (GA signaling and the nitrogen utilization key transcription factors) and nitrate transporter *TaNRT2* in mutants, and identify molecular modules that can enhance yields and nitrogen use efficiency synergistically.

The findings will contribute to the precise utilization of dwarf genes for breeding, thus providing a creative strategy to design high-yield and high-efficiency wheat varieties.

#321

Genomic prediction of resistance to wheat yellow rust in Nordic spring wheat

Min Lin[1], Jose Crossa[2], Morten Lillemo[1]
[1] Dep. of Plant Sciences, Norwegian Univ. of Life Sciences, Post Box 5003, NO-1432 ÅS, NORWAY
[2] Biometrics and Statistics Unit, International Maize and Wheat Improvement Center (CIMMYT), Km 45 Carretera México-Veracruz, Texcoco, Edo. de México, CP 56100, México

min.lin@nmbu.no

Yellow Rust (YR) in wheat, caused by *Puccinia striiformis* f. sp. *tritici*, can significantly affect global wheat production, especially in cool and humid regions. Genomic-assisted breeding, utilizing genetic markers, is effective in selecting breeding lines with high breeding values and enhanced disease resistance. In this study, 300 Nordic spring wheat lines were genotyped using the wheat 25K SNP chip, and their resistance to YR was tested across 17 trials conducted on four continents over seven years.

The objective of this study was to apply genomic prediction for yellow rust resistance and compare the prediction ability (PA) of different models. Three genomic prediction models were assessed: Model 1 utilized only significant markers from the Genome-Wide Association Study (GWAS) as genomic information (Gs), alongside environments (E), and the genomic by environment interaction (Gs × E) to fit the Genomic Best Linear Unbiased Prediction (GBLUP) model. Model 2 employed the entire genomic data (G) and fitted the GBLUP model with E and G × E. Model 3 was a deep learning model that utilized complete genome data and environments.

Cross-validation (CV) was performed by randomly selecting 20% of observations as the test set and using the remaining data as the training set. This procedure was repeated five times. Through CV, the PA for each environment varied, ranging from 0.32 to 0.84 for Model 1, 0.41 to 0.87 for Model 2, and 0.45 to 0.97 for Model 3.

Additionally, we predicted four trials from 2021 using data from thirteen trials conducted from 2015 to 2020 for training. The PAs with Model 1 were 0.54, 0.47, 0.64, and 0.65; with Model 2 were 0.63, 0.59, 0.70, and 0.73; and with Model 3 were 0.61, 0.56, 0.72, and 0.73, respectively. These results indicate that a reasonable PA can be achieved even when only significant markers are utilized as genomic information for prediction.

However, the inclusion of complete genomic information in Model 2 could further enhance the PA up to 70%. Furthermore, although there were observable differences in the PA of individual trials between Model 2 and Model 3, these models yielded comparable results.

#177

Compound extreme heat and drought stress alter the spatial gradients of protein and starch in wheat grains

Jiangyi Shi [a, #], Zhifeng Ding [a,b, #], Xin Ge [a], Xiaolei Qiu [a], Junhao Xu [a], Liujun Xiao [a], Leilei Liu [a], Liang Tang [a], Weixing Cao [a], Yan Zhu [a], Bing Liu [a, *]

[a] National Engineering and Technology Center for Information Agriculture, Key Laboratory for Crop System Analysis and Decision Making, Ministry of Agriculture, Jiangsu Key Laboratory for Information Agriculture, Jiangsu Collaborative Innovation Center for Modern Crop Production, Nanjing Agricultural University, Nanjing, Jiangsu 210095, P. R. China.
[b] College of Civil Engineering, Nanjing Forestry University, Nanjing, Jiangsu 210037,PR China
[#] These authors contributed equally to this work.

[*]**Correspondence:** Bing Liu, **E-mail**: bingliu@njau.edu.cn

Spatial gradients of protein and starch in wheat grains and determine grain milling characteristics, and flour utilization. The increasing compound heat stress and drought stress (HDS) due to global climate change threaten wheat grain yield and quality parameters, but the impacts of extreme climate events on the gradients of protein and starch in wheat grains remain unclear.

In this study, two-year environment-controlled experiments with four heat stress levels (17/27, 21/31, 25/35, and 29/39 °C) and three drought stress levels (25%, 55%, and 75% of field capacity), were conducted to investigate effects of HDS on the gradients of protein and starch concentration within the five grain layers.

The results show that HDS resulted in significantly higher protein concentration, whilst resulting in lower starch concentration in wheat grain layers. Among the five layers, the endosperm layers had the highest increase in protein concentration under HDS, but the decrease of starch concentration under HDS ranked as husk > aleurone > endosperm layer. HDS altered the protein and starch concentration of the five grain layers unevenly.

There were significant linear relationship between relative protein and starch concentration with accumulated heat degree days (AHDD). With a 1°C·d increase in AHDD, the protein concentration in the five grain layers increased by 0.42-3.76%, while the starch concentration decreased by 0.02-1.65%, depending on the drought stress levels.

Our results indicate that HDS will significantly impact the grain quality parameters for flour milling process and human diet, and will provide important insights on adapting wheat quality to climate change.

#410

Discovery of key genes regulating wheat leaf stomatal traits and their superior sites

Dezheng Liu[1,2,*], Liang Chen[1,2], Yin-gang Hu[1,2]

[1] College of Agronomy, Northwest A&F University, Yangling 712100, China. [2] State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, Northwest A&F University, Yangling 712100, China
Dezheng Liu, 2020050071@nwafu.edu.cn; Yin-gang Hu, huyingang@nwafu.edu.cn

Stomata represent the primary conduit through which plants facilitate the exchange of water and gases with the environment and serve a pivotal role in this process. In order to identify stomatal trait characteristics and screen for genes related to stomatal traits with drought tolerance and water-saving properties in wheat, four stomatal traits were determined under two environmental conditions in two growing seasons using 184 wheat populations as a natural population.

A genome-wide association analysis of stomatal traits was conducted using wheat 660K SNP microarrays in conjunction with four models: MLM, Blink, CMLM, and FarmCPU were employed to identify 18 key quantitative trait loci (QTL) intervals on the wheat chromosomes. Nine candidate genes regulating stomatal traits were identified through homologous gene annotation.
Furthermore, new chloroplast localization protein family members, CoA ligase, FLOWERING LOCUS T protein, NAC transcription factors, and 2-oxoglutarate and Fe(II)-dependent oxygenase family proteins were identified. Bayesian Ridge Regression was employed for genomic prediction, resulting in a prediction accuracy of 71% with a subset of 180 SNP markers.

This underscores the potential of genomic prediction with a limited number of markers in practical breeding applications. In addition, three pairs of KASP markers were developed in AX-110069905, AX-111486660, and AX-111646728, respectively, and validated in natural populations.
These findings contribute to a more comprehensive understanding of wheat stomatal traits and provide a reference value for germplasm screening and the innovation of wheat stomatal traits.

#632

Breeding and application of wheat starch branching enzyme *tasbeiia* gene editing germplasm

Jiansheng Chen[1,*], Qier Liu[1],Hui Chong[1], Dewei Kong[1], Haixia Yu[1], Weidong Zhang[1]

[1] Shandong Agricultural University, Tai’an 271018, China

* Correspondence: jshch@sdau.edu.cn (J.C.)

Wheat is the second largest food crop in the world, and as living standards improve, the demand for wheat varieties rich in specific nutrients is gradually increasing. Foods with high amylose content have great potential to improve human health, lower glycemic index, and improve gut health.

However, amylose accounts for 15%-26% of the total starch content in common wheat varieties. In this study, we focused on the starch branching enzyme gene *TaSBEIIa*, using CRISPR/Cas9 gene editing technology to knock out the gene in spring wheat Fielder cultivars. We aim to validate the gene function and develop a new wheat variety with high amylose levels. A total of 68 regenerated seedlings were successfully obtained, with a transformation efficiency of 51.47% and an editing efficiency of 48.53%.

Most of the mutant editing types obtained were 20-58bp large fragment deletions, and a few were single-base insertions. The non-transgenic triple deletion mutants were successfully isolated through screening of progeny, and the amylose content of the mutants increased from 15.41% to 30.47%. The activity of starch branchase (SBE) was found to be significantly lower at 7, 14, and 21 days after flowering compared to the wild type.

The morphology of the mutant starch granules has changed from smooth, spherical, or oval to a highly irregular shape. Most of the A-type starch granules (>10 μm in diameter) now appear sickle-shaped. Currently, gene editing of TaSBEIIa is being conducted on Shannong 44 and Shannong 67, which are new high-quality, high-yield winter wheat varieties. The goal is to develop new non-transgenic wheat germplasm with significantly higher amylose content.

This will enable the production of more cereal products with high amylose content to meet consumer demand. This study also provides a theoretical basis for the feasibility of using gene editing technology to improve the amylose of cereal crops.

#45

Genetic effects of agropyron cristatum 2P chromosome translocation fragments in a wheat background

Shirui Xu [1,*], Bo Jiang [1,*], Xiajie Ji[1], Haiming Han[1], Jinpeng Zhang[1], Shenghui Zhou[1], Xinming Yang[1], Xiuquan Li[1], Lihui Li[1], Weihua Liu[1]

[1] National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China

* These authors contributed equally to this work.
Weihua Liu, E-mail: liuweihua@caas.cn

Agropyron cristatum ($2n = 4x = 28$, PPPP) is a wild relative of common wheat which contains a large number of desirable genes that can be exploited for wheat improvement. Wheat–A. cristatum 2P alien translocation lines exhibit many desirable traits, such as small flag leaves, a high spikelet number and density, and a compact plant type.

An agronomic trait evaluation and a genetic analysis were carried out on translocation lines and backcross populations of these lines carrying different translocation fragments. The results showed that a translocation fragment from 2PT-3 (2PL) reduced the length of the flag leaves, while translocation fragments from 2PT-3 (2PL) and 2PT-5 (2PL (0.60–1.00)) reduced the width of the flag leaves.

A translocation fragment from 2PT-13(2PS (0.18–0.36)) increased the length and area of the flag leaves. Translocation fragments from 2PT-3 (2PL) and 2PT-8 (2PL (0.86–1.00)) increased the density of spikelets. Translocation fragments from 2PT-7 (2PL (0.00–0.09)), 2PT-8 (2PL (0.86–1.00)), 2PT-10 (2PS), and 2PT-13 (2PS (0.18–0.36)) reduced plant height.

This study provides a scientific basis for the effective utilization of wheat–A. cristatum translocation lines.

#641

TaBAP-7A confers sharp eyespot resistance in bread wheat

Caiyun Liu[1], Yanjiang He[2], Ying Liu[1], Qiaofeng Zhang[1], Bisheng Fu[1], Wei Guo[1], Jin Cai[1], Wenling Zhai[1], Jizhong Wu[1, *]

[1] Institute of Germplasm Resources and Biotechnology, Jiangsu Academy of Agricultural Sciences, Nanjing 210014, Jiangsu, China
[2] School of Life Sciences, Jiangsu University, Zhenjiang, 212013, China

*Corresponding author: wujz@jaas.ac.cn

Wheat sharp eyespot, a disease mainly caused by the soilborne fungus *Rhizoctonia cerealis*, becomes a threat to wheat production worldwide. Wheat's genetic resistance to this disease is a potential approach to reducing the application of fungicides and farming practice inputs. In the present study, a genome-wide association study using 90K SNP identified a stable QTL on chromosome 7A (*Qse.jaas-7A*) for sharp eyespot resistance in a population comprising 255 wheat accessions across three environments.

Only a C2 domain-containing gene (*TaBAP-7A*) in the LD block of *Qse.jaas-7A* was induced by *R. cerealis* through comparative transcriptome analysis. A 107 bp deletion in the exon region of *TaBAP-7A* was identified in the susceptible lines compared to the resistant lines by sequence analysis.
Overexpression lines and ethyl-methanesulfonate (EMS) mutants revealed *TaBAP-7A* positively regulating resistance to *R. cerealis*. A subcellular localization assay suggested that *TaBAP-7A* localizes to the nucleus, cytoplasm, and cytomembrane.

Thus, this study proposes *TaBAP-7A* as a promising gene for improving wheat resistance to sharp eyespot in bread wheat.

#564

Genetic dissection of complex traits and genomics-assisted breeding in wheat

Shubing Liu[1], Yunlong Pang[1], Qiang Yan[1], Hailiang Zhao[1], Guihua Bai[2]

[1]National Key Laboratory of Wheat Improvement, College of Agronomy, Shandong Agricultural University, Tai'an 271018, China. [2]USDA–ARS, Hard Winter Wheat Genetics Research Unit, Manhattan 66506, USA

E-mail: sbliu@sdau.edu.cn

Wheat is one of the major staple food crops around the world, the genetic dissection of important traits is essential to identify and use novel genes to continuously increase grain yield to ensure the global food security.

In this study, a large-scale genome-wide association study (GWAS) was conducted using a panel of 768 wheat cultivars and breeding lines that were genotyped by genotyping-by-sequencing and 599 genomic loci for 18 important traits including 12 yield related traits, resistance to stripe rust, leaf rust and powdery mildew, yellow mosaic virus, cold tolerance and pre-harvest sprouting were identified.
Among which, some of the QTLs modulate plant height, spike and grain shape and size were validated in bi-parental populations, and the candidate genes underlying the QTL were cloned with the function validated by genome editing. KASP assays were also developed for some of the major QTLs identified by GWAS, which can facilitate the fine mapping and marker assisted selection of the targeting QTLs.

Genomic prediction models for each trait were constructed based on the significant SNPs that were highly associated with those traits with high prediction accuracies. Results from this study demonstrated that high resolution GWAS using a large population and high-density marker can not only lay a solid foundation for large-scale QTL fine mapping and candidate gene identification, marker assisted breeding, but also increase the prediction accuracy for genomic selection in wheat.

#580

Decouple the pleiotropic effects of wheat vrt-a2 gene on spike development and grain weight formation

Jing Liu, Zhaoyan Chen, Zhihui Wang, Lingling Chai, Yingyin Yao, Weilong Guo, Zhongfu Ni, Qixin Sun & Jie Liu

Frontiers Science Center for Molecular Design Breeding, China Agricultural University, College of Agronomy and Biotechnology, Beijing, 100193, China

Elongated outer glume characterizes polish wheat (Triticum polonicum) as a unique tetraploid wheat species. The genetic control of long-glume trait by a single semi-dominant P1 (from Polish wheat) locus has been established over 100 years ago, whereas its causal gene and the precise molecular details remain elusive.

Based on map-based cloning, we isolate VEGETATIVE TO REPRODUCTIVE TRANSITION 2 (VRT-A2), an SVP-clade MADS-box transcription factor (TF) coding gene, as P1 candidate. Genetic evidence suggests that in T. polonicum, a naturally occurred sequence rearrangement in the intron-1 region of VRT-A2 leads to its ectopic expression in floral organs, whereby the long-glume phenotype appears.

More interestingly, we demonstrate that the intron-1 region is a key ON/OFF molecular switch for VRT-A2 expression, due to its sequence features for not only recruiting transcriptional repressors, but also conferring intron-mediated transcriptional enhancement. Genotypic analyses using wheat accessions indicate that P1 locus is likely derived from a single natural mutation in tetraploid wheat, which later has been inherited by hexaploid T. petropavlovskyi.

It should be noted that overdose activation of VRT-A2 significantly enhances grain weight but also triggers increased rudimentary basal spikelets and decreased grain number per spike. This yield penalty has presented challenge for the use of VRT-A2 in high-yield wheat breeding practice.

Aiming to decouple the pleiotropic effects of VRT-A2 on different tissues, we further make efforts to characterize the upstream regulatory modules for fine-tuning VRT-A2 expression, and confirm that: an AP2/ERF-type transcription factor multi-floret spikelet1 (TaMFS1) represses VRT-A2 expression by recruiting transcriptional co-repressor and histone deacetylase; while a structure-specific recognition protein TaSSRP1 facilitates VRT-A2 activation by assembling Mediator and RNA polymerase.

By deleting TaMFS1, we enable a moderate upregulation of VRT-A2 expression, resulting in significant increase of grain weight without yield penalty. Our study provides a feasible strategy for overcoming the trade-offs of pleotropic genes by editing their upstream transcriptional regulators.

#253

Validation of QTLS for resistance to pre-harvest sprouting in a panel of european wheat cultivars

Ana Lovrić[1,*], Bruno Rajković[1], Marko Maričević[1], Ivica Ikić[1], Hrvoje Šarčević[2]

[1] Bc Institute for Breeding and Production of Field Crops, 10370 Dugo Selo, Croatia
[2] Faculty of Agriculture, University of Zagreb, 10000 Zagreb, Croatia

*ana.lovric@bc-institut.hr

Pre-harvest sprouting (PHS), which refers to the germination of grains in the spike before harvest in response to rain, can affect the yield and quality of wheat grain. PHS is a quantitative trait controlled by a large number of genes, and QTLs for resistance to PHS have previously been found on almost all wheat chromosomes. Despite the large number of published QTLs, there is a lack of information on their presence and effect in wheat germplasm relevant for breeding programs in Southeastern Europe.

The aim of this study was to evaluate the phenotypic variation in PHS resistance of 200 winter wheat cultivars adapted to the growing conditions in Southeastern Europe and to investigate the allelic impact of 38 SNP markers previously reported to be associated with PHS resistance on the PHS phenotype. Cultivars were evaluated for PHS phenotype in four environments using germination tests from which germination index (GI) was calculated.

Combined ANOVA across environments revealed that genetic (G), environmental (E) and G × E interaction variance accounted for 46, 22 and 27% of the total phenotypic variance, respectively, with an estimated broad-sense heritability of 0.86. After excluding markers with an extremely high rate of missing data and those that are monomorphic, 21 polymorphic SNP markers were analyzed for their effects on GI, considering only homozygous genotypes that have either a tolerant or a susceptible allele.

Analysis of variance across environments revealed that GI was significantly affected by the environment (E) for 21 markers, by the genotype of the marker (G) for 12 markers, and by the G × E interaction for eight markers.

Of the 12 markers that showed a significant genotypic effect on GI, two were from chromosome 3A, two from 3B, six from 4A and one each from chromosomes 5A and 7B. The functional marker TaMKK3 from chromosome 4A showed the largest effect on PHS resistance and explained 17.4% of the phenotypic variance for GI across four environments. The results of the present study could be useful in the selection of markers for marker-assisted selection for PHS resistance in wheat.

#171

Revealing the genetic architecture of frost tolerance by gwas and genomic prediction in bread wheat

Shan Lu[1, 2], Qiumei Lu[1, 2], Renmei Tian[1, 2], Liang Chen[1, 2, *] and Yin-gang Hu[1, 2,*]

[1] State Key Laboratory of Crop Stress Resistance and High-Efficient Production and College of Agronomy, Northwest A&F University, Yangling, Shaanxi, China. [2] Institute of Water Saving Agriculture in Arid Regions of China, Northwest A&F University, Yangling, Shaanxi, China.

Frost, a critical stress factor for wheat, can significantly impact yield and quality. Understanding the genetic basis of frost tolerance is essential for developing resilient wheat varieties. This study presents a comprehensive genomic analysis integrating genome-wide association studies and genomic prediction to illustrate the genetic architecture of frost tolerance in wheat.

A diverse panel of 238 wheat varieties was genotyped with the wheat 660K SNP chip, and their frost damage scores were taken in two environments across two growing seasons. GWAS identified 52 key candidate QTL regions which contained at least two significant marker-trait associations (MTAs), revealing the fifth homologous group of chromosomes as pivotal for cold resistance.
Further analysis identified 26 key candidate genes, including MADS box transcription factors, CRT-binding factors, Glutathione S-transferase and cyclic nucleotide-gated ion channel, potentially integral to the frost stress response.

Utilizing Bayesian Ridge Regression for genomic prediction, a prediction accuracy of 79% was achieved with a subset of 102 SNP markers, underscoring the potential of genomic prediction with a limited number of markers in practical breeding applications.

Additionally, two pairs of KASP primers for MTAs AX-110387252 and AX-109056556 were developed and validated in a natural population, marking a step towards marker-assisted selection. Collectively, these results offered valuable insights for uncovering the genes and regulatory networks conferring frost resistance, facilitating the selection of genotypes with enhanced frost resistance.

#172

Mapping of dwarfing gene rht4 in bread wheat and its effect on plant height

Qiumei Lu[1], Shan Lu[1], Renmei Tian[1], Yue Qiao[1], Chunge Cui[1], Liang Chen[1], Hu Yin-Gang[1, 2]

[1] State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy, Northwest A&F University, Yangling, Shaanxi, China. [2] Institute of Water Saving Agriculture in Arid Regions of China, Northwest A&F University, Yangling, Shaanxi, China

Email: Qiumei Lu, luqiumei@nwafu.edu.cn; Hu Yin-Gang, huyingang@nwafu.edu.cn

Plant height is an important factor affecting wheat yield, dwarf and semi-dwarf genes significantly increased wheat yield by reducing plant height and enhancing lodging resistance. *Rht4*, a gibberellin-responsive (GAR) and recessive dwarfing gene from the x-ray-induced wheat variety Burt, had no negative effects on coleoptile length and seedling vigor, was located in the chromosome 2BL and linked to SSR marker WMC317.

In this study, Rht4 markedly decreased plant height by 21.29%~24.35%. Combining the analysis of 660k SNP chip and whole genome re-sequencing, 8 KASP markers were developed, Rht4 was mapped to a 1.48 Mb region between markers KASP33 and InDel18 in chromosome 2BL.

Combining the bulked segregant RNA-Seq (BSR-Seq) analysis of the segregating population in two years, there were 7 differential expression genes in the mapping region, one candidate gene which encodes a serine/threonine protein kinase had large fragment deletions in Burt ert937 mutant and dwarf lines through the whole genome re-sequencing analysis, the genomic sequence amplification and CDS amplification confirmed the deletion.

Further works is going on to understand the mechanism of Rht4 on regulating plant development, which will promote the functional characterization of Rht4 dwarfing mechanism and its rational utilization for bread wheat improvement.

#594

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

Ming Luo[1], Aihua Wang[1], Breton Brooks[1], Mick Ayliffe[1].

[1]CSIRO A&F

Fungal rust diseases are a major production constraint in wheat with the combined annual global cost of stem rust, stripe rust and leaf rust estimated to be more than $3 billion. The most cost effective and environmentally sustainable approach to controlling rust diseases is via genetic resistance. However, these pathogens evolve rapidly and can quickly overcome resistance genes, particularly when they are deployed individually.

Polygenic resistance is believed to provide more durable resistance, however selection of multiple unlinked resistance genes in breeding programs is difficult and expensive and gene combinations quickly separate in future breeding efforts. A more effective strategy for strong, longer-lasting resistance is to combine multiple genes into a single locus with multi-gene cassettes. We have developed wheat lines containing multi-gene cassettes encoding up to five different stem rust resistance genes at single loci.

Furthermore, two separate resistance gene stacks, each encoding five wheat stem rust resistance transgenes, have been combined by conventional breeding to generate wheat plants containing an unprecedented level of polygenic stem rust disease resistance. Next generation five gene stacks have now been produced in wheat using a Precision Engineering approach that incorporates only wheat DNA sequences i.e. no selectable markers, cloning scars or *Agrobacterium* border sequences.

These entirely cisgenic plants have the potential for reduced regulatory burden given recent changes to GM legislation in some countries. Cisgenic resistance gene stacks are now also being developed for wheat stripe rust and leaf rust diseases. This cisgenic technology is applicable to other crops for improving disease resistance and for developing other polygenic traits of agronomic significance with single gene inheritance.

#448

Metabolite profiling on resistant and susceptible wheat varieties infected with *puccinia striiformis* f. sp. *tritici*

Makhubu Fikile Nelly[1*], Madala Ntakadzeni Edwin[2], Hlongoane Tsepiso[3], Rauwane Molemi Evelyn[4], and Figlan Sandiswa[1]

[1]Department of Agriculture and Animal Health, University of South Africa, Florida 1709, South Africa.
[2]Department of Biochemistry, University of Venda, Thohoyandou 0950, South Africa.
[3]Agricultural Research Council - Small Grain Institute, Bethlehem 9700, South Africa.
[4]Department of Botany, Nelson Mandela University, Qheberha, South Africa

Bread wheat (*Triticum aestivum* L.) has been commercially cultivated in South Africa for more than a century and currently ranks the second most significant cereal crop after maize. The production of wheat in the country remains under threat from various pathogens, with fungal diseases, notably rusts such as *Puccinia* spp., which continues to present the most significant challenge to wheat cultivation in South Africa.

Therefore, it is important to understand how wheat regulate its defense against pathogen attacks. Thus, our current study aimed to evaluate differences in the metabolites that play important role in metabolic responses to *Puccinia striiformis* f. sp. *tritici* (*Pst*) infection in two wheat varieties at different times using an untargeted metabolomic approach.

The differential changes of metabolites in two wheat varieties, Senqu (resistant) and Morocco (susceptible), caused by the infection with two *Pst* races (6E22A+ and 6E22A-) at 14- and 21- days post-inoculation (dpi) were analysed using untargeted ultra-high performance liquid chromatography coupled with a quadruple time of flight mass spectrometry (UHPLC-qTOF-MS) analysis.

Multivariate statistical analysis (MVDA) tools, *viz.* principal component analysis (PCA) and the orthogonal projection to latent structures-discriminant analysis (OPLS-DA) loading scatter plot were used to identify the metabolites that are positively and negatively correlated to *Pst* infection. PCA and OPLS-DA analysis showed cluster separations between the varieties, infection races and the time-points, thus indicating distinct biochemical changes.

Metabolites were identified belonging class of phenolic acids, carbohydrates and fatty acids among other classes. The study provided the insights on biochemical changes in wheat metabolite expressions following stripe rust infection, thus offering valuable insights for potential applications in crop improvement.

#405

Genome-editing of a circadian oscillator taprr95 facilitates wheat peduncle growth and heading date

Mingxue Fu[1,*], Shaoshaui Liu[1,*], Yuqing Che[1,*], Dada Cui[1], Zhongyin Deng[1], Yang Li[1], Xinyu Zou[1], Xingchen Kong[2], Guoliang Chen[3], Min Zhang[3], Yifan Liu[4], Xiang Wang[4], Wei Liu[5], Danmei Liu[5], Shuaifeng Geng[1,*], Aili Li[1,*], Long Mao[1,*]

[1]State Key Laboratory of Crop Gene Resources and Breeding and National Key Facility for Crop Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China. [2]College of Life Sciences, Henan Normal University, Xinxiang, Henan 453007, China. [3]College of Agronomy, Sichuan Agricultural University, Chengdu, Sichuan 610106, China. [4]College of Agronomy, Henan Agricultural University, Zhengzhou, Henan 450002, China. [5]School of Life Science, Shanxi University, Taiyuan, Shanxi 030006, China

Emails: maolong@caas.cn

Plant height and heading date are important agronomic traits in wheat (*Triticum aestivum* L.) that affect final grain yield. In wheat, knowledge of *pseudo-response regulator* (*PRR*) genes on agronomic traits is limited. Here, we identify a wheat *TaPRR95* gene by genome-wide association study (GWAS) associated with plant height.

The CRISPR/Cas9-editing *prr95*^{aabbdd} mutant plants show increased plant height, particularly at the peduncle, with an earlier heading date. The longer peduncle is mainly caused by the increased cell elongation at its upper part, whilst the early heading date is accompanied with elevated expression of flowering genes, including *TaFT* and *TaCO1*.

A peduncle-specific transcriptome analysis reveals up-regulated photosynthesis genes and down-regulated IAA/Aux genes, key negative regulators of auxin signaling, in *prr95*^{aabbdd} plants, which may act as a regulatory mechanism to promote robust plant growth.

A haplotype analysis identifies a *TaPRR95-B* haplotype (Hap2) closely associated with reduced plant height and the increased thousand-grain weight. Moreover, the Hap2 frequency is higher in cultivars than in landraces, suggestive of the presence of artificial selection of the allele during wheat breeding.
These findings suggest that *TaPRR95* is a key regulator of plant height and heading date, thereby providing an important target for yield improvement in wheat.

#249

Genome-wide association study for seed and embryo dormancy in European winter wheat

Marko Maričević[1,*], Bruno Rajković[1,], Ana Lovrić[1], Ivica Ikić[1], Jerko Gunjača[2], Hrvoje Šarčević[2]

[1] Bc Institute for Breeding and Production of Field Crops, 10370 Dugo Selo, Croatia. [2] Faculty of Agriculture, University of Zagreb, 10000 Zagreb, Croatia

*marko.maricevic@bc-institut.hr

Pre-harvest sprouting (PHS) can reduce the grain yield and bread-making quality of wheat. The main component of wheat resistance to PHS is the level of seed dormancy, which can result from two mechanisms, seed coat imposed dormancy and embryo dormancy. The biochemical mechanisms of seed dormancy include embryo sensitivity to abscisic acid (ABA).

In the present study, a genome-wide association study (GWAS) was conducted by evaluating a panel of 200 European wheat cultivars for seed dormancy (SD), embryo dormancy (ED), and embryo response to ABA (E-ABA).

The panel was genotyped using the 25K Single Nucleotide Polymorphism (SNP) array. The field trials were conducted in four environments in Croatia and SD, ED and E-ABA were evaluated by germination tests with whole grains in water, half grains in water and half grains in 20 µM ABA, respectively. Based on the LOD threshold (–log10(p) > 3.5) GWAS identified a total of 35 quantitative trait nucleotides (QTNs) significantly associated with SD, which were located on chromosomes 1D, 3A, 3D, 4A, 4B, 5B and 7D and explained 6.4−14.1% of the phenotypic variance.

Among them only QTNs from chromosomes 4A (1) and 5B (4) were stable, occurring in two or more environments. For ED, a total of 33 significant QTNs were found on chromosomes 3A, 3B, 3D, 4A, 4B, 5B, 6A, 6B, 7B and 7D, which explained 6.4−8.9% of the phenotypic variance. Stable QTNs for ED were located on chromosomes 3A (3), 3B (1), 3D (6), 4A (1) and 7D (1).

For E-ABA, a total of 33 significant QTNs were identified on chromosomes 1B, 1D, 3A, 3D, 4A, 4D, 5A, 5D, 7B and 7D explaining 7.9−12.6% of the phenotypic variance, of which stable QTNs were located on chromosomes 1D (1), 4A (6) and 5D (1). Nine significant QTNs (all from chromosome 4A) were common to SD and E-ABA, 12 significant QTNs were common to ED and E-ABA (of which 9 from the homoeologous group 3 chromosomes), and 4 QTNs (all from chromosome 4A) were common to all three traits in single environments.

Of the stable QTNs only one, located on chromosome 4A, was common to SD and E-ABA, and another, also from chromosome 4A, was shared by ED and E-ABA.

#121

Enhancing crispr/cas mutations analysis in multicopy gene families with a breakthrough pipeline: wheat alpha-gliadins case

Miriam Marín-Sanz[1] (mmarin@ias.csic.es), María H. Guzmán-López[1] (mhguzman@ias.csic.es), Susana Sánchez-León[1] (ssanchez@ias.csic.es), Marta Gavilán-Camacho[1], (mgavilan@ias.csic.es), Francisco Barro[1] (fbarro@ias.csic.es)

[1] Institute for Sustainable Agriculture-Spanish National Research Council (IAS-CSIC), Córdoba 14004, Spain

Gluten-encoding genes have drawn the attention of CRISPR/Cas technology due to their implication in certain human pathologies, such as Celiac Disease (CD), an autoimmune enteropathy in genetically predisposed individuals.

However, the complexity of the genes encoding for gluten proteins poses challenges for developing CRISPR-based gene editing tools, mutation analysis and further selection of edited lines. Particularly, the alpha-gliadin gene family, arranged in multiple copies in tandem across the three wheat subgenomes, has been edited using one single guide RNA (sgRNA) per construct.

Now, we are progressing the approach by multiplexing CRISPR constructs, harboring multiple sgRNAs targeting the same genes. The previous workflow relied on phylogenetic tree construction for characterizing insertions/deletions (InDels) and required human intervention.

It shows the mutation profiles of CRISPR lines and how they are inherited across three generations of plants. Now, we have introduced a new automated pipeline based on reference genes family alignment, enabled by deep amplicon sequencing, and implemented in high-performance computing (HPC) clusters.

This pipeline facilitates the detection of sgRNA-specific InDels edits and covers large deletions using multi-sgRNAs in different sample types. Additionally, next-generation sequencing (NGS)-based analyses provide insights into alpha-gliadins in wild-type lines, allowing the characterization of the natural variation of the family genes and the diversity of their immunogenicity.

Altogether, the integration of bioinformatics tools accelerates the development of low-immunogenic wheat, making faster the screening of edited lines and facilitating the identification and selection of optimal CRISPR targets for this purpose.

#151

ZIP4 is required for normal progression of synapsis and for over 95% of crossovers

Martin Azahara C[1], Draeger Tracie[2], Alabdullah Abdul Kader[2], Hayta, Sadiye[2], Smedley Mark[2], Moore Graham[2]

[1] Institute for Sustainable Agriculture (IAS-CSIC), Córdoba, Spain. [2] John Innes Centre, Norwich, UK.

Tetraploid (AABB) and hexaploid (AABBDD) wheat have multiple sets of similar chromosomes, with successful meiosis and preservation of fertility relying on synapsis and crossover (CO) formation only taking place between homologous chromosomes. In hexaploid wheat, the major meiotic gene TaZIP4-B2 (Ph1) on chromosome 5B, promotes CO formation between homologous chromosomes, whilst suppressing COs between homeologous (related) chromosomes.

In other species, ZIP4 mutations eliminate approximately 85% of COs, consistent with loss of the class I CO pathway. Tetraploid wheat has three ZIP4 copies: TtZIP4-A1 on chromosome 3A, TtZIP4-B1 on 3B and TtZIP4-B2 on 5B. Here, we have developed single, double and triple zip4 TILLING mutants and a CRISPR Ttzip4- B2 mutant, to determine the effect of ZIP4 genes on synapsis and CO formation in the tetraploid wheat cultivar 'Kronos'.

We show that disruption of two ZIP4 gene copies in Ttzip4-A1B1 double mutants, results in a 76-78% reduction in COs when compared to wild-type plants. Moreover, when all three copies are disrupted in Ttzip4-A1B1B2 triple mutants, COs are reduced by over 95%, suggesting that the TtZIP4-B2 copy may also affect class II COs. If this is the case, the class I and class II CO pathways may be interlinked in wheat. When ZIP4 duplicated and diverged from chromosome 3B on wheat polyploidization, the new 5B copy, TaZIP4-B2, could have acquired an additional function to stabilize both CO pathways.

In tetraploid plants deficient in all three ZIP4 copies, synapsis is delayed and does not complete, consistent with our previous studies in hexaploid wheat, when a similar delay in synapsis was observed in a 59.3 Mb deletion mutant, ph1b, encompassing the TaZIP4-B2 gene on chromosome 5B.

These findings confirm the requirement of ZIP4-B2 for efficient synapsis, and suggest that TtZIP4 genes have a stronger effect on synapsis than previously described in Arabidopsis and rice. Thus, ZIP4-B2 in wheat accounts for the two major phenotypes reported for Ph1, promotion of homologous synapsis and suppression of homeologous COs.

#153

Zip4 is required for normal progression of synapsis and for over 95% of crossovers

Martin Azahara C[1], Draeger Tracie[2], Alabdullah Abdul Kader[2], Hayta, Sadiye[2], Smedley Mark[2], Moore Graham[2]

[1] Institute for Sustainable Agriculture (IAS-CSIC), Córdoba, Spain
[2] John Innes Centre, Norwich, UK.

Effective chromosome synapsis and crossover formation during meiosis are essential for fertility, especially in grain crops such as wheat. These processes function most efficiently in wheat at temperatures between 17-23 °C, although the genetic mechanisms for such temperature dependence are unknown.

In a previously identified mutant of the hexaploid wheat reference variety ‘Chinese Spring’ lacking the long arm of chromosome 5D, exposure to low temperatures during meiosis resulted in asynapsis and crossover failure. In a second mutant (*ttmei1*), containing a 4 Mb deletion in chromosome 5DL, exposure to 13 °C led to similarly high levels of asynapsis and univalence. Moreover, exposure to 30 °C led to a significant, but less extreme effect on crossovers.

Previously, we proposed that, of 41 genes deleted in this 4 Mb region, the major meiotic gene *TaDMC1-D1* was the most likely candidate for preservation of synapsis and crossovers at low (and possibly high) temperatures. In the current study, using RNA-guided Cas9, we developed a new Chinese Spring CRISPR mutant, containing a 39 bp deletion in the 5D copy of *DMC1*, representing the first reported CRISPR-Cas9 targeted mutagenesis in Chinese Spring, and the first CRISPR mutant for *DMC1* in wheat.

In controlled environment experiments, wild-type Chinese Spring, CRISPR *dmc1-D1* and backcrossed *ttmei1* mutants were exposed to either high or low temperatures during the temperature-sensitive period from premeiotic interphase to early meiosis I. After 6-7 days at 13 °C, crossovers decreased by over 95% in the *dmc1-D1* mutants, when compared with wild-type plants grown under the same conditions. After 24 hours at 30 °C, *dmc1-D1* mutants exhibited a reduced number of crossovers and increased univalence, although these differences were less marked than at 13 °C.

Similar results were obtained for ttmei1 mutants, although their scores were more variable, possibly reflecting higher levels of background mutation. These experiments confirm our previous hypothesis that *DMC1-D1* is responsible for preservation of normal crossover formation at low and, to a certain extent, high temperatures. Given that reductions in crossovers have significant effects on grain yield, these results have important implications for wheat breeding, particularly in the face of climate change

#548

Identification of genomic regions associated with micronutrient content (fe, zn, mn, cu) in durum wheat grains

Lourdes María Martínez[1,5], Diana Martino[2], Juan Manuel Rodrigo[1,3], Juan Manuel Rivera[2,4], Ana Clara Fernández[3], Ana Laura Achilli[3], Lisardo González[2], Viviana Echenique[1,3], Pablo Federico Roncallo[1,3]

[1]Departamento de Agronomía, Universidad Nacional del Sur (UNS), Bahía Blanca, Buenos Aires, Argentina. [2]Buck Semillas S.A., Necochea, Buenos Aires, Argentina. [3]Centro de Recursos Naturales Renovables de la Zona Semiárida (CERZOS), Departamento de Agronomía, Universidad Nacional del Sur (UNS)- CONICET, Bahía Blanca, Buenos Aires, Argentina. [4]Facultad de Agronomía, Universidad Nacional de Mar del Plata (UNMDP), Balcarce, Buenos Aires, Argentina. [5]Comisión de Investigaciones Científicas (CIC) de la Provincia de Buenos Aires, Bahía Blanca, Buenos Aires, Argentina.

e-mail: roncallo@cerzos-conicet.gob.ar, dmartino@bucksemillas.com.ar

Durum wheat (*Triticum turgidum* var *durum* L.) is used in Argentina mainly for dry pasta production and is an important source of micronutrients. The amount of micronutrients in pasta is directly related to the quality of the grain. Genetic biofortification of micronutrients in wheat grains is a sustainable and low-cost strategy to improve nutritional status in the population.

The study of the genetic basis associated with variation in micronutrient content in wheat grains using biotechnology tools, such as association mapping methodology, can contribute to increase their concentration in grains and products. The objectives of this work were to analyze i) the genetic variability for the content of iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) in grains of two durum wheat collections; ii) to identify associated SNP markers using an association mapping strategy. Micronutrient content (mg/kg) was analyzed in whole wheat flour samples using a plasma spectrometer with atomic mass detector.

Samples were taken from the CERZOS collection (CRZ) and the Global Durum Panel (GDPv1), using 170 and 140 genotypes respectively, in three field trials (Cabildo 2014, Barrow 2017 [CRZ] and La Dulce 2021 [GDPv1]). The CRZ panel was analyzed using a 35K SNP microarray (Affymetrix) and the GDPv1 panel using a 90K microarray (Illumina). Marker-trait associations (MTA) were obtained in TASSEL 5.0 software using GLM and MLM (PCA+K) models.

A high phenotypic variability was found for the four micronutrients analyzed, but the observed range was greater in the CRZ panel. A significant negative correlation was observed between Zn content and grain yield, using the data available in the CRZ panel. Marker-trait associations were identified on all chromosomes. In particular, common regions affecting both panels were identified in 4B for Zn content (two regions linked between 65-98 Mb), in 5B associated with Fe and Zn (550-560 Mb) and in 7B affecting the content of Fe and Zn (679-684 Mb).

One genomic region was strongly associated with Mn content in 6BS (15-17 Mb) and two epistatic regions were identified for Cu content in 7A (RAC875_c35270_234 x Kukri_c9728_1171). SNP markers have been selected to validate them in four segregating F2 populations using KASP technology.

#593

Enhancing wheat productivity through deficit irrigation, osmoregulators and suitable sowing window in dicoccum wheat

Thaimadam Girwani[1] and Ganajaxi Math[1]

[1]Department of Agronomy, College of Agriculture, Dharwad, UAS, Dharwad

Email: *ganajaxi@uasd.in

Amidst climate change and declining water resources, deficit irrigation has become pivotal for water conservation in agriculture. The osmoregulators offer an innovative approach to enhance crop adaptation and productivity under abiotic stress conditions especially for global crop like wheat.
Therefore, the study was conducted during rabi 2022-23 and 2023-24 at MARS, Dharwad, to understand the response of dicoccum wheat to deficit irrigation and osmoregulators under different sowing dates.

The experiment utilized strip-split plot design with three sowing windows (D1: November II fortnight, D2: December I fortnight, D3: December II fortnight) as main-plots, two deficit irrigation levels (I1: Irrigation at 1.0 ETc, I2: Irrigation at 0.5 ETc) as sub-plots, and four osmoregulators as foliar spray treatments (O1: Salicylic acid @ 200 ppm, O2: Thiourea @ 400 ppm, O3: Kaolin as antitranspirant at 5%, O4: Water spray (at tillering and ear-emergence stage) as sub-sub plots and replicated thrice.

The pooled data revealed that, November II FN sowing window recorded significantly higher grain yield (3708 kg ha-1), dry matter production at harvest (108 g at 0.3m row length), test weight (37.50 g), flag leaf area (FLA) of 17.60 cm2 and membrane stability index (MSI) of 76.5% compared to other sowing windows. Similarly, irrigation at 1.0 ETc recorded 19.56 per cent higher grain yield, higher FLA and MSI over 0.5 ETc.

The root length increased by 16 per cent in 0.5 ETc irrigation indicating deeper proliferation of roots in search of water. However, the grain yield was significantly higher with thiourea @ 400 ppm (3170 kg ha-1) and salicylic acid @ 200 ppm (3068 kg ha-1) over water spray (2735 kg ha-1).
Thiourea recorded significantly higher FLA (16.42 cm2) and test weight (36.97 g). The interaction effect of D1×I1×O2 saved water by 45 per cent over recommended irrigation practice (560 mm) with higher productivity.

Keywords: ETc (Crop evapotranspiration), Osmoregulators, deficit irrigation, flag leaf area (FLA), Membrane stability index (MSI)

#472

The negative impact of the triple-mutation of gene tagw2 on grain number of wheat

Iván Matus[1], Dalma Castillo[1], Alejandro del Pozo[2], Daniel F. Calderini[3]

[1] CRI-Quilamapu, National Institute of Agriculture Research (INIA), Chillán, Chile. [2] Plant Breeding and Phenomic Center, Faculty of Agricultural Sciences, Universidad de Talca, Talca, Chile. [3] Institute of Plant Production and Protection, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile

Grain weight (GW) is a key trait of wheat affecting grain yield and quality. Advancements in the understanding of physiological and molecular control of grain weight and size have been significant over the past decade in wheat. Among others, the triple mutation of the *Ta*GW2 gene, a known negative regulator of grain weight, resulted in a successful increase of grain weight both in rice and wheat.

However, the decrease of grain number (GN) was reported in wheat, potentially offsetting yield gains. The objective of this study was to evaluate the response of a triple mutant line of the *Ta*GW2 gene and its wild type on grain yield, grain weight, grain number and the likely causes of the trade-off between both main yield components. A field experiment was conducted with two spring wheat genotypes: a triple mutant line of the gene *Ta*GW2 and its wild type (WT) in Chillán, Chile.

These lines were arranged in a complete randomised block design with four replicates in field plots under optimal management. Data were analysed using ANOVA. As expected, GW was increased ($P< 0.05$) in the triple mutant by 20% over the WT. This positive impact was recorded in both main stems and tillers. On the other hand, grain yield was similar ($P> 0.05$) between the lines as GN was negatively affected in the triple mutant line showing similar decreases of GN between main stems (16.5%) and tillers (15.9%).

The trade-off between GW and GN was found due to the reduction of grains per spike and spikes per plant. These results supports that the effect of the triple mutant line of *Ta*GW2 occurs during a long time of the wheat cycle, at least between tillering and anthesis.

The tillering capability of wheat was found affected in the line surprising the action of the *Ta*GW2 gene. Additionally, the effect of the triple mutation was found across the spikelets of the spikes on both GW and GN. Differences in dry weight of spikes was found a week after anthesis, which is proposed as likely cause of the lower grain number per spike in the triple mutant line.

#237

Michay-inia, spring durum wheat variety for Chile

Matus Iván[1], Castillo Dalma [1], Jobet Claudio[2], Alfaro Christian[3]

1.- Instituto de Investigaciones Agropecuarias, Centro Regional de Investigaciones Quilamapu, Casilla 426, Chillán, Chile. imatus@inia.cl; dalma.castillo@inia.cl. 2.- Instituto de Investigaciones Agropecuarias, Centro Regional de Investigaciones Carillanca, Casilla 58-D, Temuco, Chile. cjobet@inia.cl. 3.-Instituto de Investigaciones Agropecuarias, Centro Regional de Investigaciones Rayentue, Casilla 13, Rengo, Chile. calfaro@inia.cl

Michay-INIA is a spring durum wheat variety (Triticum turgidum var. durum L.) originates from a cross by the Agriculture National Research Institute (INIA-Chile) National Wheat Breeding Program in 2012. The cross and pedigree is Lleuque-INIA/ Cirno C2008. Lleuque-INIA is high yielding elite variety from Chile, and the most planted variety.

Cirno C2008 is also a high yielding elite variety from Mexico. This variety has an upright growth habit in the seedling stage; the adult plant is of medium height and varies between 85 and 95 cm. The spike is white, and exhibits long whitish owns along its full length. The grain is large-sized, elongated, golden yellow, and vitreous. The thousand-kernel weigh varies between 45 and 52 g. When sown in mid-August at the Santa Rosa Experimental Station (36°31’ S; 71°54’ W), Chillan, head emergence occurred 97 to 103 days after sowing.

Mean yield across all 4 locations and four seasons was 10.05 t ha^{-1}, and varied from 9.22 t ha^{-1} to 11.67 t ha^{-1}. Michay-INIA was evaluated in field experiments between 2019 and 2022, under irrigated environments: Los Tilos (33°70’S, 70°70’W), Santa Rosa (36°31’S, 71°54’W), Yungay (37°14’S, 72°01’W) and Humán (37º43’S, 72°41’W). Michay-INIA yielded in Los Tilos, 2.7%, in Santa Rosa 17.38%, in Yungay 10.28% and in Human 16.31%, more than the control variety Lleuque-INIA.

Up to the 2023-2024 season, Michay-INIA has shown resistance to moderate resistance to stripe rust (Puccinia striiformis West. f. sp. tritici), leaf rust (Puccinia triticina Erikss.), and resistance to powdery mildew (Blumeria graminis DC. f. sp. tritici Marchal). Michay-INIA variety is a type of wheat with good hectoliter weight, with values between 82.84 to 84.81 kg hL^{-1}, with SDS values between 10.9 ml to 13.4 ml and protein values fluctuating between 9.7% and 12.9%. Color b fluctuates between 19.2 and 25.5.

#385

Molecular mapping and distribution of two new homeologous leaf rust resistance genes *LrCen* AND *LrMar*

Brent D. McCallum[1], Mingzhe Z. Che[2], Marley Boyce[3], Curt McCartney[4], Colin W. Hiebert[1]
[1] Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden, Manitoba R6M 1Y5, Canada. Email: brent.mccallum@agr.gc.ca
[2] Department of Plant Pathology, China Agricultural University, Beijing, 100193, People’s Republic of China.
[3] Global Edible Oil Solutions - Specialties, Cargill Ltd. Canada, 701 Central Ave. Aberdeen, SK, Canada, S0K 0A0.
[4] Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada

The Thatcher-*Lr1* near isogenic wheat line (RL6003) was noticed to have a mixture of resistant and susceptible plants when inoculated with a small number of Canadian leaf rust (*Puccinia triticina* Eriks). Genetic analysis of the resistant plants revealed the presence of a second unlinked resistance gene. This gene, mapped to 7AL and given the temporary designation *LrCen*, is characterized by an unusual mesothetic infection type.

A similar infection phenotype, in response to the same small set of *P. triticina* isolates, was found for a gene in RL6071 (Prelude/8*Marquis*2/3/Prelude//Prelude/8*Marquis), however this gene mapped to 7BL and was temporarily named *LrMar*. These two resistance genes appear to be homeologous and condition resistance to the same small group of isolates.

When Canadian wheat cultivars were tested phenotypically and genotypically for the presence of these genes *LrCen* was widely distributed, whereas *LrMar* was only found in Marquis, Red Fife and closely related cultivars. *LrCen* also appears to be widely distributed worldwide in wheat germplasm including otherwise susceptible cultivars like Chinese Spring. Given that *LrCen* only conditions resistance to a very small group of isolates, it is interesting that it seems to be so widespread in wheat cultivars throughout the world, which may indicate another function for this gene that is leading to selection for *LrCen*.

#342

Towards winter wheat cultivars with low grain cadmium content: developing breeding tools for low grain cadmium accumulation

Haftom Brhane[1*], Inés Berro[2*], Anders S. Carlsson[1], Johanna Holmblad[3], Marwan Alamrani[1], Mats Söderström[4], Ortrud Jäck[5], Tina Henriksson[3], Lucía Gutiérrez[2] and Therése Bengtsson[1]

[1] Swedish University of Agricultural Sciences (SLU), Department of Plant Breeding, Alnarp, Sweden. [2] University of Wisconsin-Madison, Department of Plant and Agroecosystem Sciences, Madison, USA
[3] Lantmännen Lantbruk, Svalöv, Sweden. [4] Swedish University of Agricultural Sciences (SLU), Department of Soil and Environment, Skara, Sweden. [5] Swedish University of Agricultural Sciences (SLU), Department of Crop Production Ecology, Uppsala, Sweden

Cadmium (Cd) is a toxic heavy metal found naturally in the soil. In humans it primarily enters the body through food consumption, posing risks to organs like the lungs, liver, kidneys, and bones. Wheat genotypes might accumulate different levels of Cd in the grain and therefore, breeding for low grain-Cd accumulation is important to ensure human health.

This project aims to identify winter wheat breeding lines with low grain-Cd content and develop breeding tools for a more precise and affordable selection strategy. For this purpose, 324 winter wheat breeding lines will be evaluated for grain-Cd content across three locations in south of Sweden (Bollerup, Svalöv, Kölbäck) and three years. It has been documented that Cd content in the soil can also influence the Cd accumulation in the grain. Therefore, 25 soil samples per location were evaluated for soil-Cd and ten for pH, clay, and soil organic matter (SOM).

In addition, all fields were scanned with an EM38 gamma ray sensor. Using this soil information a model was developed to predict the soil variables of each plot. The grain-Cd, soil and phenotypic and genotypic data were used to develop linear mixed model genomic prediction models and perform genome-wide association studies. The highest grain-Cd content was found in the lines in the location with the highest soil-Cd level. The Cullis broad sense heritability for grain-Cd across the three locations was high ($H^2 = 0.86$).

Using the GBLUP model, the predictive ability was moderate to high across locations (r=0.54), in Bollerup (r=0.56) and Svalöv (r=0.45) whereas a lower predictive ability was seen for Kölbäck (r=0.37). Incorporating either single or multiple soil variables as covariates doesn't change the predictive ability of the models both across and at specific locations.

Because of low genotype by environment interaction and high predictive ability and heritability in Bollerup, breeders can screen their material in this location and use genomic selection. GWAS across locations resulted in four marker-trait associations on chromosomes 3D, 4A, 5D, and 6D explaining between two and six percent of the variance.

The developed genomic prediction models and identified markers will help breeders to develop simple and affordable prediction models and diagnostic markers for selection of low grain-Cd accumulating wheat genotypes.

#176

Investigation of genetic background of fusarium head blight resistance in martonvásár wheat gene bank collection

Katalin Puskás[1], Mónika Cséplő[1], Kamirán Áron Hamow[1], Zsuzsanna Ambrózy[1], András Cseh[1], Ádám Horváth[1], Judit Bányai[1], Gyula Vida[1], Csaba Éva[1], Klára Mészáros[1]

[1] Agricultural Institute, HUN-REN Centre for Agricultural Research, Martonvásár 2462, Hungary

meszaros.klara@atk.hun-ren.hu

Improving the resistance of cultivated wheat varieties to Fusarium head blight (FHB) due to the extremely harmful mycotoxins produced by the fungus is a constant great challenge for breeders. The genetic regulation of FHB resistance is very complex, involving many regions of the wheat genome, therefore the number of often unfavorable linked traits is also large. For this reason, the use of exotic, spring sources with the best FHB resistance in winter wheat breeding is limited.

FHB resistance of 188 wheat genotypes were investigated using three inoculation methods in three growing seasons (2020-2022) under field conditions. The wheat varieties originated from gene bank collection, and 80% of it are winter growing type, while three quarters of it was bred in Martonvásár (MV). Genotyping was carried out using the Illumina 25K wheat array.

Average seed infection rate for spray inoculation were: 2020 – 47%, 2021 – 7%, 2022 – 33% respectively. Every year, the spawn method (infected seeds scattered on soil surface) simulating the natural disease process proved to be the less efficient, with the average seed infection rate: 35%, 3% and 11%, respectively. The average ear infections on the 21st day after inoculation were 24%, 44% and 34% in consecutive years by single spikelet injection method.

FHB severity of the offspring lines from crosses between Far-Eastern and MV genotypes was the mildest. Some old Hungarian varieties, Bánkúti 5, Béta Bánkúti and BKT-9158-95, were among the best 25 genotypes representing the winter grow habit using spray and spawn inoculation methods. Considering the results of all three inoculation techniques, two Romanian genotypes, F12056G2-1FZ-2 and F12056G2-01, as well as the MVF44-17 and MVF24-20 lines from Martonvásár represented excellent resistance.

As a result of the CMLM analysis, MTA were identified on each wheat chromosome, however, the number of markers that proved to be effective in each year was much lower. The analysis of the database of the spray and spawn methods both confirmed the role of the markers located at the positions of chromosome 1A 48648359 and 6A 610349860.

The effect of the 1A locus was strongest by spray inoculation in 2020. The seed infection of the lines carrying the G allele was 12.55%, and the DON contamination was 22561 ppb less than of the T types lines. The effect of marker 6A was detectable in both ear and seed infection, which were decreased in average with 20.01% and 16.59% by T allele, respectively. Relevance of marker position 414957067 of 2D chromosome was revealed by the analysis of FHB severity after single spikelet injection (type II resistance).

The role of the chromosomal regions identified so far will be further investigated using bioinformatics methods and gene editing.

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#455

Exploring alien introgressions from wild germplasm utilizing genomics approach to enhance rusts resistance in wheat

Dhanashree Mhatre[1], Bukke Kutti Bai[1], Balihar Kaur[1], Mandeep Kaur[1], Jaspal Kaur[2], Achla Sharma[2], Parveen Chhuneja[1] and Satinder Kaur[1]

[1] School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana 141004, India. [2]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, India.

dhanashree-2106002@pau.edu

The biotic stresses diminished the production and quality of wheat crop, an important staple food crop for 40% of the world's population. Among other diseases, the group of three rusts diseases are prominent causing losses as high as 10 –100% by stem rust (Puccinia graminis), 10 –70% by leaf rust (P. triticina) and 20 –100% by stripe rust (P. striiformis).

The monoculture of few wheat cultivars at global level makes these susceptible against prevalent rust races thus forcing to look for novel sources which could provide resistance. The wild species of wheat on other hand are goldmine of novel alleles due to their development under natural environmental conditions for multitude of years. About 1500 accessions of these wild wheat from 22 progenitor and non-progenitor species are being maintained at Punjab Agricultural University, Ludhiana and are being utilized for transferring and characterizing rust resistance in elite backgrounds.

Here we present the characterization and mapping of leaf rust (LR)/ stripe rust (YR) resistance genes transferred from four different species of Aegilops kotschyii, Aegilops peregrina, Aegilops speltoides, and Triticum dicoccoides. The introgression lines (ILs) carrying LR and YR resistance genes from each of these species was crossed with corresponding susceptible wheat line to examine the genetics of these transferred resistances.

The $F_{2/3/4/5}$ mapping populations were screened against Indian predominant pathotypes of LR and YR at seedling and adult plant stage and genetic ratios were calculated. The IL from Ae. kotschyii (Lr^{kots}) contain single LR resistance gene while those from Ae. speltoides (Lr^{sp3603} & Yr^{sp3603}), Ae. peregrina (Lr^{per} & Yr^{per}) and T. dicoccoides (Lr^{dic} & Yr^{dic}) carry one LR and one YR resistance gene each. Mapping of these gene was done using combination of bulked segregant analysis with sequencing and/or 35K SNP chip. Lr^{kots} was mapped to chromosome 3DL, with flanking SNP markers AX-94443154 (0.32cM) on proximal end and SSR Xbarc71 (7.9cM) on distal end defining a 3 Mb region containing eight candidate genes.

The Lr^{sp3603} and Yr^{sp3603}, were mapped on chromosome 6B, with Lr^{sp3603} flanked by markers Tag-SSR14(2.42 cM) and SNP AX-94542331 (3.28 cM) and Yr^{sp3603} was mapped close to marker AX-94542331 at 6.62 cM. Lr^{per} and Yr^{per} were mapped on chromosome 3B with Lr^{per} linked to marker AX-94449979 (7.5cM) and Yr^{per} linked to AX-94482301 (10.7cM). Mapping of Lr^{dic} at distance of 5cM to marker AX-95140906 on chromosome 1D while the KASP marker AX-94906888, situated at 404.1 Mb on chromosome 2D showed putative linkage with Yr^{dic} (8cM).

Key words: rust resistance, Introgression lines, progenitor species, non-progenitor species, complementary gene interaction, all stage resistance genes

#230

The farmer participatory wheat breeding activities in Hungary

Péter Mikó[1], Mihály Földi[2], Dóra Drexler[2]

[1] HUN-REN ATK, Centre for Agricultural Research, Agricultural Institute, Martonvásár, Hungary. [2] ÖMKi, Research Institute of Organic Agriculture, Budapest, Hungary

E-mail: miko.peter@atk.hun-ren.hu

Hungary's leading wheat breeding institute, ATK was the first in the country to start specific breeding program for organic agriculture, where new breeding concept had to be also implemented. An effective and low-cost method is to perform selection on the target farm in close cooperation with the farmer, which – in the end – helps to increase quickly the number of winter wheat cultivars (variety or population) suitable for organic farming, that have increasing demand from organic farmers' side.
ATK had sent its organic-suited winter wheat varieties (Mv) and two multi-parental populations to organic farmers with the aim to start participatory testing (PVS: participatory variety selection) and breeding (PPB: participatory plant breeding) on their farms. These on-farm trials were coordinated by ÖMKi and were run on 5 farms of its on-farm network between 2021 and 2023.

Cultivars were evaluated by farmers during the vegetation periods, while breeders of ATK visited the farms and they discussed the steps of observation, selection and harvest of trials with the farmers. Cultivars examined on un-replicated medium-plots (0.1 ha) were examined also in small-plot (6 m^2) replicated trial at one farm. Altogether 19 cultivars were involved in the trial system.

Beside grain yield, quality parameters (protein and gluten contents, Zeleny sedimentation value, test weight) were also analysed using near infrared (NIR) rapid quality measurement. PVS activity of the farmers was intensive resulting in the involvement of a couple of new registered varieties in each new season, while – in the same time – several underperforming cultivars were dismissed.

Most of the Mv varieties had higher grain yield than the populations at each site, while the two organic heterogeneous materials (OHM) showed more stable performance over the years with mostly the highest quality parameters. The younger population (i.e. more diverse), 'Mv Bio2020 Pop' showed higher yielding ability than 'Mv Elit CCP', while its quality parameters were only slightly lower than that of the other OHM population.

In most of the farms, the variety 'Mv Pántlika' has overperformed the trial average regarding most of the measured parameters. This result is in line with that of the Hungarian organic post-registration trial run for 3 years on 7 sites (mostly on-station) showing this variety to have outstanding yield and quality stability over the years.

Based on these results, ATK has already organized the official organic sowing seed production of 'Mv Pántlika', and offered 'Mv Bio2020 Pop' to more farms, where its adaptation to their cropping conditions could start. The two diverse OHM populations are also part of a PPB program, where 3 organic farmers have made their own positive spike selection in the populations and, after threshing them into bulks, the resulted 6 new subpopulations were examined in small plot trials at two sites.

Regarding plant height and earliness, specific farmer preferences were revealed: most of the farmers selected towards earliness, while one of them selected also for shorter stem and the others for taller plants. Based on the average of the 2 sites, only the subpopulations of the younger population ('Mv Bio2020 Pop') could yield more than their original population, especially at one site this yield gain was 18.1%. These results were presented in farm days and downloadable as a part of ECOBREED Participatory Trial Bulletins (www.ecobreed.eu).

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#296

Comparative transcriptomics analysis explains late-maturity amylase trait unpredictability in magic tall lines across generations

Shahida A. Mitu[1], Utpal Bose[1,2], Ronald Yu[3], Malcolm Blundell[3], Crispin A. Howitt[3], Michelle L. Colgrave[1,2], Jean-Philippe Ral[3], Angéla Juhász[2]

[1]Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, School of Science, Edith Cowan University, Joondalup WA 6027, Australia; [2]CSIRO Agriculture and Food, 306 Carmody Rd, St Lucia QLD 4067, Australia; [3]CSIRO Agriculture and Food, GPO Box 1700, Canberra ACT 2601, Australia

Late maturity amylase (LMA) is a recessive trait with increased alpha-amylase enzyme expression (TaAMY1 and TaAMY4) triggered by abiotic stress like cold shock. This leads to a decrease in the falling number and downgrading of wheat grains.

Here, we performed transcriptomics analysis on dormant (0h) and germinated (48h) seeds from two multi-parent advanced generation inter-cross (MAGIC) population subsets of tall lines (MT9 and MT10) growing for two generations (Generations 3 and 4) in glass house using the same conditions, which showed unpredictable LMA phenotypes between the two generations. The preliminary assessment showed that MT9 has higher LMA phenotype expression than the MT10 lines.

However, the LMA phenotype is unpredictable in these lines, with generations 3 and 4 showing high variability. Generation 3 had very high LMA expression, whereas LMA was absent in generation 4, but the underlying molecular mechanism for this unpredictable LMA phenotype remains unknown.

The comparison between two germination time points, 0h and 48h, for one generation (MT9-generation 3) showed the upregulation of 19,239 genes and the downregulated 10,767 genes (Fold change = 2, adj. p-value< 0.05). In the germinated grains (48h), phenylpropanoid biosynthesis, metabolic and amino and nucleotide sugar pathways were enriched. The comparison between two genotypes, MT9 and MT10, on dormant grains (0h) from generation 3 resulted in a limited change (~8%) of genes that were differentially expressed.

As expected, the seed dormancy breakdown-related pathways, such as metabolic processes and sugar metabolism pathways, were downregulated, while the spliceosome pathway was significantly enriched, indicating their critical role in maintaining seed dormancy through the catalysis of mature mRNA processing.

The comparison of dormant grains between two generations, 3 and 4, showed that the generation 4 seeds of MT9 were involved in strigolactone biosynthesis and metabolism processes and shoot system morphogenesis, indicating the underlying role of these genes' molecular functions in defining the LMA phenotype characteristics between two generations.

Overall, the enrichment of dormancy-breakdown-related pathways, increased ion transporters and sucrose metabolism between the two generations for the same genotype further elucidate the underpinning mechanism for the unpredicted LMA expression.

Key Words: Genotype, Phenotype, LMA resistance, RNA-Seq, Temporal expression profiles

#419

Wheat farmers’ perspective about climate change impacts, adaptation strategies and associated factors in Punjab, Pakistan

Jaffar Tayyar[1], Muhammad Rafay Muzamil[2, *]

[1] Institute of Agric. Extension Education and Rural Development, University of Agriculture Faisalabad, Pakistan

Corresponding author: rafay.muzmil@uaf.edu.pk

Agriculture is the most important sector of Pakistan’s economy with wheat as the main crop of food production systems across the nation. Crop production is being impacted by changing weather and wheat is one of the most affected crops. Wheat crop production is being affected by changes and declines in rainfall patterns which require significant changes in farm management practices.

This research examined wheat farmers’ perspective about climate impacts, adaptation strategies and associated factors, and the institutional support they receive to cope with the effects on wheat production. The study was conducted in Gujranwala district in Punjab, Pakistan during 2022-23 with a survey conducted in total of 24 villages engaged in wheat cultivation.

Descriptive statistics were used to identify mean values against recorded responses on a five-point Likert scale. The findings revealed that the most experienced climate impacts perceived by wheat growers are increased summer heat (mean 2.66), insufficient water for wheat crop (mean 2.37) and low wheat yield (mean 2.25) which ultimately exacerbate food insecurity in the region.

This food insecurity situation is further aggravated by factors such as rising food prices, availability, and affordability of agricultural inputs crucial for farmers’ living. In such circumstances, farmers are compelled to adapt changing weather conditions. In this vein, the findings highlight prioritised adaptation strategies such as increased use of pesticides (mean 2.82), cultivation of recommended varieties (mean 2.51), and enhanced irrigation (mean 2.01) as top three important strategies.

In addition, the results highlight factors influencing wheat farmers’ decisions to adapt, with limited education (mean 4.37), environmental factors contributing to soil infertility (mean 2.25), and credibility of agri. Information (mean 2.81), emerging as the top three influential factors.

Finally, this study assessed the institutional support available to farmers and subsequently identified the use of mass media in brining awareness (mean 2.79) and regular farmer meetings facilitated by extension personals (mean 2.49) as the top two institutional strategies to cope with challenges associated with changing climate conditions.

#213

Mapping of a hybrid incompatibility gene located on chromosome 2b in tetraploid wheat

Sotaro Nakanishi[1,*], Kazusa Nishimura[1], Maho Okuma[1], Tetsuya Nakazaki[2], Yuki Monden[1], Kenji Kato[1], and Hidetaka Nishida[1]

[1] Graduate School of Environmental, Life, Natural Science and Technology, Okayama University, Okayama 700-8530, Japan. [2] Graduate School of Agriculture, Kyoto University, Kizugawa, Kyoto 619-0218, Japan

The AABB genome donor for bread wheat, tetraploid wheat (Triticum turgidum L.) could serve as a valuable genetic resource, because this group include genetic variations not presenting in hexaploid wheat species. We then crossed tetraploid F_1 hybrids and a diploid wild wheat, Aegilops tauschii Coss., to rapidly construct synthetic hexaploid wheat NAM populations (SHN population) to detect useful tetraploid-derived genes.

During the development of the NAM population, we observed hybrid incompatibility in a triploid generation. Identification of causal genes and molecular genetic mechanisms for this hybrid incompatibility are essential for the efficient utilization of ancestral wheat species as germplasms. We aimed to identify the causal genes through QTL analysis with dpMIG-seq (Nishimura et al. 2024), mapping with DNA markers, and revealing their expression through RNA-seq analysis.

We crossed two tetraploid wheat lines, Langdon (LDN) and NP29. A triploid wheat population was developed by crossing the tetraploid F_1 individual with an Aegilops tauschii Coss. line, KU-2098. This triploid population was named SHN29. In this population, we observed 28 plants of incompatible type and 24 plants of normal type. This ratio fit a 1:1 ratio in the chi-square test ($p=0.579$). In addition, the QTL analysis using dpMIG-seq revealed a significant peak on chromosome 2BL. These results suggest this hybrid incompatibility is regulated by a single gene located on chromosome 2BL.

For narrowing down the QTL region, indel markers were developed in the QTL region based on whole genome resequencing data of LDN and NP29. Using these markers, 2 plants recombined in the QTL region were selected from 28 incompatible plants of SHN29. Next, using the newly developed indel markers, we were able to narrow down the candidate region, where the causal gene were located, within a 5 Mb region of chromosome 2B.

For RNA-seq, another triploid wheat population was developed by the same procedure as SHN29. Twenty-four plants, 12 normal and 12 incompatible, were selected by MIG-seq. Total RNA was extracted from their crown tissue and used for RNA-seq analysis.

As a result, 58 genes were located within the candidate region, and 5 of them were identified as differentially expressed genes (DEGs) with more than a 4-fold change in expression levels. Functional mutations in proteins within the candidate region were examined using SnpEff, and several mutations were found to significantly impact protein function, such as the occurrence of premature stop codon and frameshift mutations.

#133

Introgressions of *Aegilops* chromosome fragments into Common wheat using a *pairing homoeologous 2* mutant

Masahiro Tabuchi[1], Adam Izzuddin Nasir[1], Shoya Komura[1], Moeko Okada[2], Asami Michikawa[2], Yoshihiro Inoue[1], Kazusa Nishimura[1], Shigeo Takumi[2], Nobuyuki Mizuno[3], Fuminori Kobayashi[3], Kentaro Yoshida[1*]

[1] Graduate School of Agriculture, Kyoto University, Kyoto, Japan
[2] Graduate School of Agricultural Science, Kobe University, Kobe, Japan
[3] Institute of Crop Science, National Agriculture and Food Research Organization, Ibaraki, Japan

Introduction of agriculturally beneficial traits from the gene pool of wild relatives into wheat cultivars can contribute to wheat breeding. Although wild relatives of wheat, *Aegilops* spp., are used as genetic resources of abiotic stress tolerance and disease resistance, the use of *Aegilops* ssp. in wheat breeding is still limited. Introgression from wild relatives into cultivated wheat is achieved using a mutant of *Pairing homoeologous* (*Ph*) gene, which promotes homoeologous recombination between different genomes. *Ph1* and *Ph2* are known as *Ph* genes.

The *ph1* mutant is commonly used for introgression of alien chromosomes, whereas the *ph2* mutant is not. In this study, to evaluate introgression by homoeologous recombination caused by *ph2* mutant, we used the synthetic polyploid lines generated by crossing *Triticum turgidum* L. subsp. *durum* cv. ‘Langdon’ (AABB genome) as female and four *Aegilops* species: *Ae. umbellulata* Zhuk. (UU genome), five lines of *Ae. geniculata* Roth. (UUMM genome), *Ae. biuncialis* Vis. (UUMM genome), *Ae. peregrina* (Hackel in J. Fraser) Maire & Weiller. (UUSS genome).

These synthetic polyploid lines were crossed with the *ph2* mutant of *T. aestivum* ‘Chinese Spring’. The F_1 hybrids of the synthetic polyploids and the *ph2* mutant were repeatedly backcrossed with the wild type ‘Chinese Spring’ to generate backcrossed lines. We confirmed chromosomal rearrangements including translocations of *Aegilops* chromosomal fragments, intragenomic recombination, and aneuploidy in the backcrossed lines by multiplexed inter-simple sequence repeats genotyping by sequencing and chromosome karyotyping with fluorescence *in situ* hybridization.

Intragenomic recombination and translocation were more frequently observed between the wheat D-genome and *Aegilops* chromosomes than between the wheat A- or B-genome and *Aegilops* chromosomes. Aneuploidy with deletions or additions of whole chromosomes was also frequently observed. In addition, since synthetic hexaploid with U-genome showed salt stress tolerance at the time of seed germination, we screened the U-genome chromosome introgression lines harboring salt stress tolerance.

We identified introgression lines with U-genome chromosomes exhibiting more salt tolerance than the control wheat lines. These results suggest that the *ph2* mutant can be used for *Aegilops* chromosome introgression into wheat cultivars, but several backcrosses with wheat cultivars will be necessary to avoid aneuploidy and to obtain euploid plants with agriculturally beneficial traits derived from the wild relatives.
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#531

Comparative genome wide analysis of mcm dna helicase family in setaria italica and triticum aestivum

Ashima Nehra[a,b], Kiran Nehra[b], Ritu Gill[a]

[a] Centre for Biotechnology, Maharshi Dayanand University, Rohtak, 124 001, Haryana, India. [b] Deenbandhu Chhotu Ram University of Science and Technology, Murthal, 131 039, Haryana, India

Email (aashimanehra786@gmail.com)

The mini-chromosome Maintenance (MCM) proteins belongs to the AAA+ superfamily and plays pivotal role in DNA replication and genome stability across eukaryotic organisms. Like other eukaryotic MCMs, plant MCM helicases also form hexameric ring structure composed of six subunits containing MCM 2-7. Despite its significant importance, comparative studies of the MCM family in crop species remain limited. Therefore, this study planned to report the genome-wide analysis of MCM protein family in Setaria italica (foxtail millet) and Triticum aestivum (wheat), two agriculturally significant species.

Using in silico approaches, we identified the MCM gene family members in the both genomes, and analysed their phylogenetic relationships, conserved motifs, gene structures, and chromosomal localizations, protein-protein interaction and physiochemical properties. Genome-wide collation resulted in the identification of 9SiMCM and 23TaMCM proteins in S. italica and T. aestivum, respectively. The multiple sequence alignment revealed the conservation of MCM motifs in S. italica but five proteins in T. aestivum contains incomplete MCM motifs.

The protein length of MCMs varied from 417-958 and 66-956aas in S. italica and T. aestivum respectively. GRAVY value analysis of SiMCMs and TaMCMs showed that all the proteins were found to be hydrophilic in nature, except one TaMCM. Further, phylogenetic tree of SiMCMs and TaMCMs were clustered as per their subfamilies.

Comparative protein-protein interactions revealed that MCM interact with different proteins involved in DNA unwinding and replication process. The present study provides the detailed insight into SiMCMs and TaMCMs protein family which may be further exploited for genetic engineering/genome editing studies for crop improvement and stress resilience.

Keyword: Genome wide analysis, MCM family, Mini-chromosome Maintenance proteins, Setaria italica, Triticum aestivum

#611

Map-based cloning and mechanistic analysis of the male sterility gene MsgN13910 in barley

Qi Liu[1], Guojun Liu[1], Xuecong Du[1], Jianjun Ding[1], Juan Qi[1], Fei Ni[1,*]

[1] National Key Laboratory of Wheat Improvement, College of Agronomy, Shandong Agricultural University, Tai'an, Shandong 271018, China

Male sterility is a valuable trait for studying anther and pollen grain development, as well as for harnessing hybrid vigor in crops. Although a large number of genic male-sterility (GMS) genes have been reported in barley (*Hordeum vulgare* L.), only a few have been successfully cloned. Here, we cloned a barley GMS gene Msg_{N13910} using BSR-Seq combined with forward genetics methods.

The Msg_{N13910} locus was finally mapped to a 0.19 cM genetic interval, which corresponded to a 325 Kb physical region. According to the RNA-Seq data, only two genes in this region were specifically expressed in anthers. Compared with the wild type, one of the genes had a G to A mutation, which altered the normal splicing pattern and generated premature termination. CRISPR/Cas9-based knockout of $TaMsg_{N13910}$ conferred complete male-sterile hexaploid wheat.

Histological and cytological analyses showed that the msg_{N13910} mutant exhibited delayed degradation of anther tapetum, abnormal ubisch body development, defective anther cuticle development, and loss of bacula of pollen wall, which eventually led to pollen abortion. Metabolomic analysis showed that the gene was mainly involved in the glycerophospholipid metabolic pathway, indicating that Msg_{N13910} controls pollen fertility by regulating lipid metabolism in anthers.

Yeast two-hybrid, bimolecular fluorescence and luciferase complementation assays showed that $\mathrm{TaMsg}_{\mathrm{N13910}}$ interacted with NUS1 and cys protease in vivo. Yeast one-hybrid and Dual-luciferase reporter assays showed that MADS, Lim domain protein, PHD finger-like domain-containing protein and Heat shock transcription factor interact with the $TaMsg_{N13910}$ promoter and repress $TaMsg_{N13910}$ expression.

Taken together, this study cloned the barley male sterility gene Msg_{N13910} and preliminarily explored its male sterility mechanism, providing clues for further elucidating the function of Msg_{N13910} in another development.

#630

Regulation of tillage practice and residues returning on root growth and metabolism of winter wheat

Hongxiang Zhao[1], Zhen Liu[1], Geng Li[1], Yakov Kuzyokov[1,2], Tangyuan Ning[1,*]

[1] National Key Laboratory of Wheat Improvement, Key Laboratory of Crop Water Physiology and Drought–Tolerance Germplasm Improvement, Ministry of Agriculture and Rural Affairs, College of Agronomy, Shandong Agricultural University, Tai'an, P.R. China. [2] Department of Agricultural Soil Science, Department of Soil Science of Temperate Ecosystems, University of Gottingen, Gottingen 37077, Germany.

* Corresponding author: ningty@163.com (Tangyuan Ning)

Low crop water productivity and unsustainable tillage practices impede agricultural development globally. This study, initiated in 2002, leverages long-term experimental data to analyze wheat root morphology, distribution, and metabolic pathways under various tillage practices and residue management techniques.

Our findings indicate that at the seedling stage, wheat roots under rotary and subsoiling treatments exhibit a compact angle and uneven distribution within the soil profile, contrasting with the more uniform root architecture observed in plowed plots. In the top 0-10 cm soil layer, plowing and rotary tillage significantly enhance root dry matter accumulation compared to subsoiling.

However, the 10-40 cm layer shows a reverse trend, with rotary tillage maintaining higher values, particularly in the absence of residue return. Root length density trends closely mirror those of dry matter weight, with an average root diameter in the 0-40 cm layer that surpasses other treatments. Relative measures of root length, dry weight, and surface area exhibit consistent trends with increasing soil depth.

The ATP-BC transporter regulatory network, influenced by tillage practices, significantly modulates water and nutrient uptake by wheat roots. Subsoiling impacts xyloglucan metabolism and histidine kinase activity, which in turn affects plant hormone regulation. Salicylic acid, an endogenous hormone, is influenced by the signal transduction pathway involving benzoic acid and modulated by amidase and acyl phosphatase, playing a crucial role in plant development, stress resistance, and cell differentiation.

Subsoiling directly impacts ethylene biosynthesis, methionine degradation, and cysteine biosynthesis, mediated by methionine adenosyl transferase. This leads to metabolic differences in cysteine and methionine, essential for ethylene synthesis and polyamine precursors, with cysteine also serving antioxidant functions. The biosynthesis of glycosphingolipids, linked to plasma membrane construction, is influenced by the regulatory networks of subsoiling and rotary tillage.

Variations in purine metabolism may relate to root cell division and differentiation. Furthermore, the study reveals alterations in the metabolic pathways of starch, sucrose, and galactose, reflecting the complex interplay between tillage practices and plant metabolism. While tillage treatments significantly increase root dry matter, no significant differences in the root-to-shoot ratio were observed among treatments.

Notably, subsoiling with residue return yields an average of 8.28 Mg ha^{-1} for wheat and 11.83 Mg ha^{-1} for maize, surpassing plowing and rotary tillage by 6.13% and 7.57%, and 13.23% and 6.70%, respectively. These insights are instrumental for refining wheat cultivation strategies to enhance sustainability and productivity in agriculture.

#290

Genetic analysis for heading date of durum wheat with loss-of-function in two homoeologous *PCL1*

Kazusa Nishimura[1], Akimasa Fujioka[2], Maho Okuma[1], Yuki Monden[1], Kenji Kato[1], and Hidetaka Nishida[1]

[1] Graduate School of Environmental, Life, Natural Science and Technology, Okayama University, Okayama City, Okayama Prefecture 700-8530, Japan
[2] Graduate School of Environmental and Life Science, Okayama University, Okayama City, Okayama Prefecture 700-8530, Japan

Durum wheat (*Triticum turgidum* L. ssp. *durum*) is grown on about 8-10% of the world's wheat planted area, with the Mediterranean region and North America accounting for 60% of production. On the other hand, in Japan, durum wheat is rarely grown because the maturing period coincides with the short rainy season, which causes preharvest-sprouting and/or Fusarium head blight.

Therefore, it is essential to breed early-heading durum wheat varieties that can be harvested before the rainy season to expand the cultivation of durum wheat in Japan. Then, we tried to breed an extremely early heading durum wheat variety by introducing the non-functional alleles of *PCL1 (pcl1-A1* and *pcl1-B1*) derived from a hexaploid wheat line ‘Chogokuwase’.

During the breeding process, some lines did not become extremely early heading, despite possessing both early flowering alleles of *PCL1*. This suggests the presence of novel heading time-related genes that interact with *PCL1*. In this study, genetic analysis was performed on several populations to determine the cause of the lack of extremely early-heading phenotypes in some lines.

We used three F_2 populations (they were named as DF20, DF21, and DF22; 267 individuals in total) derived from crosses between extremely early-heading lines and non-extremely early-heading lines. The seed of populations was sown in the field of Okayama University on November 18, 2020. Genome-wide SNP information was obtained by Multiplexed ISSR genotyping by sequencing (MIG-seq; Nishimura et al., 2022). GWAS on heading date with 56,620 SNPs showed no SNPs with FDR<0.05.

Among these SNPs, we focused on the ones showing relatively higher peaks on chromosomes 3B and 5B. ANOVA using these peak SNPs showed that their genotypes significantly affected heading date. To perform BSA with higher density markers, 32 DF22 individuals (16 early- and 16 late-heading individuals) were subjected to degenerate oligonucleotide primer MIG-seq (dpMIG-seq), an improved method of MIG-seq (Nishimura et al., 2024).

Using 76,127 SNPs detected by dpMIG-seq, small peaks were observed on chromosomes 6B and 7A; markers near the 6B peak had a significant effect on heading date but this effect was small, while markers at the 7A peak had no significant effect on heading date.
Based on these results, it is expected that many genes with small effects, including QTLs on chromosomes 3B, 5B, and 6B, are segregating in the F_2 populations in this study, and that their pyramiding effects are causing segregation of heading date.

#80

Map-based cloning and functional analysis of tarl-7b controlling root length in wheat

Yanxiao Niu[1], Jiewen Xing[1], Liqiang Song[2], Shuzhi Zheng[3], Junming Li[3], Zhongfu Ni[1]

[1] Frontiers Science Center for Molecular Design Breeding, China Agricultural University, Beijing 100094, China. [2] State Key Laboratory of North China Crop Improvement and Regulation, College of Agronomy, Hebei Agricultural University, Baoding 071000, China. [3] Hebei & Ministry of Education Key Laboratory of Molecular and Cellular Biology, College of Life Sciences, Hebei Normal University, Shijiazhuang 050024, China

Roots are essential organs for wheat to absorb water and mineral nutrients. Exploring key genes that control root morphology and deciphering the underlying regulatory mechanisms are of great significance for genetic improvement of root architecture and nitrogen-use efficiency (NUE) in wheat.

Epigenetic regulations play a crucial role in root development, especially in the adaption to dynamic environmental cues. N6-methyladenine (m^{6}A), one of the most important RNA modifications in eukaryotes, is catalyzed by methyltransferase complexes, recognized by N6-methyladenine-binding proteins, and removed by demethylases. To date, there have been no reports of m^{6}A demethylase in the regulation of nitrogen-dependent root development in wheat.

In our study, a stable major QTL (qMrl-7B) associated with the maximum root length was finely located in the 0.26 Mb interval on chromosome 7B of Kenong 9204. Based on expression analysis and sequence alignment, the candidate gene of qMrl-7B was identified as TaRL-7B, which encodes an m^{6}A demethylase and positively regulates root length.

Loss of function of TaRL-7B resulted in reduced seedling root length and plant height, while its overexpression enhanced these traits, particularly under nitrogen-deficient conditions. Significantly, TaRL-7B expression was positively correlated with tiller number, grain width and grain weight, indicating its great potential for improving crop yield.

These results proved that the m^{6}A demethylase synergistically regulates root length, tiller number and yield potential, providing elite gene resources for improving wheat root system and breeding high-yielding varieties under various nitrogen regimes.

#633

Production vulnerability to wheat blast disease under climate change

Diego N. L. Pequeno[1,*], Thiago B. Ferreira[2], Jose M. C. Fernandez[3], Pawan K. Singh[1], Willingthon Pavan[2], Kai Sonder[1], Richard Robertson[4], Timothy J. Krupnik[5], Olaf Erenstein[1], Senthold Asseng[6]

[1] International Maize and Wheat Improvement Center (CIMMYT), Texcoco, EdoMX, Mexico
[2] University of Florida (UF), Gainesville, FL, USA
[3] Brazilian Agricultural Research Corporation (Embrapa Trigo), Passo Fundo, RS, Brazil
[4] International Food Policy Research Institute (IFPRI), Washington, DC, USA
[5] International Maize and Wheat Improvement Center (CIMMYT), Dhaka, Bangladesh
[6] Technical University Munich (TUM), School of Life Science, Freising, Germany
*Presenting author

Wheat blast is a devastating disease caused by the fungal pathogen *Magnaporthe oryzae* pathotype *Triticum* that has spread to both neighbouring and distant countries following its emergence in Brazil in the 1980s. Under climate change conditions, wheat blast is predicted to spread primarily in tropical regions.

Here we coupled a wheat crop simulation model with a newly developed wheat blast model, to provide quantitative global estimates of wheat blast vulnerability under current and future climates.
Under current climatic conditions, 6.4 million hectares of arable land is potentially vulnerable to wheat blast.

A more humid and warmer climate in the future (Representative Concentration Pathway 8.5) is likely to increase the area suitable for wheat blast infection, particularly in the Southern Hemisphere, and reduce global wheat production by 69 million tons per year (13% decrease) by mid-century. Impacts of climate change could be further exacerbated and food security problems increased.

#622

Conservation agriculture in central Asia: status and prospects

Aziz Nurbekov[1*], Alisher Mirzabaev[2], Amir Kassam[3], Nurali Asozoda[4], Nasriddin Khalilov[5], Dossymbek Sydyk[6].

[1]Tashkent State Agrarian University, Uzbekistan
[2]ZEF-University of Bonn, Germany
[3]Tajik Academy of Agricultural Science, Tajikistan
[4]Samarkand Institute of Veterinary Medicine, Uzbekistan
[5]South-Western Research Institute of Livestock and Crop Production. Kazakhstan.

*Corresponding author – Nurbekov2002@yahoo.com and Aziz.Nurbekov@fao.org

Agricultural sector in Central Asian countries is facing production reduction due to soil erosion, soil salinity and desertification. Growing evidence exists of the productivity, economic and environmental benefits that can be harnessed from using conservation agriculture (CA) practices in Central Asia, but several challenges need to be addressed.

These challenges include the need to: mobilize policy and institutional support, facilitate the change of the tillage mind-set, develop the skills to operate CA equipment, make CA equipment available and accessible, and to develop the expertise and knowledge for residue and weed management.

Several collaborative research and development projects have been implemented to promote CA in Central Asia by both ICARDA and FAO. No-till and raised-bed planting practices tested in the Karakalpakstan and Tashkent provinces of Uzbekistan have proven to be technically and economically suitable for local conditions, and can provide similar or higher crop yields while saving considerable production resources and costs including fuel, seeds and labour.

These practices are ready to be disseminated more widely in Uzbekistan. Current evidence from Central Asian countries indicates that CA practices are suitable for the existing major cropping systems. CA is not a single or uniform technology that can be immediately applied anywhere in a standard manner.

Rather, it represents a set of principles that encourage the formulation of locally adapted practices, approaches and methods, which need to be tested, evaluated and then adopted or implemented under various biophysical and socio-economic conditions.

#621

Effect of tillage on the productivity of winter wheat under rainfed conditions in central Asia

Aziz Nurbekov[1,2*], Nurali Asozoda[3], and Rukhangiz Nurbekova[4]

[1,2*]Tashkent State Agrarian University, Tashkent, Uzbekistan.
[2]Food and Agriculture Organization of the United Nations, Uzbekistan office.
[3]Research Institute of Crop Husbandry, Tajik Academy of Agricultural Sciences, Dushanbe, Tajikistan,
[4]International Strategic Center for Agri-Food Development (ISCAD).

*Corresponding author: nurbekov2002@yahoo.com and aziz.nurbekov@fao.org

The on-going degradation of soil resources in Central Asia is widespread because of science and technological development through intensification of agriculture, but represents a direct threat to the productive capacity and sustainability of the agricultural production base all over the Central Asia. The main objective of this trial was to study the effect of different tillage methods on the productivity of winter wheat in the rainfed conditions of Southern part of Central Asia.

The study areas are located in the rainfed areas of Uzbekistan and Tajikistan. There were three different tillage options; conventional till (CT), minimum till with disking (MTD) and no-till (NT). Analysis of variance (ANOVA), using replicated trials in randomized complete block design, was used to study the relationship between tillage and location.

The study describes the first analysis of no-till systems research in Uzbekistan and Tajikistan under rainfed conditions, and shows that there is potential for introducing no-till practice in the region. No-till winter wheat results in an increase in crop production and higher grain yield as compared to conventional and minimum till.Winter wheat yield under no-till system was numerically higher than conventional till practice.

Overall, the adoption of no-till cropping at farm level is dependent on the technology offering overall cost savings through reduced need for weed management and disease control (or different methods to do so) and/or the achievement of higher stable yields over the years.

#629

Growing and sequencing Triticum Monococcum in the polar region: thoughts from 10 years in the Arctic

Alexander Oeynes[1,2]

[1] Troms Oblat, analyzing and sequencing arctic wheat, Tromsø 9007 Norway.
[2] NELS, Norwegian e-infrastructure for Life Sciences

alexander@tromsoblat.no

The Einkorn, Triticum Monococcum, from which this sequence originates, has been grown under the constant light of the midnight sun, from germination on and past flowering. The strain is the product of 10 years, (2014-2024) of organic growth in the arctic sub polar area of Troms, Northern Norway.
This wheat is probably the northernmost wheat ever grown, and for a fact the northernmost grown wheat ever sequenced.

The test area where this strain has been grown has for several years, every week during growing season, had satellite data plotted to computerized topology models of the field. The positioning of the fields, which all have been smaller than 250 square meters, have been so that they are in alignment with shorelines and lines in the landscape, making the nominal satellite data more easily appliable, and the phenotype less likely to alter, due to immediate environmental factors.

The purpose of sequencing this model organism has been, all along to secure and document the genetic, kinetic and the logic algorithms of it, as host and as A gene contributor to the tetraploid bread wheats. It is also likely that this data can he used in the construction of a framework for understanding and simulating genetic and biological processes.

Landraces share this very slowly developed genetic origin naturally and special characteristics of endemic species are variants of code embedded in the Monococcum. Introduction and reintroduction of this valuable material into the genetic libraries of modern and more strongly developed strains can make for a healthier plant and a better product, probably without any loss of the key qualities of the strains harvested today.

#317

Towards Septoria nodorum blotch disease resistance: cloning of novel SNB disease susceptibility genes in wheat

Asiri Anushka Padukka Vidanalage[1], Huyen Phan[1], Mark Gibberd[1]

[1]Centre for Crop and Disease Management, Curtin University, Bentley, WA 6102

asiri.padukkav@postgrad.curtin.edu.au

The yield loss of wheat due to Septoria nodorum blotch (SNB) is up to 31% worldwide and it is ranges from 5-15% in Western Australia. Causative agent of the disease is a necrotrophic fungal pathogen, *Parastagonospora nodorum*, does not fit well into the traditional plant defence theory, but rather to the inverse gene-for-gene model.

Therefore, resistance to SNB is complicated and manipulated by numerous genes/QTL. Interactions occurring between small proteins known as necrotrophic effectors produced by the pathogen and matching dominant susceptible genes in wheat lead to the infection. In this study, RNAseq was used to identify candidate genes.

These genes have been characterised and partially validated using fine mapping of a large Mace x Lancer F2 population. EMS was also deployed to confirm the gene functions. Two candidate genes which interact with a novel *P. nodorum* toxin, SnTox8 have been identified based on the RNAseq data. Further, fine mapping data from the Mace X Lancer F2 progeny revealed that both genes interact with the toxin and the Gene1 is more significant than the Gene 2.

Finally, a near perfect marker was created but perfect markers are being developed for marker assisted selection tools in wheat disease resistance breeding programs.

The study is on the identification, characterisation, and validation of novel SNB susceptible genes for the first time in Australia for a marker-assisted selection program in wheat. Markers developed from the cloned gene by the end of 2024 could be used for the development of SNB-resistant wheat varieties by breeders.

#521

Modulating gibberellin biosynthesis for wheat improvement

Andy Phillips[1], Alison Huttly[1], Rocío Alarcón Reverte[1], Suzanne Clark[1], Steve Thomas[1], Peter Hedden[1,2], and Stephen Pearce[1*].

[1]Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK. [2]Laboratory of Growth Regulators, Institute of Experimental Botany, Czech Academy of Sciences and Palacky University, Šlechtitelů 27, CZ-78371 Olomouc, Czech Republic.
Stephen.pearce@rothamsted.ac.uk

The phytohormone gibberellin (GA) is an important regulator of plant growth and development. Bioactive GA levels are tightly regulated, in part by the activity of GA20oxidase and GA3oxidase enzymes that catalyze the final stages of GA biosynthesis. In wheat, both enzymes are encoded by multi-gene families that exhibit distinct expression profiles, allowing for precise spatial and temporal control of GA levels in different tissues.

We characterized the function of GA20ox and GA3ox genes in wheat using a collection of chemically-induced loss-of-function mutants. In replicated field experiments, we found that while *GA20ox1* plays a minor role in wheat stem elongation, *ga20ox2* mutants exhibit a reduction in height comparable to the *Rht-D1b* allele that may be useful as alternative semi-dwarfing alleles.

We found that *GA3ox3* and *GA1ox1* are highly expressed in grain tissues and act in concert to regulate bioactive GA levels during grain development.

In different mutant lines, bioactive GA levels were positively correlated with grain size and height. We characterized the natural genetic diversity in these genes among wheat landraces and identified haplotypes carrying major promoter rearrangements that confer an altered expression profile.

Our work sheds light on the function of different GA biosynthesis genes during wheat development and highlights the potential to select both natural and induced genetic variation in this pathway for crop improvement in breeding programs.

#462

A durum wheat NAM population by the founder cultivar cappelli for identification of yield-related loci

Nida Mushtaq[1,2], Giovanni Laido[2], Salvatore Esposito[2,3], Vanessa De Simone[2], Ivano Pecorella[2], Fabio Fania[2,4], Salvatore Colecchia[2], Pasquale De Vita[2], Nicola Pecchioni[2,1]

[1] Dipartimento di Scienze della Vita, UNIMORE - Università di Modena e Reggio Emilia, Reggio Emilia, Italy. [2] Centro di Ricerca Cerealicoltura e Colture Industriali, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economica Agraria (CREA), Foggia, Italy. [3] National Research Council of Italy, Institute of Biosciences and BioResources, (CNR-IBBR), Portici, Italy. [4] Department of Agricultural Sciences, Food, Natural Resources and Engineering, University of Foggia, Foggia, Italy

320383@studenti.unimore.it, giovanni.laido@crea.gov.it, salvatore.esposito@crea.gov.it, vanessa.desimone@crea.gov.it, ivano.pecorella@crea.gov.it, fabio.fania@unifg.it, salvatore.colecchia@crea.gov.it, pasquale.devita@crea.gov.it, nicola.pecchioni@crea.gov.it

Yield stability poses a critical challenge in cultivating durum wheat, with significant implications for global food security and agricultural sustainability. Thus, understanding the genetic basis of yield stability in conditions of climate change is imperative for optimizing breeding programs and ensuring robust crop productivity.

Our research aims to dissect the genetic architecture underlying complex traits, such as grain yield and related traits, in durum wheat using a Nested Association Mapping (NAM) population, specifically by identifying genomic regions associated with spike and kernel traits. Here, we introduce a large durum wheat NAM population developed at Research Centre for Cereal and Industrial Crops (CREA-CI, Foggia, Italy) by crossing thirty-one (31) diverse donor parents to a recurrent parent "Cappelli," yielding 3,720 F6 recombinant inbred lines (RILs) with 120 lines per family.

Utilizing the inherent genetic diversity within this population, we conducted phenotypic evaluations for kernel and spike traits, including spike length (SL), spike weight (SW), spikelet number per spike (SNS), fertile spikelet number per spike (FSNS), kernel number per spike (KNS), kernel weight per spike (KWS), kernel per spikelet (KPS), and spikelet density (SD).

Analysis of variance for each measured trait showed considerable variation among all genotypes. In addition, the genotyping of the NAM population is currently underway, by a targeted genotyping-by-sequencing (GBS - wheat 5K SNP) approach, allowing for comprehensive analysis of genetic variations across the entire population.

The ongoing project will advance durum wheat breeding strategies, ultimately enhancing crop productivity and resilience in diverse agricultural systems. Acknowledgment: This research has been funded by AGRITECH PNRR, Spoke 1.

#48

Identification of two QTL for hessian fly resistance in tetraploid wheat

Amanda R. Peters Haugrud[1], Kirk Anderson[1], Marion Harris[2], Justin D. Faris[1], Steven S. Xu[3]
[1]USDA-Agricultural Research Service, Cereal Crops Research Unit, Edward T. Schafer Agricultural Research Center, Fargo, ND 58102

[2]Department of Entomology, North Dakota State University, Fargo, ND 58102
[3]USDA-Agricultural Research Service, Western Regional Research Center, Albany, CA 94710

amanda.peters_haugrud@usda.gov

North Dakota is the largest durum (*Triticum durum* Desf.) producing state within the United States. Hessian fly (HF, *Mayetiola destructor*) is a major pest on wheat, and is often observed in wheat growing regions worldwide. We evaluated a recombinant inbred line population (referred to as BP025) consisting of 200 lines derived from a cross between the North Dakota durum wheat variety Ben and the cultivated emmer wheat accession PI 41025 for resistance to the Great Plains biotype of HF.

The BP025 population was evaluated under greenhouse/growth chamber conditions and 14 plants per line were scored 10 to 14 days post egg laying. Seedlings were evaluated for stunting score, larvae mortality, and percent of resistant plants. No significant quantitative trait loci (QTLs) were associated with stunting score.

Larvae mortality and percent of resistant plants were highly correlated (0.93), with the same two QTL regions associated with each trait. *QHf.fcu-2A*, located on the distal end of chromosome arm 2AL, explained 28.67 and 33.47 percent of the phenotypic variation in larvae mortality and precent resistant plants, respectively. PI 41025 alleles at *QHf.fcu-2A* resulted in increased HF resistance.

The other QTL, *QHf.fcu-3B*, mapped to the distant end of chromosome arm 3BS and explained 21.76 and 19.41 percent of the phenotypic variation in larvae mortality and precent resistant plants, respectively. Ben was the donor parent at *QHf.fcu-3B* for increased resistance.

The QTL on 3BS is within the same region as *H35*, originally mapped in winter wheat, and the *H35* KASP markers *KASP-3B3797431* and *KASP-3B4525164* were added to the BP025 map and mapped to the distal end of chromosome arm 3BS and are within the confidence region of *QHf.fcu-3B*. Further work is needed to determine if *QHf.fcu-3B* is *H35* or a novel HF resistance gene.

The QTL on chromosome arm 2AL mapped in the same region as *QH.icd-2A,* which was also identified in cultivated emmer. The flanking SNP markers for *QH.icd-2A* and the proximal marker for *QHf.fcu-3B* have been converted to KASP to be used for screening and we have genotyped the Global Durum Panel (GDP) with these three markers.

Currently, screening of the GDP for HF resistance to the Great Plains biotype is ongoing. The work here presents two HF resistant QTL, *QHf.fcu-2A* and *QHf.fcu-3B*, which can be used in durum breeding programs and the tools to screen for these QTL and introgress them via marker-assisted selection.

#105

Characterising wheat lines for rhizosheath size in acidic soil with high aluminium content

Huyen Thi Thanh Pham[1,2*], Jiayin Pang[1,3], Jairo A. Palta[1,2], Sasha N. Jenkins[1,2], Manny Delhaize[4], Kadambot H.M. Siddique[1,2]

[1] The UWA Institute of Agriculture, The University of Western Australia, Perth, WA 6001, Australia
[2] School of Agriculture and Environment, The University of Western Australia, Perth, WA 6001, Australia
[3] School of Biological Sciences, The University of Western Australia, Perth, WA 6001, Australia
[4] Research School of Biology, College of Science, Australian National University, Canberra 2601, Australia

Soil acidity poses a significant challenge to wheat cultivation in Western Australia, affecting over 75% of the wheatbelt soils. This study investigated the performance of four wheat (*Triticum aestivum* L.) lines with putative large (L_1, L_3) and small (S_1, S_2) rhizosheath size, and the commercial cultivar Mace (P). Plants were grown in pots filled with slightly acidic or highly acidic soil with high aluminium (Al^{3+}) content, in a temperature-controlled glasshouse, from June to October 2023.

We assessed various parameters including seedling emergence, tiller number, plant height, rhizosheath size, and root and shoot biomass. Tiller numbers ranged from 3.3 to 4 per plant in the slightly acidic soil, and 1 to 2.3 per plant in the highly acidic soil with high Al^{3+}. Under the highly acidic soil with high Al^{3+} content, L_1 and L_3 exhibited significantly greater rhizosheath soil dry and fresh weights, as well as root biomass compared to S_1 and S_2 at tillering (Z15/Z22) ($P < 0.05$); however, no such difference was observed under slightly acidic soil.

All wheat lines displayed larger rhizosheath size under the slightly acidic soil than under the highly acidic soil with high Al^{3+} content. The root: total plant biomass ratio remained consistent across the four lines and the cultivar Mace in both soil types, indicating no impact of soil type or rhizosheath size on biomass allocation to the root system.

Overall, these results confirmed the variation in rhizosheath size among the wheat lines, highlighting genotype-specific responses to soil acidity with high Al^{3+} content. Such findings have implications for crop growth and yield under acidic soil conditions.

Further investigations are warranted to elucidate the underlying mechanisms driving these responses and the dynamics of rhizosheath development and root traits for wheat genotypes cultivated in highly acidic soils with high Al^{3+} content.

#201

Association mapping of stem rust resistance in winter wheat

Kaitlyn A. Pidherny[1], Jim G. Menzies[2], Colin W. Hiebert[2], Harwinder S. Sidhu[3], Curt A. McCartney[1]

[1]Department of Plant Science, University of Manitoba, 222 Agriculture Building, Winnipeg, MB, Canada R3T 2N2; [2]Morden Research and Development Centre, Agriculture and Agri-Food Canada, 101 Route 100, Morden, MB, Canada, R6M 1Y5;[3] Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, 5403 1st Ave S., Lethbridge, AB, Canada, T1J 4B1

The fungal pathogen Puccinia graminis f. sp. tritici (Pgt) is the causative agent of stem rust on wheat (Triticum aestivum). Pgt has the potential to cause significant losses to wheat production in Canada, making it an economically important disease. No stem rust epidemics have occurred since the 1950s in western Canada due to the cultivation of resistant varieties.

The genetic basis for stem rust resistance in most Canadian wheat cultivars is not fully understood. The objective of this study is to identify resistance genes present in a winter wheat association mapping population, consisting of approximately 300 hard red winter wheats from western Canada, and 100 hard red winter wheats from other regions (United States, eastern Canada, and Europe).
The population was phenotyped for stem rust resistance in field trials and seedling assays and has been tested with single nucleotide (SNP) markers spanning the wheat genome.

The 25K Infinium SNP array and the Wheat Barley 40K Infinium SNP array were utilized in this study. Statistical analyses will identify quantitative trait loci (QTL). Field stem rust nurseries were grown at two locations, Winnipeg and Carman, Manitoba in 2023.

Field trials were randomized as alpha lattice designs with two replicates per trial. Plots were 1m long and inoculated with a mixture of Pgt races. Nurseries were mist irrigated to promote disease development. Data were collected on plant height, stand, heading date, and stem rust field severity and infection response.

Field trials will be repeated in 2024. Infection type data was recorded from indoor seedling inoculations with individual races. Preliminary results from genome-wide association study (GWAS) analysis suggest there are QTL of interest for resistance to stem rust located on chromosomes 1B, 2A, 2B, 2D, 3D, and 7B.

#149

Impact of waterlogging and high temperatures on wheat growth and yield

Rocío A. Ploschuk[1], Roxana Savin[1], Gustavo A. Slafer[1,2]

[1] Department of Agricultural and Forest Sciences and Engineering, University of Lleida - AGROTECNIO-CERCA Center, Av. R. Roure 191, 25198 Lleida, Spain
[2] ICREA, Catalonian Institution for Research and Advanced Studies, Spain

The risk of having extreme weather events, such as intense rainfalls or heat waves, has increased over the last decades. As the higher frequency of storms is associated with increased temperatures, the possibility of crops being exposed to waterlogging and high temperatures will increase. Wheat is crucial for food security and will be more frequently exposed to these constraints, even in Mediterranean regions, leading to substantial economic damage.

The aim of this study was to find out whether a previous event of waterlogging might reduce crop tolerance to a subsequent high temperature event or if it can confer tolerance to reduce the impact of another stress later. Thus, we conducted an experiment sowing three bread (Artur Nick, Santaella, Acorazado) and three durum (Euroduro, Don Ricardo and Athoris) spring wheats individually in plastic tubes (8.5 cm diameter and 100 cm length) outdoors.

Four treatments were imposed: control, waterlogging (for 15 days at stem elongation, DC30), heat stress (for 15 days at booting, DC40) and a treatment combining both stresses. At the end of each treatment, plants continued growing in unstressed conditions until maturity, when yield and its components (biomass and harvest index; numerical components) were quantified.

We found genotypic variability in yield responses to the stresses but not related to the different species. Yield was reduced compared to controls in all treatments by 33.7±7.3, 24.7±7.8, and 44.9±6.4% (average of 6 genotypes) when affected by waterlogging, heat stress, and both stresses combined, respectively. In general terms, a previous expose to waterlogging conferred a sort of stress acclimation to subsequently face high temperatures (resulting in an antagonistic interaction between stresses (i.e., yield reductions in the combined treatment were lower than the sum of the decreases provoked by each stress separately).

Considering the effects of individual stresses, yield seemed more sensitive in durum than in bread wheat. Treatments reduced yield mainly through decreasing grain number (mostly though the number of grains per spike) with more exceptional effects through grain weight.

Additionally, the stresses seemed to have a greater effect on overall plant growth than on yield partitioning. Understanding the impact of these stresses and their interactions is crucial not only due to genotypic variability, which aids in selecting more stress-tolerant varieties, but also because of the interactions between these successive constraints.

The antagonistic interaction observed in our experiment offers insights into mitigating the impact of concurrent waterlogging and high temperatures in wheat cultivation scenarios.

#591

An anther-specific gdsl lipase is potentially responsible for cutin polymerization in barley and wheat

Juan Qi[1,#] , Guojun Liu[1], Xiao Wang[1, 3], Meng Sun[1, 4], Jiajie Wu[1], Daolin Fu[1, 2,*], Fei Ni[1,*]

[1] State Key Laboratory of Wheat Improvement, College of Agronomy, Shandong Agricultural University, Tai'an, Shandong 271018, China. [2] Spring Valley Agriscience Co., Ltd., Jinan, Shandong 250300, China. [3] Present address: Zhaoyuan Housing and Urban Rural Development Bureau, Yantai, Shandong 265400, China. [4] Present address: Academy of Agricultural Sciences, Suzhou, Anhui 234000, China.

[*]Emails: [#]qijuan@sdau.edu.cn, [*]dlfu@sdau.edu.cn, [*]nifei1998@sdau.edu.cn.

Plant male sterility is a valuable trait, that is widely exploited in hybrid seed production. This trait can be classified into genic male sterility (GMS) and cytoplasmic male sterility (CMS). Poor genetic diversity, unreliable restoration, and a potential cytoplasmic penalty on heterosis limited the application of CMS to hybrid seed production.

The use of GMS can overcome these drawbacks. The barley accession 'GSHO3002' is a spontaneous male sterile mutant in barley variety 'Freja/Nepal' (CIho 15839). The barley sterility in GSHO3002 was controlled by a recessive GMS gene, *msg3002* (originally named as *msg,,by*). In this study, by genetic mapping, we mapped *MSG3002* to a 0.2 cM interval between two flanking marker *SP5M21* and *SP5M14* on the chromosome arm 1HL.

The *SP5M21*-*SP5M14* interval corresponds 371.2 kb in the Morex reference sequence. This interval involves four high-confidence candidates, and sequence analysis reveals that one of them, a gene encoding the GDSL lipase (named *HvGDSL64460*), has a premature stop codon in the GSHO3002, which identified as the most plausible candidate for *MSG3002*. Afterwards, the expression profiles revealed that *HvGDSL64460* was specifically expressed in anther.

The anther-specific GDSL lipase was localized in endoplasmic reticulum (ER). For validation experiments, allelic variations of *HvGDSL64460* were identified from barley EMS-mutagenized population and combined with genetic analysis revealed that this anther-specific GDSL lipase is responsible for male fertility in barley. Furthermore, widely untargeted metabolomics analysis revealed that MSG3002 may function through cutin synthases/acyltransferase activity to modulate lipid metabolism.

Meanwhile, we demonstrated the evolutionary conserved role of MSG3002 in male fertility control between barley and wheat by knocking out the MSG3002 orthologs in the A, B and D subgenomes of allohexaploid wheat. Taken together, our study suggests that MSG3002 is an indispensable evolutionary conserved member in anther development of barley and wheat that could be a valuable target for GMS-based system for hybrid breeding barley and wheat.

#600

Field performance of the hexaploid hybrid wheat ‘quanmai 01’

Xizhen Guan[1], Jifa Zhang[1,2], Weidong Zhang[2], Genshen Ren[1], Junhua Peng[1], Daolin Fu[1,2*]

[1] Spring Valley Agriscience Co., Ltd., Jinan 250300, China
[2] Shandong Agricultural University, Taian 271018, China

World population continues to grow at around 73 million people per year, approaching nine billion by 2037. There is a direct need for more cereal production to match the increasing population in the world. Wheat is the No. 1 cereal crop in terms of its global harvested area, approximately 220 milliion hectares in 2023. To date, traditional pure breeding has promoted wheat production to an extreme high level, and how to further improve its yield is a challenging task.

A more promising strategy is to harness the untapped hybrid vigor, which is successfully applied in two other major cereal crops, corn and rice. Here, we report field trials of a hexaploidy hybrid wheat ‘Quanmai 01’ (QM01), which was a hybrid between an advanced male-sterile line and the male pollen donor ‘Shannong 22’ (SN22). In 2022-2023 growing season, under a regular sowing rate of 225 kg/ha, QM01 displayed a 20% yield increase in comparison to SN22; with a reduced sowing rate, ranging from 37.5kg to 187.5kg per hectare with an increment of 37.5kg per hectare, the sowing rate was negatively correlated with the yield increase rate, reflecting a yield hybrid vigor of 11% to 31.8%.

In 2023-2024 growing season, we performed a split-plot trial by considering nitrogen fertilizer, sowing rate and genotypes. Under a sowing rate of 22.5 kg/ha, QM01 had an 8.7% yield increase in comparison to SN22, reaching approximately 9.6 tons/ha; with a sowing rate of 37.5 kg/ha, QM01 had a 3.7% yield increase in comparison to SN22, reaching approximately 9.6 tons/ha. Certainly, there is substantial yield potential in hybrid wheat.

In future, global efforts should be given to deliver a high-throughput, high-quality, economic, and environmentally friendly approach to produce hybrid wheat.

#598

The development and survival of spikelets and florets: fertile grounds for improving wheat yield potential

Yue Qu[1], Donna Glassop[2], Ben Trevaskis[2] and Scott A. Boden[1]*

[1] School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Glen Osmond, SA, 5064, Australia.
[2] Commonwealth Scientific and Industrial Research Organisation, Agriculture and Food, Black Mountain Science and Innovation Park, Canberra, ACT, 2601, Australia.

Emails: Yue Qu (yue.qu@adelaide.edu.au); Scott Boden (scott.boden@adelaide.edu.au)

Cereal grains are pivotal to the global diet, with wheat alone providing 20% of the world's calorie and protein intake. With an expected global population of 9.8 billion by 2050 and climate change impacting crop productivity, enhancing wheat yield without expanding arable land is crucial. Yield component traits, particularly grain number, offer a sustainable route for yield improvement. Grain number is determined by the number of tillers, spikelets, and florets per plant, and spikelet number and floret primordia survival are key traits for improving yield potential.

Here, we aim to assess and characterize induced and natural genetic variation for spikelet and floret fertility in bread wheat, using germplasm adapted to Australian environments. This work involves identifying and generating variant alleles for genes that modify the survival and fertility of florets and spikelets, including *GNI1*, *HOX2*, *FT2*, *FT3*, *TB1*, *VRT-2*, *SVP-1*, and *DUO1*.

Near-isogenic lines (NILs) and CRISPR-Cas9 gene-edited lines are being developed in backgrounds with different photoperiod sensitivity to test the effect of the mentioned gene alleles on floret and spikelet fertility related traits in the greenhouse and field The best alleles in adapted backgrounds will be distributed to Australian breeders.

Gene molecular functional studies are also being conducted through techniques such as RNA-seq and spatial transcriptomics to further elucidate the roles of these genes of interest. Together, this work will offer promising targets for breeders to increase grain production through enhanced floret and spikelet fertility.

#454

Genetic dissection of resistance to three rust diseases in Kenyan wheat cultivar, Kasuko

Naeela Qureshi[1,*], Sridhar Bhavani[2]

[1] International Maize and Wheat Improvement Center (CIMMYT), Carretera Mexico-Veracruz Km. 45, El-Batan, Texcoco 56237, Mexico
[2] International Maize and Wheat Improvement Center (CIMMYT), ICRAF Campus, United Nations Avenue, Gigiri, Nairobi P.O. Box 1041-00621, Kenya

Emails: n.qureshi@cgiar.org; s.bhavani@cgiar.org

Changing climate patterns across the globe poses various challenges necessitating new wheat varieties that are resilient to transboundary pests and diseases to ensure future agriculture sustainability. Wheat rusts remain economically important diseases causing substantial yield losses to wheat production worldwide. Mining rust resistance genes remains an integral component in several wheat breeding programs.

Understanding the complex genetics of resistance is crucial for varietal development and deployment. The current study focuses on unraveling the genetic basis of rust resistance in CIMMYT line, “Kasuko”. A RIL population comprising 181 lines was developed using resistant variety Kasuko and a triple rust susceptible parent Apav. This population was phenotyped under artificial epidemics of leaf rust (LR) and yellow rust (YR) in Mexico and yellow rust and stem rust (SR) in Kenya.

Quantitative trait loci (QTL) mapping studies were conducted using the DArTSeq genotyping to map the QTL associated with rust resistance. The analysis revealed two significant pleotropic loci associated with leaf rust, yellow rust and stem rust resistance, *QLrYr.cim-1BL* and *QLrYr.cim-2AS* on chromosomes 1BL and 2AS, respectively. *QLrYr.cim-1BL* was identified as *Lr46/Yr29*, whereas *QLrYr.cim-2AS* represented *Yr17/Lr37* region.

Another consistent and stable minor leaf rust QTL, *QLr.cim-2DS* was observed on chromosome 2DS explaining 9-11% phenotypic variation (PVE). Two minor QTLs were identified for yellow rust resistance in Kenya on chromosomes 3DS and 6BS, *QYrKen.cim-3DS* and *QYrKen.cim-6BS*, respectively with minor effects of less than 10% PVE.

In addition to these, four minor QTLs were detected for the stem rust resistance on chromosomes 2BS (*QSr.cim-2BS*), 5AL (*QSr.cim-5AL*), 6AS (*QSr.cim-6AS*) and 6AL (*QSr.cim-2BS*). The comparison of average severities among RILs carrying these QTL in various combinations indicated significant disease reduction.

These QTL and their closely linked markers are being developed which will be useful for fine mapping and marker-assisted selection (MAS) in breeding for durable resistance to multiple rust diseases.

#432

Enabling interoperable data infrastructure for wheat genebank genomics

Mahdi Rahimi[1], Kenny Yu[1], Don Isdale[1], David Chisanga[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]

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

The rapidly growing availability of genotype data from global wheat genebanks – including the Australian Grains Genebank – presents major challenges for data management and interoperability. Each genebank accession has associated passport information which is stored in centrally managed global databases such as Genesys-PGR. Users accessing the genotype data from the genebank will often want to filter accessions based on passport information, but this traditionally needs both types of information in a single database, which necessitates duplication of data and leads to potential synchronisation issues and overheads.

To tackle this problem, we developed a middleware solution called *Genolink* to seamlessly integrate genotype databases and genebank passport repositories like Genesys-PGR. *Genolink* allows users, for example, to filter accessions in Genesys-PGR based on passport data or specified lists, retrieve related genotype data from multiple genomic platforms, and to consolidate the datasets effectively. Our approach significantly reduces data redundancy and enhances data accessibility and interoperability across systems, ensuring requested data is always current.

Genolink fits into a wider ecosystem of tools and interfaces that support genebank genomics, with support for the Breeders API (BrAPI). In addition, *Genolink* provides interfaces for independent user-facing tools such as the AVR-developed web-based genomic visualisation tool *Pretzel* to exploit its functionality.

#65

Dissection of the molecular mechanism of glutathiones - transferase gene TAGSTF1 regulating se in wheat

Ren Yan, Liyan Wang, Mengjuan Gao, Heying Zhao, Jiahui Chen, Xiaoqian Chen, Mengkang Niu, Ning Zhang, Congwei Sun, Xia Yang, Dan Pei, Zhongdong Dong, Feng Chen*

National Key Laboratory of Wheat and Maize Crop Science / CIMMYT-China Wheat and Maize Joint Research Center /Agronomy College, Henan Agricultural University, Zhengzhou 450046, China

Corresponding author: fengchen@henau.edu.cn (86-0371-56990337)

Wheat sharp eyespot (SE), caused by *Rhizoctonia cerealis* (*Rc*), is one of the most devastating soil-borne diseases in the worldwide and frequently occurred in multiple wheat major regions in China in recent years. Here, we mapped a new and stable QTL, *QSe.hnau-3BS*, for SE resistance in the Pingyuan50/Mingxian169 doubled haploid (DH) population. Based on the fine mapping of QTL, BSR-Seq, and gene sequencing, *TaGSTF1* was determined as a candidate gene.

Through the virus-induced gene silencing (VIGS), ethyl methane sulfonate (EMS) mutants and overexpression transgenic lines, we found that *TaGSTF1* positively regulated SE resistance through removing the reactive oxygen species (ROS) in wheat plants. Moreover, we screened a wheat protein kinase TaSRK could interact with TaGSTF1, and confirmed the interaction between them in vitro and in vivo.

Transient overexpression experiments in tobacco leaf cells and wheat protoplasts both indicated that TaSRK sharply increased the protein level of TaGSTF1, and this promotion was possibly resulted from Phosphorylation of TaSRK to TaGSTF1. EMS mutants and overexpression lines showed that *TaSRK* positively regulated wheat SE resistance and reduced ROS accumulation.

Taken together, we brought forth a TaSRK-TaGSTF1 model mediated SE resistance by scavenging the ROS in wheat plants. The results will provide gene resources and material germplasm for improvement of SE resistance in wheat breeding program.

#57

Inter-specific breeding programs for increasing carotenoid content in wheat

Rodríguez-Suárez C[1], Requena-Ramírez MD[1], Hornero-Méndez D[2]; Atienza SG[1]

[1] Institute for Sustainable Agriculture, CSIC, Avda, Menéndez Pidal s/n, E-14004, Córdoba, Spain
[2] Department of Food Phytochemistry, Instituto de la Grasa, CSIC. Campus Universidad Pablo de Olavide, Edificio 46. Ctra. de Utrera, Km 1, E-41013, Sevilla, Spain

Color and color stability are key characteristics for wheat grain quality. The yellow color of the endosperm is a consequence of the accumulation of carotenoids, which are bioactive compounds of major importance for human nutrition and health. Thus, grain carotenoid content has historically been an important trait considered in wheat breeding following different strategies. Bread wheat has generally been selected for whitish endosperm, whereas durum wheat breeding has focused on increasing grain yellowness.

Inter-specific variability is a valuable resource for broadening the genetic base of wheat because hybrid crosses, although difficult in some cases, are feasible. Here, we present two strategies based on inter-specific breeding for wheat biofortification which are currently being implemented in our research group to increase grain carotenoid content in common wheat (*via* increasing biosynthesis) and in durum wheat (*via* increasing biosynthesis, accumulation and stability).

Due to the selection for white endosperm, common wheat harbors alleles for carotenogenic genes associated with low carotenoid content. *Psy1* is known to be the main rate-limiting gene in carotenoid biosynthesis. The *Psy1-A1* and *Psy1-B1* genes from durum wheat were then transferred to common wheat to evaluate their combined effect on grain carotenoid content, considering that there is an emerging market for yellow-pigmented breads due to the renewed interest in the nutritional aspects of cereal-derived food. To date, pre-breeding materials have been obtained with increases in grain carotenoid content ranging from 16 to 23 %.

A second inter-specific breeding program is aimed to incorporate into durum wheat the *Hordeum chilense* genes *Psy1-H*^{ch} (for increasing carotenoid biosynthesis) and *XAT-7H*^{ch} responsible for carotenoid esterification. Carotenoid esterification has emerged as a new target for cereal biofortification, as this process increases both carotenoid accumulation and stability.

Prior to the identification of both the phenotypic variability and the gene responsible for carotenoid esterification (*XAT-7A*) in durum wheat, we considered this approach to increase grain carotenoid content in this species. Based on a marker-assisted selection strategy, durum wheat pre-breeding materials harboring both *Psy1-H*^{ch} and *XAT-7H*^{ch} have been obtained, which can be used for pyramiding with *XAT-7A* to putatively increase the carotenoid biosynthesis and esterification capacity in this species.

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#574

Genetic diversity analysis of *BIPOLARIS SOROKINIANA* (Sacc.) shoemaker causing spot blotch disease in barley

CAUSING SPOT BLOTCH DISEASE IN BARLEY

Aradhna Sagwal[1], Rajender Beniwal[2] and Ravindra Kumar[3]

[1]Department of Agriculture, NIILM University, Kaithal, 136027, Haryana, India
[2]Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, 125004, Haryana, India
[3] Department of Crop Protection, Indian Institute of Wheat and Barley Research, Karnal, 132001, Haryana, India

aradhnasagwal31@gmail.com, rsb1965@gmail.com and ravindrakumarbhu@gmail.com

Spot blotch is most catastrophic disease in barley caused by hemi-biotrophic and aggressive fungus *Bipolaris sorokiniana* (Sacc.) Shoemaker. The disease is most damaging under warm temperature and intermittent rains. The diseased barley leaves across the locations in India were collected, the isolates of pathogen *Bipolaris sorokiniana* was isolated and purified.

Genetic variability of 30 *Bipolaris sorokiniana* affecting barley was studied using ITS gene rDNA sequencing. The positive amplicons were purified by using FavorPrep™ GEL/PCR Purification kit. The purified product was sequenced with Sanger's method DNA sequencing. The sequences obtained were blast on NCBI, upon confirmation sequences were submitted to nucleotide data base of NCBI. The same sequences were processed for multiple sequence alignment using clustal W and phylogenetic tree was constructed using Neighbors joining method in MEGA-X software.

The dendrogram generated grouped them to clusters and revealed diversity among isolates. Bs 29 isolate collected from Ludhiana, Punjab (Accession No. OQ955738) was found to be the most diverse among the isolates. It was also found that Bs 29 was most virulent among all isolates having shortest incubation and latent period.

The sequences of most pathogenic isolates submitted to nucleotide data base of NCBI were given Accession No. (OQ955726, OQ955727, OQ955728, OQ955729, OQ955730, OQ955731, OQ955732, OQ955733, OQ955734, V OQ955735, OQ955736, OQ955737, OQ955738, OQ955739). Thus, the understanding of pathotypes diversity helped in identifying the genes that are to be deployed for development of resistant barley cultivars.

Keywords: Barley, *Bipolaris sorokiniana*, diversity, Spot blotch

#475

Germplasm and data exchange to expedite solutions for farmers

Carolina Saint Pierre[1,*], Carolina Sansaloni[1], Ana Luisa Ordaz Cano[1], Guillermo S. Gerard[1], Leonardo Crespo-Herrera[1], Velu Govindan[1], Sridhar Bhavani[1], Pawan K. Singh[1], Richard Trethowan[2], Matthew Reynolds[1]

[1] International Maize and Wheat Improvement Center, CIMMYT, Texcoco, 56237, Mexico
[2] School of Life and Environmental Sciences, Plant Breeding Institute, Sydney Institute of Agriculture, University of Sydney, Narrabri, NSW, 2390 Australia

c.saintpierre@cgiar.org

The development of wheat cultivars adapted to various wheat-growing regions worldwide has been largely facilitated by the exchange of germplasm and data. The CIMMYT International Wheat Improvement Network (IWIN) has played a pivotal role in fostering a global network for wheat innovation, focusing on germplasm development and sustainable agricultural productivity, particularly in the Global South.

Collaborating with local breeding programs and other CGIAR networks, IWIN annually supply CIMMYT improved wheat lines as well as genebank accessions to about 90 countries.

As a consequence, improved wheat varieties developed using CGIAR breeding lines cover more than 100 million hectares globally. In regions like South Asia and Sub-Saharan Africa, CIMMYT-derived varieties dominate, illustrating the significance of germplasm sharing. Genetic gains have been documented, as evidenced by analyses of the Semi-Arid Wheat Yield Trials (SAWYT) conducted across 740 locations in 66 countries from 2002 to 2014, revealing an average yield increase of 1.8% in low-yielding environments and 1.41% in medium-yielding environments.

CIMMYT's partnerships extend beyond breeding networks, collaborating with academia through initiatives like HeDWIC and IWYP to deterministically improve quantitative traits like radiation use efficiency and climate resilience making use of genetic resources to accumulate complementary alleles and thereby broaden wheat breeding genepools. These efforts are complemented by international programs like the Wheat Initiative and the CIMMYT Australia ICARDA Germplasm Evaluation program, which further strengthen global food security and crop productivity. The seamless exchange of information and data between CIMMYT and national breeding programs reinforces the effectiveness of these networks.

Integration of historical breeding trial data with advancements in machine learning and deep learning techniques offers promising strategies to address challenges posed by climate change in agriculture. However, the sustainability of these efforts depends on continued collaboration and information sharing.

#645

CropSustaiN: A BNI-WHEAT MISSION

Carolina Saint Pierre[1]

[1] International Maize and Wheat Improvement Center, CIMMYT, Texcoco, 56237, Mexico

c.saintpierre@cgiar.org

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 solutions. CIMMYT has secured a grant from the Novo Nordisk Foundation to lead this multidisciplinary initiative, in collaboration with multiple partners.

The long-term vision is for farmers to reduce nitrogen fertilizer use and its associated environmental impacts by adopting BNI technology while maintaining crop productivity and soil health. The BNI mission 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 wheat lines for both spring and winter wheat. 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 has therefore the potential to significantly impact global food security and enhance environmental sustainability.

#441

Optimizing pollen tube observation in bread wheat pistils

Shun Sakuma[1,*], Sora Matsumoto[1], Kohei Mishina[1], Daisuke Maruyama[2], Hiroshi Hisano[3], Shuhei Nasuda[4]

[1] Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan
[2] Kihara Institute for Biological Research, Yokohama City University, Yokohama, 244-0813, Japan
[3] Institute of Plant Science and Resources, Okayama University, Kurashiki, 710-0046, Japan
[4] Graduate School of Agriculture, Kyoto University, Kyoto, 606-8502, Japan

*ssakuma@tottori-u.ac.jp

Global warming significantly threatens wheat yield production. The viability of pollen and pistil is pivotal for successful grain sets. However, the methods for observing pollen tube elongation, a key process in fertilization, are yet to be optimized. In the present study, we developed an improved simple method. An intergenic cross, rather than self-pollination, was performed to discriminate the obvious pollen tube signals from other factors in the pollen tube pathway. A bread wheat cultivar was utilized as a female parent and a rye cultivar as a pollen parent.

Two days after emasculating bread wheat, rye pollen was applied on wheat pistils by hand. Pistils were collected two hours after pollination. Pollen tubes were visualized by conventional aniline blue staining. The stained pistil was observed using a fluorescence microscope. Due to the thickness of the ovary of bread wheat (ca. 189 µm), the fluorescence of pollen tubes could not be detected by observation of intact ovary.

When the ovary and pistil were cut in half with a razor before staining, fluorescence from pollen tubes was detected in 74.7% of ovary examined. Chemical clearing of the ovary before staining did not provide any improvement. “Focus stacking” greatly improved the clarity of an image.
The proportion of rye pollen tubes reaching the style base was 97.1 ± 9.7%, and the proportion reaching the inside of the ovary from the base of the pistil was 90.6 ± 11.1%. The seed setting rate in the intergenic cross was 76.6 ± 15%.

This method allows observing pollen tube germination and elongation in the style and ovary, which is useful for analyzing the viability of pollen and pistils.

#635

Novel methods of year-round multi-pathotype phenotyping for adult plant resistance to rust diseases of wheat

Karanjeet S. Sandhu and Robert F. Park

Plant Breeding Institute, University of Sydney, Private Bag 4011 Narellan, NSW 2567, NSW

Email: karanjeet.sandhu@sydney.edu.au , robert.park@sydney.edu.au

Bread wheat provides an important source of food and nutrition, and it's cultivation covers more than 218 million hectares worldwide. Demand of wheat is on the rise and to meet this requirement wheat production must increase, whereas rust diseases continue to be an ongoing significant constraint to wheat production.

Epidemics of rust diseases can cause major yield losses worlwide. Rust diseases can be managed using adult plant resistance (APR) as APR genes are considered durable, for example *Yr18*/*Lr34*/*Sr57*, *Yr29*/*Lr46*/*Sr58* and *Sr2* have been commonly used across the wheat breeding programs. Expression of APR can be dependent on a number of factors like genotype, growth stage and climatic conditions. The knowledge of expression of APR is crucial for making selections and breeding for rust resistance to achieve the integrated management of rust diseases.

Phenotyping for APR using multiple pathotypes is not practical under field conditions, and only one cycle of APR screening can be achieved during crop season. Comparative experiments conducted in the greenhouse (GH) vs LED growth rooms indicated the early expression of APR to wheat stripe/yellow rust (WYR) in LED growth rooms. Different genotypes carrying seedling resistance or APR for WYR, or leaf rust (WLR), or stem rust (WSR) were grown under 240W LumiLED lights, at an average temperature of 23.21℃ and Day/Night cycle: 20hrs light / 4hrs dark.

Plant growth was much faster under LED lights as compared to the plants grown in the GH under natural Day/Night cycle. Replications of plants grown in LED growth room for one week, and for six weeks were inoculated on same day using different pathotypes of WYR, WLR, and WSR causing pathogens. Post incubation, plants were moved to LED growth rooms set for Day/Night cycle: 13hrs light / 11hrs dark, for WYR development at 19℃ (average 20.48℃), *and* for both WLR and WSR development at 23℃ (average 23.21℃). *The* expected expressions of seedling resistance and APR were recorded after 12–14 days of inoculation, and cycle of APR phenotyping was completed in less than eight weeks.

Additionally, near isogenic lines with known genes for APR to WYR were grown in GH for 10 weeks and Flag-1 leaves were point inoculated to quantify the expression of APR. Cycle of APR quantification under GH conditions was completed in 12 weeks. Results from the point inoculations of multi-pathotypes indicated pathotype specific response of APR to WYR.

Developed methodology can allow the phenotyping of APR throughout the year by eliminating the wait for crop season, however, we suggest the validation of selections under field conditions. These novel methods of high-throughput & cost-effective multi-pathotype phenotyping for APR in LED growth rooms, allowing 5–6 cycles per year can fast track the breeding of wheat cultivars with durable rust resistance.

#305

Genome wide association study for grain-yield and protein content in wheat grown under different environments

Jatinder S. Sangha[1,*], Weiwei Wang[1], Yuefeng Ruan[1], Richard D. Cuthbert[1], Ron Knox[1,†], Firdissa Bokore[1], Brad Meyer[1], Curtis Pozniak[2], Amidou N'Diaye[2]

[1] Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, Swift Current, SK, Canada, S9H 3X2
[2]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada
† Retired

*Jatinder.sangha2@agr.gc.ca

Improving grain yield (GY) and grain protein concentration (GPC) under variable climates are currently two important objectives of wheat breeding programs. Variability in environmental conditions can affect the reliability of these traits leading to uncertainty to growers and the industry. Marker trait associations (MTAs) are desirable to improve the selection procedure in wheat breeding for complex traits which are difficult to predict and phenotype.

We created 30 multi-environment datasets for grain yield and grain protein analysis using a set of 198 doubled haploid (DH) lines, derived from a Carberry/AC Cadillac cross, that were grown for six years (2014-2019) under rainfed and irrigated field environments. Approximately 11K polymorphic markers with from the 90K iSelect SNP array were deployed in a genome wide association study (GWAS) for high threshold MTA detection. A total of 71 MTAs were identified to be significantly associated to GY and GPC in different environments, of which 22 overlapped MTAs detected in both irrigated and rainfed environments.

The phenotypic variance explanation of each combined GY and GPC MTA was estimated using ridge-regression best linear unbiased prediction (rrBLUP), of which 19 MTAs had the same favourable/unfavourable alleles for GY and GPC. Gene identifications of 70 out of 71 combined MTAs were found in the NCBI and 54 in the EnsemblPlants databases, of which 55 genes had characterized functional annotations, including disease resistance, spike characteristics, zinc finger related domains, kinases, root traits and others.

Best linear unbiased estimate (BLUE) analysis used to detect DH lines having an ideal combination of favourable/unfavourable alleles differentiated the two parents, three high GY lines and three low GY lines with variation in physiological traits such as carbon isotope discrimination, stomatal conductance, and stomatal numbers.

Further research on the genetic basis of these SNP markers with GY, GPC, and various physiological traits is required to understand the benefits of this study to wheat breeders for improving wheat cultivars for variable environments.

#338

Dynamic responses associated with chloroplast integrity and heat resilience in wheat following PGPR primimg

Jayanwita Sarkar [1*], Usha Chakraborty [2], Bishwanath Chakraborty[3]

[1] Plant physiology and biochemistry Laboratory, Department of Botany, Cooch Behar Panchanan Barma University, Cooch Behar, WB, India – 736101
[2]Plant Biochemistry Laboratory, Department of Botany, University of North Bengal, Siliguri, 734013, West Bengal, India
[3]Immunophypathology Laboratory, Department of Botany, University of North Bengal, Siliguri, 734013, West Bengal, India

Changing climate scenario is posing a major threat to agriculture worldwide. Wheat, a climate sensitive crop, is envisaged to face significant yield loss in coming decades due to accelerating global average temperature. In recent years Plant Growth Promoting Bacteria (PGPR) have been successfully used in sustainable crop improvement programme and abiotic stress alleviation.

However, PGPR mediated thermal acclimation and underlying crosstalk of diverse signalling networks have mostly remained obscure. Hence, this study aims to focus on the adaptive modulations of PGPR primed wheat plants at biochemical and gene expression levels which make them more resilient to high temperature.

Our findings suggested that *B. safenisis* priming mostly dealt with shielding the cellular functions and particularly chloroplast structural integrity from heat induced oxidative injury. *B. safensis* priming positively modulated expressions of a number of HSPs including small HSPs before and during heat exposure. Better performance of various redox enzymes and antioxidants associated with ascorbate - glutathione cycle together with low proline profusion due to high PDH expression provided competent heat adaptive path without generation of excessive ROS.

Bacterial priming elicited expression of ADC1 and ADC2 linked to putrescine synthesis and also significantly enhanced accumulation glycine betain and phenols in response to high temperature. Positive effect of priming can be observed in seed quality also. *B. safensis* priming induced multilevel systematic modulation minimizes heat injury, chloroplast damage and improved heat endurance.

#586

Transcriptomic responses of wheat anthers at the early meiosis stage to water stress and antitranspirants

Misbah Sehar[1*], Peter Kettlewell[1], Philippa Borrill[2], Laura Vickers[1]

[1]Crop Science Group, Centre of Crop and Environmental Science, Harper Adams University, Newport, Shropshire, TF10 8NB, United Kingdom
[2]John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom

*Email: msehar@live.harper.ac.uk

Drought is severely affecting the yield of numerous food crops worldwide, especially wheat. The application of antitranspirants, substances that minimise water loss from plants, has demonstrated the ability to enhance crop yield in wheat, associated with pollen viability under water stress conditions when applied before meiosis. To understand the mechanisms through which antitranspirants promote pollen viability, transcriptomic responses were investigated in wheat anthers obtained from polytunnel-grown plants sprayed with antitranspirants and compared with droughted unsprayed and well-watered plants.

Film-forming (Vapor Gard – VG) and metabolic (S-Abscisic Acid – ABA) antitranspirants were sprayed at the flag leaf stage, and anthers were collected during the early meiosis stage (leptotene-zygotene). Gene expression of hormonal responses and the processes related to carbohydrate/sugar metabolism were significantly affected in the anthers of treated plants, with a notable downregulation of genes involved in these processes compared to well-watered. The application of antitranspirants significantly altered the expression patterns of genes in the early meiotic anthers under drought stress.

The majority of differentially expressed genes (DEGs) in the anthers of droughted, antitranspirant-treated plants (ABA and VG) exhibited transcriptional downregulation (over 3,900 genes) compared to unsprayed droughted plants. In contrast, the number of upregulated genes was lower (around 800 genes). Additionally, the number of downregulated genes was higher in VG-treated plant anthers (3,325 genes) compared to ABA-treated plant anthers (634 genes).

Thus, under drought stress antitranspirants suppress transcriptional responses of genes involved in various biological processes. However, despite these major transcriptomic changes, antitranspirants did not show any improvement in crop yield, and no significant differences were observed for improved pollen viability between plants treated with antitranspirants and unsprayed ones.
This suggests that male reproductive parts are more sensitive at the transcriptional level than subsequent physiological processes determining yield.

#407

Establishing the role of HKT in driving salinity tolerance and yield in water limited environments

Alex Seward [1, 2], Caitlin Byrt [2, 3], Scott Sydenham [4], Stuart Roy [1, 2]

[1] University of Adelaide Waite Campus, Urrbrae, SA, 5064, Australia
[2] ARC Training Centre for Future Crops Development, University of Adelaide, North Terrace, SA, 5005, Australia
[3] Australian National University, Acton, ACT, 2601, Australia
[4] LongReach Plant Breeders, York, WA, 6302, Australia

Alex Seward: alex.seward@adelaide.edu.au, Caitlin Byrt: caitlin.byrt@anu.edu.au, Scott Sydenham: ssydenham@longreachpb.com.au, Stuart Roy: stuart.roy@adelaide.edu.au

67% of Australian agricultural area is at risk of transient salinity[1], inhibiting plant growth and causing estimated agricultural losses of $519 million per annum[2]. The Portuguese wheat landrace Mocho de Espiga Branca (Mocho) has been found to accumulate high levels of sodium (Na^+) in leaves and shoots, whilst maintaining healthy growth, indicating novel Na^+ tolerance mechanisms not found in commercial wheat. High Na^+ accumulation was found to be caused by a naturally occurring SNP in *TaHKT1;5* that results in increased flux of Na^+ from root to shoot.

The aim of this work is to further elucidate the mechanisms behind the increased salt tolerance and investigate whether increased leaf Na^+ content leads to greater performance in water limited environments.

To investigate mechanisms behind increased sodium tolerance, a Mocho x Gladius recombinant inbred line (RIL) population was characterised under salt stress and control conditions, identifying 465 novel loci relating to growth performance. To determine whether enhanced ion accumulation in the shoot helps plants to grow better in low to moderate water conditions, a selection of RILs were grown in a mildly saline field ($Ece_{1-5} = 2.35$).

Three RILs were identified which accumulated >10x leaf Na^+ with 5-28% yield improvement when compared to the parent Gladius. This poster will introduce current and future work, further investigating the potential benefits of Na^+ accumulation in water limited environments and the tolerance mechanism behind it.

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#638

Identifying SNPS associated with novel leaf traits in wheat for enhanced self-irrigation and climate resilience

Safia Khushi[1], Muhammad Awais[1], Sadia Hakeem[2], Shoaib Ur Rehman[1], Mahmood Alam Khan[1], Babar Farid[1], Sabah Merrium[1], Humayun Raza[2], and Muhammad Ali Sher*[1]

[1] Institute of Plant Breeding and Biotechnology, MNS University of Agriculture Multan, Pakistan.
[2] Cotton Research Institute Multan, Pakistan.
[3] Department of Plant Breeding and Genetics, The Islamia University Bahawalpur, Pakistan.

Corresponding Author* ali.sher@mnsuam.edu.pk

Wheat (*Triticum aestivum* L.) is a staple crop in Asia and is crucial in nourishing humanity. Regarding food security. The changing climate and diminishing water sources require the use of scarce natural water supplies, such as air moisture, for irrigation. Wheat production is threatened by climate variability and extreme climate events, particularly heat waves and drought.

Several studies have been conducted on the self-irrigating properties of the wheat plant. This study aimed to find the SNPs associated with novel leaf traits (NLTs) in wheat. This would help in the selection of self-irrigating wheat plants by optimizing leaf architecture and surface properties for precise self-irrigation. Wheat germplasm of 220 genotypes was characterized for Novel Leaf Traits i.e. leaf angle, leaf rolling, grove type, and prickle hairs.

The analysis of variance depicted significant difference among the genotypes for the NLTs. GWAS was conducted to find the SNPs linked with Novel Leaf Traits to mitigate the water shortage problem. Based on the 37K wheat SNP assay, a genome-wide association study (GWAS) identified 50 stable and significantly associated SNPs for the above mentioned traits. The SNPs were found on chromosome 2A, 2B, 3A, 4B, 5A, 2D and 7A. Most of the SNPs were found on chromosome 2B.

This study establishes the theoretical foundation for future research into the genetic mechanisms underlying wheat novel leaf traits and the efficient breeding of varieties with desired plant architecture through Genomic selection.

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#566

MicroRNAs from the wheat-Russian wheat aphid interaction.

Phumzile Sibisi[1]

[1]Department of Agriculture and Animal Health, University of South Africa, Private Bag X6,
1710 Florida, South Africa

sibispp@unisa.ac.za

Triticum aestivum L. is one of the most dominant human and livestock feed crops. Yields of wheat have declined worldwide due to pathogens and pests. Russian wheat aphid is the most devastating aphid pest affecting wheat cultivation in South Africa and other regions. Feeding by this insect causes severe symptoms, including necrosis, streaking and trapping of the heads of the wheat plant. This reduces crop yield and can lead to the death of susceptible cultivars.

The emergence of resistance-breaking biotypes negates the use of resistant cultivars against the RWA. Feeding by the RWA on wheat induces differential expression of microRNA genes. Thus, this study used next-generation sequencing to identify a larger pool of microRNAs and further characterise them and their putative targets.Twelve microRNA libraries from Tugela uninfested, Tugela Dn uninfested, Tugela infested and Tugela Dn infested were constructed respectively. The expression of candidate miRNAs and their targets was determined by quantitative real-time PCR.

The predicted target genes were analysed for their gene ontology placement to determine their biological role in plants. Five hundred three miRNA candidates were obtained; only 87 matched the known *Triticum aestivum* miRNA. The identified miRNAs seem to target known resistance gene family members and previously identified resistance responses from wheat after infestation by the RWA.

The gene ontology results indicated that most of the identified targets in this study play a role in regulating some biological pathways known to be regulated during wheat-RWA interaction.The use of the next-generation sequencing method has boosted small RNA discoveries at an unprecedented scale.

However, a few of several thousand discovered small RNAs have been functionally characterised. The field of small RNA functional genomics research is still in its early phase, and in the case of complex crops like *Triticum aestivum*, the progress is even slower. More work needs to be done to characterise more miRNAs. Identifying and characterising more small RNA and their target genes will contribute to our understanding of wheat and RWA interaction.

Once a better understanding of this interaction is achieved, then this knowledge can be utilised in the future production of crops with better resistance against RWA.

Keywords: microRNA, Next-generation sequencing, Russian wheat aphid, Triticum aestivum

#634

The use of pcr machine for testing pollen tolerance to heat stress in wheat

Abu Bakar Siddique[1], Chenchen Zhao[1], Meixue zhou[1]

[1] Tasmanian Institute of Agriculture, University of Tasmania, Prospect, TAS 7250, Australia

Pollen viability is crucial for plant fertilization and is highly susceptible to temperature changes, particularly to heat stress. A fast and reliable method for screening pollen tolerance to heat stress is critical regarding improving plant's heat stress tolerance. Previous research disclosing heat stress effects on pollen viabilities were usually less efficiency, given that it required large space, but also predominantly examined only one single heat temperature, thereby inadequately reflecting the dynamic viabilities of pollens under a range of temperatures.

The objectives of this study were to 1) develop an easy and reliable method for investigating wheat pollen viability under different temperatures and 2) identify the best temperature range for screening pollen heat stress tolerance. To accomplish this, mature wheat pollens were harvested and distributed to a 96-well PCR plate filled with pollen culture medium. The PCR plate was then placed in a PCR machine operating under a gradient PCR program to manipulate temperatures.

After four-hour incubation under a series of temperatures (ranging from 21.9°C to 47°C), the pollens were examined under an upright microscopy. A specific image analysis pipeline was employed to quantitatively assess the impact of temperatures on pollen morphological traits, germination rates and tube growth. This method facilitates the screening of a large amount of pollen samples simultaneously, enabling rapid, reliable, and precise determination of pollen viability, germination, and developmental characteristics in response to temperatures.

This system can be employed to screen germplasm and identify quantitative trait loci (QTL)/genes for pollen heat stress tolerance and therefore can be effectively used in breeding programs.

#266

Can einkorn hold the key to fighting wheat curl mite?

Paula Silva[1*], Byron Evers[2], Jon Raupp[2], Dal-Hoe Koo[2], Jesse Poland[3], and Vijay Tiwari[4]

[1]Instituto Nacional de Investigación Agropecuaria (INIA), La Estanzuela, Ruta 50 km 11, Colonia 70006, Uruguay. [2] Department of Plant Pathology and Wheat Genetics Resource Center, Kansas State University, 1712 Claflin Road, Manhattan, KS 66506, USA. [3] Center for Desert Agriculture, King Abdullah University of Science and Technology (KAUST), Thuwal 23955, Saudi Arabia. [4] College of Agriculture and Natural Resources, University of Maryland, 4291 Fieldhouse Drive.
College Park, Maryland 20742, USA

*mpsilva@inia.org.uy

Triticum monococcum ($2n = 2x = 14$, A^mA^m), commonly known as einkorn wheat, represents a reservoir of untapped genetic diversity with promising implications for enhancing wheat breeding aimed at bolstering resistance to pests and diseases. Among these threats, the wheat curl mite poses a significant challenge to wheat production, primarily through its role as a vector for various viral diseases, including wheat streak mosaic virus.

While only five resistance genes against the curl mite have been identified thus far, notably, three of these genes were sourced from a wild relative species of wheat. Given the absence of widespread genetic resistance within wheat germplasm, our study explored the genetic architecture of curl mite resistance within T. monococcum. We tested 214 accessions, comprising 159 from T. monococcum subsp. aegilopoides and 55 from subsp. monococcum.

Under controlled conditions, accessions were infested with Aceria tosichella biotypes 1 and 2. Curl mite symptoms were recorded at the two-leaf stage, utilizing a 0-4 visual scale, where 0 denoted no symptoms (indicative of resistance), while scores ranging from 1 to 4 reflected increasing degrees of curliness or leaf entrapment (indicative of susceptibility).

Phenotypic assessment revealed the presence of both resistant and susceptible accessions within both T. monococcum subspecies, with a broad spectrum of phenotypic variability observed across the entire accession set. Notably, a greater proportion of accessions exhibited susceptibility compared to those displaying resistance, with biotype 1 demonstrating heightened aggressiveness relative to biotype 2. Mean phenotypic values for subsp. aegilopoides were 1.52 and 0.96 for biotypes 1 and 2, respectively, while for subsp. monococcum, mean values for biotypes 1 and 2 were 1.87 and 0.91, respectively.

Our findings highlight significant levels of resistance present within the tested T. monococcum accessions, underscoring the potential of this wild species as a reservoir of novel resistance against curl mite.
Ongoing association analyses, integrating phenotypic data with whole-genome sequencing data, aim to provide further insights. The findings of our study have broad implications for ongoing efforts in wheat breeding, aiming to create wheat varieties that are more resilient against the challenges presented by the wheat curl mite, thus advancing sustainable agricultural production.

#275

Genetic determinants of wheat adaptability in Uruguay: Allelic variations and phenotypic responses

Ana McAlister[1], Fernando Pereira[1], Noelia Pérez[1], Richard García[1], Ramiro Ahunchain[1], Federico Pieroni[1], Monika Kavanová[1], Paula Silva[1*]

[1]Instituto Nacional de Investigación Agropecuaria (INIA), La Estanzuela, Ruta 50 km 11, Colonia 70006, Uruguay

*mpsilva@inia.org.uy

Wheat (*Triticum aestivum* L.) is a globally distributed crop due to its wide adaptability which in turn is a consequence of the large variance in phenology among cultivars. The genetic diversity governing phenological traits, such as days to flowering, enables the selection of optimal combinations of cultivar and sowing date for each environment, crucial for maximizing yield potential in local conditions.

Additionally, morphological characteristics, especially reduced plant height, have played a fundamental role in the "Green Revolution," enhancing grain yield by mitigating lodging and optimizing partitioning to reproductive organs. Understanding the effect of allelic variation in phenological and morphological traits, including the *VERNALIZATION* (*VRN*), *PHOTOPERIOD* (*PPD*), *EARLINESS PER SE* (*EPS*) and *REDUCED HEIGHT* (*RHT*) gene family pathways, is essential for breeding programs to target specific environments.

In this study, we analyzed 19 wheat genotypes, including cultivars and advanced breeding lines, with allelic variations in *VRN-1, PPD-1, EPS-D1, RHT-1,* and *RHT-2* genes from the Wheat Breeding Program of the National Institute of Agricultural Research (INIA-Uruguay). Field trails with seven sowing dates in the 2023 growth season were used to assess the combined effects of the allelic variation on days to flowering, days to physiological maturity and grain yield.

A wide range of sowing dates was explored, from early to late, resulting in a range of days to flowering from 321 to 728 degree days, with variation among genotypes being explained by the interaction between allelic combinations and environment.

This work presents the findings of our investigation, aimed at advancing the development of wheat germplasm with heightened adaptability to Uruguayan environmental conditions.

#570

Integrative initiatives for transition to a new mode of wheat breeding for sustainable future

Satinder Singh[1], Rohit Sharma[1], Achla Sharma[1], Praveen KumarA[1], Tarun Kapoor[1], GS Mavi[1], Puja Srivastava[1], VS Sohu[1]

[1] Punjab Agricultural University, Ludhiana, Punjab, 141004, India

satinder-pbg@pau.edu

Wheat (Triticum aestivum) stands as a cornerstone of global food security, yet confronts escalating pressures from climate change and population growth, necessitating the expedited development of high-yielding, climate-resilient, and nutritionally enhanced wheat varieties. The upgradation of wheat breeding system for catering to these threats may be structured around two functional units- one aimed at acceleration of the genetic and breeding processes and the other at high throughput precision phenotyping using artificial intelligence tools.

To address the first component, a cost-effective speed breeding chamber, measuring 3 m × 4.5 m × 3 m, was constructed using Polyurethane Foam (PUF) panels, strengthened by eight 100W and four 200W LED light boxes and two 1.5 hp split type domestic air conditioners. This innovative chamber operates on a 7KW solar panel system, highlighting a novel approach to accelerate plant growth and development via solar-powered artificial lighting and temperature control.

Using these advancements, a ‘Recombinant Inbred Line’ (RIL) population, derived from a cross between 'PBW 1 Chapati' and ‘Aegilops kotschyii introgression line’, was rapidly developed under optimized growth conditions, culminating in a 365-to-377-day developmental cycle of five generations. The F6 generation from this was under field evaluation for precision phenotyping in the main crop season 2023-24.

The duration of the seed-to-seed cycle ranges from 72 to 83 days, depending upon the genotype involved for accelerated breeding in the low-cost speed breeding chamber. Alongside, hybridization has also been prioritized in the speed breeding chamber and fully viable crossed seeds during the summer, when outdoor temperatures range between 38°C to 45°C were harvested, showcasing the chamber's adaptability to diverse breeding exigencies. This accelerated development of germplasm necessitates the integration of novel precision phenotyping tools to expedite the delivery of elite varieties to farmers.

The cost-effective, user-friendly algorithm and workflow tailored to stage- and stress-specific phenotyping consisting ofa computer algorithm was devised to determine tiller numbers, grain dimensions, and morphology, using size and shape descriptors including minimum bounding rectangle (MBR), area (A), perimeter (P), solidity, minor diameter (m), and major diameter (M).

Employing digital image processing techniques, this algorithm, developed using the open-source Python language and OpenCV library, promises to revolutionize high-throughput field phenotyping, facilitating the characterization of advanced germplasm through big-data methodologies. Combined incorporation of speed breeding with precision phenotyping tools holds a transformative shift in wheat breeding towards sustainable agriculture and food security.

#137

The velvet transcription factor pnvea regulates effectors and secondary metabolism in a wheat fungal pathogen

Shota Morikawa[1], Callum Verdonk[1], Evan John[1,2], Leon Lenzo[1], Nicolau Sbaraini[3], Chala Turo[1], Hang Li[3,4], David Jiang[1], Yit-Heng Chooi[3] and Kar-Chun Tan[1]

[1]Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Perth, Australia
[2]Institute of Plant and Microbial Biology, Academia Sinica, Taipei, 115201, Taiwan
[3]School of Molecular Sciences, University of Western Australia, Perth, Australia
[4]Present address: School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, 510006, China

Kar-Chun.Tan@curtin.edu.au

The fungus *Parastagonospora nodorum* causes septoria nodorum blotch (SNB) on wheat. The phytopathogen secretes proteinaceous necrotrophic effectors to cause necrosis on wheat carrying a dominant susceptibility phenotype leading to SNB. The coordination of effector and pathogenicity gene expression by signalling pathways is a topic of intense investigation.

In this study, we used a reverse genetic approach to dissect the role of the fungal Velvet-family transcription factor VeA in *P. nodorum* development and virulence on wheat. *P. nodorum* mutants carrying VeA (*PnVeA*) deletion displayed vegetative growth abnormalities, altered pigmentation, lost the ability to produce asexual spores and demonstrated a strong reduction in its ability to infect wheat. Comparative RNA-Seq and RT-PCR analyses revealed that the deletion of PnVeA decoupled the expression of major necrotrophic effector genes.

In addition, the deletion of PnVeA resulted in an up-regulation of four predicted secondary metabolite (SM) gene clusters. Using liquid-chromatography mass-spectrometry, it was observed that an increase in the expression of the alternariol SM gene cluster led to an accumulation of the mycotoxin.

Our study has shown that PnVeA is essential for asexual sporulation, full virulence, secondary metabolism and necrotrophic effector regulation.

#212

Revisiting critical nitrogen dilution curve of wheat

Bo Yao[1], Xiaolong Wang[1], Syed Tahir Ata-Ul-Karim[2], Yancheng Wang[1], Tianyang Ye[1], Yanling Li[1], Gilles Lemaire[3], David Makowski[4], Qiang Cao[1], Xiaojun Liu[1], Xia Yao[1], Bing Liu[1], Leilei Liu[1], Yan Zhu[1], Weixing Cao[1], Enli Wang[5], Liang Tang[1, *]

[1] National Engineering and Technology Center for Information Agriculture, Engineering Research Center for Smart Agriculture, Ministry of Education, Key Laboratory for Crop System Analysis and Decision Making, Ministry of Agriculture and Rural Affairs, Jiangsu Key Laboratory for Information Agriculture, Jiangsu Collaborative Innovation Center for Modern Crop Production, Nanjing Agricultural University, Nanjing 210095, Jiangsu, PR China
[2] The University of Tokyo Graduate School of Agricultural and Life Sciences / Faculty of Agriculture, Bunkyo-Ku 113-8657, Tokyo, Japan
[3] Honorary Director of Research, INRA, 86600 Lusignan, France
[4] University Paris-Saclay, INRAE, AgroParisTech, UMR MIA 518, 75231 Paris, France
[5] CSIRO Agriculture and Food, Cluniess Ross Street, Black Mountain, ACT, Australia

Liang Tang tangl@njau.edu.cn

Critical nitrogen dilution curves (CNDC) have been widely used for plant N status diagnosis, N management, crop modeling, and remote sensing. The CNDC can be described by a power function of critical N (Nc) and dry matter (W) ($Nc = A_1 \cdot W^{-A2}$), where parameters A1 and A2 control the starting point and slope of the curve, respectively. Wheat is a major crop worldwide, and its CNDCs developed under different conditions (genotype×environment×management, G×E×M) show large parameter variations.

This study aimed to investigate the uncertainty and drivers of wheat CNDCs for organ-specific and shoot based on 23 field experiments (environments) involving different varieties, ecological sites, and years. The results show that parameters A_1 and A_2 are highly correlated. Although the variation of parameter A_1 was less than that of A_2, the values of both parameters can change significantly in response to G×E×M.

The drivers of curve parameters include maximum N concentration, maximum biomass, and accumulated growth degree days (AGDD) during the vegetative growth period and planting density. Though the Nc curve differences between G×E×M were statistically significant, the NNI differences remained relatively small.

NNI calculated using organ-specific CNDC is in general consistent with NNI estimated with overall shoot CNDC, indicating that a simple organ-specific CNDC may be used for wheat N diagnosis to assist N management. However, the significant differences in organ-specific CNDC across G×E×M conditions imply potential errors in Nc and crop N demand estimated using a general CNDC in crop models, highlighting a clear need for improvement in Nc calculations in such models.

These results provide new insights into N diagnosis and management, modeling and prediction in wheat.

#684

Assessing salinity tolerance in two wheat varieties under moderate saline conditions

Rongrong Tao[1,2], Ce Guo[2], Jianbo Chen[2], Chenchen Zhao[2], Wenshan Guo[1,3], Min Zhu[1,3], Meixue Zhou[2*]

[1] Jiangsu Key Laboratory of Crop Genetics and Physiology, and Jiangsu Key Laboratory of Crop Cultivation and Physiology, Agricultural College of Yangzhou University, Yangzhou 225009, China,
[2] Tasmanian Institute of Agriculture, University of Tasmania, Launceston, TAS 7250, Australia
[3] Co-Innovation Centre for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou 225009, China

* Correspondence: Meixue Zhou; Email: Meixue.zhou@utas.edu.au

Improving wheat production efficiency on saline-alkali land is of long-term strategic importance in addressing food security and worsening soil salinization issues. Screening genotypes which can complete their life cycle under saline conditions is the key to breeding wheat varieties for salinity tolerance.

Most of the previous screenings are mainly on the seedling stage. In our preliminary screening trial under a high level of salinity (320 mM NaCl), and H-093 showed significant difference in salinity tolerance in the seedling stage with H-135 being more tolerant than H-093. In this study, these two genotypes were grown under a continuous moderate salinity stress (150mM NaCl) for the entire life cycle.

Salt stress inhibited the growth performance of wheat, as evidenced by the reduced leaf area index (LAI), number of tillers, dry matter accumulation (DW), NDVI and harvest index (HI). Under moderate salinity stress, there were no significant differences in salinity tolerance between these two genotypes at the flowering stage with both genotypes showing similar reductions in plant height, tiller number and biomass production.

The significant differences between genotypes were primarily observed during the late growth (grain filling) stage. The grain number per spike (GN) declined 32% in H-093 while there was no significant decrease in H-135 due to reduced fertility of H-093.

Thousand-grain-weight (TGW) showed the greatest reduction with H-093 showing nearly 60% reduction while H-135 only having 27% reduction. H-093 showed a much greater yield reduction (79%) than H-135 (38%).

#495

Integral nitrogen fertilization management of wheat crops in France: recent advances enabled by CHM-conduite

François Taulemesse[1,*], Benoît Piquemal[1], Guy Deshayes[1], Thierry Denis[1], Anaïs Bonnard[1]

[1] ARVALIS, 3 rue Joseph et Marie Hackin, 75116 Paris, France

For several decades in France, nitrogen (N) fertilization reasoning for wheat crops has been based on the principle of the "balance sheet" method. This method comes up against strong implementation limitations (e.g. difficulty of estimating grain yield objective a priori), and a lack of adaptability in the face of climatic hazards. This observation has motivated the emergence of a new concept of "integral management" of N fertilization, which avoids a priori estimates of forecast N rate in favor of reasoning based on instantaneous plant needs. Since 2017, ARVALIS has been developing an integral management tool of N fertilization for bread wheat, called CHN-conduite, which relies on a mechanistic crop model (CHN) to access plant N nutrition levels in real time and forecast crop N requirement.

The innovative near-real-time coupling of CHN with data from on-board satellite sensors provides a highly accurate diagnosis of crop's N nutrition status, further improving the accuracy of crop N requirement projections. An on-farm French experimental network composed of 27 field trials of winter bread wheat (Triticum aestivum L.) was conducted in 2023 to evaluate the agronomic performance of CHN-conduite under field conditions.

Compared with the balance sheet method, integral management of N fertilization significantly improved grain yield by 0.25 tha^{-1} (p-value = 0.03) without increasing the nitrogen rate applied (-12.1 kgNha^{-1}, p-value = 0.12) and without penalizing grain protein concentration (+0.2%, p-value = 0.23). Producing more grain with higher protein concentration using less nitrogen fertilizer mechanically improves the nitrogen net profit margin.

Depending on the combination of fertilizer purchase price and grain selling price scenarios, the average gain ranges from 77 to 109 €ha^{-1}. The results confirm the potential of this new approach. It also opens prospects for future developments, making it possible to optimize fertilization strategies by integrating new optimization constraints, such as crop's carbon footprint.

#420

Haplotype and transcriptome analyses reveal genes and gene regions that promote fhb resistance in spring wheat

Sittal Thapa[1,2*], Bikash Poudel[5], Guihua Bai[3], Steven Xu[4], Jason Fiedler[2]

[1]Department of Plant Pathology, North Dakota State University, Fargo, ND 58102, USA. [2]Cereals Crops Research Unit, Edward T. Schafer Agriculture Research Agriculture Research Center, USDA-ARS, Fargo, ND 58102, USA. [3]4008 Throckmorton Hall USDA/ARS/HWWGRU, Kansas State University, Manhattan, KS 66506, USA. [4]USDA-ARS, Western Regional Research Center, Albany, California, USA. Donald Danforth Plant Science Center, 975 N Warson Rd, St Louis, Mo 63146, USA

Fusarium Head Blight (FHB) is a devastating disease of small cereal grains that affects all growing regions throughout the world. Apart from yield and economic losses associated with the disease, toxicity of the deposited mycotoxins in the infected grains impact human and animal health. Control of this disease involves spraying of costly, biologically, and ecologically hazardous chemicals, and identifying and deploying genetic resistance.

Several quantitative trait loci (QTLs) associated with Fusarium head blight (FHB) resistance have been identified, of which seven of them are well-characterized (Fhb1...Fhb7). Chinese spring wheat, Sumai3 is the source for three major QTLs for FHB viz., Fhb1, Fhb2 and Fhb5. Interestingly, this FHB-resistant cultivar was developed by crossing two moderately susceptible parents, “Funo” and “Taiwanxiaomai”, which suggests that the resistance was inherited by appropriate combinations for favorable haplotype blocks.

This study aims to decipher those specific combinations of haplotype blocks in Sumai3 and trace the movement of resistance-associated gene regions across various derivatives. The whole genome sequencing of the Sumai3 along with resequencing of thirty lines harboring Sumai3 background was carried out and the haplotype blocks were characterized.

Coupled with RNASeq information, we aim to explore expression patterns of resistance-associated genes and their conjugation to these defined haplotype blocks. Preliminary analysis has shown that the entire 3B chromosome in Sumai3 harboring Fhb1 was inherited by a single parent, Taiwanxiaomai. But contradictorily, Taiwanxiaomai possessing this chromosome is moderately susceptible, suggesting Funo-derived genes might be necessary for strong resistance.

Furthermore, Fhb1 introgression into adapted germplasm does not always result in lines with the expected level of resistance. Hence this study aimed for identification of those co-inherited gene regions. This comprehensive analysis of the genomes of these important sources of FHB-resistance will expand our knowledge on what is required to combat disease and help with the development of molecular markers to track important linkage blocks in breeding germplasm.

#597

Agronomic fortification through biosynthesized fe nanoparticle, a sustainable approach for enhancing the productivity of wheat (*triticum aestivum)*

Shaik Nazma[1] and T. Sudha[2]

1. Ph. D Scholar, Department of Agronomy, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India.
2.Professor, Department of Agronomy, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka, India.

Email.ID: sudhat@uasd.in

Mineral deficiencies, mainly Zn and Fe, affect more than half of the world's population due to their reliance on cereal crops, particularly wheat, for their daily diet. Wheat (*Triticum aestivum* L.), a widely cultivated and consumed cereal staple crop, can be used as a suitable target for Zn and Fe bio-fortification to fill nutritional gaps in cereal diet-based countries. Agronomic biofortification of wheat crop has gained popularity as an efficient alternative technique for achieving nutritional improvements.

Nanotechnology has the potential to contribute to a new technology-based agricultural revolution and provide possible solutions to several drawbacks of traditional biofortification.Biological synthesis of nanomaterials is the ideal option since it is cost effective and eco-friendly.

The experiment was conducted during *Rabi* season 2022 at Main Agricultural Research Station, University of Agricultural Sciences, Dharwad, Karnataka, India with an objective of studying the effect of biosynthesised Fe nanoparticles and in comparison with commercial Fe nanoparticles on yield, yield parameters and grain and straw Iron content. The experiment was laid out in randomized complete block design with three replications comprising of twelve treatments.

The results of the experiment revealed that seed priming @250ppm and foliar spraying @500ppm at panicle initiation stage with biosynthesised Fe nanoparticles through actinobacteria recorded significantly higher grain yield (4.56 t/ha) and found on par with seed priming @250ppm and foliar spraying @500ppm at panicle initiation stage with biosynthesised Fe nanoparticles through *Pseudomonas (*4.53 t/ha) and commercial Fe nanoparticles (4.49 t/ha).

The yield attributing characters viz., productive spikes (253.6m2), number of grains per spike (48.23), grain weight per spike (1.80g) and 1000 grain weight (42.19 g) were recorded significantly higher with seed priming @250 ppm and foliar spraying@500ppm at panicle initiation stage with biosynthesised Fe nanoparticles through actinobacteria and found on par with seed priming @250ppm and foliar spraying@500ppm at panicle initiation stage with biosynthesized Fe nanoparticles through *Pseudomonas* (251.6m2, 47.33, 1.79g and 42.05) and commercial Iron nanoparticles(249.6m2, 47.57, 1.76g and 41.87) respectively.

The higher grain and straw Iron concentration (76.85ppm,159.87) was noticed with seed priming @250ppm and foliar spraying@500ppm at panicle initiation stage with biosynthesised Zn nanoparticles through actinobacteria and found on par with seed priming @250ppm and foliar spraying@500ppm at panicle initiation stage with biosynthesised Fe nanoparticles through *Pseudomonas*(76.56 ppm 158.63*) and* commercial Iron nanoparticles (75.94 ppm,157.59ppm)

#418

Anthocyanin-rich biofortified black wheat mitigates inflammatory responses and improves metabolic parameters in HFD/STZ-induced T2DM model

Vandita Tiwari [1, 2], Archana Bhatnagar[2] and Monika Garg[1]*

[1]National Agri-Food Biotechnology Institute-Mohali (India)
[2]Panjab University-Chandigarh (India)

bhatnagar.archana@gmail.com, vanditatiwari2017@gmail.com, mkajgarg@gmail.com

Type 2 diabetes mellitus (T2DM) is characterized by β-cell dysfunction, insulin resistance, and chronic inflammation leading to hyperglycemia. Anthocyanins, known for their potent antioxidant properties, show promising role in alleviating T2DM-related complications. This study aims to explore the impact of anthocyanin-rich black wheat on glucose metabolism, insulin sensitivity, lipid profile, oxidative stress, inflammation, and organ protection in HFD-STZ induced T2DM rats.

Rats were subjected to a high-fat diet (HFD) for 8 weeks to induce a Type 2 Diabetes Mellitus (T2DM) model, followed by a single intraperitoneal injection of streptozotocin (STZ). Subsequently, throughout the 12-week study duration, the rats were provided with a HFD supplemented with isoenergetic diet of both black and white wheat. Compared to white wheat, black wheat demonstrates a lower glycemic index and stronger inhibitory activities against α-amylase and α-glucosidase.

Consumption of black wheat leads to significant reductions in blood glucose and HbA1c levels, improved insulin sensitivity, oral glucose tolerance, and insulin tolerance. Additionally, it enhances antioxidant enzyme activities, ameliorates atherogenic dyslipidemia, and reduces inflammatory markers, while increasing adiponectin levels. Black wheat activates adiponectin-AMPK and PI3-AKT pathways, resulting in increased expression of glucose transporters (GLUT-2, GLUT-4). H

Histopathological examinations reveal protective effects on the liver, kidney, and spleen. Anthocyanin-rich black wheat emerges as a promising functional food for managing T2DM and its associated complications exerting its effects through the modulation of essential pathways and glucose transporters.

#651

Delivering sustainable wheat (DSW) – UK institute strategic programme

Gary Barker [1], Malcolm Bennett [2], Rahul Bhosale [2], Philippa Borrill [3], Elizabete Carmo-Silva [4], Hannah Cooper [2], Laura Dixon [5],Cathrina Edwards [6], Gary Frost [7], Wilfried Haerty [8], Anthony Hall [8], Kim Hammond-Kosack [9], Keywan Hassani-Pak [9], Malcolm Hawkesford [9], Brittany Hazard [6], Phil Howell [10], Paul Kroon [6], Christophe Lambing [9], Richard Leggett [8], Alison Lovegrove [9], Sacha Mooney [2], Vladimir Nekrasov [9], Paul Nicholson [3], Matthew Paul [9], Stephen Pearce [9], Michael Pound [2], Tony Pridmore [2], Alexandra Przewieslik-Allen [1], Jason Rudd [9], Diane Saunders [3], Mikhail Semenov [9], David Seung [3], Peter Shewry [9], Craig Sturrock [2], Cristobal Uauy [3], Martin Urban [9], Fred Warren [6], Martin Warren [6], Simon Griffiths [3]

[1] University of Bristol
[2] University of Nottingham
[3] John Innes Centre
[4] University of Lancaster
[5] University of Leeds
[6] Quadram Institute
[7] Imperial College London
[8] Earlham Institute
[9] Rothamsted Research
[10] National Institute for Agricultural Botany
Email: cristobal.uauy@jic.ac.uk

Wheat is an indispensable global crop and the major crop of the UK and Western Europe. With a projected population of 10 billion by 2050, the need for sustainable wheat production is urgent. The Delivering Sustainable Wheat (DSW) research programme is a cross-institute strategic programme that will run from 2023 – 2028.

It brings together the complementary skills of four research institutes (John Innes Centre, Rothamsted Research, The Quadram Institute and Earlham Institute), five universities (Bristol, Imperial College, Lancaster, Leeds and Nottingham) and the National Institute of Agricultural Botany, to address critical challenges in wheat health, yield, and production.

The DSW programme aims to address these challenges by enhancing wheat production sustainably and improving human nutrition, by applying our biological knowledge of key traits for the benefit of wheat consumers.

The programme also invests in pre-breeding to ensure that the new traits, genes, knowledge, and new types of wheat are fed into breeding, farming, and food production, to help safeguard this vital crop for the future.

More information can be found at https://designingfuturewheat.org.uk/.

#648

Wheat blast - chemical control and correlation of genetic resistance in the spike and leaf

Camila Turra[1,*], Silvia Barcellos Rosa[1,2], Igor Pires Valério[1], Rafael Nornberg[1], Amarilis Labes Barcellos[1]

[1] OR Melhoramento de Sementes Ltda., Passo Fundo, Rio Grande do Sul, Brazil
[2] CÉROM, Saint-Mathieu-de-Beloeil, QC, Canada

[*] camila@orgenetica.com.br

Wheat blast, caused by the fungus *Pyricularia oryzae Triticum*, can infect all the green tissues of the plant, such as leaves, stem, spikelets, awns and especially the rachis, where the disease is most destructive. Currently, the principal method to control the disease in wheat spikes is to incorporate the 2NS/2AS translocation, which comes from *Aegilops ventricosa.*

In Brazil, in 2023, of the 30 cultivars in the performance wheat cultivar trial, where the most important commercial wheat cultivars are tested, around 80% presented the 2NS translocation. However, the presence of 2NS does not correlate well with the resistance to the leaf lesions caused by *Pyricularia*. In the 2023 wheat season, the wheat crop in the South of Brazil was subjected to the El Niño system with high precipitation (> 1500mm Sept-Nov) and warmer temperatures, favoring the occurrence of diseases, including spike and leaf blast.

The elliptical lesions with a dark border and a silvery center appeared between seven days after infection and showed rapid sporulation, increasing the pressure of the disease in the field. The aim of this study was to test fungicides for controlling blast on wheat leaves, given that there are no specific fungicides recommended for controlling these symptoms.

The work was conducted in greenhouse with the cultivar “BRS 264”, which is susceptible to leaf blast. The experimental design was randomized with five replications and a total of 25 plants per treatment. It was tested 15 fungicides (application 24 hours before inoculation). Inoculation of conidia from infected rachis was carried out 35 days after sowing (Stage 13 of the Zadoks). The control of the disease was evaluated five and seven days after inoculation, scoring the severity of the disease on the leaves and the disease control index (%) – comparing the fungicide treatments with values of the control treatment (water application).

The fungicides composed of tricyclazole, commercial name Bim 750 and Bim Max (Tricyclazole and Tebuconazole), fungicides indicated for control of leaf and panicle rice blast, reduced wheat leaf blast severity by 97 and 98%, respectively.

The results showed that preventive application of fungicides enabled control of almost 100% of the blast lesions on leaves, which can be beneficial to reduce the inoculum pressure at heading stage in the field.

#647

Breeding efforts and management to control recent and evolving diseases in Brazil

Camila Turra[1,*], Silvia Barcellos Rosa[1,2], Igor Pires Valério[1], Josemere Both[1], Rafael Nornberg[1], Amarilis Labes Barcellos[1]

[1] OR Melhoramento de Sementes Ltda., Passo Fundo, Rio Grande do Sul, Brazil
[2] CÉROM, Saint-Mathieu-de-Beloeil, QC, Canada

[*] camila@orgenetica.com.br

Wheat crop in Brazil is threatened by high disease pressure yearly, which leads to the identification of good sources of resistance. In 2023, extreme weather conditions in the south of Brazil (1500 mm rainfall accumulation from September to November and increased temperatures) contributed to optimum conditions for disease development from seedling stage, affecting all green tissue of the plants.

Serious epidemics were observed: Fusarium head blight (FHB), leaf and spike blast (another poster), leaf rust, powdery mildew (*Pm*), leaf spots, bacterial diseases (*Xanthomonas sp)*. The impacts were extreme, with a reduction of 50 to 70% in the production of grains and certified seed, besides the reduced germination caused by bacteria and Fusarium infection, among other causes. Breeding for resistance is a priority of OR Genetics since the 90s.

Studies are conducted to determine (1) pathogen population evolution; (2) screening (phenotypically and genotypically) of segregating populations, advanced lines and cultivars; (3) biological and synthetic fungicide efficiency. Brazilian *Puccinia triticina* populations are phenotyped yearly and the most predominant races are TFT-RT and TPT-LT.

In case of powdery mildew, near-isogenic lines were tested at seedling to determine the genes still efficient in Brazil. PM severity ranged from 4 to 50% in cultivars with only *Pm2* and *Pm8* and from 0 to 15% in lines with *Pm2*, *Pm8* plus unknown genes. In the cultivar “ORS Soberano”, which only has *Pm8*, the severity of the disease was 60% and it was 0-5% in “BRS Belajoia” (*Pm2*, *Pm8* and *Pm17)*. Concerning FHB, a study was conducted to assess the genetic resistance of 20 wheat cultivars in Coxilha/RS in 2023 and 2024.

A randomized design (four replications) with and without fungicide was used to assess disease severity, protein content, grain yield and mycotoxin levels (deoxynivalenol - DON). There was no variation in the protein content of the grains when fungicide was applied compared to the treatment without application.

In terms of productivity, the cultivars with fungicide application had average yields between 3.8 and 1.5 ton/ha and without application between 2.2 and 0.7 ton/ha, with a positive correlation in relation to FHB severity. DON levels ranged from 170 ppb to 1520 ppb in the treatment without fungicide application (Brazilian maximum DON limit: 750 ppb in white flour).

Ongoing research in the search for cultivars with genetic resistance to multiple disease and integrated management to reduce risk in wheat cultivation is fundamental to guarantee profitability to wheat growers.

#161

Breeding for improving abiotic stress tolerance of winter wheat under the changing climatic conditions in the Carpathian Basin

Balázs Varga, Zsuzsanna Farkas, Márton György and Ottó Veisz

HUN-REN Centre for Agricultural Research - Agricultural Institute, Martonvásár, Hungary

The Carpathian Basin is especially exposed to the changes in the climate because the impacts of the terrain strengthen the consequences of extreme weather events as well as the long-term climatic changes. It was confirmed that the rise in the yearly average temperature was two times faster in this inner lowland area than the global average.

The weather conditions of the region are formed by three major climatic regimes, Continental, Oceanic and Mediterranean. The overdominance of the Mediterranean climate is observable which resulted in more humid and mild winters and warm and moderately wet summers with more frequent extreme heatwaves. Conventional breeding of cereals is even a very long process therefore, the adaptation ability of the released cultivars to the challenges posed by the abiotic stressors becomes a critical issue. However, the basin is rich in above and belowground freshwater resources, but irrigation of small grain cereals is unrealistic from an economic point of view.

The efficient utilization of the available soil water in combination with a good drought tolerance is the only possible way to increase the sustainability of cereal production. In the Centre for Agricultural Research, the focus is on the determination of the water demand and water use efficiency as well as the rooting habits of cereal species under controlled environments and real field conditions.

A model experimental system was developed by using pots with a volume of 10 litres to determine the water uptake and its trend during the vegetation period while simulation of drought is possible at different phenophases. Generally, it was concluded that early-ripening varieties are especially sensitive to drought in the vegetative growth stages while the impacts of the water shortage were more severe in the generative stages by late-ripening genotypes. The dynamics of the daily water use of winter wheat genotypes is under investigation in a scientific lysimeter system (Meter Group, Germany) consisting of 12 soil columns (1 meter in diameter and 2 meters in depth).

The root system has key functions in determining the adaptation ability of plants to the water-limited environment. The root development and its morphological features are investigated under controlled conditions in climate chambers. Large containers (volume: 1 m^3) filled with soil were applied for in situ monitoring of the root development and turnover by using a CI-600 (CID Bioscience, USA) root scanner at different soil depths and the photos were analyzed by the RootSnap software.

A sand tube system (diameter 10 cm, depth: 75 cm) is applied to analyze the impacts of the water shortage simulated at various developmental stages. At the end of the treatments or the vegetation period the complete root system in its original structure was carefully washed out of the sand and the root properties were determined by the WinRHIZO Pro (Regent Instruments, Canada) analytical system.
It was concluded that the presence of the dwarfing genes (Rht1 and Rht2) significantly reduced the root length and root biomass compared to the control (without dwarfing genes) but the presence of the Rht2 was more favourable.

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#348

Allelic variation in *PSY-A1* and *lpx-B1.1* genes affects yellow pigment-content and loss in durum wheat

José G. Vázquez-García[1,*]Naima Bouabdellah[1], Virginia García-Calabres[2], Carlos Guzman[2], Patricia Giraldo[1], Laura Pascual[1]

[1] Department of Biotechnology-Plant Biology, School of Agricultural, Food and Biosystems Engineering, Universidad Politécnica de Madrid, Madrid, Spain.
[2] Departament of Genetics, Escuela Técnica Superior de Ingeniería Agronómica y de Montes, Edificio Gregor Mendel, Campus de Rabanales, Universidad de Córdoba, CeiA3, ES-14071 Cordoba, Spain.

jg.vazquez.garcia@upm.es

Triticum turgidum L. spp *durum* is a cereal crop mainly grown in the Mediterranean basin. Among durum wheat quality related traits, the grain protein content, and the yellow color of the semolina and pasta products are the most valued by breeders and consumers. In the present study we focused on semolina color. A bi-parental HIFs (Heterogeneous Inbred Families) population was characterized for the yellow color of the semolina during two crop seasons (2019/20 and 2020/21).

This population was developed from two ancient durum wheat cultivars: Endural released by Benoist in 1989, with good rheological properties; and Aldura, an Italian landrace that presents good semolina color. One-hundred seven HIFs were evaluated for the Yellow Pigment Content (YPC) and the Yellow Pigment Loss (YPL) after exposure to oxygen and light. With these two parameters it was possible to determine the percentage loss of the yellow pigment (% of loss) in semolina samples.

Besides, the population was genotyped for two pasta color-related genes which were polymorphic in the parental lines, *Psy-A1* and *Lpx-B1.1*. *Psy-A1* is related to the initial carotenoid content for which Endural and Aldura carried the alleles *Psy-A1a* and *Psy-A1I,* respectively. The *Lpx-B1* gene encodes a lipoxygenase which affects the carotenoids and consequentely the yellow color of the pasta at post-cooking stages.

For that gene, Endural carries the *Lpx-B1.1c* allele carrying a deletion associated with a 4.5 reduction in lipoxygenase activity. Statistical analyses revealed that the lines carrying the *Psy-A1I allele* presented a higher yellow pigment content compared to the lines carrying the *Psy-A1a allele*. On the other hand, lines which did not carry the deletion for *Lpx-B1.1* presented a lower YPL and a higher percentage loss compared to lines with the *Lpx-B1.1c* allele.

No epistatic effects between both genes were detected, as it was observed that differences lines with higher YPC and carrying the allele *Psy-A1l* were maintained after cooking even when the fully functional allele, *Lpx-B1.1a,* was present.

Finally, a fixed effect model confirmed that the best allele for pasta color was *Psy-A1l* combined with *Lpx-B1.1c* allele.

The characterization of these materials polymorphic for one carotenoid synthesis related gene (*Psy-A1*) and for one carotenoid degradation related gene (*Lpx-B1.1*) through molecular markers facilitates the study of the effect of each allele in the carotenoid synthesis and in the final color of the pasta products and while aid MAS in wheat breeding programs.

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#626

Genome-wide association study for fusarium head blight resistance in southern Brazilian wheat

Jéssica Rosset Ferreira[1], Gabriela Soster Santetti[1], Marilia Hoffmann[1], Felipe Moreira[1], Rodrigo Celer[1], Ana Silvia de Camargo[1], Diego Maciel Trevizan[1], Igor Gonçalves de Paula[1], Leo Gustavo Cantoni[1], Ana Carolina Ojeda Fermoselle[2], Lucia Daniela Montenegro[2], Ernandes Manfroi[1], Ivana Sabljic[1]

[1]GDM, Passo Fundo, Rio Grande do Sul, 99052-160, Brazil
[2]GDM, Chacabuco, Buenos Aires, B6740, Argentina

Fusarium head blight (FHB), caused mainly by the fungus *Fusarium graminearum*, is an important wheat disease in several producing areas worldwide, including in Southern Brazil. The pathogen causes yield losses and also qualitative losses, once the infected grains get contaminated with mycotoxins, especially the Deoxynivalenol.

The use of molecular markers is a proven strategy to increase the efficiency in breeding programs. The aim of this study is mapping through GWAS, new QTL responsible for FHB resistance in a panel of Brazilian varieties evaluated at Southern Brazil. The panel was composed by 252 genotypes. The lines were genotyped with the technologies Silico DArT and DArTSeq. After a filtering procedure, a total 5154 markers remained for the analysis. The panel was assayed in two field seasons, 2021 and 2023, at the municipality of Coxilha, Rio Grande do Sul, Brazil.

Three traits were evaluated: i) FHB general score; ii) Fusarium Damaged Kernels (FDK); iii) Deoxynivalenol (DON) contend (only in 2023), totalizing 7 variables, as single and combined (average) analyses were performed. The Mixed Linear Model (MLM) implemented in Tassel V.5 was applied. Principal Components Analysis (PCA) (6 components) and the Kinship Matrix by Centered IBS method were used to correct population structure. Only FDK and DON in 2023 presented significant SNPs. For FDK, the significant SNPs were in the chromosomes 4A and 7A.

On the other hand, for DON, the significant SNPs were found in chromosomes 3A, 3D, 4A, 4D and 5A. The SNP with largest effect is the one in chromosome 4A, for FDK, which reduces 19% of damaged kernels in resistant genotypes. The same SNP has shown effect for DON reduction.

The SNPs will be converted to routine markers. As a conclusion, the panel presented important genetic variability, which allowed mapping QTLs for Fusarium head blight resistance.

#625

Enhancing wheat blast resistance in tropical Brazil: phenotyping under controlled conditions and 2NS MAS

Flávio Chupel Martins[1], Eduardo Venske[1*], Francisco Saccol Gnocato[1], Gustavo Mazurkievicz[1], Juan Ignacio Severo[2], Jéssica Rosset Ferreira[1], Gabriela Soster Santetti[1], Marilia Hoffmann[1], Felipe Moreira[1], Rodrigo Celer[1], Ana Silvia de Camargo[1], Diego Maciel Trevizan[1], Igor Gonçalves de Paula[1], Leo Gustavo Cantoni[1], Ana Carolina Ojeda Fermoselle[2], Lucia Daniela Montenegro[2], Ernandes Manfroi[1], Ivana Sabljic[1]

[1]GDM, Passo Fundo, Rio Grande do Sul, 99052-160, Brazil
[2]GDM, Chacabuco, Buenos Aires, B6740, Argentina

Wheat blast, caused by the fungus *Magnaporthe oryzae* pathotype *Triticum* is a fast-acting and devastating disease of the crop in the tropical producing areas of Brazil. The pathogen attacks the wheat leaves; however, the most severe damage occurs at the spikes, causing up to 100% loss when environmental conditions are favorable and the genotype is susceptible.

The major resistance gene for wheat head blast comes from the 2NS chromosomal segment, introgressed from *Aegilops ventricosa*. Chemical control measures have shown limited effectiveness in managing the disease. GDM, a private global multi-crop breeding company, operates three main wheat breeding programs in Brazil: Rio Grande do Sul, Paraná, and the tropical Cerrados region, where blast resistance improvement is a critical objective.

The aim of this study is to show and discuss the results of a middle-term effort on the increase of the genetic resistance of GDM's wheat germplasm to wheat head blast. Two main selection strategies were applied: i) extensive phenotyping in controlled conditions; and ii) selection for the 2NS chromosomal segment through marker assisted selection, both complementing the field selection. Every year all lines which advance from nursery to first year field trials, pass through both selection strategies, being approximately one thousand lines per year.

As a result, the frequency of the 2NS segment has increased over the last five years, in all three GDM's wheat breeding programs in Brazil, diagnosed in the lines in third year field trial. For the Cerrados program, the frequency of the segment has increased from 81%, in 2020, to 100%, in 2024 (R^2=0.75), comparing to from 78% to 95% (R^2=0.65) in Rio Grande do Sul and from 80% to 95% (R^2=0.40) in the Paraná program.
The general mean of the Area Under the Disease Progress Curve (AUDPC) in controlled conditions from 2021 to 2023 (data from 2024 is not available at the date of this study), was taken as a measure of the success of the strategies described.

The AUDPC average has diminished from 450 in 2021 to 150 in 2023, which represents a three-times decrease in a curse of three years. As a conclusion, phenotyping in controlled conditions and selection for the 2NS segment resulted in important improvement in wheat blast resistance in GDM's Cerrados' germplasm.

#54

Association of rht8 and ppd-d1 genes with agronomic traits in croatian wheat cultivars

Tihomir Čupić[1], Sonja Petrović[2], Sunčica Kujundžić[2], Vlado Guberac[2], Sonja Vila[2]

[1] Institute of Agriculture Osijek, Južno predgrađe 17, 31 000 Osijek, Croatia. [2] Faculty of Agrobiotechnical Sciences Osijek, University J.J. Strossmayer Osijek, 31 000 Osijek, Croatia,

tihomir.cupic@poljinos.hr, spetrovic@fazos.hr, suncica.kujundzic@fazos.hr, vlado.guberac@unios.hr, sonja.vila@unios.hr

Genetic variability and functional roles of the Rht8 and Ppd-D1 genes have been crucial in the evolution of Croatian wheat cultivars, impacting important agronomic traits. This study aimed to investigate the association of Rht8 and Ppd-D1 alleles with key agronomic characteristics in Croatian wheat genetic resources over time. A total of 40 winter bread wheat cultivars registered in Croatia from 1931 to 2008 were evaluated for seven agronomic traits. These cultivars originated from Croatia, Austria, France, Italy, and Russia, reflecting a diverse set of genetic resources used in Croatian wheat breeding programs. Significant genetic variability was found for alleles of Rht8 and Ppd-D1 genes.

The analysis found that certain alleles of these genes have a statistically significant influence on agronomic traits such as plant height, spike length, number of spikelets, thousand grain weight and grain yield. Among the researched accessions, the most represented allele was Rht8 with a length of 192 base pairs (bp), which contributed to the development of cultivars with lower plant height, faster spike development and higher grain yield.

On the other hand, the Ppd-D1 allele also showed an influence on these characteristics, particularly accelerating spikelet development and shortening the plant's life cycle. These results highlight the important role of Rht8 and Ppd-D1 genes in wheat breeding and provide valuable insights for further selection programs aimed at improving agronomic characteristics in Croatian wheat cultivars.

Understanding the genetic variability of these genes allows better management of wheat genetic resources to achieve higher productivity and adaptability under different growing conditions. In addition, research has shown that the combination of certain alleles of the Rht and Ppd group genes can influence the optimization of agronomic properties in new wheat cultivars that are more resistant to extreme conditions and adapted to changing climatic conditions.

#77

Geographical distribution of ppd and vrn alleles in diverse wheat germplasm

Suncica Kujundžić[1], Sonja Petrović[1], Tihomir Čupić[2], Andrijana Rebekić[1], Sonja Vila[1]

[1] Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia. [2] Institute of Agriculture Osijek, Južno predgrađe 17, 31 000 Osijek, Croatia

The aim of this research was to examine geographical distribution of Ppd and Vrn alleles in diverse set of wheat genotypes. Research was conducted on 120 hexaploid wheat varieties originating from 25 countries (Hungary, Japan, USA, Italy, France, Austria, United Kingdom, Australia, Mexico, Argentina, Canada, Russia, India, Serbia, Chile, North Macedonia, Croatia, Czech Republic, China, Sweden, Germany, Ukraine, Romania, Bulgaria and Slovakia).

For research purposes, varieties were grouped into geographical regions – North America, Central and South America, Asia and Australia, West Europe, East Europe, Central Europe, South Europe, Croatia-Serbia. Genomic DNA was extracted from the young leaves according to CTAB extraction method. PCR analysis was conducted using functional markers specific for Ppd-B1, Ppd-D1, Vrn-A1, Vrn-B1, Vrn-B3 and Vrn-D1 loci.

Amplification products were analyzed using horizontal gel electrophoresis. At Ppd-B1 locus, Ppd-B1b allele prevailed over Ppd-B1a allele in all geographical regions. The highest frequency of Ppd-D1a allele (85 %) was recorded in varieties from South Europe, while the highest frequency of Ppd-D1b allele (75 %) was recorded in varieties from North America.

The highest frequency of dominant Vrn-A1a allele (50 %) and Vrn-A1b/c allele (25 %) was observed in varieties from North America. The recessive vrn-A1 allele was the most abundant in varieties from South Europe (100 %). At Vrn-B1, Vrn-B3 and Vrn-D1 loci recessive alleles prevailed over dominant alleles in all geographical regions.

The lowest frequency of vrn-B1 allele was observed in varieties from Central and South America, and the lowest frequency of vrn-D1 allele in varieties from Asia and Australia.

#500

Breeding progress of photosynthesis acclimation strategies to light and temperature fluctuations in winter wheat

Emilio Villar Alegria[1], Tsu-Wei Chen[1]

[1]Albrecht Thaer Institut für Agrar- und Gartenbauwissenschaften, Humboldt Universität zu Berlin, Berlin, Germany

emilio.villar.alegria@hu-berlin.de, tsu-wei.chen@hu-berlin.de

Understanding how plants acclimate their photosynthetic capacity to fluctuating environments is essential for developing crop varieties for future climatic conditions. Here, we propose a theoretical model of photosynthetic protein turnover to explain photosynthetic acclimation to light and temperature. This model considers the effects of light and temperature on the synthesis and degradation rate of photosynthetic proteins in three functional pools: carboxylation, electron transport, and light harvesting.

Using this model, photosynthetic protein abundancies and carbon assimilation rate can be simulated in leaf from its emergence until senescence. To assess how breeding has affected photosynthetic acclimation, we conducted a series of parameterizing experiments in growth chamber with different combinations of varying and constant light and temperature conditions using 60 winter wheat (*Triticum aestivum*) genotypes covering the recent German breeding history.

The maximum carboxylation rate, maximum electron transport rate, and chlorophyll concentration were periodically estimated using a combination of gas exchange, chlorophyll fluorescence, and pigment content measurements. Additionally, this ground-truth information was correlated to leaf hyperspectral data to achieve non-destructively estimation of three protein pool dynamics with high throughput.

The experiments enabled us to adapt our theoretical model by refining it with genotype-specific data, ensuring accurate simulations of photosynthetic responses under the tested environmental conditions. The experimental results suggest that there is variability in the acclimation strategies of our genotype panel in response to the different light and temperature regimes.

This highlights the importance of incorporating dynamic environmental responses into breeding programs to optimize the photosynthetic performance and sustainability of crop production systems.

#565

Evaluation of wheat lines for zinc and iron content from CIMMYT germplasm's advanced nursery trails

Karthikeyan Thiyagarajan[1], Manish Kumar Vishwakarma[1], Pradeep Kumar Bhati[1], Arun Kumar Joshi[1], Velu Govindan2*

1. Borlaug Institute for South Asia-CIMMYT, Ludhiana, Punjab, India.
2. International Wheat and Maize Improvement Center, Texcoco, Mexico.

Wheat is one of the major cultivated staple food crops and is consumed by over 2.5 billion people globally as its main source of energy. Besides, wheat is also a good source of macro- and micronutrients, according to a USDA report. Besides, there is a need to focus on nutritional quality in wheat as an important criteria to meet the requirements of micronutrients as well.

Among the micronutrients, zinc and iron play a vital role in a number of metabolic activities; hence, biofortification is necessary to increase the concentration of Fe and Zn content in wheat through breeding aspects. In this regard, CIMMYT-BISA is implementing a number of wheat breeding initiatives for the biofortification of zinc and iron through selection, introgressions, and advancement of the number of wheat lines through international and national field evaluations across the country and beyond.

Here we present the Zn and iron evaluation results of around 6,000 wheat lines from various trials, such as SABWGPYT, HZAN, HZWYT, ESWYT, SAWYT, SATYN, etc. The results suggested that there is a positive correlation and synergy between zinc and iron, and some of our wheat lines revealed the presence of zinc and iron at concentrations of more than 60 ppm and 70 ppm, respectively.

Our analysis further revealed that the accumulation of zinc and iron is based on genotype and environmental interactions, as some of the specific locations derived lines showed relatively better zinc and iron content than other lines, signifying the importance of the epigenetic mechanism behind the biofortification of these microelements.

#592

Enhancing genomic prediction accuracy for grain yield in wheat breeding by leveraging CIMMYT historical data

Paolo Vitale[1], Osval Montesinos-Lopez[2], Guillermo Gerard[1], Zerihun Tarekegn[1], Govindan Velu[1], Abelardo Montesinos-Lopez[3], Keith Gardner[1], Jose Crossa[1], and Leonardo Crespo-Herrera[1]

[1]International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Edo. de Mexico, Mexico. [2]Facultad de Telemática, Universidad de Colima, Colima, México. [3]Centro Universitario de Ciencias Exactas e Ingenierías (CUCEI), Universidad de Guadalajara, Guadalajara, Jalisco, Mexico

Genomic selection is a powerful tool for enhancing genetic gain in wheat breeding. However, prediction accuracy (PA), which is directly proportional to genetic gain in genomic-based breeding programs, remains low for complex traits such as wheat grain yield (GY). This study aimed to increase PA for GY in different selection environments (SEs) by leveraging CIMMYT's historical dataset.

Ten years of GY data observed in six different SEs for elite breeding lines were used. The Genomic Best Linear Unbiased Prediction (GBLUP) model assessed PA using five years as the test population (from 2018-2019 to 2022-2023) and all available years back up to 2013-2014 as the training population. Generally, we observed that as the number of training years increased, PA tended to improve or stabilize.

For instance, by testing the year 2022-2023, the SE BLHT (late heat stress) showed a notable increase in PA, reaching 0.23 when using five years back for training compared to 0.11 with only one year back, then PA was found stable by training from five to nine years back (from 0.23 to 0.26). Similarly, in B2IR (intermediate drought) SE exhibited an improvement from 0.12 to 0.21 using one and four years back, subsequently no significative improvements were observed by training from four to nine years back (from 0.21 to 0.24).

Conversely, some traits such as F5IR (flat optimal environment) did not show a significant increase, with PA fluctuating around 0.09-0.14 regardless of the training years used. This indicates that while increasing the number of training years generally enhances PA for GY, the degree of improvement can vary significantly across different SEs.

Our findings suggest that leveraging an extended historical dataset generally enhances genomic prediction accuracy of complex traits like wheat grain yield. These insights provide valuable guidance for optimizing genomic selection strategies in wheat breeding, ultimately contributing to the development of high-yielding, resilient wheat varieties.

#417

Use of *Thinopyrum* species to improve salt tolerance in bread wheat

Authors: Jack Walker[1,2,4,*], Stella Edwards[2], Caiyun Yang[2], Duncan Schofield[2], Stephen Ashling[2], Jonathon Atkinson[1,3], Malcolm Hawkesford[4], Darren Wells[1,3], Ian King[2], Julie King[2], Surbhi Grewal[2].

[1]Future Food Beacon, University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdon
[2]Nottingham BBSRC Wheat Research Centre, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdom
[3]School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, United Kingdom
[4]Rothamsted Research, West Common, Harpenden, United Kingdom

Email: Jack.Walker2@nottingham.ac.uk Surbhi.Grewal@nottingham.ac.uk

Soil salinization negatively effects plant growth. Twenty percent of cultivated land and thirty-three percent of irrigated land are salt affected and degraded. To increase food security, an increase in food production on saline soils is particularly important. Bread wheat is slightly salt tolerant, however several of its wild relatives are highly salt tolerant, particularly *Thinopyrum species.*

In this work, a high throughput hydroponic system for screening salt tolerance was established to assess the salt tolerance of wheat and its wild relatives during the vegetative growth stage. This system exposed plants to varying levels of salt stress over approximately three weeks, upon where the shoot dry weight, tiller number, SPAD values and ion content in leaf was recorded.

During high salt stress, *Thinopyrum* species and *Thinopyrum* addition lines outperformed wheat in terms of whole shoot dry weight, low leaf sodium content, SPAD values and tiller number.

This screening system will be employed in the future to screen for salt tolerance in introgression lines developed at the WRC, primarily focusing on *Thinopyrum* linkage group 5, which have demonstrated a superior ability to tolerate salt stress.

#605

A kinase fusion protein from Aegilops longissima confers resistance to wheat powdery mildew

Huagang He[1], Zhaozhao Chen[1], Renchun Fan[2], Jie Zhang[3], Shanying Zhu[4], Jiale Wang[1], Qianyuan Zhang[1], Anli Gao[5], Shuangjun Gong[6], Lu Zhang[8], Yanan Li[2], Yitong Zhao[2], Simon G. Krattinger[7], Qian-Hua Shen[2], Hongjie Li[9,10], and Yajun Wang[7,8*]

[1] School of Life Sciences, Jiangsu University, Zhenjiang 212013, China.
[2] State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.
[3] Institute of Biotechnology and Nuclear Technology Research, Sichuan Academy of Agricultural Sciences, Chengdu 610061, China.
[4] School of Environment and Safety Engineering, Jiangsu University, Zhenjiang 212013, China.
[5] School of Life Sciences, Henan University, Kaifeng 475004, China.
[6] Institute of Plant Protection and Soil Science, Hubei Academy of Agricultural Sciences, Wuhan 430064, China.
[7] Plant Science Program, Biological and Environmental Science & Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, 23955-6900, Kingdom of Saudi Arabia.
[8] National Key Laboratory of Plant Molecular Genetics, Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China.
[9] Institute of Biotechnology, Xianghu Laboratory, Hangzhou 311200, China.
[10] National Engineering Laboratory for Crop Molecular Breeding, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China.

Many disease resistance genes have been introgressed into wheat from its wild relatives. However, reduced recombination within the introgressed segments hinders cloning of the introgressed genes. *Pm13* originated from Aegilops longissima, confers a broad-spectrum resistance to 108 wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) isolates collected from China and the European countries.

We cloned Pm13 using a method combining physical mapping with radiation-induced chromosomal aberrations and transcriptome sequencing analysis of ethyl methanesulfonate (EMS)-induced loss-of-function mutants. *Pm13* encodes a kinase fusion protein, designated MLKL-K, with an N-terminal domain of mixed lineage kinase domain-like protein (MLKL_NTD domain) and a C-terminal serine/threonine kinase domain bridged by a brace. The resistance function of *Pm13* was validated by transient and stable transgenic complementation assays.

Transient over-expression analyses in Nicotiana benthamiana leaves and wheat protoplasts revealed that the truncated fragment containing brace plus kinase domain of MLKL-K was capable of inducing cell death, which is dependent on a functional kinase domain and the three α-helices in the brace region close to the N-terminus of the kinase domain.

Collectively, our results reveal that powdery mildew broad-spectrum resistance gene *Pm13* encodes a novel kinase fusion gene, and shed light into the mechanism underlying broad-spectrum resistance.

#631

TaCRG1 plays an important role in tiller development of winter wheat

Yonghong Wang[1, 2*], Yuqi Song[1,], Wenguang Wang[1,], Han Zhang[1], Jiajia Cao[1], Zihao Wang[1], Bo lv[1]

[1] State Key Laboratory of Wheat Improvement, College of Life Sciences, Shandong Agricultural University, Tai’an, Shandong 271018, China.
[2] Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China.

[*]Corresponding author: Yonghong Wang yhwang@genetics.ac.cn

Wheat (*Triticum aestivum* L.) is a major staple food for more than one-third of the world’s population. Tiller number is an important agronomic trait determining panicle number and grain yield in wheat, and the development of tillers in winter wheat requires overwintering. Currently, understanding of the underlying mechanisms is very limited.

Here, we identified a cold response gene *TaCRG1* (*Cold Response Gene 1*) that plays an important role in tiller development of winter wheat. We generated *TaCRG1* knockout transgenic lines (*Tacrg1*) and *TaCRG1* overexpressed transgenic lines (*TaCRG1-B-OE*) under Fielder background, respectively. We found that the tiller number of *Tacrg1-2* was significantly lower while *TaCRG1-B-OE1* were significantly higher than that in wild type when they planted in autumn.

However, there were no significant differences in tiller number of these *TaCRG1* transgenic plants planted in spring. Moreover, we also found that the freezing survival rate was significantly higher in *TaCRG1-B-OE* plants while lower in *Tacrg1-2* plants compared with that in the wild type, demonstrating that *TaCRG1* also promotes cold tolerance of wheat.

In order to further understand the molecular mechanism of *TaCRG1* in regulating tiller number and cold tolerance of wheat, the TaCRG1 interacting protein TaCRG1IP1 was screened by yeast two-hybrid assay and interaction was further verified by using double luciferase complementary assay.

Further illustrating the molecular mechanism of *TaCRG1* will be helpful to breed wheat cultivars with ideal plant architecture and cold tolerance.

#577

A K-mer based approach to detect sequence variations and define haplotypes in bread wheat

Jesus Quiroz-Chavez[1], Ricardo H Ramírez González[1], Xiaoming Wang[2]*, Cristobal Uauy[1]*

[1] John Innes Centre, Norwich Research Park, Norwich, UK. [2] State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy, Northwest A&F University, Yangling, Shaanxi, China.

The release of the wheat whole-genome reference sequence has significantly accelerated the association studies between genetic variations and agricultural traits. Currently, the workflow for identifying genetic variations in wheat is derived from those established for rice and maize by mapping the resequencing sequences to the reference genome.

However, the allohexaploid nature and high sequence similarity among subgenomes of wheat challenge the conventional workflows. Moreover, bread wheat has many wild relatives, which are an important gene pool for expanding its genetic diversity, yet the larger genetic distances between them hampers the identification of sequence variations with conventional workflows.

This study employs a strategy that directly queries the presence/absence of the K-mers within a given 50 Kb window of the reference genome sequence in resequencing data and records the number of reference-specific K-mers to assess the extent of sequence variations between them.

Compared to conventional workflows, this strategy:

1. Eliminates the need to align resequencing sequences to the reference genome;
2. Requires no sufficient sequence alignment coverage, enabling detection of genetic variation between distant genotypes.
3. Reduces sequence complexity by partitioning the reference genome into smaller windows.
4. Integrates various types of sequence variation into a single value, simplifying downstream data analysis. Building upon this, unsupervised clustering analysis using genetic variation values from consecutive 20 windows (1 Mb) allows for rapid haplotype assignment within the 1 Mb interval.

Based on the identified genetic variation values and haplotypes, the following applications have been developed:

1. Direct identification of introgressions by comparing genetic variation values between hexaploid accessions and the wild relatives;
2. Uncover the parental origins for a given progeny genome segment;
3. Identification of the donor for a given accession's genome segment within the investigated population, enabling pedigree reconstruction independent of original documents; 4) Develop a haplotype-GWAS model to associate haplotypes and agricultural traits.

#326

Development of broad-spectrum antifungal resistance in common wheat using a newly defined molecular module

Liuhui Qiao[1#], Kunpu Zhang[1,2#], Jinyan Li[1], Ziyue Li[1], Nannan Ni[1], Ziming Zhang[1], Jianhui Zhao[1], Xiao Sui[1], Wenming Zheng[3*], Daowen Wang[1,2*], Zhenqing Fu[4*] and Huan Wang[1*]

[1] State Key Laboratory of Wheat and Maize Crop Science, Center of Crop Engineering, College of Agronomy, Longzi Lake Campus, Henan Agricultural University, Zhengzhou 450046, China
[2] National Wheat Engineering Research Center, College of Agronomy, Henan Agricultural University, Longzi Lake Campus, Zhengzhou 450046, China
[3] College of Life Science, Longzi Lake Campus, Henan Agricultural University, Zhengzhou 450046, China
[4] Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

Correspondence: dwwang@henau.edu.cn (Daowen Wang), zfu@mailbox.sc.edu (Zhenqing Fu), and hwang@henau.edu.cn (Huan Wang)

Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating foliar diseases of wheat. Deployment of resistant varieties is an efficient way to control stripe rust in wheat. RING-type E3 ubiquitin ligases play vital role in regulating of disease resistance responses against multiple pathogens.

By analyzed RNA-seq data, we found a gene encoding a RING-finger E3 ubiquitin ligase, designated as *TuRFE*, was up-regulated significantly after *T. urartu* was inoculated with stripe rust and positively regulates the stripe rust resistance in wheat. TuCSP, a protein in the SA pathway, can interacts with TuRFE and ubiquitinated and degraded by TuRFE via the 26S proteasome. Downregulation of TuCSP by TuRFE enhanced the expression of downstream immune regulators. CRISPR-Cas9 inactivation of TaCSP in wheat confers broad-spectrum resistance against stripe rust and powdery mildew.

Taken together, TuRFE positively regulate the resistance against stripe rust by interacting with TuCSP to mediate its ubiquitination and degradation via 26S proteasome pathway. Our study provides a theoretical basis for utilizing the TuRFE-TuCSP module to develop broad-spectrum disease resistance against multiple pathogen species in wheat.

#576

Intra-subgenome regulation induces unbalanced expression and function among bread wheat homoeologs

Xiaoming Wang[1*], Peng Zhao[1], Yuxiu Liu[1], Wenyang Hou[1], Mingzhu Cheng[1], Philippa Borrill[2], Xue Shi[1], Wanquan Ji[1], Shengbao Xu[1]

[1] 1State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy, Northwest A&F University, Yangling, Shaanxi, China.
[2] John Innes Centre, Norwich Research Park, Norwich, UK.

The differential expression of homoeologous genes confers expression plasticity and facilitates the adaptation and domestication of major polyploid crops. However, how this homoeolog expression bias (HEB) is regulated remains elusive. Here, transcriptome analysis of 406 worldwide wheat (*Triticum aestivum*) accessions revealed great variation in HEB among accessions.

We identified 14,727 QTLs regulating HEB (hebQTLs) by developing a region-based model to quantify HEB among accessions, indicating that HEB is genetically regulated and can be predicted using genotyping data. The hebQTLs only regulate the expression of homoeologs in the same subgenome and downregulate their expression to result in HEB, suggesting that intra-subgenomic rather than inter-subgenomic interactions drive subgenome expression bias.

Furthermore, non-hebQTL-regulated homoeologs have stronger biological functions, are under higher selection pressure and exhibit lower genetic diversity than hebQTL-regulated homoeologs. Significantly, the downregulations of 38.38% hebQTL-regulated homoeologs are compensated for by the upregulation of other homoeologs within a triad.

Our findings reveal how homoeolog expression is coordinated at the genetic level and provide an avenue for leveraging HEB to improve agronomic traits in polyploid crops.

#216

Unraveling the genetic and molecular basis of sr43-mediated stem rust resistance in wheat

Yan L. Wang[1], Karthick Gajendiran[1], Oliver R. Powell[1], Renjie Chen[1], Guotai Yu[1], Brande B. H. Wulff[1]

[1]King Abdullah University of Science and Technology, Biological and Environmental Science and Engineering Division, Thuwal, Saudi Arabia

yan.wang.1@kaust.edu.sa

Kinase fusion proteins (KFPs) are an emerging cluster of resistance proteins identified in Triticeae. In our previous work, we cloned the stem rust resistance gene *Sr43* from *Thinopyrum obtusiflorum*, which encodes a KFP containing a kinase domain and two domains of unknown function. In the current study, we have identified four *Sr43* extragenic EMS mutants which have lost resistance to the stem rust UK01 isolate.

Following backcrossing, we are attempting to identify the extragenic suppressor of *Sr43*. Meanwhile, we are conducting IP-MS on transgenic wheat expressing GFP-Sr43/HA-Sr43 to identify the physical interactors from wheat and the effectors from stem rust. Identification of the effector and the host interactors of *Sr43* will allow us to further study the mechanism of *Sr43*-mediated resistance in wheat, thereby contributing to crop breeding against stem rust.

#271

Molecular evidence for adaptive evolution of drought tolerance in wild cereals

Yuanyuan Wang[1], Guang Chen[2], Fanrong Zeng[3], Zhigang Han[4], Cheng-Wei Qiu[5], Zujun Yang[6], Peter Franks[7], Eviatar Nevo[8], and Zhong-Hua Chen[1]

[1]School of Science, Western Sydney University, Penrith, NSW, 2751, Australia
[2]Central Laboratory, Zhejiang Academy of Agricultural Sciences, Hangzhou, 310021, China
[3]Collaborative Innovation Centre for Grain Industry, College of Agriculture, Yangtze University, Jingzhou, 434025, China
[4]State Key Laboratory of Subtropical Silviculture, Zhejiang A&F University, Lin'an, Hangzhou, 311300, PR China
[5] College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China
[6]School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, Sichuan 611731, China
[7] School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW 2006, Australia [8]Institute of Evolution, University of Haifa, Mount Carmel, 34988384 Haifa, Israel

The considerable drought tolerance of wild cereal crop progenitors has diminished during domestication in the pursuit of higher productivity. Regaining this trait in cereal crops is essential for global food security but requires novel genetic insight.

Here, we assessed the molecular evidence for natural variation of drought tolerance in wild barley (*Hordeum spontaneum*), wild emmer wheat (*Triticum dicoccoides*), and model grass allotetraploid *Brachypodium hybridum* and diploid progenitor species *Brachypodium stacei* collected from contrasting dry and moist habitats at Evolution Canyon, Israel (ECI). We report that prevailing moist vs. dry conditions have differentially shaped the stomatal and photosynthetic traits of these wild cereals in their respective habitats. Evidence of genomic landscape of mutation profiles accounting for the differences in slope-specific DEGs in *Triticum dicoccoides* and *Hordeum spontaneum*, driven by the xeric site conditions on the African Slope at ECI.

We observed that the allopolyploid *B. hybridum* employs a drought escape strategy via the maintenance of significantly higher photosynthetic capacity and a shorter flowering time than the aridic *B. stacei*. Co-expression gene module 'circadian rhythm' was linked to significant drought-induced delay in flowering time in *Brachypodium* genotypes.

The biased homeolog expression pattern and transferable dominance of subgenomes of *B. hybridum* illustrated that the allopolyploid species may utilize the expression patterns of both progenitor species under drought conditions to survive.

Our results provide new genetic information for the breeding of resilient wheat and barley in a changing global climate increasingly frequent drought events.

#624

Cell-type specific metabolomics and proteomics analysis of early and late wheat grain filling stages

Shuang Zhang[1], Palak Chaturvedi[1], Arindam Ghatak[2], Wolfram WECKWERTH[1,2]

[1] Molecular Systems Biology (MOSYS), Department of Functional and Evolutionary Ecology, University of Vienna, 1030 Vienna, Austria. [2] Vienna Metabolomics Center (VIME), University of Vienna, 1030 Vienna, Austria.

Email: wolfram.weckwerth@univie.ac.at

The nutritional value of wheat grains, particularly their protein and metabolite composition, is a result of the grain-filling process, especially in the endosperm. Here, we employ tissue-specific sampling and laser microdissection (LMD) combined with shotgun proteomics and metabolomics to generate a tissue- and cell type-specific proteome and metabolome inventory of developing wheat endosperm at different grain-filling stages [1,2].

We identified altogether more than 15000 proteins from four different tissues and cell types seed coat, embryo, endosperm and cavity fluid, aleurone cells, sub-aleurone cells, starchy endosperm cells, and endosperm transfer cells.

Cell-type accumulation of specific SUT and GLUT transporters, sucrose converting and starch biosynthesis enzymes correlate well with the respective sugar metabolites, suggesting sugar upload and starch accumulation. Changes in various protein levels between AL, SA and ETC support this metabolic switch from 15 to 26 DAA.

The distinct spatial and temporal abundances of proteins and metabolites revealed a contrasting activity of nitrogen assimilation pathways, e.g. for GOGAT, GDH and glutamic acid, in the different cell types from 15 to 26 DAA, which can be correlated with specific protein accumulation in the endosperm.

The integration of cell-type specific proteome and metabolome data revealed a complex metabolic interplay of the different cell types and a functional switch during grain development and grain-filling processes.

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#604

Role of mitochondrial 2-oxoglutarate dehydrogenase in wheat tissue tolerance to salinity

Wijeweera, R.W.S.N.[1], Duncan, O.[1], Millar, A.H.[1]

1. ARC Centre of Excellence in Plant Energy Biology, School of Molecular Sciences. University of Western Australia, Australia.

Wheat is one of the major staples worldwide, yet it is significantly affected by salt stress. Exploring the natural genetic diversity in wheat is the key to improving salinity tolerance. Five wheat genotypes, differing in salt tolerance, were subjected to 150 mM NaCl stress for 8 days. Changes in biomass, photosynthesis, chlorophyll content and Na^+/K^+ content were measured.

Despite the higher tissue salt levels, wheat genotype Mocho showed greater physiological tolerance to salinity. Shotgun proteomics and metabolomics of the leaf samples confirmed the differential regulation of proteins located in mitochondria and metabolites generated in mitochondria in the 5 genotypes under salt stress. Subsequent mitochondrial complexome profiling identified 2-oxoglutarate dehydrogenase (OGDC) and a plant hydroxyglutarate synthase orthologue (HglS) as enzymes in complexes that significantly lose structural stability and dissociate into subunits under salt stress.

In vitro activity assays and proteome profiling showed Mocho's ability to retain higher OGDC activity and higher OGDC subunit abundance under salt stress. *In planta* and leaf disc assays using the OGDC inhibitor succinyl phosphonate (SP), salt-like symptoms of decreased chlorophyll content and plant biomass were observed.

These effects were significantly more apparent in the tissue-tolerant Mocho landrace than in other excluder varieties, indicating an enhanced role of OGDC in its metabolism. Intriguingly, OGDC exhibited the capacity to oxidize both 2-oxoglutarate (OG) and 2-oxo adipate (OA), which is also the substrate of HglS in the lysine degradation pathway.

Mocho's OGDC demonstrated a heightened catalytic rate using OA under salt stress compared to salt-excluding varieties, showcasing its robust ability to sustain both the TCA cycle and lysine degradation functions. This altered substrate preference of OGDC in Mocho underscores its importance in the metabolic landscape of the variety, suggesting a putative role in salt tissue tolerance.

The enhanced performance of OGDC in tissue-tolerant Mocho under salt stress would be a promising prospect for developing salt-tolerant wheat varieties, enhancing global wheat production.

#131

Multi-target genome editing to reduce free asparagine concentration in wheat (*Triticum Aestivum* l.) Grains

Forrest Wold-McGimsey[1], Caitlynd Krosch[1], Rocío Alarcón-Reverte[1,2] , Karl Ravet[1] , Andrew Katz[1] , John Stromberger[1] , Richard Esten Mason[1] , Stephen Pearce[1,2]
[1]Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO, United States
[2]Sustainable Soils and Crops, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom

Forrest.Wold-McGimsey@colostate.edu, Caitlynd.d@hotmail.com, Rocio.Alarcon-Reverte@rothamsted.ac.uk, Karl@tesorogenetics.com, Andrew.Katz@colostate.edu, John.Stromberger@colostate.edu, Esten.Mason@colostate.edu, Stephen.Pearce@rothamsted.ac.uk

Acrylamide is a processing contaminant formed during high-temperature cooking processes of starchy foodstuffs, including bread products, and is a potent neurotoxin and probable carcinogen. There is now a growing movement among regulatory agencies to limit the permissible amount of acrylamide in food products sold to the public.

The amino acid asparagine provides the carbon backbone for acrylamide formation in baked products. Because free asparagine concentration in wheat grains is highly correlated with acrylamide levels in bread products, the development of bread wheat lines with low free asparagine content in their grains is a favorable method of producing end-use wheat products with low acrylamide levels.

A common wheat line carrying a chemically induced null allele in one of the three *ASPARAGINE SYNTHETASE 2* (*ASN2*) homoeologues exhibited reductions in free asparagine content in their grains between 9 and 34% compared to wild-type sister lines. We hypothesize that loss of function in all three *ASN2* homoeologues will confer more extreme reductions in free asparagine content.

To do this, a CRISPR/Cas9 construct with one single guide RNA was used to introduce knockout edits in all three *ASN2* genes in five elite bread wheat varieties. In the greenhouse, edited wheat plants exhibit no reduction in germination rate or overall vigor. We will measure free asparagine content in the edited wheat grains using mass spectrometry coupled with ultra-performance liquid chromatography.

We expect these results from at least one edited variety to be available by June of 2024 and field trials for edited lines will be conducted within the next two years. It is our hope that this method can be used in complement with traditional breeding efforts to produce healthier, higher quality bread products with no loss to the favorable qualities exhibited in existing elite lines.

#130

Multi-target genome editing reduces polyphenol oxidase activity in wheat Triticum Aestivum l.) Grains

Forrest Wold-McGimsey[1], Caitlynd Krosch[1], Rocío Alarcón-Reverte[1,2] , Karl Ravet[1] , Andrew Katz[1] , John Stromberger[1] , Richard Esten Mason[1] , Stephen Pearce[1,2]

[1]Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO, United States
[2]Sustainable Soils and Crops, Rothamsted Research, Harpenden, Hertfordshire, United Kingdom

Forrest.Wold-McGimsey@colostate.edu, Caitlynd.d@hotmail.com, Rocio.Alarcon-Reverte@rothamsted.ac.uk, Karl@tesorogenetics.com, Andrew.Katz@colostate.edu, John.Stromberger@colostate.edu, Esten.Mason@colostate.edu, Stephen.Pearce@rothamsted.ac.uk

In wheat (*Triticum aestivum* L.), polyphenol oxidase (PPO) enzymes released from the aleurone layer of the grain during milling results in the discoloration of flour, dough, and end-use products, reducing their value. Loss-of-function mutations in the *PPO1* and *PPO2* paralogous genes on homoeologous group 2 chromosomes confer reduced PPO activity in the wheat grain. However, limited natural variation and the proximity of these genes complicates the selection of extremely low-PPO wheat varieties by recombination.

A CRISPR/Cas9 construct with one single guide RNA targeting a conserved copper binding domain was used to edit all seven *PPO1* and *PPO2* genes in the spring wheat cultivar 'Fielder'. Five of the seven edited T_1 lines exhibited significant reductions in PPO activity, and T_2 lines had PPO activity up to 86.7% lower than wild-type. The same construct was transformed into the elite winter wheat cultivars 'Guardian' and 'Steamboat', which have five *PPO1* and *PPO2* genes. In these varieties PPO activity was reduced by >90% in both T_1 and T_2 lines.

In all three varieties, dough samples from edited lines exhibited reduced browning. Using this method, we hope to develop elite wheat lines with near-zero PPO grain content with no loss to their favorable qualities.
This study demonstrates that multi-target editing at late stages of variety development could complement selection for beneficial alleles in crop breeding programs by inducing novel variation in loci inaccessible to recombination.

#53

Metabolic responses to early-drought stress in Nordic spring wheat

Ronja Wonneberger**[1]**, Kerstin Neumann[10], John Charles D'Auria[10], and the CResWheat Consortium[1-9]

[1] Ronja Wonneberger, Therése Bengtsson, Rodomiro Ortiz & Marwan Alamrani, Ahmed Jahoor, Swedish University of Agricultural Sciences, Department of Plant Breeding, SWEDEN
[2] Mogens Støvring Hovmøller & Mehran Patpour, Aarhus University, Department of Agroecology, DENMARK
[3] Merja Veteläinen, Outi Manninen & Tarja Niemelä, Boreal Plant Breeding Ltd, FINLAND
[4] Muath Alsheikh, Jon Arne Dieseth & Susanne Windju, Graminor AS, NORWAY
[5] Pernilla Vallenback & Firuz Odilbekov, Lantmännen ek. för., SWEDEN
[6] Marja Jalli & Annika Johansson, Natural Resources Institute Finland (Luke), FINLAND
[7] Morten Lillemo & Min Lin, Norwegian University of Life Sciences, Department of Plant Sciences, NORWAY
[8] Ahmed Jahoor, Jeppe Reitan Andersen, Pernille Bjarup, Janni Hedensvang Jørgensen & Jihad Orabi, Nordic Seed A/S, DENMARK
[9] Rasmus Lund Hjortshøj, Linda Kærgaard Nielsen & Charlotte Damsgård, Sejet Plant Breeding, DENMARK
[10] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)**,** GERMANY

Ronja.wonneberger@slu.se, therese.bengtsson@slu.se

The CResWheat project is a collaborative pre-breeding initiative involving researchers and spring wheat breeders from the Nordic and Baltic countries. Its primary goal is to enhance climate resilience of Nordic spring wheat by improving yield, yield stability, and resistance to pests and diseases as well as abiotic stresses.

The cultivation of spring wheat (*Triticum aestivum* L.) plays a vital role in the climatic conditions of northern Europe, especially in regions where winter wheat cultivation encounters challenges. Historically, this region has relied on its temperate climate for successful wheat production. However, climate change is increasingly altering the dynamics of precipitation, leading to erratic rainfall patterns and prolonged dry spells during critical growth stages of wheat.

To identify sources of drought stress resistance in Nordic spring wheat, twelve selected Nordic spring wheat lines were grown under controlled conditions at the LemnaTec high-throughput phenotyping facility at IPK Gatersleben, Germany. Three week-old plants were subjected to either regular watering (up to 90% plant available water (PAW)) or reduced watering (up to 10% PAW) to induce a moderate early-season drought stress for 22 days, followed by a five-day regeneration phase.

During this time, imaging-based phenotyping of the plants and soil water measurements were performed daily, photosynthetic measurements were taken at four time points and leaf samples for metabolomics analysis were taken at four time points during and after the drought treatment. After harvest, grain and spike traits and tiller number gain were recorded for each plant.

Biomass, tiller number gain, plant weight, thousand grain weight and traits associated with grain size and shape were most strongly affected by drought. Biomass was reduced between 66 and 79% in the different genotypes. Differential metabolomics responses to drought became apparent at ca. 13 days after the start of the drought treatment.

We identified several differentially accumulated metabolites between control and drought-treated plants that may be used as biomarkers in breeding for drought stress tolerance. In total, 94 metabolites were significantly accumulated and 102 metabolites were significantly reduced under drought stress in at least one of the four time points.

In addition, we were able to identify metabolites that are strongly correlated with different phenotypes and should therefore be an interesting starting point for further studies on how metabolic changes affect phenotypes and yield components and on their predictive ability for plant performance.

#656

A Glu209Lys substitution in DRG1/TaACT7 reduces plant height and grain length in bread wheat

Zhencheng Xie[1] , Lichao Zhang[1] , Qiang Zhang[1] , Yan Lu[1], Chunhao Dong[1] , Danping Li[1] , Xu Liu[1], Chuan Xia[1] and Xiuying Kong[1]

[1]State Key Laboratory of Crop Gene Resources and Breeding, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

xiachuan@caas.cn

Plant height and grain size are two important agronomic traits that are closely related to crop yield. Numerous dwarf and grain-shape mutants have been studied to identify genes that can be used to increase crop yield and improve breeding programs. We characterized a dominant mutant, dwarf and round grain 1 (drg1-D), in bread wheat (Triticum aestivum L.). drg1-D plants exhibit multiple phenotypic changes, including dwarfism, round grains, and insensitivity to brassinosteroids (BR).

Cell structure observation in drg1-D mutant plants showed that the reduced organ size is due to irregular cell shape. Using map-based cloning and verification in transgenic plants, we found that a Glu209Lys substitution in the DRG1 protein is responsible for the irregular cell size and arrangement in the drg1-D mutant.

DRG1/TaACT7 encodes an actin family protein that is essential for polymerization stability and microfilament (MF) formation. In addition, the BR response and vesicular transport were altered by the abnormal actin cytoskeleton in drg1-D mutant plants.

#270

Single-pollen RNA-SEQ analysis of male gametophytes with the induced chromosome breakage by a gametocidal gene

Koichi Yamamori[1], Kazuki Murata[1], Hiroyuki Kakui[2], Takanori Yoshikawa[3], Shuhei Nasuda[1*]

[1] Graduate School of Agriculture, Kyoto University, Japan
[2] Graduate School of Agricultural and Life Science, The University of Tokyo, Japan
[3] National Institute of Genetics, Japan

*email: nasuda.shuhei.5z@kyoto-u.ac.jp , yamamori.koichi.2k@kyoto-u.ac.jp

Gametocidal (Gc) genes in wheat are selfish genetic factors that preferentially transmit themselves to progeny by inducing chromosome breakage in the gametes lacking them. Gc genes are carried by the Gc chromosomes derived from *Aegilops spp.*, and induce chromosome breakage during the first pollen mitosis when introduced hemizygously into the bread wheat genome. So far, at least five Gc genes have been reported and none of their responsible genes have been identified.

In this study, single-cell RNA-seq (scRNA-seq) analysis was performed on single pollen grains isolated from anthers at a post-meiotic stage. By comparing the expression profiles of each pollen, we intended to detect changes in the expression patterns related to the chromosome breakage induced by the Gc gene. In total 32 pollen grains from the *Gc2* nulli- (hereafter, *Gc2*-null, n = 8), hemi- (*Gc2*-hemi, n = 16) and homo-zygous (*Gc2*-homo, n = 8) lines in the 'Fielder' background were analyzed by scRNA-seq. Chromosome breakage is expected to occur in a half of the pollen derived from *Gc2*-hemi, but not in *Gc2*-null and *Gc2*-homo.

Analysis of transcriptome patterns by UMAP (uniform manifold approximation and projection) for dimension reduction indicated that gene expression patterns of the pollen grains were largely classified into two groups; pollen grains from *Gc2*-null and *Gc2*-homo formed a group and all the pollen grains from *Gc2*-hemi formed another group. Within the *Gc2*-hemi group, pollen grains were further classified into two subgroups. We presume one subgroup corresponds to the pollen with chromosome breakage and the other to the ones without it.

To examine the characteristics of the pollen groups indicated by UMAP, we screened for the differentially expressed genes (DEGs) among the groups. In a comparison between pollen of *Gc2*-hemi and the other lines, the DEGs related to histone and genome stability were detected. Moreover, differences in expression levels of the histone-related genes were observed in a comparison between the two subgroups of pollen from *Gc2*-hemi. This study was in part supported by Cabinet Office, Government of Japan, Moonshot R&D Program.

#606

TaCRTISO dosage modulates plant height and spike number per plant in wheat

Qiang Yan[1, †], Yue Lu[1, †], Yunlong Pang[1, †], Hailiang Zhao[1], Shubing Liu[1, *]

[1]National Key Laboratory of Wheat Improvement, College of Agronomy, Shandong Agricultural University, Tai’an 271018, China

Plant height (PH) and spike number per plant (SN) are essential wheat yield-related traits, and identifying genes affecting PH and SN is important to improve wheat yield. In a previous study, a quantitative trait locus (QTL) on 1B, qSN1B.1, was detected controlling spike number per plant (SN) by genome-wide association study (GWAS).

In this study, a gene encoding a protein sharing high homology with carotenoid isomerase (CRTISO) (TaCRTISO-1B) was identified as the causal gene underlying the QTL, and the function of TaCRTISO in controlling wheat PH and SN was validated by genome editing and EMS mutant.

Single (aaBBDD and AAbbDD), double (AAbbdd) and triple (aabbdd) loci mutants in all three TaCRTISO homoeologous genes were obtained by gene editing. The four types of mutants were all significantly shorter than the wild-type with more loci knocked-out, the more decrease of PH. In greenhouse, aaBBDD and AAbbDD mutants had an increased SN, whereas the AAbbdd and aabbdd mutants had a reduced SN.

In growth chamber, the SN was higher in all mutants relative to the wild-type. Four types of natural allelic variations of TaCRTISO-1B in wheat germplasm lines were identified, accessions with Type Ⅲ alleles are 1BL/1RS translocation lines harboring the rye (Secale cereale) CRTISO locus.

Phenotypic evaluation indicated that the introgression of 1RS into the wheat genome affecting PH and SN. This study provided a targeting gene and high valuable information that can be used to modify PH in wheat breeding by gene editing and marker assisted breeding.

#35

Genome-wide association study of kernel traits using a 35k snp array in bread wheat

Delong Yang[1, 2, *], Peng Wang[1], Jingfu Ma[1], Tao Chen[1, 2], Peipei Zhang[1], Lijian Guo[1]

[1] State Key Laboratory of Aridland Crop Science, Gansu Agricultural University, Lanzhou 730070, China. [2] College of Life Science and Technology, Gansu Agricultural University, Lanzhou 730070, China.

* Correspondence: Delong Yang, yangdl@gsau.edu.cn;

Kernel size and weight are crucial components of grain yield in wheat. Deciphering their genetic basis is essential for improving yield potential in wheat breeding. In the present study, five kernel traits, including kernel length (KL), kernel width (KW), kernel diameter ratio (KDR), kernel perimeter (KP) and thousand-kernel weight (TKW), were evaluated in a panel consisting of 198 wheat accessions under six environments.

Wheat accessions were genotyped using the 35K SNP iSelect chip array, resulting in a set of 13,228 polymorphic SNP markers that were used for genome-wide association study (GWAS). A total of 146 significant marker-trait associations (MTAs) were identified for five kernel traits on 21 chromosomes ($-\log_{10}(P) \geq 3$), which explained 5.91-15.02% of the phenotypic variation.

Of these, 12 stable MTAs were identified in multiple environments, and six superior alleles showed positive effects on KL, KP and KDR. Four potential candidate genes underlying the associated SNP markers were predicted for encoding ML protein, F-box protein, ethylene-responsive transcription factor, and 1,4-α-glucan branching enzyme.

These genes were strongly expressed in grain development at different growth stages. The results will provide new insights into the genetic basis of kernel traits in wheat. The associated SNP markers and predicted candidate genes will facilitate marker-assisted selection in wheat breeding.

#655

Identification and regulation of drought resistance of wheat TaAGT2-1A

Huayan Yin, Xingcai Sun, Qian Sun, Yifan Lv, Boyu Ren, Shuying Fang, Xinyu Chen, Xiaoyan He, Ping Mu*

College of Agronomy, Qingdao Agricultural University, Qingdao 266109, Shandong, China

yinhuayan@qau.edu.cn

Alanine glyoxylate aminotransferase (AGT) is an important member of the Alanine aminotransferase (AlaAT) family, which plays an important role in plant photorespiration. Photorespiration is an important source of H_2O_2 in plant cells and an important component of cell REDOX maintenance, which affects plant response to abiotic stresses such as drought, high temperature and high salt. Wheat (*Triticum aestivum* L.) is one of the three major food crops in the world.

In actual production, abiotic stress such as drought seriously affects the formation of wheat yield and quality, and then causes huge economic losses. Therefore, the analysis of the expression of alanine-glyoxylate aminotransferase gene under drought stress and its molecular mechanism in response to drought stress is of great significance for the breeding of new wheat varieties resistant to drought.

In this study, the differentially expressed gene *TaAGT2-1A* was screened based on the transcriptomic expression profile of Qingmai 6 under drought stress, and its basic physicochemical properties, gene expression characteristics, phylogenetic relationship, gene function and drought resistance regulation pathway were analyzed.

The results showed that the full-length cDNA of *TaAGT2-1A* was 1434 bp, encoding 477 amino acids ($C_{2288}H_{3601}N_{633}O_{665}S_{18}$), molecular weight was 51.2 KDa, and theoretical isoelectric point (pI) was 8.13. TMHMM and SignalP online prediction showed that the protein encoded by *TaAGT2-1A* gene had no transmembrane region and no signal peptide, so it was inferred that the protein encoded by *TaAGT2-1A* gene was not secreted protein.

Under drought stress, compared with wild type, *TaAGT2-1A* overexpressed transgenic wheat had increased germination potential (1.92 times), POD (1.65 times), soluble sugar content (1.85 times), root length and effective tillering (2.5 times) at germination stage, and POD (1.65 times) at seedling stage.

The results indicated that overexpression of *TaAGT2-1A* could significantly improve drought resistance of wheat. PlantRegmap and transcriptomic data were used to screen the transcription factor TaDREB4A that may bind to the promoter of *TaAGT2-1A*, and the results of yeast single hybridization and EMSA tests proved that the two could bind. This study lays a foundation for further research on the regulatory mechanism of drought resistance gene *TaAGT2-1A*.

#120

Search for the genes for necrosis expressed in the synthetic-hexaploid-derived hybrids

Yuto Yoden[1], Soyoko Noguchi[2], Shoya Komura[1], Kazuki Murata[1], Kazusa Nishimura[1], Yoshihiro Inoue[1], Shigeo Takumi[2], Nobuyuki Mizuno[3], Yoshihiro Matsuoka[2], Shuhei Nasuda[1], Fuminori Kobayashi[3], Kentaro Yoshida[1]

[1] Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan
[2] Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan
[3] Institute of Crop Science, National Agriculture and Food Research Organization (NARO), Tsukuba, Ibaraki 305-8518, Japan

Triticum monococcum ssp. *aegilopoides* (Link) Thell. is a wild relative of common wheat, which is diploid of the A^m genome. Since *T. m.* ssp. *aegilopoides* has agronomically beneficial traits, it provides useful genetic resources in wheat breeding. We developed $A^uA^uBBA^mA^m$ synthetic hexaploid lines by crossing *Triticum turgidum* L. ssp. *durum* cv. ‘Langdon’ (A^uA^uBB genome) with *T. m.* ssp. *aegilopoides* (A^mA^m genome).

When the synthetic hexaploids were crossed with *T. aestivum* L. cv. ‘Chinese Spring’ (CS), 70% of the tested $A^uA^uBBA^mA^m$ synthetic hexaploids showed growth inhibition with dead leaves called hybrid necrosis under low temperatures in F_1 plants ($A^uA^uBBA^mD$). When one $A^uA^uBBA^mA^m$ synthetic hexaploid line was crossed with *T. aestivum*, all the tested accessions of *T. aestivum* showed the hybrid necrosis phenotypes. Therefore, this hybrid necrosis potentially prevents the use of A^m genome in wheat breeding.

In this study, we aim to characterize the expression of hybrid necrosis and to identify genomic regions responsible for the hybrid necrosis. In the F_1 plants ($A^uA^uBBA^mD$), hybrid necrosis gradually proceeded. Plant growth was arrested around 30 days after germination. Differences in the number of leaves and tillers between the necrotic line and the wild-type line were obvious around 50 days after germination. By linkage analyses with $A^uA^uBBA^mA^m$ F_2 population between necrotic line and wild-type line based on MIG-seq markers and deletion mapping with a gametocidal gene, a causal region in the synthetic hexaploid was mapped near the centromere of chromosome $1A^m$.

Another causal region in common wheat was located at the short arm of chromosome 5D by cross experiments using CS nulli-tetrasomic lines and CS 5D chromosome deletion lines. These results indicated that the hybrid necrosis was caused by the interaction between genes located at $1A^m$ and 5D chromosomes. Genome sequencing of CS 5D chromosome deletion lines narrowed down to 25.4 Mbp.

Currently, we have performed simultaneous CRISPR-Cas9 knockout of candidate genes using the bread wheat cultivar ‘Fielder’ and successfully obtained genome-edited plants with mutations in the multiple candidate genes, some of which were crossed with the $A^uA^uBBA^mA^m$ synthetic hexaploid line. Identification of the causal genes could contribute to expanding the use of A^m genome and understanding post-reproductive isolation by genome-genome interaction.

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#616

Integration of genomic and environmental characteristics to enhance durum wheat breeding for climate resilience

Meryem Zaim[1], Zakaria Kehel[1], Miguel Sanchez-Garcia[1], Bouchra Belkadi[2], Abdelkarim Filali Maltouf[2], Ayed Al Abdallat[3] and Filippo M. Bassi[1]

[1]ICARDA. Biodiversity and Integrated Gene Management. P.O. Box 6299 Rabat Institutes. Rabat, Morocco.
[2]Laboratory of Microbiology and Molecular Biology, Faculty of Sciences, University Mohammed V in Rabat, Morocco.
[3]Faculty of Agriculture. The University of Jordan. Amman 11942. Jordan.

Email: m.zaim@cgiar.org

Durum wheat is mostly cultivated under rainfed conditions in the Mediterranean Basin, where the precipitation is irregular across years and locations resulting often to moisture stress. Therefore, the identification of loci controlling the capacity of genotypes to convert moisture into grain yield is quintessential to stabilize production despite climatic variations.

In a first study, four recombinant inbred lines (RIL) populations were used to conduct quantitative trait loci (QTL) discovery for grain yield (GY). A total of 576 individuals were sown at three locations in Morocco and Lebanon. These individuals were genotyped by sequencing with 3,202 high-confidence polymorphic markers, to derive a highly dense consensus genetic map. Six QTLs were found to be associated with GY and independent from flowering time, explaining high phenotypic variation. To confirm our findings, a global panel of 384 accessions was tested across eighteen environments in Morocco, Lebanon, and Jordan representing a vast range of moisture levels. The accessions were assigned to water responsiveness classes.

Genome wide association studies (GWAS) revealed that six loci explained the majority of variation, among them one QTL previously found in the RIL populations. A second validation panel tested under moisture stress confirmed that carrying the positive allele at three loci generated a high average water productivity gain. These loci were tagged by Kompetitive Allele Specific PCR (KASP) markers, and these were used to screen a third independent validation panel composed by elites tested across moisture stressed sites.

These loci are now ready for molecular pyramiding and transfer across cultivars to improve the moisture conversion of durum wheat. Additionally, the material was tested for resistance to various disease, including leaf and yellow rust and tan spot. Interestingly, the results showed promising levels of resistance.

#76

Global crotonylatome and gwas revealed a tasrt1-tapgk model regulating wheat cold tolerance through mediating pyruvate

Ning Zhang[1], Sisheng Wang[1], Simin Zhao[1], Daiying Chen[1], Hongyan Tian[1], Jia Li[1], Lingran Zhang[1], Songgang Li[1], Lu Liu[1], Chaonan Shi[1], Xiaodong Yu[1], Yan Ren[1], Feng Chen[1*]

[1]National Key Laboratory of Wheat and Maize Crop Science/CIMMYT-China Wheat and Maize Joint Research Center/Agronomy College, Henan Agricultural University, Zhengzhou, China

Here, we reported the complete profiling of the crotonylation proteome in common wheat. Through a combination of crotonylation and multi-omics analysis, we identified a TaPGK associated with wheat cold stress. Then we confirmed the positive role of TaPGK modulating wheat cold tolerance.

Meanwhile, we found that cold stress induced lysine crotonylation of TaPGK. Moreover, we screened a lysine de-crotonylase TaSRT1 interacting with TaPGK and found that TaSRT1 negatively regulated wheat cold tolerance. We subsequently demonstrated TaSRT1 inhibiting the accumulation of TaPGK protein, and this inhibition was possibly resulted from de-crotonylation of TaPGK by TaSRT1.

Transcriptome sequencing indicated that overexpression of TaPGK activated glycolytic key genes and thereby increased pyruvate content. Moreover, we found that exogenous application of pyruvate sharply enhanced wheat cold tolerance. These findings suggest that the TaSRT1-TaPGK model regulating wheat cold tolerance is possibly through mediating pyruvate.

This study provided two valuable cold tolerance genes and dissected diverse mechanism of glycolytic pathway involving in wheat cold stress.

#615

Production of spontaneous translocations for plant architecture by transferring agropyron cristatum chromosome 1p into wheat

Jinpeng Zhang[1,2*], Bohui Han[1], Xiao Wang[1], Xilu Kang[1], Yangyang Sun[3], Meng Zhang[1], Jiawen Luo[1], Yuqing Lu[1], Haiming Han[1], Shenghui Zhou[1], Weihua Liu[1], Xinming Yang[1], Xiuquan Li[1], Lihui Li*[1]

[1] State Key Laboratory of Crop Gene Resources and Breeding, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China. [2] Zhongyuan Research Center, Chinese Academy of Agricultural Sciences, Xinxiang, Henan, 453519, China. [3] Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao 266101, China

[*]Email: zhangjinpeng@caas.cn; lilihui@caas.cn

Agropyron cristatum is a valuable wild relative for wheat improvement. Our previous study confirmed that the A. cristatum chromosome 1P carries small flag leaf and dwarf traits in wheat backgrounds. Here, we reported that T1AL·1PS and T1AS·1PL Robertsonian translocations (RobTs) were produced by breakage-fusion mechanism based on wheat-A. cristatum 1P(1A) substitution line II-3-1c.

Combining molecular markers and cytological analysis, we identified 16 spontaneous RobTs from 911 F_2 individuals derived from the cross of commercial cultivar Jimai22 and II-3-1c. Fluorescence in situ hybridization was applied to detect the fusion structures of the centromeres in wheat and A. cristatum chromosomes. The whole genome re-sequencing results indicated that the chromosomal junction point was located at the physical position of Triticum aestivum chromosome 1A (212 Mb) and A. cristatum chromosome 1P (230 Mb).

Genomic in situ hybridization in pollen mother cells showed that the produced translocation lines could form stable ring bivalent. Introducing chromosome 1PS translocation fragment into wheat significantly increased the number of fertile tillers, grain number per spike, and grain weight, and reduced the flag leaf area.

However, introducing chromosome 1PL translocation fragment into wheat significantly reduced flag leaf area and plant height with a negative effect on yield components. The pre-breeding two types of spontaneous RobTs T1AL·1PS and T1AS·1PL pave the way for wheat dense planting architecture improvement.

#613

Identification of a paired wheat resistance and leaf rust avirulence genes contributing to LR41 resistance

Yi Ding[1], Peng Zhang[1], Benjamin Schwessinger[3], Bayantes Dagvadorj[3], Simon G. Krattinger[2], Brande Wulff[2], Yuqi Wang[1], Xiaohui Yu[1], Evans Lagudah[4], Li Huang[5], Robert F Park[1]

[1] Plant Breeding Institute, School of Life and Environmental Sciences, Faculty of Science, The University of Sydney, Cobbitty 2570, Australia
[2] Biological and Environmental Science & Engineering Division, King Abdullah University of Science and Technology, Thuwal 23955-6900, Kingdom of Saudi Arabia
[3] Division of Plant Science, Research School of Biology, College of Science, The Australian National University, Canberra ACT 2601, Australia
[4] Agriculture and Food, Commonwealth Scientific and Industrial Research Organisation, Canberra ACT 2601, Australia
[5] Department of Plant Sciences and Plant Pathology, College of Agriculture, Montana State University Bozeman MT 59717-3150, United States

Leaf rust, caused by the fungus *Puccinia triticina*, continues to be the most damaging disease of wheat globally. The resistance (R) - avirulence (Avr) interaction model is widely exploited in wheat breeding of leaf rust resistance because this simple mode of action can be selected based on clear phenotypes in specific wheat cultivars and *Pt* isolates and mostly offers resistance at all plant growth stages.

The lack of knowledge of resistance mechanisms and the host and pathogen *R* and *Avr* genes that modulate pathogen ingress has often led to “blind” selection of disease resistance, and due to fast adaptation of pathogens in one-on-one relationships, rapid breakdown of resistance. Till now, more than 80 loci conferring resistance to *Pt* (“*Lr*” genes) have been designated in wheat, however, few of the actual *Lr* genes have been identified and no corresponding *Avr* gene has been isolated from *Pt*.

This study identified a race-specific all-stage wheat *R* gene *Lr41*, which is among the first known alien introgression from *Aegilops tauschii* on chromosome 2DS. The presence of *Lr41* as a non-*NLR* gene was confirmed with comparative genomics and transcriptomics, mutagenesis and functional validation.

We further showed that *Pt* virulence on *Lr41* was due to haplotype sequence variations in a specific genomics region affecting *AvrLr41*. Experimentally confirmed with native- and hetero-expression, this study provided the first model illustrating direct and unconventional R-Avr interaction in wheat leaf rust resistance.

We anticipate the current findings would help understand how wheat recognises *Pt* or even other pathogens through race-specific modes. Identification of the paired *Lr41* and *AvrLr41* may be directly fed into wheat breeding programs for engineering disease resistance.

#589

TaMYB72 activates the TaFT gene to promote heading and enhance grain yield traits in wheat

Lifen Wu[1,2,†], Zhencheng Xie[1,†], Danping Li[1], Yaoyu Chen[1], Chuan Xia[1], Xiuying Kong[1,*], Xu Liu[1,2,*] Lichao Zhang[1,*]

[1] Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China. [2] College of Agronomy, Hebei Agricultural University, Baoding 071001, Hebei, China.

[*] Address correspondence to Lichao Zhang (zhanglichao@caas.cn), Xiuying Kong (kongxiuying@caas.cn) and Xu Liu (liuxu03@caas.cn)
[†] These authors contributed equally to this work

Heading date affects yield and adaptability in wheat (Triticum aestivum L.). Characterizing the genes that regulate heading date would provide insight into the molecular mechanisms underlying heading date regulation and thereby facilitate efforts to breed high-yielding wheat varieties. We previously identified a heading regulatory gene in wheat, TaMYB72, and established its role in promoting flowering through heterologous expression analysis in rice.

In this study, we further demonstrated the role of TaMYB72 in regulating heading date and yield traits in wheat. TaMYB72, a MYB-related transcription factor, was shown to transactivate transcription in yeast. Compared with wild-type plants, the heading and maturation dates of TaMYB72 knockout mutants were delayed by about 11 and 6 days, respectively.

Moreover, the mutants exhibited increased plant height, spike length, grain number per spike, grain length, and grain weight—all traits associated with increased yield. Furthermore, an electrophoretic mobility shift assay, dual-luciferase reporter, yeast one-hybrid, and chromatin immunoprecipitation-qPCR assays suggested that TaMYB72 directly activates expression of the heading date gene TaFT and thereby promotes heading and enhances grain yield traits in wheat.

Together, our results indicate that TaMYB72 is an important factor in the regulation of wheat grain yield and that mutants harboring defects in this gene can be used as germplasm resources for wheat improvement.

#68

***TaCOCO1* regulated cold tolerance by mediating *TaMYB4* involving in *TaCBF12d* pathway in common wheat**

Lei Zhao[1#], Jinyuan Liu[1#], Yaoyao Jiang[2], Congwei Sun[1], Ning Zhang[1], Jian Yang[2], Feng Chen[1*]

[1] National Key Laboratory of Wheat and Maize Crop Science / CIMMYT-China Wheat and Maize Joint Research Center /Agronomy College, Henan Agricultural University, Zhengzhou 450046, China
[2] State Key Laboratory for Managing Biotic and Chemical Threats to the Quality and Safety of Agroproducts, Key Laboratory of Biotechnology in Plant Protection of MOA of China and Institute of Plant Virology, Ningbo University, Ningbo 315211, China

Emails: Lei Zhao (leizhao2016@163.com), Feng Chen (fengchen@henau.edu.cn)

Low temperature as one of the most important abiotic stresses has caused serious wheat production loss worldwide. Here, we reported genetic identification and functional analysis of the *Cold Controlling 1* (*TaCOCO1*) gene affecting cold tolerance in wheat. *TaCOCO1*-overexpressed transgenic wheat plants significantly enhanced cold tolerance, whereas EMS mutant and CRISPR/Cas9-mediated knockout plants were more sensitive to cold response, indicating that *TaCOCO1* positively regulates cold tolerance in wheat.

Interaction analysis showed that TaCOCO1 strongly interacted with TaMYB4 and reduced the protein level of TaMYB4 during co-expression. We subsequently found that the BSMV-mediated silenced wheat plants and EMS mutants of *TaMYB4* possessed significantly increased wheat cold tolerance. Furthermore, we revealed that *TaMYB4* bound to the promoter of *TaCBF12d* to repress its expression, and BSMV-mediated *TaCBF12d*-silenced wheat plants possessed significantly decreased wheat cold tolerance.

Luciferase complementation assay showed that *TaCOCO1* alleviated the inhibition of *TaMYB4* on the transcriptional activity of *TaCBF12d*. Therefore, *TaCOCO1* regulated wheat cold tolerance possibly through mediating *TaCBF12d* expression.
This study offered a new insight into the molecular mechanism of wheat cold tolerance involved in CBF pathway, and also provided valuable genes for the improvement of cold tolerance in wheat breeding program.

#139

A method for keeping wheat stomata open for physiological studies

Chenchen Zhao[1], Ce Guo1, Rongrong Tao[1,2], Meixue Zhou[1*]

[1]Tasmanian Institute of Agriculture, University of Tasmania, Launceston, TAS 7250, Australia
[2]Jiangsu Key Laboratory of Crop Genetics and Physiology, Agricultural College of Yangzhou University, Yangzhou, China.

High sensitivity and rapid closure of wheat stomata to environmental stimuli make it difficult and inconvenient when investigating stomatal physiology and morphology using epidermal peels. This is due to inevitable mechanical stress to stomatal guard cells when separating epidermis from mesophyll cells, which induced a vast majority of stomatal closure in wheat.

Stomata are more open and active in detached leaves than in epidermal peels. Based on these observations, we proposed a simple method, which promotes stomatal opening using detached leaves rather than epidermis for physiological observations. Stomatal response to stimuli was significantly increased when using intact leaf segment.

The method was used to investigate stomatal behaviours of two wheat genotypes with contrasting salt tolerance to salinity stress. The effects of salt stress and exogenous abscisic acid (ABA) treatment on stomatal behaviours were also assessed. The salt-tolerant genotype, H-135, demonstrated a greater stomatal closure rate than the salt-sensitive genotype, H-093, in response to exogenous ABA under salt stress, highlighting the potential of stomatal responsiveness as an indicator for breeding salt-resistant crops.

This method not only facilitates the effective initiation of stomatal opening but also ensures the continued responsiveness of stomata to subsequent treatments in wheat.

#140

Understanding physiological and molecular insights into stomatal kinetics linked with wheat salinity tolerance

Chenchen Zhao[1], Ce Guo1, Rongrong Tao[1,2], Meixue Zhou[1*]

[1]Tasmanian Institute of Agriculture, University of Tasmania, Launceston, TAS 7250, Australia
[2]Jiangsu Key Laboratory of Crop Genetics and Physiology, Agricultural College of Yangzhou University, Yangzhou, China.

Salinity represents a big abiotic threat to global agricultural sustainability. Na^+ accumulation in plants significantly reduced stomatal conductance and wheat crop yield. However, the regulatory networks underlying Na^+ induced stomatal kinetics changes remain unclear. Here, we investigated stomatal morphological and physiological responses using two wheat cultivars, SaltY055 (salinity tolerant) and Salty 038 (salinity susceptible), upon long- and short-term salinity treatment to dissect the stomatal regulatory networks underlying wheat salinity tolerance.

Long-term salinity (150 mM salt in soil) led to reduced stomatal conductance but enhanced stomatal sensitivity to exogenous abscisic acid (ABA) in Salty055. Feeding the wheat roots transiently with 150 mM NaCl significantly enhanced stomatal closure and reduced stomatal response time only in SaltY055. Additionally, sorbitol failed to induce same amount of stomatal closure in both cultivars, excluding the osmotic stress from 150 mM NaCl treatment.

Further molecular investigations suggest that differences in salinity tolerance among cultivars are highly linked with expression levels of genes involving ABA biosynthesis, ROS metabolites and ion channel activities. Our research adds evidence to understand Na^+ derived stomatal regulations, offering clues in screening germplasm and speeding up breeding of crops for salinity tolerance.

#392

Mapping of dwarf gene Rht15 and its effect on agronomic traits in durum wheat

Zhangchen Zhao[1], Liang Chen[1*], Yin-Gang Hu[1, 2*]

[1]State Key Laboratory for Crop Stress Resistance and High-Efficiency Production, College of Agronomy, Northwest A&F University, Yangling, Shaanxi, China.[2]Institute of Water Saving Agriculture in Arid Regions of China, Northwest A&F University, Yangling, Shaanxi, China.

Email: Zhangchen Zhao, zhaozhangchen@nwafu.edu.cn; Yin-Gang Hu, huyingang@nwafu.edu.cn

Wheat is an important food crop in the world. Lodging resistance is one of the key traits for achieving maximum yield in wheat breeding. The current production is mainly based on the dwarf genes Rht1 and Rht2, with a relatively single genetic background. Therefore, it is very important to map the new wheat dwarf gene Rht15 and clarify its genetic effects.

The F_2 population was obtained by crossing the Rht15 donor parent durum wheat "Durox" (Rht15) with the tall durum wheat "Langdon" (Does not contain any dwarf genes). The recombinant was screened by previously developed molecular markers. Through the BSA strategy combined with 660K SNP chip and RNA-Seq analysis, and wheat whole genome re-sequencing technology, the homozygous differential SNPs and deletion insertion fragments between the tall and dwarf parents-lines were screened, and specific KASP markers and Indel markers were screened and developed to map the Rht15.

Based on the phenotypic and genotypic data analysis of the F_2 individual and recombination, the dwarf gene Rht15 is located between markers 6A085 and 6A101 of chromosome 6A. In addition, the effects of Rht15 on plant height, internode characteristics, lodging resistance, yield components and quality traits were evaluated by the Rht15 dwarf and tall $F_{3:4}$, $F_{4:5}$ and $F_{5:6}$ lines. Rht15 significantly reduced plant height by 31.5 % on average by shortening the cell length.

Furthermore, the diameter and wall thickness of internodes were significantly increased by Rht15, which decreased the height center of gravity (35.4 %), increased the mechanical strength of the basal internode (41.8 %), and finally improved the lodging resistance index (153.7 %). Rht15 reduced biomass plant^{-1} (-13.7 %), 1000-kernel weight (-12.9 %), grain number per spike (-2.3 %) and grain yield plant^{-1} (-10.3 %).

However, Rht15 increased the grain protein content (8.7 %), wet gluten content (9.2 %) and starch content (2.3 %). Nonetheless, Rht15 has opened up an opportunity as an alternative dwarfing gene in wheat breeding and improvement.

#268

Dynamic regulation of chitinase-based defence by a wheat ERF protein and a pathogen effector-mediated protein degradation

Hongyuan Zheng[1,*], Xingyu Zhang[1], Chenyu Zhao[1], Xiuyu Wang[1], Xuemin Zhou[1], Yu Wang[1], Daowen Wang[1]

[1] State Key Laboratory of Wheat and Maize Crop Science, College of Agronomy, and Center for Crop Genome Engineering, Henan Agricultural University, Zhengzhou 450002, China

Email: zhenghongyuan@henau.edu.cn

Wheat is one of the most important food crops in the world. Increasing wheat yield is essential for maintaining global food security. Powdery mildew (PM) is a worldwide fungal disease seriously harming wheat production. There is an urgent need to breed PM resistant varieties to ensure stable wheat production.

Chitinases are ubiquitous defense proteins in plant immunity against pathogen invasion. However, their regulation by plant and pathogen factors remains poorly understood. Here we identified a wheat ERF transcription factor (TF) that was rapidly upregulated by powdery mildew infection, which enhanced plant defense by promoting the transcription of multiple chitinase genes.

However, the protein abundance of the ERF TF declined as powdery mildew infection progressed. An E2 enzyme produced by PM interacted with the ERF and mediated its degradation through recruiting a wheat E3 ligase.

Consistent with the above findings, wheat resistance to PM was elevated in the plants overexpressing the ERF TF or with the co-opted E3 ligase downregulated by gene silencing. In sum, our work sheds light on dynamic regulation of chitinase-based plant defense by both host and pathogen genes and provides new molecular targets for engineering wheat resistance against PM through biotechnology breeding.

#265

Interaction of dynamic light, elevated CO_2 and nitrogen fertilizer impact stomatal kinetics in wheat

Yanmin Zhou[1,*], Colin Osborne[1]

[1] School of Biosciences, The University of Sheffield, Western Bank, Sheffield, S10 2TN, United Kingdom

Stomatal behavior controls the balance between carbon fixation and water loss and therefore plays a central role in crop performance. Faster stomatal responses to a fluctuating environment can improve intrinsic water use efficiency and reduce the risk of transient water deficits. The speed of stomatal responses depends on guard cell size, and recent work has highlighted that plants have rapid stomatal closure presenting higher water use efficiency, which could be explained by plants grown at high nitrogen conditions contributing to higher stomatal density and smaller guard cell size.

In this study, we used wheat with diploid, tetraploid and hexaploid ploidy levels to investigate how stomatal responses to light fluctuations at the different stages of wheat growth under elevated CO_2 and nitrogen fertilizer conditions. We measured the variation of guard cell size and density, showing that flag leaves with smaller guard cell size and higher density in diploid wheat increased the rapidity of stomatal responses than hexaploidy wheat.

Meanwhile, elevated CO_2 and nitrogen fertilizer also improved the faster stomatal opening and closing and therefore enhanced the water use efficiency in wheat. However, the effects of elevated CO_2 and nitrogen fertilizer on stomatal kinetics were diminished during the ripening stage compared to the flowering stage.

Overall, the findings indicate that stomatal rapidity in wheat is dependent on guard cell size, elevated CO_2 and nitrogen fertilizer conditions, and suggest that diversity in the speed of stomatal changes could be a potential breeding target for optimizing the physiological responses of wheat to dynamic field conditions.

#199

Concerted local genomic and epigenomic reorganizations accompany stabilization following centromere breakage in wheat

Jingwei Zhou[1†], Yuhong Huang[1†], Huan Ma[1], Yiqian Chen[1], Handong Su[1,2*]

[1] National Key Laboratory of Crop Genetic Improvement, Hubei Hongshan Laboratory, Shenzhen Institute of Nutrition and Health, Huazhong Agricultural University, Wuhan, 430070, China. [2] Shenzhen Branch, Guangdong Laboratory for Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen, China
[†] Jingwei Zhou and Yuhong Huang contributed equally to this work

[*] Corresponding authors: Handong Su (Email: shandong@mail.hzau.edu.cn)

Centromeres have unique chromatin features that play an essential role in maintaining genomic stability. Although they are intrinsically repetitive and fragile hotspots for chromosomal rearrangements, little is known about how centromeres maintain their integrity and ensure the homeostasis of nucleus when they are breakage.

In this study, we investigated the genetics and epigenetics of damaged centromeres in stocks of wheat ditelocentrics lines from centromere misdivisions. We show novel chromosome end structures with de novo telomere addition and subtelomere deletions following centromere misdivisions in common wheat. Local complex chromosome rearrangements were observed with segment deletion and copy number expansion, suggesting BFB cycle occurred at centromere break site in wheat.

Furthermore, we found maintenance, expansion and contraction centromere regions with remodeling nucleosome distributions, but preserve strict CENH3 nucleosome positioning on complex centromeric repeats. We also found that de novo centromeres were formed when the original centromere were lost. Local transcription and chromatin reorganizations were substantially associated with damaged centromere following centromere breakage in wheat.

These findings suggest how structure and function integrity of centromere is preserved in response to centromere damage. Ultimately, these results finely resolved chromosome end structures, providing knowledge about the consequences regarding repair processes after centromere dysfunction misdivision, suggesting insights into evolution of complex chromosome end structures in nature.

#607

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

Ziru Zhou[1], Yawen Yang[2], Guo Ai[1], Miaomiao Zhao[1], Baozhu Han[2], Chunjie Zhao[1], Yiqian Chen[1], Yuwei Zhang[1], Hong Pan[2], Caixia Lan[1], Qiang Li[1], Jieting Xu[2, *], Wenhao Yan[1, *]

[1]National Key Laboratory of Crop Genetic Improvement, Hubei Hongshan Laboratory, Huazhong Agricultural University, Wuhan , 430070,China
[2]WIMI Biotechnology Co., Ltd, Changzhou, 213002,China

*Correspondence: Jieting Xu (xjt@wimibio.com), Wenhao Yan (yanwenhao@mail.hzau.edu.cn)

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

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

Meanwhile, visual isolation of regeneration factors could be achieved by expression pLTP2::*DsRed* cassettes with morphological regulators together. We tested the robustness of expressing the four morphogenic regulators simultaneously in multiple genotypes and the results all showed that this combination could greatly promote genetic transformation efficiency and editing efficiency in wheat. Surprisingly, by combining the four morphogenic regulators, we succeeded in obtaining transgenic lines with approximately 13% efficiency with mature embryo from both Fielder and Kenong199 genotypes.

This method greatly reduces cost and time needed for transformation in wheat.

#71

Controlled-release nitrogen fertilizer application mitigated N losses and modified microbial community while improving wheat yield and N use efficiency

Haijun Sheng[1], Yinsen Qian[1], Qiaoqiao Yu[1], Yifan Cao[1], Rongrong Tao[1], Min Zhu[1,2], Jinfeng Ding[1,2], Chunyan Li[1,2], Wenshan Guo[1,2], Xinkai Zhu[1, 2, 3, *]

[1] Jiangsu Key Laboratory of Crop Genetics and Physiology, Agricultural College of Yangzhou University, Yangzhou, Jiangsu 225009, China
[2] Co-Innovation Center for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou, Jiangsu 225009, China
[3] Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University, Yangzhou, Jiangsu 225009, China

E-mail address: xkzhu@yzu.edu.cn.

The extensive application of traditional fertilizer has greatly contributed to wheat yield, accompanied by massive nitrogen (N) loss and environmental pollution. Controlled-release nitrogen fertilizer (CRNF) is expected to improve N use efficiency (NUE) in agricultural systems. Unfortunately, the mechanism by which CRNF reduces N loss and its response to soil microbial communities remains unclear.

In this study, common urea, polymer-coated urea (PCU), sulfur-coated urea (SCU) and urea-formaldehyde (UF) were used as materials to analyze the effects of split application of different N sources on wheat yield, NUE, soil N balance, soil bacterial diversity and functional abundance. The results showed that PCU and SCU significantly improved yield relative to urea, with an average increase of 18.20% and 15.73%, respectively. N uptake by wheat in PCU, SCU and UF was increased by 18.76%, 14.26% and 7.75% compared to that in urea, respectively.

CRNF increased the mineral N content of the topsoil (0−20 cm) but decreased the mineral content in the deeper soil (40−60 cm). CRNF was observed to significantly decrease cumulative N2O emissions, as well as apparent N loss compared with urea, which was reduced by 39.45%, 30.74% and 11.68% in PCU, SCU and UF, respectively. In addition, PCU decreased soil bacterial diversity but increased the abundance of microbes involved in N cycle, such as *Firmicutes*, *Actinobacteriota* and *Bacteroidota*, which could regulate soil nitrate concentrations.

The results indicated that split application of PCU was conducive to promoting N uptake by wheat, increasing topsoil mineral N content, reducing N leaching into deeper soil and N_2O emissions, thereby alleviating N loss while increasing NUE and wheat yield.

#69

Enhancing wheat seed germination under salinity stress: alleviating water uptake limitation with higher A-amylase activity and “cheap” osmolytes

Rongrong Tao[1], Quan Ma[1], Jinfeng Ding[1,2], Chunyan Li[1,2], Xinkai Zhu[1, 2] Wenshan Guo[1,2*] and Min Zhu[1,2*]

[1]Jiangsu Key Laboratory of Crop Genetics and Physiology, and Jiangsu Key Laboratory of Crop Cultivation and Physiology, Agricultural College of Yangzhou University, Yangzhou, China,
[2]Co-Innovation Centre for Modern Production Technology of Grain Crops, Yangzhou University, Yangzhou, China

Email: Wenshan Guo guows@yzu.edu.cn

Germination is a crucial process in the life span of plants and is one of the stages most sensitive to salinity stress. Numerous physiological mechanisms and multistage processes were found to be involved in it. In this study, the impact of salt stress on wheat germination was investigated, in terms of water uptake, ion accumulation, hormonal balance, and reserve utilization. When salt stress occurs, excessive Na$^+$ accumulates in plants, accompanied by K$^+$ extrusion.

The ABA content increased while GA$_3$ decreased, and α-amylase and cysteine protease activities were inhibited leading to a decrease in soluble sugar and protein content. Correlation analysis indicated that water uptake rate (WUR) was found to have a significant positive correlation with seed vigor index, Na$^+$ and K$^+$ contents. Salt-tolerant variety had higher Na$^+$ and K$^+$ contents and *TaNHX1* and *TaAKT1-like* expression.

We concluded that water is the main limiting factor for germination under salt stress, soluble sugar was mainly used as an osmotic adjustment substance, showing an upward trend of its content after salt treatment at 48-72h while declining under control. Besides, Na$^+$ and K$^+$ can be used as an osmotic to facilitate water uptake instead of suffering from ion toxicity, which benefits from Na$^+$ sequestration and K$^+$ retention.

Meanwhile, α-amylase activity was found to have a significant positive relationship with germination rate, GA$_3$, soluble sugar content and K/Na. Besides, there was a significant positive correlation between K/Na and soluble sugar content while GA$_3$/ABA showed a significant positive correction with WUR. Transcriptome results showed that starch and sucrose metabolism was the most enriched pathway, and six genes related to α-amylase activity were annotated.

These results indicated that α-amylase activity was the key to germination under salt stress, K/Na homeostasis and GA$_3$/ABA balance contributes to starch metabolism and water uptake by maintaining high α-amylase activity.

#509

Photosynthetic responses of wheat with different ploidy levels to high temperature

Marek Zivcak[1*], Marian Brestic[1], Marek Kovar[1], Andrej Filacek[1], Erik Chovancek[1,2], Pavol Hauptvogel[3]

[1] Slovak University of Agriculture, Institute of Plant and Environmental Sciences, Nitra, Slovakia
[2] Molecular Plant Biology, Department of LifeTechnologies, University of Turku, Finland
[3] National Agricultural and Food Centre, Research Institute of Plant Production, Piestany, Slovakia

*e-mail: marek.zivcak@uniag.sk

Heat waves are projected to become more frequent and severe in the near future, significantly impacting crop plants' physiological functions and yield. The development of technical tools for non-invasive monitoring of photosynthetic traits enables the uncovering of valuable responses and mechanisms associated with crop tolerance to stress applicable in crop plant phenotyping and screening.

In our study, we applied the non-invasive techniques to investigate the residual post-heat stress effects influencing photosynthetic responses in six diverse winter wheat genotypes (*Triticum* sp.), which vary in origin, taxonomy, and ploidy (tetraploids vs. hexaploids). After subjecting the plants to elevated temperatures (up to 38 °C) for five days, we compared their photosynthetic parameters: gas exchange analyses, simultaneous measurements of photosystem I (PSI) and photosystem II (PSII) photochemistry, electrochromic bandshift (ECS), at the beginning of recovery and after five additional recovery days compared to control plants grown under moderate temperatures. Notably, the plants belonging to hexaploid and tetraploid species exhibited distinct responses to heat stress based on CO_2 assimilation rate (A) and maximum carboxylation rates (V_{Cmax}).

Further analyses revealed that tetraploid genotypes fully recovered their photosynthetic and photoprotective functions in the later stage of recovery, while the hexaploid group showed limited recovery. The latter was associated with reduced photosystem I activity and elevated electric membrane potential in chloroplasts. A poor recovery was associated with an overly reduced acceptor side of photosystem I, as well as high values of the electric membrane potential ($\Delta\psi$) derived from ECS measurements in the chloroplast.

Our results suggest that a high $\Delta\psi$ can be associated with an excessive proton flux via the thylakoid membrane due to membrane leakiness as a symptom of membrane impairment. On the other hand, a good recovery of photosynthetic capacity and photoprotective functions were clearly associated with an enhanced proton gradient (ΔpH) obtained by ECS records, thus demonstrating a key role of efficient regulation of proton transport to produce sufficient transthylakoid proton gradient needed for photosynthesis restoration after high-temperature episodes.

In a broader sense, our results also demonstrate a diversity of photosynthetic responses to heat stress and the crucial role of photoprotective responses at the photosystem I (PSI) level that are associated with overall recovering capacity after heat stress, which deserves more attention in phenotyping and screening of crops in stress conditions. The work was supported by the research projects APVV-22-0392, APVV-20-0071, VEGA 1-0664-22 and VEGA 1-0425-23.

#618

Genotypic variations for yield and quality related traits in winter wheat and identifying superior genotypes

Zafarjon M. Ziyaev[1,2]*, Saidmurat K. Baboev[2], Abbos B. Elmurodov[2], Abdumurod E. Hakimov[2], Saidjon E.Teshaboev[1], Ozod S. Turaev[1,2], Maftuna B. Sodikova[2], Dilafruz V. Solieva[3] and Ram C. Sharma[4]

[1]Research Institute of Plant Genetic Resources under National Center for Knowledge and Innovation in Agriculture, Tashkent, Uzbekistan
[2]Institute of Genetics and Plant Experimental Biology under Academy of Sciences of Uzbekistan, Tashkent, Uzbekistan
[3]National University of Uzbekistan named after Mirzo Ulugbek, Tashkent, Uzbekistan
[4]ICARDA, Central Asia and the Caucasus Regional Office, Tashkent, Uzbekistan

*Email: zafaruzripi@gmail.com

High grain yield (GY) and improved quality are important objectives of winter wheat improvement programs in Central Asia. A field study was conducted in 2021 and 2022 in Kibray, Uzbekistan using 100 winter wheat genotypes of diverse genetic background and origin to determine genotypic variations for GY and quality related traits and identify superior genotypes for GY and quality. The 100 genotypes showed arrays of variations for heading (154–166 days) and maturity (200–212 days), plant height (79–116 cm), peduncle length (30–46 cm), spike length (7.9–12.3 cm), number of spikelet per spike (15–22), single spike weight (1.8–3.5 g), grain weight per spike (1.4–2.6 g), kernel per spike (36–66), GY (4.11–7.45 t/ha), 1000-kernel weight (TKW) (30–46 g), test weight (TWT) (665–800 g/l), protein (13.0–17.2%), gluten (25.9–36.8%), gluten deformation index (74–114), sedimentation (26–50 mL) and vitreousness.

Superior genotypes for multiple traits were determined in comparison with a widely grown cultivar Grom. Genotype #47 (20AWYT-IR-87) showed significantly higher GY, TKW, TWT, and protein and gluten contents than Grom. Three genotypes #3 (27FAWWON-IR-13), #42 (20AWYT-IR-54), #44 (20AWYT-IR-75) and #59 (20AWYT-IR-327) had significantly higher GY, TKW, TWT and protein content and gluten equal to Grom.

This study identified genotypes valuable for release as new cultivars for high GY and improved quality. One new variety “Farovon” (#59) was identified for release for commercial cultivation. Since a large number of genotypes used in this study originated from the International Winter Wheat Improvement Program, the findings have implications for national and international wheat improvement programs.

#567

Precise genome editing with programmable nucleases in wheat

Pei Ni[1], Yidi Zhao[1], Ximeng Zhou[1], Zhongfu Ni[1], Qixin Sun[1] and Yuan Zong[1,*]

[1] 1 Frontiers Science Center for Molecular Design Breeding (MOE), Key Laboratory of Crop Heterosis and Utilization (MOE), and Beijing Key Laboratory of Crop Genetic Improvement, China Agricultural University, Beijing 100193, China

*Correspondence: zongyuan@cau.edu.cn

As wheat is a pivotal staple crop globally, maintaining and enhancing its yield is a crucial task. However, the hexaploid nature and functional gene redundancy of wheat pose challenges in effectively and precisely modifying genes for critical agronomic trait improvement. The advent of genome editing technologies has ushered in new hopes for crop improvement.

Nevertheless, CRISPR/Cas9 currently primarily focuses on knocking out wheat genes, leading to functional deficiencies. To address this, we have previously established a precise editing system for single-base editing and predictable multi-nucleotide deletion in wheat, yet it still falls short of meeting the diverse needs in wheat gene function research and molecular breeding.

The Prime Editor (PE) system enables the substitution of any base, as well as the insertion and deletion of small fragments in plant genomes.

However, its editing efficiency in polyploid plants like wheat is extremely low, rendering it nearly unusable. Therefore, our team has comprehensively redesigned the PE system, encompassing the pegRNA, reverse transcriptase, and fusion protein construction, resulting in a highly efficient reengineered plant Prime Editor. This advancement significantly improved the editing efficiency of the Prime Editor system in hexaploid wheat (by an average of 33-fold) and established an efficient, precise, and stable multi-gene Prime Editing system. We have achieved precise editing of eight genes (21 gene loci) simultaneously in wheat, broadening the applicability of the Prime Editor system in plant genome editing.

This research marks the first report of utilizing the Prime Editor system to obtain wheat mutants, providing significant insights and technical support for Prime Editing and trait stacking in wheat and other polyploid plants. Furthermore, we have preliminarily achieved cross-scale editing of the common wheat genome, encompassing diverse chromosomal rearrangements such as deletions, duplications, and fusions, using genome editing technology. This offers diverse technical support for various agricultural applications, effectively advancing the process of crop molecular design breeding.

#372

Characterization and cloning of Cmc4 gene for curl mite resistance in wheat

Lanfei Zhao[1,2], Liangliang Gao[1,3], Hui Chen[1], Yujiao Gao[1], Paula Silva[3,4], Thomas Lux[5], Yuzhou Xu[1], Wei Zhao[1,2], Hyeonju Lee[3], Harold N. Trick[3], Bin Tian[3], Manuel Spannagl[5], Guorong Zhang[1], Liuling Yan[6], Jesse Poland[3,7], Guihua Bai[1,8*]

[1]Department of Agronomy, Kansas State University, Manhattan, KS 66506, USA. [2]College of Agronomy, Shandong Agriculture University, Taian, Shandong, 271018, China. [3]Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA. [4]Instituto Nacional de Investigación Agropecuaria (INIA), Estación Experimental La Estanzuela, Colonia, 70006, Uruguay. [5]Plant Genome and Systems Biology, Helmholtz Center Munich, Neuherberg, Germany. [6]Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK 74078, USA. [7]Plant Science Program, Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Kingdom of Saudi Arabia. [8]USDA, Hard Winter Wheat Genetics Research Unit, KS 66506, USA.

Wheat curl mite (WCM, Aceria tosichella Keifer) causes significant wheat yield losses in many wheat-growing regions worldwide. It not only rolls leaves to directly disrupt wheat growth, more importantly it transmits a devastating, widespread virus to cause wheat streak mosaic virus (WSMV) disease. Cmc4 is a WCM resistance gene from Aegilops tauschii and provides high WCM resistance when it was transferred into wheat.

By sequencing a Cmc4-containing Ae. tauschii accession using PacBio HiFi technology, map-based cloning, and conducting genome-wide association and gene expression studies, we successfully identified two candidate genes in the Cmc4 interval. Transformation of the candidate genes confirmed a CC-NB-LRR gene regulating Cmc4 resistance to WCM
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Comparison of the numbers and sizes of mites and their eggs on the infested leaves between Cmc4 near-isogenic lines found that the Cmc4 conferred WCM resistance by disrupting mite growth that resulted in death of most mites and production of only a few eggs in the Cmc4-resistant plants.

Analysis of geographic distribution of Cmc4-carrying accessions suggested Central Asia as the most likely origin of Cmc4. Diagnostic markers have been developed for selection of Cmc4 in wheat breeding programs. Cloning of Cmc4 is an essential step toward understanding the wheat-mite interactions and cloning of other Cmc genes for genetic control of both WCM and WSMV in wheat.

#507

Development and characterisation of wheat (Triticum aestivum L.) near-isogenic lines for metribuzin resistance

Rudra Bhattarai *, Hui Liu, Kadambot H.M. Siddique, Guijun Yan

UWA School of Agriculture and Environment, The University of Western Australia, WA 6009, Australia. The UWA Institute of Agriculture, The University of Western Australia, WA 6009, Australia

Email: rudra.bhattarai@research.uwa.edu.au

Metribuzin is a broad-spectrum herbicide that can also kill our crops from the same field while controlling the weeds, therefore metribuzin resistance identification in wheat is essential for successful controlling the weeds. One of the best approaches to identifying causal genes or their linked markers for metribuzin resistance in wheat is through the development and characterisation of near isogeneic lines (NILs).

Those causal genes or their linked markers are useful for marker-assisted breeding and selection for metribuzin resistance in wheat. Targeting a major metribuzin resistance Qsns.uwa.4A.2 genomic region, putative NILs were developed using the principle of heterogeneous inbreed family (HIF) method with the assistance of fast generation cycling system (FGCS), in a cross-population of Chuan Mai 25 (Resistance) and Ritchie (Susceptible).

NILs were confirmed through genotype-phenotype association analysis at F8 generation, with 200g ai per ha dose of metribuzin, the recommended dose to control weeds in Australian wheat fields. Pairs of contrasting NILs (NIL_3 and NIL_17) were studied using mRNA sequencing and protein sequencing (NIL_3, NIL_17 and NIL_10) to identify metribuzin resistance candidate genes and candidate proteins. Obtained candidate genes through mRNA sequencing and protein sequencing were validated using qPCR analysis. NILs targeting a major metribuzin resistance locus explaining 69% of the phenotypic variance were developed.

Seven pairs of NILs targeting Qsns.uwa.4A.2 were confirmed through genotype-phenotype association analysis. NILs (NIL_3 and NIL_17) were also validated using qPCR-based gene expression analysis revealed that nitrate excretion transporter and aspartyl protease were the major candidate genes. Novel molecular variants (SNPs at 714355066bp on 4A wheat chromosome) were identified within the gene nitrate excretion transporter (TraesCS4A03G1098900), from which a novel major allele for metribuzin resistance has been identified.

Additionally, other novel molecular variants (SNPs at 739694493bp on 4A wheat chromosome) were identified within the gene aspartyl protease (TraesCS4A03G1181200) from which a novel major allele for metribuzin has been identified within the same QTL region. Proteomic analysis using an isobaric tag (label-based) for relative and absolute quantification (iTRAQ) method identified candidate proteins WD repeats, AB hydrolase_1 and PsbP associated with metribuzin resistance.

The reason for detecting novel candidate genes through mRNA sequencing, but not detecting the same candidate genes/protein through proteomics analysis from the same QTL genomic region could be due to heterozygous novel SNPs obtained through mRNA sequence analysis from a locus linked to the candidate genes where such SNPs combination may not find through using the information of WheatRefSeq2.1 database through Proteomic analysis.

As compared to the allelic combination and their effects within genes TraesCS4A03G1098900 and TraesCS4A03G1181200, combinations of allelic variation could be different with the candidate genes TraesCS4A03G1099000 and TraesCS4A03G1181300 between contrasting isolines, respectively, though they are similar in functions. In this study, identified candidate genes from two different NIL pairs may have multiple novel alleles from a locus responsible for detecting common but some different levels of candidate gene expression between NIL pairs which can be utilised in marker-assisted wheat breeding for metribuzin resistance.

#401

Circadian influence on wheat senescence and grain nutrition

Christopher Buckley[1], Joshua Boyte[1], Robert Albiston[1], Jessica Hyles[2], Jesse Beasley[1], Alex Johnson[1], Ben Trevaskis[2], Alexandre Fournier-Level[1], Michael Haydon[1]

[1]School of Biosciences, University of Melbourne, Parkville, VIC 3010, Australia. [2]CSIRO Agriculture and Food, GPO Box 1700, Canberra, ACT 2601, Australia

The circadian clock plays a key role in regulating the timing of plant development. Despite its immense potential contribution to yield-related traits, the wheat (Triticum aestivum) clock has received little focus. Here, we measured circadian rhythms of elite Australian wheat cultivars and identified a large variation in the clock's output between genotypes. We show that the cycling pace (period) of these cultivars covaries with the timing of leaf senescence and leaf-to-grain nutrient mobilisation.

To investigate how the clock might govern these traits, we generated 48-hour 'circadian transcriptomes' in mature and senescent wheat flag leaves. The period of rhythmic transcripts shortens by an average of 0.5 h in senescent leaves, akin to observations of ageing in other organisms. Strikingly, clock genes are enriched amongst a set of transcripts that show little or no change in period with senescence (within ±0.5 h).

We clustered clock genes by specific changes in their transcript rhythms in senescent flag leaves, revealing a multifaceted transcriptional response of the clock at senescence. Genetic variation associated with each transcriptional response was used to group a 283-cultivar wheat diversity panel into six haplotypes. Circadian period, senescence and grain yield differed significantly between haplotypes.

Moreover, a deletion in the clock gene EARLY FLOWERING 3 (TaELF3-D1) within pairs of near-isogenic lines (NILs) was shown to accelerate senescence and reduce grain protein content. Our findings highlight the untapped functional potential of clock gene variation in wheat and outline a genetic basis for a connection between the clock, senescence, and grain nutrition.

#487

Role of annexin 8 in stomatal regulation of Triticeae under salinity and heat stress

Seidat Oluwadamilola Dauda[1], Michelle Mak[1], Jay Bose[1,2], Zhong-Hua Chen[1,2*]

[1]School of Science, Western Sydney University, Penrith, NSW 2751, Australia. [2]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia. First author:

o.dauda@westernsydney.edu.au

*Corresponding author: z.chen@westernsydney.edu.au

Annexins are a multigene family linked to calcium ion (Ca^{2+}) regulation under abiotic stress in plants. These genes bind to negatively charged phospholipids in membrane walls and are upregulated to signal calcium production under stress. Annexins have been studied under salinity, oxidative, drought, cold and abscisic acid stress conditions in diverse plants.

However, abiotic stresses occur simultaneously, and independent stress application is not enough to gauge plants response to these conditions thus the role of annexins in combined salinity and heat stress in wheat is unknown. In barley, we found HvANN8 confers salt tolerance in barley via the regulation of Ca^{2+} and-mediated ROS signalling. We also identified 25 annexins in wheat and this includes three annexin 8 gene (TaANN8s) in the A, B and D sub genomes.

This study evaluates the role of calcium-signalling annexin 8 gene in salinity and heat stress tolerance in wheat/barley crop (Triticeae). Here, we employed bioinformatics, physiological, biochemical, and molecular approaches to study the role of annexin in combined stress tolerance in wheat. Localisation analysis has shown diverse expression of this gene in the leaves and roots of wheat plants.

This study screened 319 wheat varieties including wild types for heat tolerance in the field at Hawkesbury River farm and greenhouse at Western Sydney University Hawkesbury campus (S35) to identify sensitive and tolerant cultivars by subjecting them to heat stress of 32°C for 5 days at the flowering stage to identify 16 heat tolerant and sensitive varieties.

The heat tolerant varieties were further screened for salinity stress against common bread wheat variety (fielder) by subjecting to salinity stress of 200 mM NaCl at the seedling stage to identify two salinity tolerant cultivars. The results showed that H-199 and H-031 were more tolerant to salt stress than the other cultivars.

These two cultivars along with fielder variety are currently being screened for combined salinity and heat stress tolerance in wheat with focus on the role of annexin 8 gene in inducing stomatal regulation under these stresses. Annexin 8 gene confers salinity and heat tolerance in wheat via calcium signalling for ROS scavenging.

#387

Deciphering wheat yield genetics and its contributing traits in rain-fed environments via multi-locus gwas

Vijay Gahlaut [1,2], Vandana Jaiswal[3], Hariendra Singh Balyan[1], Arun Kumar Joshi[4], Pushpendra Kumar Gupta[1]

[1]Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, India. [2]Department of Biotechnology & University Center for Research and Development, Chandigarh University, Mohali, Punjab, India. [3]CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India. [4]Borlaug Institute for South Asia (BISA), New Delhi, India

Email: zone4vijay@gmail.com

Multi-locus genome-wide association study (ML-GWAS) was undertaken using a set of 320 diverse spring wheat accessions, which were each genotyped for 9,626 SNPs. The association panel underwent replicated trials in four distinct environments, consisting of two irrigated (IR) and two rainfed (RF) conditions. We recorded phenotypic data for nine essential traits: days to heading, days to anthesis, days to maturity, plant height, grain filling duration, grain number per ear, grain weight per ear, thousand-grain weight, and grain yield, alongside seven stress-related indices.

Our analysis unveiled a total of 76 significant marker-trait associations (MTAs) across the nine traits, with 35 MTAs found in IR and 33 MTAs in RF environments. Additionally, eight MTAs were common to both environments. Furthermore, we identified 153 MTAs related to the seven stress-related indices. Among these, ten MTAs co-localized with previously known QTL/MTAs, while the remaining MTAs represented novel discoveries, contributing to the existing knowledge base.

In addition to MTAs, we identified three favourable haplotypes associated with agronomic traits: one for enhancing performance in RF conditions and two for improving performance in IR environments. We also pinpointed twenty promising candidate genes (CGs) associated with seven distinct biological activities using the wheat genome assembly IWGSC1.0.

Notably, the expression patterns of four out of the 20 genes, namely Trehalose-6-Phosphate, APETALA2/Ethylene-responsive factor, DNA-binding One Zinc Finger, and Gibberellin dioxygenases, demonstrated induction in response to drought stress in wheat seedlings. These MTAs, haplotypes, and CG-based markers hold significant potential for marker-assisted breeding to enhance wheat drought tolerance.

#31

Unsupervised learning algorithms empower identification of large indels from pangenomes.

Bosen Zhang[1], Haiyan Huang[1], Laura E. Tibbs-Cortes[2], Adam Vanous[3], Zhiwu Zhang[1], Karen Sanguinet[1], Kimberly A. Garland-Campbell[1, 2], Jianming Yu[4], Xianran Li[1, 2]

[1] Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164, USA. [2] Wheat Health, Genetics, and Quality Research Unit, Pullman, WA 99164, USA. [3] USDA-ARS, Plant Introduction Research, Ames, IA 50011, USA. [4] Department of Agronomy, Iowa State University, Ames, IA 50011, USA
Xianran Li: xianran.li@usda.gov

A substantial portion of characterized natural functional polymorphisms are large indels, which can alter gene structure and expression. One purpose of developing a pangenome is to accelerate gene identification and characterization by pinpointing such large indels. However, for specific genes, surveying and graphing large indels across assemblies are challenging and painstaking tasks.

To overcome the challenge, we have devised two unsupervised machine learning algorithms, CHOICE (Clustering HSPs for Ortholog Identification via Coordinates and Equivalence) and CLIPS (Clustering via Large-Indel Permuted Slopes). CHOICE autonomously retrieves the segments harboring the ortholog from each assembly for comprehensive All-vs-All comparisons, while CLIPS aggregates accessions sharing identical indels into haplotypes for concisely graphing the indel patterns.

We constructed an interactive webapp BRIDGEcereal (https://bridgecereal.scinet.usda.gov/) streamlining these two algorithms to expedite the process. With a just a gene model ID as input, BRIDGEcereal rapidly surveys the presence of large indels among pan-genomes, typically completing the task in under 30 seconds. To showcase the utility of BRIDGEcereal, we demonstrate its effectiveness in identifying potential causal genes associated with large indel polymorphisms using three QTLs (*Rc-D1*, *B1*, and *Hooded*) identified from a wheat RIL population.

#411

Genetics and physiology of leaf structure for heat stress tolerance in wheat.

Afroz Naznin[1], Jing He[1], Yuanyuan Wang[1], Asad Abbas[1], Oluwadamilola Dauda[1], Kun Ning[1], Oula Ghannoum[2], Jay Bose[1] and Zhonghua Chen[1]

[1]School of Science, Western Sydney University, Penrith, NSW, Australia. [2]Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia
a.naznin@westernsydney.edu.au, j.he4@westernsydney.edu.au, yuanyuan.wang@westernsydney.edu.au, a.abbas@westernsydney.edu.au, o.dauda@westernsydney.edu.au, k.ning@westernsydney.edu.au, o.ghannoum@westernsydney.edu.au, j.bose@westernsydney.edu.au, z.chen@westernsydney.edu.au

The constant increase in global temperatures due to climate change poses a significant threat of heat stress, affecting both plants and human livelihood. Heatwaves are becoming more frequent and intense, threatening food security and crop productivity, especially in major staples like wheat, faces substantial challenges due to fluctuating environmental conditions.

Originally, wheat is a crop in temperate regions, facing various hurdles when grown in tropical or subtropical areas. Considering this, our research undertook a comprehensive assessment of 319 wheat genotypes under both field and controlled glasshouse conditions at elevated temperatures. The aim was to evaluate phenotypic variations in leaf structure and other pertinent physiological and agronomical traits, with a focus on identifying quantitative trait locus (QTL) related to heat tolerance in wheat.

Numerous physiological, agronomic and yield related characteristics were measured. The plant growth, morphology and physiology were found to be changed under heat stress condition. An increase in leaf temperature (Tleaf), chlorophyll content, transpiration rate (Tr), leaf vapor pressure deficit (VPD) and vein density (VD) has observed under heat stress condition.

On the other hand, leaf thickness and leaf size found to be reduced under heat stress. Chlorophyll content, Tleaf, normalized difference vegetation index (NDVI), Tr and VD were found to be involved in facilitating the stress responses. We conducted a genome-wide association study (GWAS) to identify QTLs associated with leaf structural, physiological, and agronomic traits. Wheat population (189 genotypes) genotyped with Diversity Array Technology (DArT) polymorphic markers and several QTLs were identified related to leaf traits.

Our analysis revealed 166 markers linked to Tleaf, 160 markers correlated with VPD, 2 markers related to NDVI, 3 markers linked to biomass, and 1 marker related grain yield under field conditions. These QTLs are distributed across chromosomes 1A, 2B, 4A, 4B, 5A, 5B, 6A, 6D, and 7A. Notably, we identified 11 significant QTLs (LOD > 4.5) for Tleaf and 9 significant QTLs for VPD.

The phenotypic variability explained by these QTLs ranged from 17.5- 19.6% for Tleaf, 17.5- 21.1% for vpd, 10.3-11.7% for NDVI and 11.2-15.4% for biomass. Candidate genes related to heat stress tolerance, particularly from the regions of these significant QTLs, will be further identified. Subsequent validation of these QTLs and candidate genes can facilitate their utilization in marker-assisted selection and breeding programs.

This approach aims to develop wheat varieties with enhanced stability in grain yield under high temperatures, thereby contributing to sustainable crop production in challenging environments.

#404

Hyperspectral imaging and machine learning accelerate identification of grain protein content alleles in wheat landraces

Luqman B. Safdar [1,2,3], Alix Cornish [4], Elizabeth D. Goodman [2], Charlie Philp [3], Andrew Riche [5], David Steel [5], Burkhard Stuernagel [3], Iain R. Searle [6], Ehsan Abbasnejad [7], Martin Whitworth [4], Malcolm J. Hawksford [5], Rahul A. Bhosale [2], M. John Foulkes [2], Simon Griffiths [3], Ian D. Fisk [2], Scott A. Boden [1]

[1] School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Glen Osmond, SA 5064, Australia. [2] School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire, LE12 5RD, United Kingdom. [3] John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom. [4] Campden BRI, Station Rd, Chipping Campden, GL55 6LD, United Kingdom. [5] Rothamsted Research, West Common, Harpenden, AL5 2JQ, United Kingdom. [6] School of Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia. [7] Australian Institute for Machine Learning, University of Adelaide, Adelaide, SA 5005, Australia

The priority to increase grain yield has led to the loss of alleles that benefit grain quality traits of wheat, such as grain protein content (GPC). Modern germplasms either lack the favourable alleles for GPC, or the trait is heterogeneous among grain harvested from a plot or from a single spike, which presents challenges for identifying genetic variation to improve grain quality.

The sequence of a global collection of A. E. Watkins wheat landraces shows that modern cultivars have emerged from only two of the seven ancestral groups; this collection offers the opportunity to identify unique GPC alleles. In this study, we used hyperspectral imaging (HSI) to screen single wheat grains from over 600 Watkins landraces, with 12 grains per genotype. We developed a Partial Least Squares Regression (PLSR) model with R-squared value of 0.90 and Root Mean Squared Error (RMSE) of 0.77 for predicting GPC from single wheat grains.

Using this model, we predicted GPC from two field trials of Watkins population grown at the John Innes Centre and Rothamsted Research in 2020 and 2022, respectively. The predicted single GPC varied between 8–20%. Using sequenced Watkins data of over 90 million genetic variants, we performed a genome-wide association study (GWAS) and identified 22 loci for GPC, with 5 prioritised for gene identification.

Within these 5 loci, we identified 14 haplotypes from Watkins HapMap with over 5% positive effect on GPC-related traits. Taking advantage of HSI's ability to phenotype single grains, we performed a GWAS for single grain protein heterogeneity – a previously unexplored trait that has potential to select homogenous grains with high GPC.

Moreover, we used HSI and machine learning generated data to investigate loci for mean and single grain heterogeneity for grain weight, length, breadth, area, perimeter, and roundness. Our findings report novel landrace-derived alleles that influence grain quality and size, and they highlight the potential to combine HSI and machine learning with genomics to fast-track gene identification of key agronomic traits.

#175

Genome-wide association study (GWAS) and genomic selection models for leaf rust resistance in winter wheat

Anirup Sengupta[1], Brent D. McCallum[1,2], Colin W. Hiebert[1,2], Harwinder S. Sidhu[3], Curt A. McCartney[1]

[1] Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2, Canada. [2] Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden, Manitoba R6M 1Y5, Canada. [3]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta T1J 4B1, Canada

Emails: sengupt1@myumanitoba.ca, Curt.McCartney@umanitoba.ca

Leaf rust, caused by the fungus Puccinia triticina, is a prevalent disease of wheat affecting both yield and quality. Leaf rust resistance is an important trait, that is evaluated in the registration of wheat varieties in western Canada, and is an effective strategy for sustainable disease management. The genetic basis of this resistance remains unknown in Canada Western Red Winter (CWRW) wheat.

The objectives of this research were to; identify the quantitative trait loci (QTL) controlling leaf rust resistance using genome-wide association study (GWAS), and develop genomic selection (GS) models for improving leaf rust resistance in winter wheat. The wheat GWAS panel consisted of approximately 300 western Canadian winter wheat breeding lines and cultivars, and 100 winter wheat breeding lines and cultivars from the USA, eastern Canada, and Europe.

The GWAS panel was evaluated for leaf rust resistance in seedling tests, with multiple P. triticina races. This panel was also tested for resistance in inoculated field trials located in Winnipeg and Morden, Manitoba, following randomized alpha lattice designs, with two replicates per field trial, in the 2022-23 and 2023-24 growing seasons. Genotyping was done using the Illumina Infinium Wheat Barley 40K SNP array and the 25K wheat Infinium array.

The DNA marker and leaf rust datasets were used for GWAS. Significant QTLs associated with leaf rust resistance have been detected from the GWAS analyses, which indicated the presence of *Lr* genes in the GWAS panel, though the identity of these genes has yet to be determined. The leaf rust and SNP marker data were also used to develop genomic selection (GS) models for estimating leaf rust resistance in CWRW breeding germplasm.

The accuracy of these genomic selection (GS) models for predicting the leaf rust resistance of wheat germplasm was assessed via cross-validation. Improved knowledge of the resistance genes in Canadian winter wheat and DNA markers for selecting these genes will improve the efficiency of wheat breeding programs.

#555

Integrated omics approaches for understanding cold tolerance mechanisms in diverse wheat germplasm of Western Himalayas

Safoora Shafi[1], Sofora Jan[1], Rohit Kumar[1], Farkhandah Jan[1], Parthiban M[2], Mukesh Rathore[1], Soliha Gul Jabeen[1], Parvez Ahmad Sofi[1], Mohd. Anwar Khan[1], Sundeep Kumar[3], Rajeev K Varshney[4], Reyazul Rouf Mir[1]

[1]Division of Genetics and Plant Breeding, Faculty of Agriculture, SKUAST-Kashmir, Wadura, Sopore-193201. [2]Division of Entomology, Faculty of Agriculture, SKUAST-Kashmir, Wadura, Sopore-193201. [3]ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. [4]Centre for Crop and Food Innovation, WA State Agricultural Biotechnology Centre, Murdoch University, Murdoch WA 6150, Australia.

*Corresponding author e-mail: rrmir@skuastkashmir.ac.in; imrouf2006@gmail.com

Wheat is one the most important cereal crops in the world. Cold stress is a major constraint affecting wheat production, particularly in regions with low temperatures. In this study, we undertook a multifaceted approach for cold stress encompassing field screening, membrane stability assessment, biochemical profiling, metabolomic and lipidomic analysis, gene expression studies, proteomics analysis, and a genome-wide association study (GWAS).

The study evaluated 4,560 wheat genotypes over two years, identifying significant genetic diversity for cold stress tolerance. From these, 350 genotypes were selected based on field performance. Membrane stability analysis showed varied results, prompting further selection of 50 genotypes for detailed biochemical profiling. This included assessing reactive oxygen species (ROS) levels, osmoprotectants like proline, and enzymatic antioxidants such as ascorbate peroxidase, superoxide dismutase, guaiacol peroxidase, and catalase.

Correlation analysis of these biochemicals unveiled negative associations between antioxidants and ROS, shedding light on the vital role of antioxidants in alleviating oxidative damage under cold stress conditions. Two contrasting genotypes, SKUA_52 and SKUA_4301, were selected based on their performance in cold stress tolerance. Metabolomic and lipidomic analyses of these genotypes provided valuable insights into the activation of defense mechanisms, highlighting the modulation of key defense hormones such as salicylic acid, jasmonic acid, and abscisic acid in cold-tolerant genotypes.

Additionally, untargeted GC-MS metabolomic analysis revealed upregulation of osmo-protectants and stress-responsive amino acids in the cold-tolerant genotype, while lipidomic analysis underscored the importance of upregulating unsaturated lipids and downregulating saturated lipids to maintain membrane fluidity under cold stress.

Furthermore, gene expression analysis via., qRT-PCR elucidated the coordinated upregulation of specific genes involved in the ICE-CBF-COR pathway, such as COR and CBF genes, in the cold-tolerant genotype. Transcriptomics and proteomics analyses identified highly upregulated genes and proteins associated with antioxidative defense, osmotic adjustment, and signal transduction pathways, providing deeper insights into wheat's cold stress adaptation mechanisms.

Lastly, the GWAS in mini-core set by utilizing 35K Illumina iSelect single nucleotide polymorphism (SNP) array identified 35 marker trait associations and 85 candidate genes associated with cold resistance, offering valuable markers for molecular-assisted breeding efforts aimed at developing cold-tolerant wheat varieties. Overall, this comprehensive study significantly advances our understanding of the complex molecular landscape of cold stress adaptation in wheat, with implications for breeding resilient crop varieties capable of thriving in challenging environments.

#416

Candidate gene analysis for nue-related traits in common wheat based on a genome-wide association study

Gayatri[1], Ankur Poudel[1,2], Ashmita Rai[1], Karnam Venakatesh[3], Luzie U Wingen[4], Simon Griffiths[4], Amitha Mithra Sevanthi[1], Pranab Kumar Mandal[1]*

[1]ICAR-National Institute for Plant Biotechnology, Pusa Campus, New Delhi-110012, India. [2]Lamjung Campus, Institute of Agriculture and Animal Sciences, Tribhuvan University, Nepal. [3]ICAR-Indian Institute of Millets Research, Hyderabad, Telangana-500030, India. [4]John Innes Centre, Norwich Research Park, Colney Lane, Norwich, United Kingdom

Nitrogen deficiency has been identified as the most severe abiotic stress affecting the growth, development, and yield of bread wheat (Triticum aestivum L.). To investigate the genetic basis underlying the nitrogen use efficiency (NUE) in bread wheat, a genome-wide association (GWAS) study was conducted for N-use responsive morpho-physiological traits like biomass, photosynthetic pigments, and protein content; five biochemical traits (important N and C metabolizing enzyme activities) and two indexed traits (Stress Susceptible/Tolerance Index: SSI/STI) at the seedling stage under contrasting N conditions.

A panel of 317 diverse bread wheat genotypes spanning diverse ecogeographic regions was genotyped using a breeders' 35K Axiom Array, and a set of 22,650 filtered single nucleotide polymorphism (SNP) markers were used to study marker-trait associations (MTAs). Population structure and diversity analysis indicated two sub-populations with significant diversity. A large whole genome LD block size of 5.12 MB was obtained. 145 MTAs were identified using the different association mapping algorithms viz., BLINK, FarmCPU, and MLM.

Among them, 14 pleiotropic markers were also identified. We found phenotypic variance up to 24.8% in the case of GOGAT-STI followed by ICDH-SSI (16.8%), and NR enzyme activity (10.2%) in N-stress condition. A total of 12567 genes were retrieved from the 145 SNPs ± 5.12Mb region. 318 differentially expressed genes were identified by integrating GWAS and transcriptome analyses of N-efficient and N-inefficient genotypes.

Finally, nine genes (TraesCS4B02G394500, TraesCS7D02G008700, TraesCS1A02G295600, TraesCS2B02G521600, TraesCS2B02G600200, TraesCS5B02G426300, TraesCS6A02G061300, TraesCS2A02G066700, TraesCS5B02G426500) which expressed differentially in the N-efficient genotype were defined as more valuable candidate genes for low-nitrogen tolerance.

This is the first attempt to use GWAS for the identification of N-use-related biochemical and morpho-physiological traits QTLs in common wheat. This study will provide strong evidence for N regulation mechanisms, and reliable genetic resources for growth and NUE genetic improvement in wheat.

#390

Identification of genes affecting important agronomic traits based on wheat mutants

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

State Key Laboratory of Crop Gene Resources and Breeding / National Engineering Laboratory of Crop Molecular Breeding / CAEA Research and Development Centre on Nuclear Technology Applications for Irradiation Mutation Breeding, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China
Luxiang Liu: liuluxiang@caas.cn; Hongchun Xiong: xionghongchun@caas.cn

The agronomic traits such as heading date (HD) and grain-related traits are very important factors affecting yield potential in wheat. Based on an early heading (eh1) wheat mutant, we constructed an RIL population and identified a minor QTL for HD on the short arm of chromosome 2A by Bulked Segregant Analysis (BSA). Gene fine mapping using a segregating population of 4894 individuals further delimited it to a 1.70-Mb physical interval which contains 16 high-confidence genes based on IWGSC RefSeq v1.0.

Gene sequences and expression analysis indicated that TraesCS2A02G181200 encoding a C2H2-type zinc finger protein is the best candidate gene that affects HD. Screening our TILLING mutant resource (http://jing411.molbreeding.com) validated the function of TraesCS2A02G181200 in controlling HD. We also analyzed the variations of its putative regulatory sites in wheat natural accession, and identified the allele which was positively selected during wheat breeding.

Meanwhile, we identified a round grain 1 (rg1) wheat mutant with flag leaf morphology change, and found that the gene encodes von Willebrand factor type A (vWA)-/Vwaint domain-containing protein affecting grain size and weight, and flag leaf morphology by map-based cloning. Various missense mutations at sites in the Vwaint domain required for RG1 protein folding result in round grains and short flag leaves with a yellow, degraded apex.

Further protein-protein interaction experiments indicate that RG1 could interact with the key enzyme for biosynthesis of gibberellin/abscisic acid precursor, MCT, and their corresponding levels were decreased in rg1 plants, potentially contributing to round grain and short flag leaf phenotype. Moreover, we have cloned the important dwarfing gene Rht8 from wheat mutants and identified the new allele in Rht8-B1 for regulating plant height.

These studies provide important genetic resources for improving the associated agronomic traits, and therefore facilitating yield enhancement in wheat breeding programs.

#267

Molecular and physiological assessment of chloride channel b (clcb) in wheat and barley stomatal regulation under drought and heat stress.

Asad Abbas[1], Jing He[1], Yuan Yuan Wang[1], Afroz Naznin[1], Damilola Dauda[1], Jay Bose[1], Mark Jones[1], Zhonghua Chen[1*]

[1]School of Science, Western Sydney University, Penrith, 2751, Australia.

Email: A.Abbas@westernsydney.edu.au, j.he4@westernsydney.edu.au, yuanyuan.wang@westernsydney.edu.au, a.naznin@westernsydney.edu.au, ,d.dauda@westernsydney.edu.au, j.bose@westernsydney.edu.au, m.r.jones@westernsydney.edu.au, z.chen@westernsydney.edu.au.

Tonoplast destined chloride channel genes are characterized in model plant to perform multiple functions ranging from nutrient uptake assimilation and stomatal regulation by efficiently transporting NO3- /H+ and Cl- in response to biotic and abiotic stress conditions. We have done homology-based genome wide analysis and identified 23 chloride channel genes in wheat and 12 chloride channels genes in barley, based on maximum likelihood grouped into four clades.

Tissue specific gene expression analysis for Chloride Channel B gene in tolerant and susceptible wheat varieties showed its higher expression in aerial tissues, in response to heat and drought stress. There is a high confidence that Chloride Channels in wheat and barley are responsible for influx and efflux of the anions in and out of the vacuoles in the result of phosphorylation and dephosphorylation effectively regulating the opening and closing of stomata like in other species as orthologs shares high sequence similarities.

With the more genomes are now being published, enabling comparative genomic analysis for stomatal evolution and regulation under drought and heat stress conditions focusing Chloride Channels. Wild relatives of wheat and barley have evolved over time and are rich genetic resources for improving tolerance in commercially available cultivars.

Chloride channel B is our prime gene for functional studies using knockout mutation by utilising CRISPR Cas9 system and over expression mutants. To find suitable commercial candidate variety we have screened more than 300 wheat genotypes in greenhouse and field settings for their tolerance to heat and drought conditions using low-cost screening methodology which includes physiological measurements, stomatal imaging, stomatal gas exchange, biochemical analysis, and tissue specific gene expression analysis.

Chloride Channels are involved in complex plant tolerance mechanism, whereas in plants studies investigating Chloride Channels were limited to the model plants we have designed this study to elucidate the roles of Chloride Channel B in stomatal regulations of wheat and barley under drought and heat stress along with molecular and physiological networks involved in tolerance to heat and drought stress.

Key Words: Chloride Channel B, Wheat, Barley, Stomatal regulation, CRISPR Cas9, Over expression, Drought, and Heat stress.

#221

NPF2.12 acts as a nitrate transceptor homolog that coordinates root growth and nitrogen use efficiency in wheat

Md. Nurealam Siddiqui[1], Jens Léon1, Gabriel Schaaf[2], Michael Schneider[1,3], Grace Achieng Ochieng[1], Luc Gujer[1], Shalom Christopher[1], Annaliese Mason[1], Agim Ballvora[1*]

[1] Plant Breeding Department, Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Kirschallee 1, 53115 Bonn, Germany. [2] Plant Nutrition Department, Institute of Crop Science and Resource Conservation (INRES), University of Bonn, Karlrobert-Kreiten-Strasse 13, 53115 Bonn, Germany. [3] Department of Crop Sciences – Plant Breeding Research Institute of Organic Agriculture Ackerstrasse 113, 5070 Frick, Switzerland

[*] ballvora@uni-bonn.de

Breeding crop varieties with improved nitrogen use efficiency (NUE) is critically important for minimizing nutrient losses as well as reducing the costs related to excess fertilizer application. Achieving efficient N-uptake and transport relies on a beneficial root system architecture, but the genetic and molecular mechanisms underlying NUE are not yet well-explored in wheat.

We performed genome-wide association analyses using a genetically diverse panel of winter wheat to analyze root trait phenotypes under extreme N-deprivation levels in the field and under high N condition. We identified several marker-trait associations for root architecture traits with underlying candidate genes involved in N transport and metabolism. One of these genes, NPF2.12, shares homology with Arabidopsis nitrate transceptors. The expression of a rare regulatory-element-based natural allele of NPF2.12 was associated with significantly enhanced root growth and root-to-shoot nitrogen translocation at low N availability.

In agreement with this, under limited N conditions the NPF2.12 allele shows reduced expression and lines carrying this allele display increased NITRATE REDUCTASE 1 (NIA1) expression that possibly leads – through increased NR-mediated NO biosynthesis - to root growth and root-to-shoot N translocation. We intend to increase the variability at the NPF2.12 locus by searching for natural allelic variants and by developing various alleles via genome-editing approaches that will both permit to analyse the regulatory mechanisms and select more efficient allelic variants.

Similar findings were observed in barley. Our study provides a better understanding of the molecular mechanisms underlying the process of nitrate sensing and modulation through root developmental adjustments of nitrogen uptake and translocation in cereals, and suggests genes which can be exploited to improve root growth and NUE under low N availability.

#449

Genome wide association study identifies multiple qtl in spring wheat germplasm of nordic-baltic origin

Gintaras Brazauskas[1], Mara Bleidere[2], Hannes Kollist[3], Morten Lillemo[4], Ilmar Tamm[5]

[1] Lithuanian Research Centre for Agriculture and Forestry, Instituto av. 1, 58344 Akademija, Lithuania. [2] Institute of Agricultural Resources and Economics, Zinātnes Iela 2, Cēsis County 4126, Latvia. [3] Institute of Bioengineering, University of Tartu, Nooruse 1, 50411 Tartu, Estonia. [4] Department of Plant Sciences, Norwegian University of Life Sciences, Post Box 5003, NO-1432 ÅS, Norway. [5] Centre of Estonian Rural Research and Knowledge, J. Aamisepa 1, Jõgeva Alevik, 48309 Jõgeva Maakond, Estonia

European wheat yields have recently stagnated in major production areas due to abiotic and biotic stresses caused by climate change forcing future wheat production to expand towards the Northern regions, therefore breeding climate-resilient wheat varieties is an important research task for the Baltic and Nordic countries to ensure safe and sustainable food systems.

The NOBAL wheat project established a spring wheat collection of 300 genotypes originating from Baltic states and Norway and tested its genetic plasticity and adaptation capacity in 12 diverse environments (3 seasons in 4 countries). The collection was genotyped with a 25K SNP array yielding 18,467 high-quality genetic markers to perform genome-wide association study (GWAS). Analysis revealed 18 consistent QTL for plant height, grain protein content, thousand kernel weight and plant earliness.

GWAS was further conducted on the disease data, and an in-depth analysis was done on the powdery mildew resistance, identifying a highly significant QTL on chromosome 3A providing race non-specific adult plant resistance under Nordic-Baltic field conditions. This QTL was further validated on an independent set of Norwegian spring wheat breeding lines and confirmed its promising use in resistance breeding.

Furthermore, unmanned aerial vehicles (UAVs) were deployed weekly to facilitate fast acquisition of high-quality phenotypic data. Finally, superior genotypes identified in this study will be directly utilized in the breeding process of the climate-fit wheat varieties to secure yields and contribute to sustainable food production in the Nordic-Baltic region.

#83

Revealing paired NLRs driving yr84-mediated wheat stripe rust resistance

Valentyna Klymiuk[1], Krystalee Wiebe[1], Harmeet Singh Chawla[1], Jennifer Ens[1], Rajagopal Subramaniam[2], Curtis J Pozniak[1]

[1] Crop Development Centre and Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK S7N 5A8, Canada. [2] Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada.

valentyna.klymiuk@usask.ca, k.wiebe@usask.ca, Harmeet.Chawla@umanitoba.ca, jennifer.ens@usask.ca, rajagopal.subramaniam@AGR.GC.CA, curtis.pozniak@usask.ca

Rapid cloning and functional characterization of genes controlling important agronomic traits in polyploid wheat are facilitated by recent technological innovations. We have applied a combination of bulk-segregant analysis sequencing (BSA-Seq), fine mapping, PacBio genome sequencing, long-read amplicon sequencing and EMS-mutagenesis to reveal genes underlying Yr84-mediated stripe rust resistance. We have confirmed that the expression of resistance requires two nucleotide-binding and leucine-rich repeat genes (NLRs): CC-NB-ARC-LRR (CNL) and NB-ARC-LRR (NL).

In NLR pairs, one protein, designated as the sensor NLR (sNLR), detects the presence of the pathogen, while the other, known as the helper NLR (hNLR), transmits the signal to downstream cascades. In the case of Yr84, expression studies revealed that only the NL was upregulated in the presence of the pathogen, whereas CNL exhibited constitutive expression.

These results are consistent with expression of paired NLRs in rice and Arabidopsis, where the hNLR is upregulated while the sNLR is constitutively expressed. Therefore, we hypothesize that in the Yr84 NLR pair, CNL functions as the sNLR, while NL serves as the hNLR. Although these NLRs are physically linked on chromosome arm 1BS, they exhibit structural and evolutionary divergence. Several susceptible alleles have been identified in natural populations of wild emmer wheat for both genes, whereas resistance alleles were rarely detected.

An analysis of predicted CNL and NL protein structures revealed that both proteins contain conserved amino acid motifs in the NB-ARC and LRR domains, with conservation being more pronounced for the CNL compared to the NL protein. An analysis of the interactions within the Yr84 NLR pair using the yeast two-hybrid system did not confirm the general trend of direct homo- or hetero-interaction between the sNLR and hNLR observed in other pathosystems.

However, we are in the process of verifying this finding through co-immunoprecipitation experiments (co-IP) conducted using Tobacco infiltration experiments. We have developed a Yr84 functional molecular marker, located within the CNL gene, which has been utilized for introgressions into multiple durum and bread wheat genetic backgrounds, including those important for breeding programs and genetic/pathogenicity studies.

#332

Indian wheat initiatives to harness the full potential of genetic resources for developing climate-resilient cultivars

Sundeep Kumar[1]*, Sherry R. Jacob[1], Reyazul Rouf Mir[2], V. K. Vikas[3], Pawan Kulwal[4], Satinder Kaur[5], Pradeep Bhati[6], Divya Sharma[1], Shailendra Sharma[7], S.S. Gaurav[7], Ravinder Singh[8], J. B. Singh[9], Sandeep Sharma[10], V.K. Mishra[10], Anju Mahendru Singh[1], Sanjay Kumar Singh[11], Shailendra Kumar Jha[11], Renu Pandey[11], Sindhu Sareen[12], Arun Gupta[12], Arvind Kumar[13], Vijay Rana[14], Ashwani Kumar Basandrai[14], Daisy Basandrai[14], Dinesh Kumar[15], Manoj Prasad[16], Rakesh Bharadwaj[1], Jyoti Kumari[1], Amit Kumar Singh[1], M. S. Saharan[11], Monika Garg[17], S.C. Bharadwaj[18], Om Prakash Gangwar[18], Kuldeep Singh[1&19] and G.P. Singh[1]

[1]Indian Council of Agricultural Research-National Bureau of Plant Genetic Resources, New Delhi, India; [2]Division of Genetics and Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (SKUAST-Kashmir), Jammu and Kashmir, India; [3]ICAR-Indian Agricultural Research Institute, RS, Wellington, Tamilnadu, India; [4]State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri, India; [5]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, India; [6]Borlaug Institute for South Asia, Ludhiana, India; [7]CCS University, Meerut, India; [8]School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu (SKUAST-Jammu), Jammu and Kashmir, India; [9]ICAR-Indian Agriculture Research Institute Regional, Indore, India; [10]BHU, Varanasi, India; [11]ICAR-Indian Agricultural Research Institute, New Delhi, India; [12]ICAR-Indian Institute of Wheat and Barley Research, Karnal, India; [13] ICAR-CSSRI, Karnal, India; [14]CSKHPKV, Palampur, India; [15]ICAR-IASRI, New Delhi, India; [16]NIPGR, New Delhi, India; [17]National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab, India; [18]ICAR-Indian Institute of Wheat and Barley Research, Rs, Shimla, India; [19]*ICRISAT*. Patancheru, Hyderabad

Corresponding author e-mail address: Sundeep.Kumar@icar.gov.in

Wheat is crucial for India's food security, but its growth is hampered by various stresses, leading to low-quality grains and yield. To address this, an Indian wheat genomics initiative was taken to explore the genetic diversity stored in the National Gene bank of India. The National Gene bank of India houses a rich collection of wheat germplasm, including landraces, cultivars, and lines from around the world.

However, breeding stress-tolerant wheat varieties has been challenging due to genetic and environmental complexities, lack of resistant germplasm, and reliable markers. Therefore, a pioneering effort was made to incorporate innovative breeding technologies, including genome-wide association studies (GWAS) and genomic selection (GS), which consider genotype-environment interactions, can advance crop improvement by enhancing climatic resilience, integrating biotic stress resistance, and maximizing yield potential.

A set of around 7000 wheat germplasm accessions were evaluated for biotic stresses (leaf rust, stem rust, stripe rust, Karnal bunt, Fusarium head blight, spot blotch, powdery mildew, and Alternaria blight), abiotic stresses (drought, heat, salinity, and cold tolerance) and quality traits at 19 different institutions from four different wheat growing zones of India (NHZ, NWPZ, NEPZ, CZ, and PZ).

Several promising germplasm lines for different biotic and abiotic and quality traits were identified based on the preliminary evaluation. For example, IC406690 (29.1%) was identified for high amylose content. Similarly, IC15048, IC16124, IC14102 (≥16%) for high protein content, IC598274, IC336494 for high Iron content (>60mg/kg) and IC252830 and IC296727 for high Zinc content (55mg/kg) were identified.

In addition, lines having high fructan content ≥ 2.0% i.e. IC19015, IC25060, IC41056, IC11057, IC20005 and low fructan content (0.51-0.68%) IC532434, IC524220, IC0534077, IC138559, IC549410 were also identified. These accessions were genotyped using 35K Axiom array and this genotypic data along with the phenotypic data is being used for Genome-wide association studies (GWAS) of various biotic traits (Rust, Spot Blotch and Powdery mildew) and abiotic traits (heat tolerance).

We have identified novel QTLs for spot blotch, leaf rust, stripe rust and powdery mildew resistance in wheat which will be discussed during the presentation. Additionally, we are extensively assessing this large germplasm set to identify promising QTLs for wheat quality traits.

Thus, the Indian wheat germplasm genomics initiative aims to establish a complete process that links the assessment of germplasm with genomic data. This will enable faster integration of native wheat germplasm into national breeding efforts, focusing on enhancing traits related to pests, environmental stresses, and grain quality.

#203

Exploring novel beneficial alleles in primary synthetics for improving us winter wheat yield and quality

Zhen Wang[1,5], Kyle Parker[1,5] Zhiwu Zhang[2], Eduard Akhunov[3], Paul St Amend[4], Guihua Bai[4], Qingwu Xue[5], Jackie C. Rudd[5], Amir M. H. Ibrahim[1], Dirk B. Hays[1], Shannon Baker[5], Jason Baker[5], Geraldine B. Opena[1], Bryan E. Simoneaux[1], Russell Sutton[1], Shuyu Liu[1,5]

[1] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843, United States of America. [2] Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164, United States of America. [3] Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, United States of America. [4] USDA-ARS, Hard Winter Wheat Genetics Research Unit, Manhattan, KS 66506, United States of America. [5] Texas A&M AgriLife Research, 6500 Amarillo Blvd W, Amarillo, TX 79106, United States of America.

Primary synthetic is one of the major resources that has been utilized in spring wheat breeding worldwide to improve drought tolerance and pest resistance but little research has been conducted in winter wheat backgrounds. Texas A&M Wheat breeding and genetic programs have utilized synthetic hexaploid wheat to introduce novel alleles from various tetraploid durum wheat (*Triticum* turgidum L.) and diploid tauschii (Aegilops tauschii L.) into the hard red winter wheat for more than 20 years.

In this study, two adapted popular wheat cultivars TAM 111 and TAM 112 were designated as recurrent parents and backcrossed with 21 synthetic hexaploid wheat lines, then those derived lines were advanced for five generations to generate a set of 298 $BC_1F_{5:}7$ synthetic derived wheat lines (SDLs) as a multi-reference advanced-backcross nested association mapping population (MR-AB-NAM).

Took advantages on the pedigrees and population structure, genotyping-by-sequencing (GBS) and skim-seq were used to impute a large set of 28 million single nucleotide polymorphisms (SNPs) and insertion deletions (Indels) from parental whole genome sequencing (WGS) data. Yield and quality traits of the population was evaluated in 13 environments across Texas for genome-wide association analyses to identify significant marker-trait associations and performing genomic selections.

These developed SDLs provided novel genetic resources and broadened the winter bread wheat gene pool. Significant SNPs linked to target beneficial alleles were used to explore the efficient utilization of synthetic wheat in breeding programs. In addition, genomic prediction with multiple traits/environments/models provided in-depth knowledge for the breeding application of SHW.

#183

Exploring the prevalence of kinase fusion proteins as a source of disease resistance

Oliver R. Powell, Renjie Chen, Brande B. H. Wulff

King Abdullah University of Science and Technology, Biological and Environmental Science and Engineering Division, Thuwal, Saudi Arabia

oliver.powell@kaust.edu.sa, renjie.chen@kaust.edu.sa, brande.wulff@kaust.edu.sa

More than half of all cloned resistance (R) genes encode nucleotide-binding, leucine-rich repeat (NLR) intracellular immune receptors. This effort has been greatly assisted by the development of tools capable of annotating NLR loci across the genome.

However, in wheat and barley, 20% of cloned R genes encode kinase-fusion proteins (KFPs). Recently, we cloned a genetic interactor required for the function of a KFP providing mechanistic insights into the KFP immune response.

Based on this discovery, we are developing genome-wide scans to identify KFPs with their candidate signalling partners. This will allow us to quantify the prevalence of KFPs in plant immunity. The best candidates from this analysis will be selected for further analysis including in silico effector discovery and in vitro functional characterisation.

Through this work we hope to provide mechanistic insight into the emerging class of KFP immune receptors.

#402

A direct guide to stem rust resistance integration into modern european elite varieties

Philipp Schulz[1], Sophie Bouchet[2], Mehran Patpour[3], Jessica Schunke[1], Susan Bergmann[1], Anne-Kristin Schmitt[1], Jens Hansen[3], Poul Lassen[3], Hans Pinnschmidt[4], Mogens Hovmøller[3], Tiphaine Vidal[5], Biagio Randazzo[6], Kerstin Flath[1]

[1] Federal Research Centre for cultivated plants, Julius Kühn-Institut (JKI), Kleinmachnow, Germany. [2] INRAE-Université Clermont-Auvergne, UMR1095, Génétique Diversité Ecophysiologie des Céréales, Clermont-Ferrand, France. [3] Department of Agroecology, Aarhus University, Slagelse, Denmark. [4] Department of Medical Biometry and Epidemiology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. [5] Université Paris-Saclay, INRAE, UR BIOGER, 78850 Thiverval-Grignon, France. [6] AS.A.R. Società Semplice Agricola Randazzo, Sicily, Italy

Philipp.Schulz@julius-kuehn.de

Wheat stem rust is a critical disease that is currently spreading into new areas within Europe, while at the same time developing new virulence around the globe. Control of the disease relies either on pesticides or on varietal resistance, the later unfortunately being under high pressure from newly developing rust races. Currently marketed varieties are largely unprotected even against non-*Ug99* related races.

However, introgression of new resistant sources, usually from secondary or even tertiary gene pool is a timely and laborious task. The success of such process also strongly depends on the race(s) used for selection, which may not necessarily represent the current native race situations in target regions.

To identify direct usable sources, we screened 263 European elite lines in a 4-year (2019-2022), two locations trial under natural and artificial infection conditions with natural present isolates in southern and central Europe. Additionally, we used different molecular markers and seedling resistance based gene postulations finally to deliver validated directly usable resistances in elite material.

Noteworthy, only 10 out of the 263 tested varieties displayed a high and durable level of resistance across the four years and both locations. The multiple level analysis of those clearly show that Sr24 and Sr31 are the major resistance drivers in European cultivars, with some support on field level by Sr38. Our study provides a directly usable source to fortify European elite cultivars against the currently present Pgt isolates.

#219

Disease resistance modifiers: the hidden genetic background of leaf rust resistance in wheat

Lamia Aouini[1], Yajun Wang[1,2], Emile Cavalet-Giorsa[1], Michael Abrouk[1] , Esther Jung[3], Beat Keller[3], Simon G. Krattinger[1]

[1] Plant Science Program, Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia. [2] National Key Laboratory of Plant Molecular Genetics, Center for Excellence in Molecular Plant Sciences, Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, Shanghai 200032, China. [3] University of Zurich, Department of Plant and Microbial Biology, Zollikerstrasse 107, 8008, Zurich, Switzerland

Emails: lamia.aouini@kaust.edu.sa, yjwang@cemps.ac.cn, emile.cavaletgiorsa@kaust.edu.sa, michael.abrouk@kaust.edu.sa, esther.jung@botinst.uzh.ch, bkeller@botinst.uzh.ch, simon.krattinger@kaust.edu.sa

The effective deployment of disease resistance (*R*) genes is one of the most important topics in wheat disease resistance breeding. Many R genes are influenced by genetic backgrounds. Although ‘genetic background effects’ are well documented in literature, the underlying genetic and molecular mechanisms are only poorly understood.

Our study focusses on the leaf rust resistance gene Lr14a that encodes an unusual protein consisting of twelve N-terminal ankyrin repeats and a C-terminal transmembrane domain. Noticeably, Lr14a is strongly influenced by the genetic background. Certain wheat cultivars were fully susceptible to leaf rust despite the presence of Lr14a.

Here, we used two contrasting, Lr14a-containing near-isogenic wheat lines, ArinaLr14a and ArinaLrFor, which differed in their responses to leaf rust at seedling stage. While ArinaLrFor exhibited the characteristic mesothetic Lr14a-type of resistance at seedling stage, ArinaLr14a was fully susceptible. Segregation analysis in an ‘ArinaLr14a X ArinaLrFor’ mapping population indicated the segregation of a single modifier gene that was mapped to chromosome arm 1BS.

Interestingly, the identified 1BS genomic region overlapped with the previously reported Lr75 gene. Lr75 was identified as a minor adult plant resistance gene in ArinaLrFor. Our results indicate that the Lr14a seedling modifier and the Lr75 adult plant resistance gene might be identical. Our research highlights the importance of disease resistance modifiers and provides the basis to fine-tune leaf rust resistance in the future.

#371

Enrichment and diversification of the wheat genome by genomics-enabled chromosome engineering

Xiwen Cai[1,2], Tatiana Danilova[1], Ahmed Charif[2], Fang Wang[2], Gang Li[1], Satyanarayana Tatineni[1,3], Shaobin Zhong[4], Zhaohui Liu[4], Katherine Frels[2], Jeffrey Boehm Jr.[1,2], Steven Xu[5]

[1] USDA-ARS, Wheat, Sorghum and Forage Research Unit, Lincoln, NE 68583, USA. [2] Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE 68583, USA. [3] Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE 68503, USA. [4] Department of Plant Pathology, North Dakota State University, Fargo, ND 58108, USA. [5] USDA-ARS, Crop Improvement and Genetics Research Unit, Western Regional Research Center, Albany, CA 94710, USA

The allopolyploid origin of wheat led to narrow genetic variation of its genome, which has been increasingly becoming a genetic bottleneck of wheat improvement especially under the ever-changing climate. There is an urgent need to extend the genetic variability of wheat and unblock the bottleneck in wheat breeding. Tremendous genetic variation was left behind in lineages of wheat, which remains in its ancestors.

In addition, wheat has numerous relatives that contain genomes homologous or homoeologous to the wheat genome. Both ancestors and relatives represent an invaluable gene reservoir to extend the genetic variability of wheat. Recently, we developed an effective genomics-enabled chromosome engineering pipeline for alien introgression and genome study in wheat and its relatives.

Here we report the meiotic homoeologous recombination-based genome study and alien introgression from Thinopyrum elongatum, Th. ponticum, Th. intermedium, Aegilops speltoides, and Secale cereale into durum and common wheat. Hundreds of meiotic homoeologous recombinants of wheat B genome with Ae. speltoides S genome and Th. elongatum E genome have been developed using wheat ph1b mutant.

They have been used in genome mapping and gene discovery in wheat and its relatives. Several genes for resistance to Fusarium head blight (FHB), tan spot, Septoria nodorum blotch, and stem rust diseases have been integrated into the wheat genome as small translocations. A novel FHB-resistant Fhb7 allele, designated $Fhb7^{The2}$, was identified on Th. elongatum chromosome 7E and transferred to wheat chromosome 7B through a 7B-7E translocation (7BS·7BL-7EL). $Fhb7^{The2}$ has exhibited significant Type II FHB resistance and may detoxify Fusarium-produced mycotoxin deoxynivalenol (DON).

The wheat germplasm (PI 702949) containing $Fhb7^{The2}$ has been released and distributed to the wheat research community worldwide. Meanwhile, we have been deploying $Fhb7^{The2}$ into different classes of US wheats for FHB-resistant variety development using a marker-assisted backcross breeding pipeline.
Furthermore, we have identified high-level resistance to wheat streak mosaic virus (WSMV) from wheat-Th. ponticum/Th. intermedium derivatives as well as resistance to bacterial leaf streak (BLS) disease from rye (Secale cereale). Individual Thinopyrum and rye chromosomes containing the resistance genes will be identified by dissecting the alien genome of the derivatives.

Also, we are generating wheat-Thinopyrum/rye recombinants to transfer the resistance genes and potentially other genes for favorable agronomic traits into wheat using the *ph1b* mutant. In summary, meiotic homoeologous recombination-based chromosome engineering is an effective approach to expand the genetic variability of wheat and facilitate understanding of the complex genomes in wheat and its relatives.

#426

Determination of genomic regions associated with stem solidness and tolerance against wheat stem sawfly cutting

Richard D. Cuthbert[1], Firdissa Bokore[1], Ron Knox[2], Brad Meyer[1], Kirby T. Nilsen[3], Curtis J. Pozniak[4], Yuefeng Ruan[1], Samia Berraies[1], Jatinder Sangha[1]

[1] Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, Swift Current, SK, Canada. [2] Retired, Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, Swift Current, SK, Canada. [3] Brandon Research and Development Centre, Agriculture and Agri-Food Canada, Brandon, MB, Canada. [4] Crop Development Centre and Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada

Correspondence: richard.cuthbert@agr.gc.ca; firdissa.bokore@agr.gc.ca
Presenting: Richard Cuthbert

Wheat stem sawfly (Cephus cinctus Nort.) (WSS) is a destructive insect pest of spring wheat (Triticum aestivum L.) in Western Canada. Growing solid stemmed wheat cultivars to prevent insect larvae from growing inside the wheat stem is an important management strategy. Developing wheat varieties with stable stem solidness in WSS prone environments is a major breeding objective.

This study sought to (1) identify and map genes/QTL associated with stem solidness in the wheat doubled haploid populations of CDC_Hughes/AAC_Concord and Lillian/Glenlea and (2) identify QTL associated with WSS cutting tolerance in AAC_Hodge/BW5087 and BW5087/AAC_Stoughton both breeding populations fixed for major stem solidness QTL. CDC_Hughes/AAC_Concord (n=188 lines) and Lillian/Glenlea (n=190) were rated for stem solidness at maturity at a specialized WSS nursery near Swift Current, SK for three years. CDC_Hughes/AAC_Concord was additionally assessed for the stem solidness near Leeston, New Zealand for one year.

The populations AAC_Hodge/BW5087 (n=146 lines) and BW5087/AAC_Stoughton (n=125), were developed by simultaneously applying two DNA markers predictive of major solidness QTL located on chromosome 3B (Sst1) and 3D (3Ag14) on 2,000 F$_2$ plants and selecting plants homozygous at both loci.

Selected F$_{3:5}$ progeny were assessed for stem cutting near Pense, SK in 2023. The CDC_Hughes/AAC_Concord and Lillian/Glenlea populations were genotyped using the 90K Infinium iSelect SNP array. The AAC_Hodge/BW5087 and BW5087/AAC_Stoughton populations were genotyped by TraitGenetics using 7K LD Wheat array. Genetic linkage maps were constructed and a QTL analysis was completed.

The QTL analysis revealed five genomic regions associated with stem solidness in Lillian/Glenlea population, and four loci associated with the trait in CDC_Hughes/AAC_Concord population. Two Lillian derived QTL on chromosomes 2D and 3B were consistent across all the environments. AAC Concord contributed a significant QTL that was consistent across environments on chromosome 3D and CDC Hughes was found to possess a QTL on 5A.

The genes/QTL identified in both populations could synergistically enhance the expression of Sst1 (3B) and 3Ag14 (3D) loci. The analysis with AAC_Hodge/BW5087 and BW5087/AAC_Stoughton crosses revealed several QTL controlling resistance to stem toppling. Five loci were associated with tolerance to cutting in AAC_Hodge/BW5087, whereas fourteen QTL were detected in BW5087/AAC_Stoughton at Pense.

Two QTL were identified on chromosome 4B from AAC Hodge and AAC Stoughton as well as 6A from BW5087 in both crosses and shared similar physical locations. Results of this study could help breeders determine important minor loci that significantly reduce stem toppling in wheat.

#165

Breeding for wheat germplasm with improved resistance to fusarium head blight at CIMMYT

Xinyao He*, Susanne Dreisigacker, Velu Govindan, Guillermo Gerard, Pawan K. Singh

International Maize and Wheat Improvement Center, Texcoco, Mexico

Email: x.he@cgiar.org

Fusarium head blight (FHB), also called head scab, is one of the most challenging wheat diseases globally, severely affecting wheat production in warm and humid regions in East Asia, Europe, North America, Latin America, and African countries. The disease not only reduces wheat grain yield but causes mycotoxins contamination in the grain that are harmful to human and animal consumers.

Breeding for wheat germplasm resistant to FHB and its associated mycotoxin deoxynivalenol (DON) is an important component of wheat improvement at CIMMYT. Since the 1980s, CIMMYT has integrated a broad spectrum of resistant sources into its breeding program, especially introductions from China and Brazil, as well as wild relatives of wheat. Newly developed advanced breeding lines are routinely screened for FHB resistance and the results are used as an important criterion for selection.

Promising lines identified from breeding materials, germplasm bank collections, and introductions, are included in special nurseries called FHB Screening Nursery (FHBSN). These selected lines must undergo at least three years of field and greenhouse evaluation before they are distributed globally for potential use by national breeders. CIMMYT lines generally do not have very high FHB resistance, with the best ones exhibiting moderate/good resistance.

Genetic studies and haplotyping work indicated very low frequencies of Fhb1 and other major QTL, except for one on 2DL chromosome that has shown frequencies of 10-20% in recent breeding nurseries. Efforts on further increasing the frequency of Fhb1 and its combination with the 2DL QTL are ongoing, and some of such lines exhibited good resistance in field experiments.

Introduction of additional sources of resistance including the Fhb7 resistance gene is in progress, and their utilization in breeding is expected to further improve the FHB resistance of CIMMYT germplasm, which may in turn contribute to FHB resistance in African countries, where CIMMYT germplasm has been extensively utilized. Thematic area: Enhancing wheat resistance against biotic stresses.

#124

Identification of marker genes for drought stress resistance in wheat through δ13 c/12 c isotope discrimination

Sepideh Jafarian[1,2,3], Manuel Geyer[1], Franz Bügger [2], Klaus F.X. Mayer[2], Nadia Kamal[2,3], Jörg-Peter Schnitzler[2], Manuel Spannagl[2], Lorenz Hartl[1]

[1] LfL, Bavarian Institute for Agriculture, Institute for Crop Science and Plant Breeding, Freising 85354, Germany.
[2] Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg 85764, Germany.
[3] Technical University of Munich, School of life science, Freising 85354, Germany.

Contacts: Sepideh.jafarian@helmholtz-munich.de

Wheat farming, dating back millennia, prioritizes yield and disease resilience. Climate change now endangers winter wheat stability, emphasizing “drought resistance”. This trait varies among wheat varieties and populations, depending on intrinsic and extrinsic factors. Identifying key genes and molecular factors is crucial for marker availability and breeding resilience.

The Dry-Wheat project utilizes the Bavarian Magic Wheat population (BMW) to study drought stress resistance diversity and pinpoint associated genes and markers in modern bread wheat (Triticum aestivum) varieties. The project employs $\delta^{13}C/^{12}C$ isotope composition as a physiological measure of intrinsic water use efficiency (iWUE) to identify wheat lines that are more resistant to drought stress. This project systematically investigates the variability in drought resistance of the relevant elite breeding material.

In this experiment, ten extreme genotypes selected from nearly four hundred lines based on their $\delta^{13}C/^{12}C$, divided into high and low groups. Extensive phenotyping trials, consisting of 20 replications per genotype and two conditions of drought stress and control, were carried out at the Helmholtz Center. Relevant parameters such as dry biomass, plant height, leaf area etc. were systematically determined both automatically (using Phenospex system) and manually, and then compared. In the anthesis stage, flag leaves were collected for RNA sequencing. Statistically significant differences for desired traits were observed between the two conditions both for different genotypes as well as for the $\delta^{13}C$ groups.

We performed a differential gene expression analysis (DEG) to identify candidate genes in our two experimental conditions. We observed pronounced differences between the two $\delta^{13}C$ groups under drought conditions, where the number of up and down regulated genes were found significantly higher in the group of low $\delta^{13}C$ s.

In a next step, these expression data will be analyzed in correlation with the results from the phenotyping experiments and the isotope discrimination measurements.

This project is financed by the Bavarian State Ministry of the Environment and Consumer Protection in the frame of the project network BayKlimaFit 2.

#450

Additional genotyping with breedwheat-410k-array evidenced new loci associated to nitrogen allocation to grain storage proteins

Naoto SANO[1], Anne Plessis[1,2], Catherine Ravel[1], Pierre Martre[3], Jacques Le Gouis[1]

[1]Université Clermont Auvergne, INRAE, UMR1095 GDEC, 63000 Clermont-Ferrand, France. [2]School of Biological and Marine Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK. 3 LEPSE, Univ Montpellier, INRAE, Institut Agro Montpellier, 34060 Montpellier, France.

Email: naoto.sano@inrae.fr

The content and composition of glutenin and gliadin, the two major grain storage proteins (GSPs) of wheat, are the main determinants of the end-use value of bread wheat (*Triticum aestivum* L.) grain. We previously performed a genetic association study for allocation levels of grain nitrogen (N) to GSP classes/subunits by using an allometric scaling method with a worldwide core collection of 196 bread wheats cultivated in three environments.

Besides structural GSP genes, several loci putatively trans-regulating GSPs such as transcriptional factors were detected as the candidate loci, although the number of markers used in the study was limited (Plessis et al. 2013).

Here, we conducted a genome-wide association study (GWAS) on the previous phenotypic data sets with 250,129 SNPs additionally obtained by the BreedWheat 410K genotyping array, aiming to more comprehensive detection of relevant loci for quantity of GSP classes/subunits and grain N allocation. In the analysis of phenotypic data sets, Pearson correlation test and principal component analysis (PCA) on quantity of GSPs and grain N allocations revealed that glutenin and gliadin contents as well as the N partitioning among these proteins were not significantly correlated, suggesting that allocation factors are different between these major GSPs.

On the other hand, grain N allocations to high- and low- molecular weight glutenin subunits demonstrated a significant negative correlation, indicating that some common molecular mechanism underlies the nitrogen partitioning between these subunits.

In addition to a GWAS based on the allometric scaling parameters, a strategy of using PC-scores as dependent variables for the GWAS (PCA-based GWAS) on quantity of GSP classes/subunits and grain N allocation was applied expecting to extract key information from phenotypically these complex traits.
The effectiveness of these methods in revealing the factors responsible for N allocation in wheat grain and the newly detected associated loci will be discussed.

#235

Combined multi-omics and physiological studies to elucidate drought-response mechanisms in durum wheat

Osvin Arriagada[1], Claudio Meneses[2], Romina Pedreschi[3], Gerardo Núñez-Lillo[3], Patricia Cabas-Lühmann[4], Carlos Maureira[4], Samantha Reveco[4], Valentina Villarroel[4], Christian Alfaro[5], Iván Matus[6], and Andrés R. Schwember[4*]

[1]Centro de Estudios en Alimentos Procesados (CEAP), Campus Lircay, Talca 3480094, Chile
[2]Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago 8331150, Chile
[3]Escuela de Agronomía, Facultad de Ciencias Agronómicas y de los Alimentos, Pontificia Universidad Católica de Valparaíso, Quillota 2260000, Chile
[4]Departamento de Ciencias Vegetales, Facultad de Agronomía y Sistemas Naturales, Pontificia Universidad Católica de Chile, Santiago 7820436, Chile
[5]Centro Regional Investigación Rayentué, Instituto de Investigaciones Agropecuarias (INIA), Rengo, Chile
[6]Instituto de Investigaciones Agropecuarias INIA-Quilamapu, Programa de Mejoramiento Genético de trigo, Avenida Vicente Méndez 515, 3800062, Chillán, Chile

*aschwember@uc.cl

Durum wheat is a fundamental cereal in the Mediterranean region. It provides more than 20% of the nutritional demands in the form of pasta and other semolina-based products for human consumption. Plants have developed different mechanisms to cope with drought stress since water deficit affects the durum grain yields, causing farmers losses of up to 50%.

Therefore, the objectives of this study were to evaluate the yield components on a panel of 225 elite durum wheat genotypes under contrasting water regimes (optimal and water-deficit) and to understand the physiological, molecular, and metabolic aspects of the drought response in durum wheat as a basis for developing resilient and high-yielding cultivars to ensure food security.

The combined analyses of variance showed significant effects ($p < 0.05$) of genotypes (G), environments (E), and their interactions (G×E) for all traits, except for grains per spike (GPS) and spike per square meter (SPM), in which the G×E factor was not significant. The E effect explained most of the variation for yield (YD; 79.8%) and 1000-grains weight (TKW; 63.22%).

Moreover, the G×E interaction exhibited a more significant contribution to the total variability than the G effect for all traits except for TGW, which had a broad-sense heritability value of 0.882. Seven hundred seventy-two significant marker-trait associations (MTAs) distributed across the 14 chromosomes for five yield-related traits and along the 8 environments studied were identified. Among them, 9 MTAs were detected across all environments and were grouped into three QTLs according to linkage disequilibrium.

These QTL were associated with TGW and GPS and located on chromosomes 2A (*QTN_2A_TGW*/GPS.1; *QTN_2A_TGW*/GPS.2) and 2B (*QTN_2B_TGW*/GPS.1), comprising a total of 630 genes and explaining between a 5.15% and 14.29% of the phenotypic variation. Two contrasting genotypes to drought response, QUC 3678-2016 (drought-tolerant) and BRESCIA (drought-susceptible), were identified. RNA-seq analysis indicated that 697 and 900 genes were regulated in response to water deficit between these two contrasting genotypes in leaf and root, respectively, at 0-, 14-, and 30-days post anthesis (DPA).

The multi-omics integration analysis identified 30 genes and six metabolites in root and 30 genes and ten metabolites in leaf as the primary variable in the drought-tolerant genotype, confirming proline as an important compound involved in drought tolerance and opening an opportunity for the use of new biomarkers (i.e., associated with some specific carbohydrates and amino acids) in durum wheat breeding programs.

#255

Ectopic expression of *TA*EXPA6 and triple-mutant of *TA*GW2 increase resilience to higher temperature in wheat

Lucas Vicentin[1,2], Daniel F. Calderini[2,*]

[1] Graduate School, Faculty of Agricultural Science, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile
[2] Institute of Plant Production and Protection, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile

Wheat production faces challenges from the need to increase grain yield amid climate change. Additionally, a bottleneck has arisen due to the trade-off between grain weight (GW) and grain number (GN). The GW of wheat has been enhanced through both the ectopic expression of the TaExpA6 gene and a triple mutation in the TaGW2 gene. However, their impact on grain yield and quality traits under elevated temperatures remains largely unknown.

The aim of this study was to evaluate the response of lines carrying these genetic modifications to wheat traits under heat stress. A field experiment was conducted with four genotypes: a transgenic line expressing TaExpA6 in grains, its wild type (WT), a triple mutant of the TaGW2 gene, and its WT, across two thermal treatments: a control at ambient temperature and an increased temperature treatment (+4°C) from booting to 7 days post-anthesis.

The treatments were arranged in a split-plot block design with four replicates, assigning thermal treatments to main plots and genotypes to subplots. The experiment took place in field plots under optimal management, except for the increased temperature treatment. This temperature rise was achieved using polyethylene chambers equipped with heaters and automatic temperature controllers. Data were analysed using ANOVA. GW was enhanced ($P < 0.05$) in both transformed lines compared to their WTs in control conditions (TaExpA6: 10%; TaGW2: 24%) and under elevated temperatures (TaExpA6: 7%; TaGW2: 22%).

The response of GW in control and heated treatments was consistent across different grain positions within the spike. Treatment effects on GW were corroborated by tracking the time-course of individual grain weight and gene expression dynamics. As expected, the transgenic line showed no significant trade-off with GN ($P>0.05$), whereas the TaGW2 line did ($P<0.05$). Elevated temperatures adversely affected GN (6-8%) across all genotypes.

Interestingly, the negative impacts of heating on grain yield and GN observed in the WTs were mitigated in the transformed lines under the same thermal increase, demonstrating partial resilience in grain yield. This resilience was also observed in quality traits, such as the protein concentration in grains.

#142

Benefits of an obligate mutualist endophyte, *Epichloë*, in wheat

John R Caradus[1], Lisa Wood[1,3], David Hume[2], Alison Popay[2], Wade Mace[2]
[1] Grasslanz Technology Ltd, New Zealand
[2] AgResearch Ltd, New Zealand
[3] School of Agriculture, Food and Wine, University of Adelaide, Adelaide, Australia

john.caradus@grasslanz.com
lisa.wood@grasslanz.com
david.hume@agresearch.co.nz
alison.popay@agresearch.co.nz
wade.mace@agresearch.co.nz

The continued use of synthetic chemistry for the protection of crops against pests and diseases is becoming more difficult due to changes in regulations, societal concerns, and loss of effectiveness of some chemistry. New solutions are required. Over many years the use of microbial bioprotectants has been promoted and used with at times poor outcomes and inconsistent results.

These microbes have been free living and used as either a spray directly onto crops or as a seed coat. The efficacy of these bioprotectants has often been reliant on using appropriate formulations to ensure microbial viability to deliver effective protection of the crop, but again often with mixed outcomes. However, another class of microbes has been effectively used for over 20 years to provide protection of some temperate grass species to pests and pathogens and improve abiotic tolerance.

These fungal microbes are found in the genus *Epichloë*. As obligate mutualists these fungal endophytes are only found within the plant and are maternally inherited, so passed to the next generation of plants through seed. Modern wheat does not have these *Epichloë* endophytes, but wild grass relatives of modern cereals do. It has been possible using chromosome substitution and replacement lines of wheat to insert these *Epichloë* endophytes into wheat. Early results suggest significantly improved resistance to several pathogens and insect pests.

This has resulted in reduced infection by *Zymoseptoria tritici, Fusarium* head blight and *Puccinia triticina* leaf rust compared with equivalent endophyte-free wheat. Improved resistance to insect pests Argentine stem weevil, Hessian fly and wheat sheath miner have also been observed in field trials. Improved adaptation to some environmental challenges resulting from climate change may also be mitigated by these *Epichloë* endophytes.

There are some challenges related to genetic compatibility between the host plant and the endophyte, but these are being solved through breeding and will be described in another paper. Here the aim will be to highlight the benefits and value that could be derived from using *Epichloë* endophytes in modern wheats.

#143

Grain moisture content, dormancy, and environmental conditions interact to influence pre-harvest sprouting risk during grain maturation

Jeremy Curry[1,2]

[1]Dept. of Primary Industries and Regional Development, Esperance WA 6450
[2]The University of Western Australia, Perth WA 6009

Email: Jeremy.curry@dpird.wa.gov.au

Pre-harvest sprouting is a seed quality issue that occurs when grains germinate on the mother plant, usually in response to rainfall. In wheat, this precocious germination leads to the production of hydrolytic enzymes that reduce the grain's suitability for a range of food products, decreasing its value. Australia predominantly produces white wheat genotypes that exhibit low levels of grain dormancy, meaning that wet conditions near harvest can lead to significant levels of pre-harvest sprouting in some seasons.

The germination capability of grains changes significantly throughout the progression of seed development, maturation, and after-ripening. Along with these temporal changes, the environmental conditions that the mother plant is exposed to causes significant phenotypic plasticity. As a result, the incidence of pre-harvest sprouting in the field is the consequence of complex interactions between genotype, environment, and development stage that influence germination capability.

Field research conducted over multiple seasons in Western Australia has observed detrimental levels of pre-harvest sprouting occurring during the maturation drying phase (prior to harvest maturity). Increased levels of pre-harvest sprouting have been linked to cool and wet conditions that prolong the duration of grain drying, with elevated grain moisture promoting more rapid grain germination upon exposure to rainfall.

This transient period of increased risk during maturation drying challenges the idea that pre-harvest sprouting risk is simply determined by the decline in grain dormancy after physiological maturity and highlights the importance of the maturation drying phase in determination of the immediate and ongoing risk of germination.

The germination traits of seed populations from genotypes of contrasting dormancy levels produced under variable grain development and maturation conditions has been assessed to understand how grain moisture content, grain dormancy, and environmental conditions interact to determine the pre-harvest sprouting risk of wheat crops in the field. These results will be discussed in the context of trait improvement for pre-harvest sprouting under field conditions.

#492

Drought stress effect on wheat physiology and genes’ expression after anthesis

Jurica Duvnjak[1*], Hrvoje Sarcevic[2], Rosemary Vukovic[3], Valentina Spanic[1]

[1]Department for Breeding & Genetics of Small Cereal Crops, Agricultural Institute Osijek, Juzno predgradje 17, 31000 Osijek, Croatia
[2]Faculty of Agriculture, University of Zagreb, Svetosimunska cesta 25, 10000 Zagreb, Croatia
[3]Department of Biology, Josip Juraj Strossmayer University of Osijek, Cara Hadrijana 8a, 31000 Osijek, Croatia

Changing climatic conditions are becoming one of the major challenges facing agricultural production globally as demand for staple foods increases. Wheat is one of the most important foods consumed globally, grown in semi-arid and temperate environments where drought negatively affects wheat production.

This study was designed to identify and select drought-tolerant bread wheat genotypes using morpho-physiological and molecular approaches. The aim was to quantify the effects of two drought intensities by withholding watering for 45 and 65% of the volumetric soil moisture content (VSMC) for 14 days after anthesis to identify changes associated with drought tolerance. Increased malondialdehyde (MDA) accumulation resulted in extinguishing of chlorophyll that can be an indicator of stress. Drought tolerant genotypes tended to increase carotenoids that could play a vital role in resisting water shortage stress, while severe drought stress at 65% of VSMC induced a significant decrease of the chloroplyll a content in drought-sensitive genotype.

The expression of ascorbate peroxidase (APX) and glutathione S-transferases (GST) in response to drought was genotype-specific and dependent on drought intensity. Results suggested that oxidative stress was induced by drought, and that besides catalase (CAT), the enzymes of the AsA-GSH cycle (glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR)), appear to function as an important component of the antioxidative defence system under severe drought stress.

The results of gene expression of the wheat dehydrins (*DHN5* and *WZY2*) showed that at severe drought stress those two genes were highly expressed in drought-tolerant genotype (Bubnjar), compared to other genotypes. Compiling all data, it can be concluded that drought-tolerant and medium-tolerant wheat genotypes have better response to drought stress that is corresponding to decreasing oxidative injury by earlier induction of the antioxidant system, compared to drought-sensitive genotypes.

Wheat breeders should use these findings in selection by combining information from physiological, biochemical, molecular and morphological results that will help to identify the most drought-tolerant genotypes having the highest number of genes controlling drought tolerance.

#0

Temperature and photoperiod interaction: releasing aerial branching in wheat

Isabel Faci[1], Nikolai M. Adamski[1], Katie Long[1], Antony Dodd[1], Laura Dixon[2], Cristobal Uauy[1].
[1] John Innes Centre (JIC), Norwich Research Park, Norwich NR4 7UH, United Kingdom.
[2] University of Leeds, Woodhouse, Leeds LS2 9JT, United Kingdom.

Isabel.Faci-Gomez@jic.ac.uk

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

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

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

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

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

#488

Mining *AE. TAUSCHII* genetic diversity for heat stress resilience, seed dormancy and end-use quality

Yasir S. A. Gorafi[1,2], Izzat S. A. Tahir[2,3] and Hisashi Tsujimoto[3]

[1] Graduate School of Agriculture, Kyoto University, 606-8502, Japan
[2] Agricultural Research Corporation, P. O. Box 126, Wad Medani, Sudan
[3] Arid Land Research Center, Tottori University, Tottori 680-0001, Japan.
Email: seragalnor.yasir.5x@kyoto-u.ac.jp

Introducing genes from wild relatives is one of the best options to broaden the narrow genetic diversity and discover new alleles necessary for wheat improvement under the pressures of climate change and the growing world population. Among wheat wild relatives, Aegilops tauschii is believed to be an attractive and rich gene source; therefore, we developed a new strategy for fast and efficient utilization of Ae. tauschii genetic diversity for wheat breeding.

A total of 43 primary synthetic hexaploid wheat (SHW) lines were developed by crossing the durum wheat cultivar ‘Langdon’ with 43 different Ae. tauschii accessions representing the entire range of the species. A multiple synthetic derivatives (MSD) population was then developed by crossing and backcrossing the hexploid wheat ‘Norin 61’ with the 43 SWH lines. We genotyped selected lines, identified their pedigree and evaluated them across six heat-stress environments (combination of seasons and locations) in Sudan. In addition, the harvested seeds were used to examine some end-use quality attributes.

The MSD lines showed wide variation in the trait studied. Some MSD lines were superior to the adapted Sudanese cultivars and the recurrent parent ‘Norin 61’ in heat stress tolerance and end-use quality. Genome wide association analysis showed several potential marker-trait associations (alleles) that could enhance heat stress tolerance and end-use quality under heat stress conditions. For seed dormancy we followed a different strategy. First, we screened 3000 seeds from the MSD mixture, selected the dormant lines, genotyped them and identified their pedigree.

Using the pedigree information a backcross inbred lines population was constructed (BC1F6), genotyped, and evaluated for seed dormancy. QTL analysis revealed a novel QTL on chromosome 5D that might correspond to a putative alanine transferase gene. Our findings demonstrate that our strategy was efficient and successful in exploring the diversity of Ae. tasuchii in the background of bread wheat.

This work represents a rare example of systematic evaluation of wild relatives' genes in a hexaploid wheat genetic background, and it may pave the way for obtaining more useful insights that could contribute to food security. We could successfully move *Ae. tauschii* genes from the gene bank to the field and make them available for selection.

#359

Novel high throughput low-cost phenotyping methodologies for screening of heat tolerance in 319 wheat genotypes

Jing He [1,*], Yuanyuan Wang [1], Asad Abbas [1], Afroz Naznin [1], Oluwadamilola Dauda [1], Jay Bose [1,2], Oula Ghannoum [2], Zhonghua Chen [1,2,*]

[1] School of Science, Western Sydney University, Penrith, NSW, 2751, Australia
[2] Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia

Emails: Z.Chen@westernsydney.edu.au; J.He4@westernsydney.edu.au

In wheat, high temperature accelerates leaf senescence and shortens the crop cycle - limiting the opportunity for plant photosynthesis and accumulation of carbohydrates in grains. Understanding the response of wheat to heat stress in the context of source-sink relationship (e.g. plant phenotypic traits, leaf structural and biochemical traits, spikelet fertility, and seed morphology) is critical for maintaining high yields under heat stress.

In this study, we evaluated 319 wheat genotypes in both controlled greenhouse conditions and field settings during the 2023 growing season. The objectives were to examine the performance of these genotypes under heat stress and to develop rapid heat tolerance screening methods.

We found that leaf vapour pressure deficit, leaf temperature, stomatal conductance as well as stomatal density and index, linear electron flow, normalized difference vegetation index are reliable indicators that can be used for screening heat tolerance in wheat. A weighted ranking system was developed based on performance metrics across the assessed traits, facilitating the identification of genotypes with superior heat resilience.

Our findings highlighted 16 genotypes, including Aus19402, Caz53, and Vulcan, that demonstrated robust tolerance to heat stress. These genotypes exhibited significantly lower leaf temperatures and higher stomatal conductance, suggesting effective physiological adaptations for maintaining homeostasis under elevated temperatures.

These findings are critical for wheat breeding programs aimed at enhancing heat tolerance. Incorporating these insights into breeding strategies can help develop wheat varieties that maintain high yields under increasing temperatures. As the next step, the project team will is evaluating QTLs, haplotypes, and genes to understand the molecular and genetic basis of source-sink relationship in regulating heat tolerance of wheat.

#349

VRNTURING into the unknown: dissecting the role of V*ernalization2 (VRN2)* in climate adaptation

Dominique Hirsz[1], Adam Gauley[1,2], Simon Griffiths[3], Shifeng Cheng[4], Laura Dixon[1]

[1]Faculty of Biological Sciences, University of Leeds, LS2 9NL, UK.
[2]Agri-Food and Biosciences Institute, 18a Newforge Ln, Belfast, BT9 5PX, UK.
[3]John Innes Centre, Norwich Research Park, Colney Ln, Norwich, NR4 7UH, UK.
[4]Agricultural Genomics Institute at Shenzhen (AGIS), Chinese Academy of Agricultural Sciences (CAAS), Shenzhen, China.

In a changing climate, it will become an increasing challenge to ensure food security. A contributing factor to this is that cereals are highly sensitive to the more irregular and extreme temperatures. The environmental factors temperature and photoperiod are key regulators of the transition from vegetative to floral growth.

This is an essential transition which needs to be timed correctly to optimise reproductive success and is critical for high crop yields. For cereals which experience a winter growth habit, the extended period of cold exposure called vernalization is essential for floral transition to occur. The cereal-specific floral repressor *VERNALIZATION2 (VRN2)* has an integral role in this pathway, yet this locus remains poorly characterised in hexaploid wheat.

Our research suggests that the tandemly duplicated genes comprising the *VRN2* locus, *ZCCT1* and *ZCCT2*, show differences in gene expression patterns in varying environmental conditions at both a gene and subgenome level. Additionally, interactions between each ZCCT protein and the key floral promoter VERNALIZATION1 (VRN1), alongside interactions with their promoter regions, provide new insight into the vernalization network.

Different allelic variants of *VRN1* and *VRN2* have also been analysed based on selection occurring in the 'Watkins' collection comprised of landraces from across the globe, with further testing of protein interactions between these variants showing further specificity. The differing response to extreme cold temperatures across a panel of these diverse 'Watkins' landrace varieties also indicate that *VRN2* has a role in cold stress in addition to vernalization response.

Continuing to further our understanding of *VRN2* will enable the generation of more temperature-robust wheat varieties, essential to ensure high yields with a changing climate.

#345

Grain mapping wheat spikes under frost damage using three different cultivars varying in frost tolerance

Brenton Leske[1,2*], Timothy Colmer[2], Thomas Ben Biddulph[1],

[1] Department of Primary Industries and Regional Development, South Perth, WA 6151, Australia
*brenton.leske@dpird.wa.gov.au
[2] School of Agriculture and Environment, The University of Western Australia, Crawley, WA 6009, Australia

Frost-after-spike-emergence (FASE) is a major production risk to global grain production. This study evaluated if wheat cultivars with varying susceptibility to FASE have different patterns of grain set and weight contributions under field frost conditions. Field plot trials were conducted in 2018 and 2019, with three wheat cultivars sown multiple times.

Primary tillers were assessed for floret sterility, individual grain set, individual grain weight and yield components. Natural frost events (screen air temperatures ≤ 2 °C) occurred on 22 and 33 occasions from July to October in 2018 and 2019, resulting in grain yields being reduced by up to 65% relative to the control (heated plots). Without frost, cultivars Cutlass and Scout (known to be less susceptible to frost) had higher grain number per spike, lower average grain weight, with proportionally more grains formed in distal florets.

In contrast, the more FASE susceptible cultivar, Wyalkatchem had lower grain number per spike, higher average grain weight, with proportionally more grains set in proximal florets and less in distal florets. With frost, Cutlass and Scout had a higher grain number per spike but lower individual grain weights compared to more FASE susceptible Wyalkatchem.

This study identified less susceptible wheat cultivars form more grains in distal florets, have higher grain number per spike with lower individual grain weights and high grain number per unit area and thus higher grain yield under FASE compared to a more susceptible cultivar. Further detailed studies into the physiological basis for grain yield under FASE conditions are needed to improve grain yield in frost prone growing regions.

#330

Bread wheat stem characteristics and their contribution to grain size and yield under changing climate

Simeon Ntawuguranayo[1,2], Roy Sadeh[1], Marina Baizerman[2], Nadav Shalit[2], Kammal Nashef[2], Ittai Herrmann[1], Zvi Peleg[1], Roi Ben-David[2*]

[1]The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O.B 12, Rehovot 7610001, Israel.
[2]The Institute of Plant Sciences. Agriculture Research Organization (ARO) - Volcani Institute, 68 HaMaccabim Road, P.O.B 15159 Rishon LeZion 7505101, Israel.

Email: Simeon.ntawugurana@mail.huji.ac.il

Water deficit is the primary environmental constraint affecting wheat growth and production and is increasingly exacerbated due to climate change. Previously, we showed that crop investment in stem structural biomass and stem water-soluble carbohydrates promote grain filling and yield under water-stress conditions. Thus, underpinning the genetic architecture of stem traits under water stress could serve as a basis for future breeding of climate-resilience wheat.

Here, we evaluated the bread wheat diversity panel WheatMore (*n*=300) under contrasting water availabilities: Well-watered (~700mm) and water-limited (~350mm) in a rainout shelter facility in Rehovot, Israel. The focus was given to *stem morphology traits*: Internode diameter, stem solidness, peduncle length, and *yield-related traits*: Above-ground biomass, thousand-grain weight, and grain yield. Under water-limited conditions, structural stem biomass was reduced, as expressed in shorter peduncle lengths and narrow diameters.

Consequently, grain size and yield were reduced by 12.5% and 21.7%, respectively. Under both water availabilities, increased investment in stem internode diameter and peduncle length were positively associated with grain size. The panel was genotyped using 90K arrays, resulting in 26, 435 SNPs. A genome-wide association study (GWAS) was applied to detect genomic regions associated with stem traits. We identified a total of 81 highly significant markers for stem traits, with majority of 62 identified across environments (consecutive), 11 and 8 were detected only under well-watered and water-limited conditions, respectively (adaptive).

Major consecutive quantitative trait loci (QTL), both previously reported, were identified for stem solidness on chromosomes 3B and 3D, explaining 40.82% and 38.53% of the phenotypic variance under the two treatments. Adaptive QTLs were identified for internode diameter on chromosome 1D (14.74%) under well-watered and 3A (5%) under water-limited.

For peduncle length, adaptive QTLs were identified on chromosome 2B (with 4.1%) under WW and 6A (5.3% PVE) under WL environment. Out of these adaptive QTLs, only the one on 3A was previously reported.

Our results suggest that selecting spring wheat genotypes with wider internode diameter and long peduncle could be a valuable strategy to improve grain size under water stress conditions without compromising yield potential. These findings open new avenues for further research on the genetic basis underlying stem traits and for marker-assisted breeding of climate-resilient wheat.

#34

Physiological and proteomics approaches for deciphering heat-stress tolerance in bread wheat (*TRITICUM AESTIVUM* L.).

Agyeya Pratap[1,2&3]*, Nicolas L. Taylor[2,3]*, Madan Pal[4], Viswanathan Chinnusamy[4], and Kadambot H.M. Siddique[2]*

[1]School of Agriculture and Environment, The University of Western Australia, 35 Stirling Highway, Crawley, Perth 6009, Australia.
[2]The UWA Institute of Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley, Perth 6009, Australia.
[3]School of Molecular Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, Perth 6009, Australia.
[4]Indian Agriculture Research Institute, Indian Council of Agricultural Research, New Delhi 110012, India.

Heat-stress has detrimental effects on global bread wheat (*Triticum aestivum* L.) productivity. The aim of our study was to identify proteomic biomarkers for heat tolerance in wheat. We compared physiology, yield, and protein abundance changes of wheat genotypes with contrasting heat tolerance [two tolerant (RAJ3765 and HD2932) and two susceptible (HD2329 and HD2733)] under short-term and long-term heat-stress (32°C) at ear peep.

This experiment revealed that heat tolerant genotypes-maintained grain yield under both short- and long-term heat exposure by maintaining photosynthesis, membrane stability, chlorophyll content, pollen viability, and redox homeostasis. We identified 31 and 60 changes in protein abundance in flag-leaves and spike tissues respectively.

These revealed key pathways responsive to heat, including photosynthesis, RNA processing, heat-shock proteins, redox homeostasis, carbohydrate metabolism, protein breakdown, translation and translocation, stress-induced accumulation, and chromatin organization in flag-leaves. In contrast carbohydrate, lipid and secondary metabolism, cell wall organization, redox homeostasis, chromatin organization, membrane transport, methylation, protein folding, breakdown and translocation, RNA processing, lipid transfer, cell morphogenesis, heat-shock proteins and reproduction were prominent in spikes.

Co-expression analysis revealed networks of proteins co-related to agronomic traits. These proteins can be used as biomarkers for heat tolerance in future breeding programs.

Keywords
Wheat, heat stress, tolerance, protein biomarkers, protein abundance, physiology, yield.
Abbreviations - RNA: ribonucleic acid

#557

Dissecting responses of contrasting varieties for drought tolerance in Canadian bread wheat heritage panel

Subarna Sharma[1,3], R. Soolanayakanahally[2], A. Rahman[3], K. Tanino[1]

[1]Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada
[2]Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, Saskatoon, SK, canada
[3]United Graduate Agriculture School (UGAS) Iwate University, Morioka, Iwate, Japan

A novel study of diverse genotypes (founder, eastern, and western groups) in the Canadian heritage bread wheat panel was conducted for physiological traits and yield attributes. This unique study, spanning three years (2021-2023) under rainfed conditions in Saskatoon, SK, Canada, used the Stress Tolerance Index (STI) grain yield for contrasting years to establish relationships with traits that best explain drought tolerance mechanisms.

A set of variables were identified and validated over the years, revealing that newer varieties exhibited higher drought tolerance relative to founder lines. The moderate to high broad sense heritability (h^2_{bs}) for these identified traits underscores their importance as a selection criterion. Principle component analysis (PCA) and clustering approaches were applied to identify distinct groups based on these variables. In the PCA analysis, PC1 and PC2 accounted for 39.4% and 10.8% of the total trait variation, respectively. The GYT (grain yield*trait) approach was subsequently used to calculate the superiority index (SI) based on the relationship of traits with the grain yield.

This technique effectively distinguished and ranked varieties. AAC Brandon (the most widely grown cultivar across the prairies) was associated with the maximum SI value (1.74), and Red Fife (old founder line, no longer produced) displayed the minimum value (-2.21). Groups 1 and 2 were identified as drought-tolerant genotypes, whereas Groups 3 and 5 were found to be sensitive.

The five selected varieties were further examined for trait variation, which revealed significant differences for additional physiological and yield-contributing traits. Two key ratios under drought conditions were strong indicators of drought tolerance. Higher exposed: enclosed peduncle length (P_{upper}:P_{lower}) and lower Adaxial: Abaxial stomatal number during drought are features of drought tolerant variety.

#128

Wheat *Brevis Radix*: *TaBRXL2A* regulates organ size, stomatal density and improves drought tolerance in *Arabidopsis*

Sneha Tiwari[1,4] , M. Nagaraj Kumar[2] , Senthil Kumar Muthusamy[3], Aruna Kumar[4], Monika Dalal[1*]

[1]ICAR-National Research Centre on Plant Biotechnology, New Delhi-110012, India
[2]Division of Plant Physiology, ICAR- Indian Agricultural Research Institute, New Delhi-110012, India
[3]ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram-695017, India
[4]Amity Institute of Biotechnology, Amity University, Noida, Uttar Pradesh-201313, India

BRX (*BREVIS RADIX*) is a small gene family specific to plants. It serves as a key regulator of plant developmental processes, particularly root elongation, shoot growth, stomatal development and tiller angle, which have direct relevance for crop improvement. Therefore, in this study, identification and functional characterization of BRX family genes was carried out in cereal crop wheat (*Triticum aestivum*).

Five BRX family genes were identified in wheat genome.Phylogenetic and synteny analyses revealed evolutionary conservation among BRX proteins from monocot species. Expression analysis of*BRX*genes viz. *TaBRXL1* to *TaBRXL4* was carried out in different tissues at developmental stages and under abiotic stresses. *TaBRXL1* was predominantly associated with developmental processes, whereas *TaBRXL2* exhibitedextensive regulation by development, hormones, and various abiotic stresses. The remaining *TaBRX* genes displayed differential expression levels and distinct patterns during developmental stages and stress conditions.

Further, we functionally characterized *TaBRXL2A* by gain-of-function in *Arabidopsis*. Overexpression of *TaBRXL2A* resulted in longer primary roots under control and exogenous hormone treatments as compared to wild type plants. Overexpression lines also exhibited significant differences such as increased rosette size, higher leaf number and leaf size as compare to wild type. At reproductive stage, overexpression lines exhibited wider siliques and higher grain weight per plant. During drought stress, overexpression lines displayed improved tolerance in terms of higher chlorophyll retention and lower oxidative stress, thereby leading to significant recovery from drought stress.

The analysis suggests that the inherent lower stomatal density in the leaves of overexpression lines and higher stomatal closure in response to ABA might contribute to lower water loss from the overexpression lines.
The TaBRX proteins showed homotypic and heterotypic interactions with BRX domain containing proteins. TaBRXL2A protein exhibited membrane localization, presence of conserved residues at the N-terminal for palmitoylation, and phosphosites in the linker region, indicating its potential involvement in protophloem differentiation and stomatal lineage.

Thus, we identified a TaBRX family gene that participates in developmental pathways vital for plant growth and augments drought tolerance in *Arabidopsis*.

#227

Stinking smut in Sweden: insights from distribution, genetics, and resistance strategies

Therése Bengtsson[1], Marwan Alamrani[1], Eva Edin[2], Björn Andersson[3], Tina Henriksson[4], Johanna Holmblad[4] & Anna Berlin[3]

[1]Dept. of Plant Breeding, Swedish University of Agricultural Sciences, Box 190, 234 22 Lomma, Sweden
[2]Rural Economy and Agricultural Society, HS Konsult AB, Brunnby Gård 1, 725 97 Västerås, Sweden
[3]Dept. of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences, Box 7026, 750 07 Uppsala, Sweden
[4]Lantmännen Lantbruk, Udda Lundqvists väg 11, 268 31 Svalöv, Sweden

E-mail: therese.bengtsson@slu.se

Wheat is a primary provider of daily calories and protein for humans worldwide. However, the pursuit of high yield and grain quality faces persistent challenges due to biotic and abiotic stresses adversely affecting wheat performance. For instance, the expanding organic wheat production in Europe has led to an increase in the incidences of common bunt (CB) caused by *Tilletia tritici* and *Tilletia laevis.* This disease can significantly reduce grain quality and cause yield losses of up to 40%. While chemical seed treatments are effective in conventional farming, organic agriculture relies on resistant cultivars.

The primary aim of our project is to ensure the production of bunt-free organic wheat. To accomplish this, we will undertake two main objectives: 1) study the distribution, population structure and virulence spectra of the pathogen and 2) identify resistance genes in wheat to be targeted by the national breeding program.

In collaboration with the extension service, CB-infected wheat spikes were collected from farmer's fields and the bunts were examined under the microscope to identify the causal species based on teliospore morphology. Furthermore, species confirmation and population genetic studies were conducted through de-novo sequencing and Illumina sequencing of collected samples.

A virulence spectrum test of the collected *Tilletia* lineages using a CB differential set (*Bt0* to *Bt15* and *BtP*) is ongoing using accessions with one or more race-specific resistance genes (R-genes) complemented with Swedish winter wheat cultivars, including the resistant cultivars Stava, Festival and Hallfreda, and the susceptible cultivars Brons and Kranich.

Preliminary results reveal *T. tritici* prevalence across regions, with varying genetic diversity and demonstrated virulence against *Bt2*, *Bt4*, *Bt7*, *Bt8*, *Bt9*, *Bt13*, and *Bt15*. While virulence against *Bt2*, *Bt7* and *Bt13* were common among the tested *Tilletia* lineages, no virulence against *Bt1* and *Bt11* was found.

Notably, a few aggressive lineages with high infection rates in Hallfreda and Festival were observed, cultivars considered resistant to CB. The findings from this project will enhance understanding of CB epidemiology and support breeding efforts towards the development of resistant cultivars.

#400

Investigating genetic structure of powdery mildew resistance in wheat via QTL meta-analysis

Neeraj Budhlakoti[1], Dwijesh Chandra Mishra[1], Divya Sharma[2], Reyazul Rouf Mir[3], VK Vikas[4], Sundeep Kumar[2] and Girish Kumar Jha[1]

[1]ICAR- Indian Agricultural Statistics Research Institute, New Delhi, India
[2]ICAR-National Bureau of Plant Genetic Resources, New Delhi, India
[3]Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-K), Kashmir, India
[4]ICAR-Indian Agricultural Research Institute, Regional Station, Wellington, Tamilnadu, India

Email: neeraj35669@gmail.com; neeraj.budhlakoti@icar.gov.in

Powdery mildew (PM), caused by *Blumeria graminis* f. sp. tritici, presents a significant challenge to wheat farming, emphasizing the urgency of developing genetically resistant wheat varieties for sustainable management. Consequently, investigating the genetic makeup of PM in wheat to identify crucial genomic regions is a vital aspect of wheat research. In recent times, meta-QTL (MQTL) analysis has emerged as a key method for deciphering the intricate genetic architecture governing complex quantitative traits.

This study aimed to conduct a QTL meta-analysis to pinpoint specific genomic regions in wheat associated with PM resistance. By integrating data from 222 QTLs across 33 linkage-based studies using a consensus map featuring 54,672 markers, the analysis revealed 39 MQTLs, which were further refined to 9 high-confidence MQTLs (hcMQTLs) with confidence intervals ranging from 0.49 to 12.94 cM. The MQTLs spanned an average physical interval of 41.00 Mb, varying from 0.000048 Mb to 380.71 Mb per MQTL. Notably, 18 MQTLs were found to co-localize with known resistance genes such as Pm2, Pm3, Pm8, Pm21, Pm38, and Pm41.

The study identified 256 gene models within hcMQTLs, offering potential targets for marker-assisted breeding and genomic prediction programs aimed at enhancing PM resistance. These MQTLs provide a basis for fine mapping, gene isolation, and functional genomics studies, facilitating a deeper comprehension of molecular mechanisms.

The discovery of candidate genes presents exciting prospects for validating and ultimately developing PM-resistant wheat varieties.

#381

A novel class of rust resistance genes, (TNB), in the triticeae

Chunhong Chen[1], Matthias Jost[1], Megan A. Outram[1], Dorian Friendship[2], Jian Chen[1], Aihua Wang[1], Sambasivam Periyannan[1, 3], Jan Bartoš[4], Kateřina Holušová[4], Jaroslav Doležel[4], Peng Zhang[2], Dhara Bhatt[1], Davinder Singh[2], Evans Lagudah[1], Robert F. Park[2], Peter M. Dracatos[2, 5]

[1] CSIRO Agriculture and Food, Commonwealth Scientific and Industrial Research Organisation, GPO Box 1700, ACT 2601, Australia
[2] The University of Sydney, Faculty of Science, Plant Breeding Institute, Cobbitty, NSW 2570, Australia
[3] The University of Southern Queensland, School of Agriculture and Environmental Science, Centre for Crop Health, Toowoomba, QLD 4350, Australia
[4] Institute of Experimental Botany, Centre of Plant Structural and Functional Genomics, Olomouc CZ-77900,
[5] La Trobe Institute for Sustainable Agriculture & Food (LISAF), Department of Animal, Plant and Soil Sciences, La Trobe University, Melbourne, VIC 3086, Australia

Email: Chunhong.chen@csiro.au

Rust disease pose a significant threat to wheat, barley, and various crops worldwide, jeopardising food security. Plant breeders employ resistance genes to prevent or reduce the damage caused by rust disease. The typical resistance genes often include receptor kinases, which recognise the ligands outside the cell and trigger defence responses, as well as nucleotide-binding site and leucine-rich repeat (NLR) proteins and tandem kinase proteins (TKP), which detect pathogen effectors within cells and initiate defence mechanism.

In this study, we report the successful cloning of an atypical leaf rust resistance gene from barley. The barley leaf rust resistance locus *Rph7* has been shown to have unusually high sequence and haplotype divergence. Our investigation involved isolating the *Rph7* gene using a fine mapping and RNA_seq approach, subsequently confirmed by mutational analysis and transgenic complementation. Through transient expression experiments, we observed that Rph7 induces partial cell death in *Nicotiana benthamiana* leaves or Golden Promise barley protoplasts.

Rph7 is a pathogen-induced, non-canonical resistance gene encoding a protein that is distinct from other known plant disease resistance proteins in the Triticeae. Structural analysis using an AlphaFold2 protein model suggests that *Rph7* encodes a putative NAC transcription factor with a zinc-finger BED domain with structural similarity to the N-terminal DNA binding domain of the NAC transcription factor (ANAC019) from Arabidopsis.

Comparative analysis reveals intriguing parallels between *Rph7* and the recently cloned wheat stripe rust resistance gene *YrNAM* aka *Yr10*. Despite the absence of amino acid sequence similarity at the N-terminal domains between Rph7 and Yr10, structural alignment indicates shared structural features at both the N-terminal and C-terminal domains. Consequently, *Rph7* and *Yr10* are classified as a novel category of rust resistance genes with a Triticeae NAC- BED (TNB) domain architecture.

#64

A TaSRT1-TaHSP18.6 module confers resistance to Fusarium crown rot by mediating auxin content in common wheat

Ning Zhang[#]*, Lu Liu[#], Songgang Li[#], Simin Zhao, Hongyan Tian, Wanting Ren, Zhipeng Sun, Yuhui Wei, Mengyao Li, Xia Yang, Yan Ren, Feng Chen*

National Key Laboratory of Wheat and Maize Crop Science / Agronomy College, Henan Agricultural University, Zhengzhou, China

[*]Corresponding: fengchen@henau.edu.cn; zhangning88012@126.com.
Agronomy College, Henan Agricultural University, 218 Ping'An Road, Zhengzhou 450046, China

Fusarium crown rot (FCR) is one of the most serious soil-borne diseases in common wheat and have caused major wheat yield losses worldwide. It is crucial to isolate resistance genes for improving wheat FCR resistance. Here, through a combination of transcriptome, VIGS (virus-induced gene silencing) and GWAS (genome-wide association analysis) analysis, we identified an 18.6 kDa heat-shock protein gene (*TaHSP18.6*) associated with wheat FCR.

Overexpression lines and ethyl methanesulfonate (EMS) mutants revealed *TaHSP18.6* positively regulating FCR resistance. Next, we screened a lysine de-acetylase sirtuin-like (TaSRT1) to interact with *TaHSP18.6*. We further demonstrated that TaSRT1 de-acetylated TaHSP18.6, thereby inhibiting the accumulation of TaHSP18.6 protein. Overexpression lines and EMS mutants showed that the *TaSRT1* gene negatively regulated wheat FCR resistance.

Meanwhile, we identified the TaHSP18.6 interacting with an auxin-responsive protein IAA1 (TaIAA1) that negatively regulated FCR resistance. *TaHSP18.6*-overexpressed lines significantly increased auxin content. Subsequently, exogenous application of auxin sharply enhanced wheat FCR resistance. Taken together, we brought forth a *TaSRT1-TaHSP18.6* model regulating wheat FCR resistance possibly through mediating auxin signaling.

#554

Integrated physio-biochemical characterization, genomics, metabolomics and pre-breeding for understanding stripe rust resistance in wheat from western Himalayas of India

Farkhandah Jan[1], Parthiban. M[2], Mohd Anwar Khan[1], Parvaze Ahmad Sofi[1], Satinder Kaur[3], Pradeep Bhatia[4], Sundeep Kumar[5], Rajeev K Varshney[6], ReyazulRouf Mir*[1]

[1]Division of Genetics and Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-Kashmir), Srinagar, India.
[2]Division of Entomology, Sher-e-Kashmir University of Agricultural Sciences & Technology of Kashmir (SKUAST-Kashmir), Srinagar, India.
[3]School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, 141004.
[4]Borlaug Institute for South Asia (BISA), Ludhiana, India
[5]ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi
[6]Centre for Crop and Food Innovation, WA State Agricultural Biotechnology Centre, Murdoch University, Murdoch WA 6150, Australia

Email: rrmir@skuastkashmir.ac.in ; imrouf2006@gmail.com

Bread wheat is one of the most important cereal crops in the world. The production and productivity of wheat is significantly reduced by various unpredictable biotic and abiotic stresses. Among biotic stresses, diseases like stripe rust is the most important diseases posing threat to its production and productivity both locally and globally.

Therefore, we have made efforts to screen a core set of **>4500** diverse wheat genotypes from Indian gene bank harboring whole world wheat diversity for stripe rust resistance in six (06)diverse environments in India. Based on trait data of stripe rust resistance, a core set of 400 genotypes showing varying degree of response to stripe rust was selected for further trait characterization. This mini core set was used for physio-biochemical characterization for Ascorbic peroxidase, Catalase, Peroxidase, Superoxide dismutase, Polyphenol oxidase, Lipid peroxidation and Hydrogen peroxidation.

The analysis underscored heightened antioxidant activity in the resistant genotypes, indicating a stronger defense mechanism against oxidative stress. The same mini-core set was also characterized for different already known stripe rust resistance gene linked markers.

The trait data on stripe rust collected over six (06) environments across three different locations in India was used along with 35K SNP genotyping data for validation of already known and discovery of several new genes/QTLs for stripe rust resistance.

GWAS analysis identified 108 significant associations for stripe rust resistance using six GWAS models across six environments E1, E2, E3, E4, E5, E6 and data pooled over environments (E7). Among them, 20 marker-trait associations (MTAs) were found stable and promising and detected across all seven environments using multiple GWAS models.

In addition, based on trait evaluation for stripe rust, we have selected two candidate genotypes for stripe rust resistance (one highly resistant and one highly susceptible) and used them for metabolomics study.

The analysis of metabolomics data from contrasting wheat genotypes for stripe rust resistance led to the identification of several important metabolites including defense phytohormones, flavonoids and Anthocyanidins. Metabolomic profiling unveiled a notable increase in arginine biosynthesis, aldarate metabolism, and amino acid and nucleotide metabolism in the resistant genotype, fortifying its defense system against stripe rust.

Comparative analysis of phytohormones revealed the role of key regulators like salicylic acid jasmonic acid signaling cascade that activates defense genes, leading to the production of antimicrobial proteins, pathogenesis-related (PR) proteins and phytoalexins. Comparative flavonoid analysis revealed key secondary metabolites like rutin, malvidin and epigallocatechin in conferring resistance against stripe rust by providing antioxidant protection and antiomicrobial activity. We have also tried to study inheritance pattern and selection of promising segregants for stripe rust resistance using 9 bi-parental F_2 mapping populations for stripe rust.

This study will lead us to identify new sources of stripe rust resistance under Kashmir conditions, the candidate genotypes and candidate genes/QTLs/markers for stripe rust, key candidate metabolites induced by stripe rust and important segregants for future wheat breeding programs.

Key words: Wheat, mini-core set, stripe rust, biochemical, gene/QTLs, metabolomics, genomics, GWAS.

#0

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

Beat Keller[1*], Javier Sánchez-Martín[1,2], Lukas Kunz[1], Marion Müller[1,3], Ursin Stirnemann[1], Lili Yue[1], Rebecca Leber, Zoe Bernasconi[1]

[1] Department of Plant and Microbial Biology, University of Zürich, Zollikerstrasse 107, 8008 Zürich, Switzerland
[2] Department of Microbiology and Genetics, Spanish-Portuguese Institute for Agricultural Research (CIALE), University of Salamanca, 37007, Salamanca, Spain
[3] Chair of Phytopathology, TUM School of Life Sciences, Technical University of Munich, Freising, Germany, Emil-Ramann-Str. 2, 85354 Freising-Weihenstephan, Germany

[*] Email: bkeller@botinst.uzh.ch

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

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

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

Second, we are exploring several approaches to improve NLR-based resistance: one of them relies on the monitoring of avirulence genes to predict, based on pathogen-derived information, the potential durability of genes newly introgressed into wheat from wild species or wheat crop relatives.

We propose that the identification and monitoring of avirulence gene diversity in pathogen populations becomes an integral part of introgression breeding to ensure effective and durable resistance in wheat. Moreover, the knowledge of the wheat-fungal pathogen “interactome” promises to support breeding strategies for increased durability of resistance. We are also combining transgenic, overexpressed *NLR* genes to improve the durability of resistance.

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

#232

Lr34/Yr18/Sr57/Pm38 mediates defensive lignificaiton against fungal disease via transport of sinapyl alcohol in wheat

Yichen Zhang[1], Guang Chen[1], Yiming Zang[1], Sridhar Bhavani[2], Bin Bai[3], Wei Liu[1], Miaomiao Zhao[1], Yikeng Cheng[1], Shunda Li[1], Wei Chen[1], Wenhao Yan[1], Hailiang Mao[1], Handong Su[1], Ravi Singh[1,2], Evans Lagudah[4], Qiang Li[1,*], Caixia Lan[1,*]

[1] Hubei Hongshan Laboratory, National Key Laboratory of Crop Genetic Improvement, College of Plant Science and Technology, Huazhong Agricultural University, No. 1 Shizishan Street, Hongshan District, Wuhan City, Hubei Province, 430070, China
[2] International Maize and Wheat Improvement Center (CIMMYT), Km. 45, Carretera, México-Veracruz, El Batán, Texcoco CP 56237E do. de México, Mexico
[3] Wheat Research Institute, Gansu Academy of Agricultural Sciences, Lanzhou City, Gansu Province, 730070, China
[4] CSIRO Agriculture & Food, Canberra, ACT2601, Australia

*email: cxlan@mail.hzau.edu.cn, Qiang Li, e-mail: qli@mail.hzau.edu.cn

Widely known pleiotropic adult plant resistance (PAPR) gene, *Lr34* encodes an ATP-binding cassette transporter and plays an important role in breeding wheat for enhancing resistance against multiple fungal diseases. Despite its recognized significance, the mechanism underlying *Lr34* in pathogen defense remains largely elusive.

Our study demonstrated that wheat lines harboring the *Lr34res* allele exhibit thicker cell walls and enhanced resistance to fungal penetration compared to lines lacking *Lr34res*. Transcriptome and metabolite profiling revealed that the lignin biosynthetic pathway was repressed in *lr34* mutants, indicating a disruption in cell wall lignification.

Furthermore, our investigation uncovered the hypersensitivity of *lr34* mutant lines to sinapyl alcohol, a major monolignol crucial for cell wall lignification. Yeast accumulation and efflux assays confirmed that Lr34 protein functions as a sinapyl alcohol transporter. Both genetic and virus-induced gene silencing (VIGS) experiments revealed that the disease resistance conferred by *Lr34* could be enhanced with the addition of the *TaCOMT-3B* gene, which is responsible for biosynthesis of sinapyl alcohol.

Collectively, our findings provide novel insights into the role of *Lr34* in disease resistance, through mediating sinapyl alcohol transport and cell wall deposition. Moreover, *TaCOMT-3B* plays a synergistic role in the *Lr34* facilitated defensive lignification in adult wheat plants against multiple fungal pathogens.
Key words: *Lr34*; adult plant resistance; disease resistance; lignin; *TaCOMT-3B.*

#539

Mechanisms of susceptibility and resistance to necrotrophic fungal diseases of wheat

Huyen T. T. Phan, Eiko Furuki, Fiona Kamphuis, Kasia Rybak, Catherine Cupitt, Kalai Marathamuthu and Pao Theen See

Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Perth, WA, Australia

Septoria nodorum blotch (SNB) and yellow/tan spot (YS) are among the most economically important diseases of bread and durum wheat which are caused by the Dothideomycete fungal pathogen *Parastagonospora nodorum* (*P. nodorum*) and *Pyrenophora tritici-repentis* (*Ptr*). No resistance mechanism has been reported for the diseases. In this study, a broad SNB and YS resistance wheat line (56:ZWB11) was identified from CYMMIT Australian ICARDA Germplasm Evaluation (CAIGE) collection which was used for developing a double haploid population.

To characterise the resistance, Quantitative Trait Loci (QTL) mapping of the double haploid population was carried out for x SNB and YS related traits including disease, culture filtrate and effector assays; at different plant stages with different pathotypes. Results obtained have revealed the resistance observed in 56:ZWB11 to SNB and YS was complex.

The disease severity was a mixture of resistance and susceptibility conferred by different mechanisms. Physical barrier to both pathogens and susceptibility avoidance due to SnTox267 insensitivity play the major roles in the observed resistance phenotype. QTL profiles were distinct between *P. nodorum* and *Ptr* isolates. SnTox267 was found to interact with five wheat loci of which 2DL contributed the largest effect on disease incidence.

In addition, the study identified four other QTL (2A, 2B, 5B and 7B) which are common to multiple isolates and conditions; whereas six and four others only associated with culture filtrates or whole plant infection assays, respectively. Knowledge gained from this study will help to understand the resistance phenomenon and determine which QTL will be focused in the future for SNB and YS marker development. Implication of the findings for future research activities and molecular SNB resistance breeding will be discussed.

#274

Deciphering stable sources of resistance to yellow rust in wheat in north Himalayas of India

Priyanka[1], Vijay Rana*[1], Suman Bakshi[2], O. P. Gangwar[3] and Amit Rana[1]

[1]Department of Genetics and Plant Breeding, CSK Himachal Pradesh Krishi Vishwavidyalaya, Palampur (H. P.), India-176062
[2]Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, Maharashtra, India-400085
[3]ICAR-IIWBR Flowerdale, Shimla-171002

*Corresponding and presenting author E-mail: vijayrana_2005@rediffmail.com

Major biotic constraints in wheat production are wheat rusts caused by *Puccinia* species. Of the three rusts i.e. (stripe leaf and stem); stripe rust caused by *P. striiformis* f. sp *tritici* (*Pst*) is the important disease in many wheat growing regions of the world. India witnessed an outbreak of this disease during last decade on the previously known susceptible varieties.

North Hill Zone of India is strategically important for safeguarding yield potential of the rest of the wheat producing zones, as it serves as a source of primary inoculums for stripe rust. Therefore, evaluation of the newly developed strains, mutants and local landraces to stripe rust is important before releasing cultivars and use as potential sources for resistance. Therefore, the purpose of this study was to identify stable sources of resistance to yellow rust in North Western Himalayan conditions of India.

Sixty-six germplasm lines, including mutant lines from mutation breeding programme of BARC, Trombay (India), high yielding varieties, advanced breeding lines and some local landraces were evaluated in six environments at the Rice & Wheat Research Centre, Malan for three consecutive years through 2019-2022 and at off season hotspot locations; HAREC, Kukumseri during Summer 2020 and Summer 2021, while at the ICAR- IIWBR, Regional Station, Dalang Maidan during 2022. These lines were also tested for seedling resistance against four prevalent yellow rust pathotypes (78S84, 110S84, 110S119, 238S119

Results of the multi-environment evaluation revealed that out of 66 experimental lines, the genotype TYRM 2 was found consistently free from the disease over the environments, where not even a single fleck of yellow rust was observed and therefore can be considered as highly resistant. This genotype was also found resistant to all the prevalent pathotypes of the yellow rust pathogen in seedling resistance test.). PW 1908, FLW 16, *Unnat* PBW 550, which recorded low rAUDPC values (≤ 5) in all three seasons, were found to be slow rusting.

The other resistant genotypes identified on the basis of muti-environment evaluation were *Unnat* PBW 550, FLW 16, HS 562, PW1908, HPW 368, WH 1270, HTW 9, HPW 469, HIKK 05 and HI 8173. In overall testing, genotypes *viz*., TYRM 2, PW 1908 and FLW 16 were found superior for yellow rust resistance along with high grain yield potential. These genotypes can be used as potential donors for yellow rust resistance for diversifying the genetic basis of resistance of cultivars in rust prone zone.

#260

Breeding for wheat stripe rust resistance: understanding genetic architecture and evaluating genomic prediction in the Uruguayan breeding program

Venancio Riella[1,2*], Bettina Lado[2], Paula Silva[1], Richard García[1], Fernando Pereira[1], Lucía Gutiérrez[3], Silvia Germán[1]

[1] Instituto Nacional de Investigación Agropecuaria (INIA), La Estanzuela. Ruta 50, km 11, 70006 Colonia, Uruguay
[2] Facultad de Agronomía, Universidad de la República, Garzón 780, 12900 Montevideo, Uruguay
[3] Department of Plant & Agroecosystems Sciences, University of Wisconsin, 1575 Linden Dr, Madison, Wisconsin 53705, USA

Wheat stripe (yellow) rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is one of the most devastating diseases of wheat worldwide. Stripe rust was a minor disease in Uruguay until 2016. Since the severe epidemic in 2017, stripe rust has become the most economically important wheat foliar disease in Uruguay. In the short term, local management of ongoing epidemics on susceptible wheat varieties is limited to fungicide spraying. However, the deployment of resistant wheat cultivars appears as the most environmentally friendly strategy without additional cost for producers.

Until 2017, the Uruguayan National Institute for Agricultural Research Wheat Breeding Program (WBP) did not consider resistance to *Pst* as a priority, therefore little is known about the genetic architecture of the disease resistance in local germplasm, a key requirement for developing resistant varieties. With the general objective of contributing to the increase and sustainability of national wheat production through the development of stripe rust-resistant cultivars, we defined a diverse mapping population to 1) characterize wheat resistance architecture to stripe rust using genome-wide association studies (GWAS) and 2) predict wheat lines behavior to stripe rust using genomic prediction (GP) models.

The GWAS population of 368 wheat lines, including WBP and the main cultivars grown locally, was field phenotyped in La Estanzuela Experimental Station for two years (2021 and 2022) under artificial inoculations. The area under the disease progress curve (AUDPC) was the response variable based on six evaluation dates each year. Adjusted means of both years were computed due to the substantial correlation observed between consecutive years, reaching a coefficient of 0.84. Additionally, the population was genotyped using genotyping by sequencing (GBS) at the Biotechnology Center of the University of Wisconsin. SNPs calling was performed using TASSEL 5, followed by imputation of missing data using Beagle.

Over 100 thousand SNPs were obtained after filtering. GWAS analyses were performed with GWASpoly package. Seven genomic regions associated with resistance to *Pst* were identified on chromosomes 1B, 2B, 3A, 5B, 6B, and 7B, each explaining 3-6% of the phenotypic variance.

These genomic regions appear to confer quantitative resistance, a trait anticipated within a program where all-stage resistance had not been deliberately employed. The QTL identified on chromosome 1B is located closely to the previously reported *Yr29/Lr46* gene, while the remaining QTL are in the process of verification, to determine if these correspond to previously reported Yr genes or QTL. For GP, prediction accuracy was evaluated by computing the correlation between adjusted phenotypic means and predicted values using 10-fold cross-validation with the GBLUP model in the rrBLUP package.

The prediction accuracy obtained was on average 0.64, which positions the GP as a promising tool for selecting resistant lines in the WBP. These results provide valuable knowledge and tools to improve YR genetic resistance in the WBP.

#300

Predicting mycotoxin deoxynivalenol (DON) in wheat using near infrared (NIR) spectroscopy

Jatinder S. Sangha[1],* Weiwei Wang[1], Yuefeng Ruan[1], Samia Berraies[1], Ron Knox[1†], Richard D. Cuthbert[1], Angela Doane[1], Srinivas Sura[2], Barbara Blackwell[3], Amina Hussein[4]

[1] Swift Current Research and Development Centre, Agriculture and Agri-Food Canada, Swift Current, SK, Canada, S9H 3X2
[2] Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden, MB, Canada
[3] Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, ON, Canada
[4] University of Alberta, Edmonton, AB, Canada
† Retired

*Jatinder.sangha2@agr.gc.ca

Cost-effective high throughput screening for deoxynivalenol (DON) mycotoxin in grains, especially during early generations, in the breeding process is essential for selecting Fusarium head blight (FHB) resistance in wheat. We demonstrated the application of near infrared (NIR) spectroscopy with a wavelength ranging from 350-2500 nm to test DON concentrations in wholemeal flour from different durum wheat populations.

Chemometrics analysis involved using the GRAMS/AI software (Thermo Fisher) and R software to build models between DON concentration obtained from an enzyme linked immunosorbent assay (ELISA) and reflectance spectrum data obtained from NIR spectrometer (Labspec4). A small amount (~6 g) of wholemeal flour with DON concentration ranging from 0 to 70 ppm was prepared using nine different genetic populations (136 to 400 lines) of durum grown in FHB nurseries and scanned with NIR spectrometer.

The sample population was partitioned into a training population for model building and a test population for external validation (EV). Second derivative pre-processing of the spectral data using the Savitzky–Golay smoothing with 17 data points using GRAMS/AI provided the highest coefficient of determination (R^2= 0.90) that predicted DON concentrations (R^2=0.62) in test samples during EV. With R software based prediction models, both partial least squares (PLS) and orthogonal partial least squares (OPLS) methods generated reliable models than principal components regression (PCR) to predict DON concentration in wheat, with a predictive relevance (Q^2) ranging from 0.78 to 0.89. The loading plots show wavelength peaks for DON specific signatures in the near infrared zone around 1926 nm and 1439 nm wavelengths.

These prediction results are potentially useful in screening FHB resistance by detecting and discarding wheat lines with high DON concentrations during early generation selections in the breeding process, which is higher throughput and more economical than ELISA or mass spectrometry. Further work aims to use these prediction models for evaluating unknown wheat populations for DON prediction.

#166

Wheat blast: a growing threat to global wheat production

Pawan K. Singh[1,*], José L. Acarapi[2], Muhammad R. Kabir[3], Krishna K. Roy[3], Aakash Chawade[4], Xinyao He[1]

[1]International Maize and Wheat Improvement Center, Texcoco, Mexico
[2]Instituto Nacional de Inovacion Agropecuaria y Forestal, La Paz, Bolivia
[3]Bangladesh Wheat and Maize Research Institute, Dinajpur, Bangladesh
[4]Swedish University of Agricultural Sciences, Alnarp, Sweden

Email: pk.singh@cgiar.org

Wheat blast is an emerging disease caused by the fungus *Magnaporthe oryzae* pathotype *Triticum* (MoT) and has shown growing threat to global wheat production. Its epidemics had been confined to the Southern Cone region of South America before 2016, the year when a major outbreak occurred in Bangladesh, marking its movement from South America to South Asia. In 2018, the detection of wheat blast in Zambia signaled its initial appearance in Africa.

Predictive models, which consider agro-climatic factors, have identified vast areas with warm and humid conditions as vulnerable, a risk that could increase with climate change and the pathogen's potential to adapt to cooler and drier environments. Since mid-2010s, CIMMYT has worked closely with international partners on germplasm screening, resistance breeding, as well as integrated blast management.

Sources of resistance have been limited, and genetic studies have indicated the importance of the 2NS/2AS translocation, with minor effects contributed by other loci. Breeding efforts are challenged by lack of resistant sources and the rapid mutation of MoT populations threatening the fungicide and host resistance management strategies. Seed treatments effectively prevent seedling infections but do not protect against infections in the spikes.

Although fungicides provide some preventive advantages, their effectiveness is reducing as MoT rapidly develops resistance. Altering planting times is the most frequently adopted cultural method in regions impacted by wheat blast, but the use of bio-control agents remains minimal and is not yet a standard practice in agriculture. Overall, addressing wheat blast requires a complex, multi-disciplinary strategy and international collaboration to mitigate its harmful effects.

#129

Genetic analysis of leaf rust resistance in a doubled haploid winter wheat population

Kamalpreet Singh[1], Brent McCallum[2], Curt A. McCartney[1]

[1] Department of Plant Science, University of Manitoba, 222 Agriculture Building, Winnipeg, MB, Canada R3T 2N2
[2] Morden Research and Development Centre, Agriculture and Agri-Food Canada, 101 Route 100, Morden, MB, Canada, R6M 1Y5

Email: Curt.McCartney@umanitoba.ca, singhk1@myumanitoba.ca

Canada is the sixth largest wheat producer in world with Western Canada responsible for over 90% of Canadian wheat production. Abiotic and biotic factors limit wheat production with rust diseases (caused by *Puccinia spp.*) being one of the main biotic constraints. Leaf rust (also known as brown rust), caused by the fungus *Puccinia triticina* (*Pt*), is a prevalent rust disease in North America and is a Priority One disease in Western Canadian wheat breeding.

Leaf rust is controlled with conjunction of multiple strategies of which genetic resistance is most reliable and desirable method. The objective of this study is to identify leaf rust resistance genes in a doubled haploid winter wheat population developed by crossing Emerson (susceptible to leaf rust) and W538 (resistant to leaf rust). Phenotypic data was collected at the seedling stage from a greenhouse trial located in AAFC Morden, and field trials located in Winnipeg and Morden for collecting adult plant resistance data in summer (June-July) 2024.

The genotypic data based on single nucleotide polymorphism (SNP) markers will be collected via a 25K Infinium SNP array developed by SGS TraitGenetics. Linkage and QTL analysis based on the phenotypic and the genotypic data will indicate markers that are closely linked with leaf rust resistance and further studies will follow to develop KASP markers from the markers of interest.

The results of this study will broaden our understanding of leaf rust resistance in Western Canadian winter wheat.

#103

Identification of novel regulators for spot blotch resistance in bread wheat through association mapping and expression analysis

G Mahendra Singh[1], Sandeep Sharma[1], VK Mishra[1], Pradeep Bhati[2], Manish Vishwakarma[2], Dinesh Saini[3], AK Joshi[24].

[1]Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India.
[2]Borlaug Institute of South Asia (BISA), NASC Complex, DPS Marg, New Delhi, India.
[3]Texas Tech University, USA.
[4]CIMMYT, NASC Complex, DPS Marg, New Delhi, India

Email: ngmmahi.singh865@gmail.com

Spot blotch disease (caused by *Bipolaris sorokiniana*) results in significant yield loss in wheat. To identify the genetic regulators for spot blotch resistance, we evaluated 1500 elite wheat lines in two diverse environments. A highly significant negative association was observed between the area under the disease progress curve (AUDPC) and stay-green traits, indicating that the disease drastically affects the total greenness of the plant. Additionally, a negative association was observed between the AUDPC and days to heading (DH), and a positive association between stay green traits and DH, underscoring the substantial impact of DH on disease dynamics.

Further, we performed genome-wide association mapping using three different GWAS models: MLM, FarmCPU, and BLINK, which identified a total of 180 SNPs associated with the area under the disease progress curve (AUDPC) and 148 SNPs with stay-green traits.

There were 7 marker-trait associations (MTAs) that remained stable across environments for AUDPC, and out of those, 3 MTAs (3B_6127880, 5B_546704556, and 5B_546132836) were shared between AUDPC and other stay-green traits. However, a separate supporting GWAS in genotypes with similar DH, showed only MTA 3B_6127880, stable for AUDPC.

Time course analysis of the candidate genes associated with these MTAs in susceptible and resistant genotypes under spot blotch conditions prioritized genes which are expressed differentially and associated with early defense signaling and late defense response to spot blotch. Notably two CGs *TraesCS3B02G014400* (coding for expansin) *and TraesCS3B02G015000* (uncharacterized protein) were induced significantly against SB disease.

These findings can enable efforts to selectively breed genotypes with favorable alleles, superior haplotypes or prioritized candidate genes for improving resistance against spot blotch in wheat.

#379

Pattern recognition receptors and cytoplasmic receptor kinases involved in resistance against *fusarium graminearum*

Maryam Nourimand[1,] Valentyna Klymiuk[2], Curtis Pozniak[2], Sean Walkowiak[3], Gopal Subramaniam[1]

[1] Agriculture and Agri-Food Canada (AAFC), Ottawa, Ontario, Canada
[2] Crop Development Centre, University of Saskatchewan, Saskatoon, Saskatchewan, Canada
[3] Canadian Grain Commission, Winnipeg, Manitoba, Canada

Email: Rajagopal.subramaniam@agr.gc.ca

Fusarium graminearum is a pathogen of small grain cereal crops such as wheat, maize, and barley. As functional genomics resources in cereals continue to improve, Arabidopsis remains an attractive non-host model for plant-microbe interaction studies. Resistance to *F. graminearum* (Fg) does not follow the classic gene for gene interaction; therefore, atypical resistance components must be involved. Previously, a reverse genetics screen in Arabidopsis identified several pattern recognition receptors (PRRs) with a role in *F. graminearum* resistance.

Two of the genes identified in our screen, APEX, and the danger-associated molecular patterns (DAMP) receptor RLK7 as negative regulators of defense against Fg infection. Accordingly, the double mutant *rllk7/apex* displayed enhanced resistance to Fg and therefore, was used as a screening platform to functionally characterize *RLK7* and *Apex* alleles from wheat.

The results clarified the role of Apex as the negative regulator, whereas RLK7 is one of the several PRRs that are activated after infection in Arabidopsis. The family of PBL cytoplasmic receptor kinases are the immediate downstream targets of fungal PRRs, such as CERK1. We were also interested in the role of these kinases in Fg resistance.

A combination of genetic and chemical studies directly implicated PBL27 and its corresponding resistance gene *Zar1* in Fg resistance. Together, these studies suggest a role for both membrane and cytoplasmic receptor kinases in the resistance to Fg that holds a prospect of pyramiding resistance genes to improve resistance against FHB in wheat.

#301

Leaf water relations and osmotic adjustment of Canadian wheat cultivars subjected to drought

Gopal Sharma[1], Thorsten Knipfer[1], Gurcharn S. Brar[1,2]

[1]The University of British Columbia (UBC), Vancouver, Canada
[2]University of Alberta, Edmonton, Canada

gopals@student.ubc.ca; tknipfer@ubc.ca; gurcharn.brar@ualberta.ca

For wheat (*Triticum aestivum*), in previously published literature, sustained crop yield at limited soil water has been linked to osmotic adjustment (OA) as one of the main drivers to minimize drought-induced reductions in leaf hydration status and growth. Canada Western Red Spring (CWRS) cultivars are typically grown in rainfed areas in northern plains region with milder climates, but ongoing climate change has increased the frequency and intensity of drought event questioning how successful they are in tolerating drought.

The extent of OA and its relation to stomatal behavior, leaf roll, and kernel development under periods of drought remain elusive for CWRS. For several commercially used Canada Western Red Spring (CWRS) wheat cultivars (‘Superb’, ‘Stettler’, ‘AAC Viewfield’), OA was not found to be a mechanism contributing to drought tolerance. In contrast, we found that sustained kernel weight during periods of relatively low soil water content was linked to ‘tight’ stomatal behavior (i.e., efficient transition from onset to full stomatal closure) and ‘early’ leaf roll (i.e., reductions in flag leaf width).

Moreover, leaf hydration status (Θ_{RWC}) marked the onset of drought-induced losses in kernel weight in all three cultivars. Among cultivars, ‘Superb’ was most successful in employing these strategies which also prolonged the onset of severe leaf dehydration under drought to a soil relative water content (i.e., % of field capacity) as low as 36% (defined as threshold Θ_{RWC}); ‘Stettler’ at a Θ_{RWC} of 48%, and ‘AAC Viewfield’ at a Θ_{RWC} of 51%.

Moreover, Θ_{RWC} marked the onset of drought-induced losses in kernel weight in all three cultivars. Leaf epicuticular waxes exhibited differences in chemical composition between cultivars, which will be discussed in the context of leaf water loss beyond stomatal regulation under drought.

In conclusion, Canadian hard red spring wheat lacks OA but both leaf stomatal behavior and leaf rolling aid in securing leaf hydration status and kernel weight under drought.

#117

Soluble sugars have greater role than cell wall polysaccharides for attaining freezing tolerance in wheat

Sushan Chowhan[1,2*], Tatsuya Kutsuno[1], Hiroto Handa[1], Toshihisa Kotake[1], Daisuke Takahashi[1]

[1] Division of Life Science, Graduate School of Science and Engineering, Saitama University, 255 Shimo-okubo, Sakura-ku,Saitama 338-8570, Japan
[2] Adaptive Research and Extension Division, Bangladesh Institute of Nuclear Agriculture, BINA Sub-station Ishurdi 6620, Pabna, Bangladesh

*corresponding & presenting author's email: sushan04@yahoo.com

Due to freezing temperatures, wheat production is seriously affected. Wheat acquires freezing tolerance through exposure to low, non-freezing temperatures (cold acclimation, CA). Under CA, many plants respond to extracellular freezing and dehydration by accumulating soluble sugars and modifying cell wall properties, in addition to altering membrane lipids.

However, changes in soluble and cell wall sugars during CA and their role in the freezing tolerance of wheat are still unclear. We investigated the growth, freezing tolerance, changes in soluble sugar, and cell wall composition, followed by the activity and gene expression of key enzymes associated with sugar metabolism in two winter cultivars (Yumechikara and Norin-61) and one spring cultivar (Haruyokoi) during CA. Under CA for 4 weeks, Yumechikara showed higher freezing tolerance and accumulated more soluble sugars, including fructan, than other cultivars.

Changes in cell wall composition during CA were not obvious among the cultivars. Moreover, invertase and α-amylase activity showed no difference between Yumechikara and Haruyokoi during CA, consistent with the results of gene expression. Conversely, the expression of fructan exohydrolase at CA for 4 weeks in Yumechikara was also linked to changes in fructan content. We assume that the varying degrees of freezing tolerance in wheat cultivars are mostly caused by differences in soluble sugars rather than polysaccharides in the cell wall.

#217

Exploring the adaptation of wheat plants to inter-plant competition

Guy Golan[1], Ragavendran Abbai[1] , Thorsten Schnurbusch[1,2]
[1] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), OT Gatersleben, 06466 Seeland Germany
[2] Martin Luther University Halle-Wittenberg, Faculty of Natural Sciences III, Institute of Agricultural and Nutritional Sciences, 06120 Halle, Germany

Presenting author: Guy Golan; email: golan@ipk-gatersleben.de

The genetic heritage of wheat (*Triticum spp.)* crops has been shaped by millions of years of pre-domestication natural selection, often driven by competition among individuals. However, agricultural production evaluates collective population performance, measured as yield per unit area, which may conflict with individual competitiveness. Active competition among crop plants uses resources that could be used for grain yield formation. Thus, yield production will benefit from less 'selfish' and more 'cooperative' resource acquisition behaviors.

Recent studies have shown a strong correlation between responses to plant density and to low light, indicating that the scarcity of light is often a limiting factor in high-density crop communities. These observations suggest that studying the basis of wheat responses to changes in the intensity and spectrum of light due to competition from neighboring plants will advance our understanding of adaptation to the high-density crop environment.

We used Recombinant Inbred Lines and backcrossed Introgression lines originating from a cross between a wild emmer genotype and an elite durum wheat cultivar to study the individual fitness of wheat grown under sunlight and simulated canopy shade and its relationships to the high-density monoculture community performance in the field. We also focused on genotype-by-environment interactions in biomass allocation among organs and identified loci underlying the investment strategies of wheat plants when facing shading by neighboring plants.

Our approach provides prospects for studying and selecting single plants under a canopy-like environment and will be useful in predicting the social behaviors of individuals in a crop community.

#119

Unravelling the potential of Indian genebank *Khapli* wheat germplasm for climate resilience and quality traits

Jyoti Kumari[1,*], Pavan Kumar Naik[2], Udaya Bhanu Angirekula[2,] Rakesh Bharadwaj[1,] Yashvantha Kumar[3], Sudhir Navathe[3], Dwijesh C Mishra[4], Amit Kumar Singh[1], Shailendra Kumar Jha[5], Vasudha Jadon[1], Priya Pardeshi[1], Sherry Rachel Jacob[1], Arun Gupta[6], Achla Sharma[7], Girish Kumar Jha[4], Mahesh C Yadav[1], Velu Govindan[8], P V Varaprasad[9], Raj Kumar Gautam[1,] and Gyanendra Pratap Singh[1]

[1]ICAR-National Bureau of Plant Genetic Resources, New Delhi-110012, India
[2]The Graduate School, ICAR-Indian Agricultural Research Institute, New Delhi-1100012, India
[3]Agharkar Research Institute, Pune, Maharashtra, 411004 India
[4]ICAR-Indian Agricultural Statistics Research Institute, New Delhi-1100012, India
[5]ICAR-Indian Agricultural Research Institute, New Delhi-1100012, India
[6]ICAR-Indian Institute of Wheat & Barley Research, Karnal, Haryana-132001, India
[7]Punjab Agricultural University, Ludhiana, Punjab, 141027, India
[8]International Maize and Wheat Improvement Center (CIMMYT), Apdo postal 6-641, 06600 Mexico
[9]Department of Agronomy, Kansas State University, Manhattan, Kansas-66506, USA
*Corresponding author e-mail: Jyoti.kumari@icar.gov.in

Among cereal crops, wheat being indispensable staple crop globally, plays an important role in combating challenges related to food and nutritional security. It has been reported that every 1°C rise in average global surface temperature will lead to wheat yield reduction from 4.1 to 6.4% world-wide and 8.0% in India.

Enhanced tolerance to heat stress, increase in yield potential and gene discovery using wild genepool should remain priorities for the genetic improvement of wheat. Emmer wheat (*Triticum turgidum subsp. dicoccum*) is getting attention due to its nutritional benefits and adaptability to varying climates. Recent advancements in genomic research are being used to unravel the genetic architecture of target traits in different species of wheat including tetraploid wheat.

In this study, we have evaluated ~ 200 diverse set of tetraploid emmer wheat germplasm from the national genebank (NGB) of India for terminal heat stress tolerance and grain quality traits. Evaluation for terminal heat tolerance was conducted using multi-environmental phenotyping of 192 accessions using 15 agro-physiological traits at two locations (Delhi and Pune) for two consecutive years in alpha lattice design (ALD).

Grain quality traits were estimated from grain harvest of multi-environmental trial conducted at Delhi, Karnal, Pune, Ludhiana. Analysis of variance (ANOVA), Correlation, Principal Component Analysis, Clustering, Population Structure was performed. GWAS analysis was done using phenotyping data and 35 K SNP genotyping data to identify Marker Trait Association (MTA) for the studied traits. All the traits were significantly different across the environment.

Regarding terminal heat tolerance, five most tolerant genotypes were identified namely, IC535302, IC47037, EC06912, IC535082 and IC534587 based on heat susceptibility index for grain yield. Similarly, multi trait accessions EC577932, IC35119, EC06910 and IC535301 having high zinc and protein content and, accession IC535079 with high iron, zinc and protein were identified. For terminal heat stress, a total of 180 significant MTAs were identified under different environment for all the traits using multi-locus GWAS model, out of that 24 were identified as stable MTAs for DSE, DF, NDVI, FLL, PH, PDL.

For grain quality traits, 125 genomic regions were identified using three multi-locus models, 68 for TGW followed by 25 for GPC. Marker Affx-92926240, PDL_LP2022 codes for pentatricopeptide repeat-containing protein, has role of high temperature tolerance. Thus, we can conclude that NGB emmer wheat germplasm possesses huge genetic diversity for the studied traits and the promising germplasm and trait linked markers identified could be deployed in wheat improvement program.

#316

A Bayesian machine learning framework for genomic selection incorporating genomic and environmental interactions

Jia Liu[1,4*], James Walter[2], Julian Taylor[3], Shannon Dillon[1]

[1] CSIRO Agriculture and Food, Acton, Canberra 2601, Australia
2 Australian Grain Technologies, Roseworthy, South Australia 5371, Australia
3 University of Adelaide, School of Agriculture, Food and Wine, Waite Research Institute, Glen Osmond, SA, 5064, Australia
4. BDSI, College of Science, Australian National University

The global wheat industry is threatened by variability in the rate of productivity growth due to factors such as climate change, biotic and abiotic stress. Designing new wheat varieties with genetics tailored to target environment and management will provide flexibility for growers aiming to maximise productivity and contribute to the resilience and profitability of wheat crops.

This can be achieved by leveraging both genomic and interaction effects, including epistasis (G#G), environment (GxE) and management (GxExM) in evaluating genomic merits for breeding selection. In practice, understanding the correlation from these interactions, particularly the impact weight of each environment covariate is difficult and has been less considered. We have developed a new statistical and machine learning framework to address this challenge.

Specifically, we extended the traditional linear mixture model, GBLUP, by leveraging the flexibility and scalability of Bayesian stochastic Gaussian Processes (GP) to integrate genomic, environmental, and non-additive genomic interaction effects from data collected at farm and landscape scales to robustly evaluate genetic merits in different environments.

The model also provides biological insights into how the genome together with environment (weather, soil and disease) interact to drive wheat yield efficiently. We demonstrate the approach using a large-scaled data collected across an Australian wheat breeders' trial network (AGT) consisting of ca. 20K genotypes with matched genomic SNP data and two-stage yield estimates (ca. 75K), together with environmental, soil and biotic covariates spanning max. 79 environmental trials (ET: site + year).

Model performance was evaluated against four breeder scenarios, predicting observed genotypes into observed environments; unobserved genotypes into observed environments; observed genotypes into unobserved environments and unobserved genotypes into unobserved environments.

By directly incorporating environmental covariates, this framework enables breeders to predict genotype merit into new ET, helping to extend evaluations beyond their current sites and make effective decisions to support continued genetic gain under environmental uncertainty.

#88

Understanding over a century of Australian wheat leaf energy metabolism and reproductive responses to nocturnal warming

Pratima Rana Shahi[1*], Andrew Scafaro[2,3], Romina Rader[1] and Onoriode Coast [1,2]

[1] School of Environmental and Rural Sciences, Faculty of Science, Agriculture, Business, and Law, University of New England, Armidale, NSW 2351, Australia
[2] ARC Centre of Excellence in Plant Energy Biology, Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia
[3] Division of Plant Science, Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia

Increase in night temperature of 1 °C reduces wheat yields by 6-9%. Yield losses may be linked to capacity of physiological processes to acclimate to warm nights. To understand the mechanisms of wheat responses to warm nights we used 12 Australian wheat cultivars released between 1901 and 2012 (over a century of breeding) to assess whether leaf photosynthetic and respiratory processes acclimate to warm nights, and if rates of pollen germination and pollen tube growth were influenced by warm nights. Net CO_2 assimilation rate (A_n) increased by 5.12% under warm nights, driven by higher rates of maximum Rubisco carboxylation and electron transport.

Rates of dark respiratory O_2 consumption and CO_2 release (R_{dark}) acclimated to warm nights. However, the type of acclimation differed: Type I for R_{dark} on O_2 basis; and mostly Type II for R_{dark} on CO_2 basis. These results suggest that acclimation was underpinned by changes in substrate availability, membrane fluidity, and mitochondria capacity and density. Warm nights did not alter the ratio of R_{dark} to A_n (R_{dark}/A_n).

This means representation of carbon fluxes in earth systems models can assume homeostasis of R_{dark}/A_n. Warm nights also reduced ($P<0.05$) pollen germination and pollen tube growth which consequently reduced spikelet fertility and grain weight. We conclude that leaf A_n and R_{dark} can acclimate to warm nights, and this is more pronounced in modern cultivars maintaining carbon balance and a more efficient energy economy. Breeding for resilient wheat cultivars should exploit variation in both physiological acclimation capacity and reproductive tolerance to warm nights.

#242

Genetic mechanisms behind resistance to preharvest sprouting in norwegian spring wheat line T7347

Anja Karine Ruud[1], Most Champa Begum[1], Tatiana Belova[2], Ellen Færgestad Mosleth[3],Ennian Yang[4], Jon Arne Dieseth[5], Muath Alsheikh[5], Anne Kjersti Uhlen[1], Morten Lillemo[1],

[1]Department of Plant Sciences, Norwegian University of Life Sciences, Post Box 5003, 1432, Ås, Norway
[2]Centre for Molecular Medicine Norway, Faculty of Medicine, University of Oslo, 0318, Blindern, Norway
[3]NOFIMA AS, Ås, Norway
[4]Crop Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu, 610066, Sichuan, China
[5]Graminor AS, Hommelstadvegen 60, 2322, Ridabu, Norway

Preharvest sprouting (PHS) in wheat is the untimely germination of mature seed on the mother plant before harvest that can destroy the baking quality. Hence, such wheat lots will be degraded to feed. Higher temperatures combined with high humidity between grain ripening and harvest decrease the dormancy level and promotes preharvest sprouting (PHS). However, some genotypes, including the Norwegian breeding line T7347, show a high level of dormancy even under elevated temperatures.

In this study, we investigated the genetic mechanisms behind the high dormancy in T7347. A population of 233 recombinant inbred lines (RILs) was developed by crossing T7347 with Saar, a CIMMYT line with moderate to low level of dormancy. The population was grown at three different locations in Norway and Chengdu, China in a total of 13 trials, and screened for germination index (GI) and falling number (FN). Red grain is known to be associated with higher level of dormancy than white grain, and we measured color with NIR-VIS on dry seeds.

The population was genotyped with the TraitGenetics 25 K SNP chip and QTL mapping revealed five stable PHS QTL on chromosomes 1A, 3A, 3B, 7A and 7B. Among these, the largest proportion of phenotypic variation of GI and FN was explained by QTL overlapping with the known red color loci on chromosomes 3AL and 3BL, with the alleles conferring dormancy contributed by T7347 and Saar, respectively.

To further investigate genes underlying the QTL, a transcriptome study was carried out on a subset of extreme dormant and non-dormant RILs grown in the greenhouse. RNA was extracted from embryo, aleurone and starchy endosperm 30 days after anthesis. Transcriptome analysis allowed us to identify several candidate genes within the QTL regions. Current work includes establishment of a gene editing protocol for functional validation of these.

#155

Phase-specific light fluctuations double the reduction of kernel number and yield potential in winter wheat

Khadija Sabir[1], Tsu-Wei Chen[2], Hartmut Stützel[1]
[1]Institute of Horticultural Production Systems, Leibniz University Hannover, Germany
[2]Group of Intensive Plant Food Systems, Albrecht Daniel Thaer Institut of Agricultural and Horticultural Science, Humboldt University Berlin, Germany

* Corresponding and presenting author: sabir@gem.uni-hannover.de

Wheat yield is predicted to decrease globally because of climate change. Short-term environmental conditions during critical growth stages may have significant impact on grain yield. In addition to the physiological sub-phases of the plant, genotypic variation may also determine the magnitude of these effects.

A better understanding of the three-way interactions between cultivar, phenology and environmental conditions may help to reduce the gap between potential and actual yields under enhanced climatic variability. We analyzed multiple environment field trials data from three seasons (2014-2017) at six different sites throughout Germany under three different cropping intensities using a novel statistical approach to estimate the sensitivities of three yield components, to short-term variations in global radiation, temperature and precipitation in 220 cultivars across 81 time windows ranging from double ridge to seed desiccation.

The most sensitive yield component was found to be kernel number per spike (KpS) affected by short-term fluctuations of light and temperature, especially during the sub-phase between yellow anther and tipping and at pre-grain filling. We further examined the light effects on yield components of 16 cultivars during identified specific sub-phases in a growth chamber experiment under five light treatments and confirmed sub-phase and genotypic-specific sensitivity to light fluctuations. Sensitivity of KpS to light fluctuations during the yellow anther and tipping phases is explained by effects on floret development and increases kernel abortion, especially on the basal and middle spikelets.

Based on these responses, genotypic resilience or sensitivity to abiotic stress (i.e. light) was identified. Furthermore, another growth chamber experiment with seven light treatments and four cultivars was used to test if the effects of fluctuation in different sub-phases could be additive. KpS was reduced by 17% if fluctuation occurred in only one sub-phase but by 40% in two consecutive sub-phases (yellow anther and heading), showing significant additive effects. Similarly, reduction in florets per spike, kernel weight per spike and abortion rate per spike were found.

Moreover, photosynthetic parameters and carbohydrate levels were measured to interpret how the light fluctuations affect the source availability that physiologically reduces the development of sink, and therefore a two-fold reduction in yield potential.

Our results offer deep insights into complex genotype x environment interactions and highlight the significance of phase-specific sensitivity to environmental fluctuations. This knowledge can be used to mitigate effects of climate variability and maximize grain yield.

#299

Validation of molecular markers and detection of translocated segments in hybridized cultivated wheat varieties

Manish K Vishwakarma[1]*, Pradeep K Bhati[1], Karthikeyan Thiyagarajan[1], and Arun K Joshi[1]

[1]Borlaug Institute for South Asia (BISA), NASC Complex, New Delhi 110012 India

With the aim to introduce translocated genomic segments carrying BNI properties into cultivated wheat varieties, the backcross approach was used, taking chromosome addition lines (CAL) as a donor and recently released Indian varieties as a recipient.

The CAL was developed through the crosses of *L. recemosus* and *T. aestivum* due to harbouring BNI quality and other favourable traits for wheat improvement. We validated the molecular markers developed from de Novo sequencing of Leymus species to identify translocated and non-translocated lines of wheat and for detecting the introgressed segment in the backcrossed progenies in different cross combinations.

Molecular markers were utilized to confirm the introgression of the BNI segments instead of GISH. While GISH would be used to verify the translocation region in the genome of introgressed lines.

#373

From genomics to phenomics: integration of genomic prediction and crop models for wheat phenology

Bangyou Zheng[1], Hamish Brown[2], Zhigan Zhao[3], Enli Wang[3], Neil Huth[4], Shannon Dillon[3], Jeremy Whish[1], Julianne Lilley[3], Jessica Hyles[3], Tina Rathjen[3], Maxwell Bloomfield[5], Corinne Celestina[6], James Hunt[6], Ben Trevaskis[3], Kenton Porker[7], Felicity Harris[8], Ben Biddulph[9]

[1] CSIRO Agriculture and Food, Queensland Biosciences Precinct 306 Carmody Road, St Lucia, QLD, 4067
bangyou.zheng@csiro.au
[2] The New Zealand Institute for Plant & Food Research, Private Bag 4604, Christchurch, New Zealand
[3] CSIRO Agriculture and Food, GPO Box 1700, Canberra, ACT 2601, Australia
[4] CSIRO Agriculture and Food, PO Box 102, 4350 Toowoomba, Australia
[5] Department of Animal, Plant and Soil Sciences, La Trobe University, Bundoora, VIC 3086, Australia
[6] School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, University of Melbourne, Melbourne, VIC 3010, Australia
[7] CSIRO Agriculture and Food, Urrbrae, South Australia 5064, Australia
[8] School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, New South Wales 2678, Australia
[9] Department of Primary Industries and Regional Development, East Perth, Western Australia 6004, Australia
bangyou.zheng@csiro.au

Wheat phenology is a major determinant of the adaptation of wheat to diverse environments and management, and lowering risk of yield reducing climatic factors (e.g. frost, heat and drought). However, it is impacted by interactions of genotype, environment and management (GxExM) from agronomy perspectives or interaction of genotype and environment (GxE) from genomics perspectives.

Over the decades, genomic predictions and crop models have been separately developed to deliver genetic gain within limited environments, or determine environmental responses within limited genotypes. Here we describe an integrated model combining genomic prediction (GP) and a crop growth model (CGM) to predict wheat phenology (i.e. timing of flag leaf emergence, heading and flowering) in diverse environments across the Australian grain belt.

A wheat population was selected to represent the current phenological diversity of cultivars in Australia and genotyped for single nucleotide polymorphisms (SNPs) with the wheat 90K Illumina SNP array.

A random forest model was used to predict genotypic parameters for the crop model APSIM Next Generation, which was used to further predict wheat phenology. The observed datasets are assembled from controlled environment and field experiments and historical datasets used to calibrate and validate the new hybrid model (GP-CGM) in four scenarios with one calibration and three validations for unobserved genotypes and/or environments.

GP-CGM can accurately predict wheat phenology in unobserved environments and genotypes with prediction accuracy of more than 0.7. The new GP-CGM model can now be used to predict phenology for current cultivars across environments and new cultivars with just SNP and controlled environment validation to explore the complex interaction between genotype and environment. It can also be extended to other crop models, -omics prediction and phenotypic data sourced from remote and proximal sensing.

#512

GBS mediated exploration of genetic diversity and trait potential of novel mutant lines of wheat

Sana Zulfiqar[1, 5], Sayyad Ali Raza Bukhari[2], Susanne Dreisigacker[3], Bradley J Till[4], Mehboob Ur Rehman[1]

[1] Plant Genomics and Molecular Breeding Laboratory, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, 38000, Punjab, Pakistan
[2] Discipline of Biological Sciences, University of Tasmania, Tasmania
[3] International Maize and Wheat Improvement Center (CIMMYT), Km. 45, Carretera Mex-Veracruz, El Batan, CP 56237, Veracruz, Mexico
[4] Veterinary Genetics Laboratory, University of California, Old Davis Road, Davis, CA 95616, USA
[5] Pakistan Institute of Engineering and Applied Sciences (NIBGE-C, PIEAS), Faisalabad, 38000, Punjab, Pakistan

Email: sanazulfiqar3103@gmail.com

With global population growth and declining cropland availability, enhancing wheat productivity is critical for food security. Strategic trait-based improvements emerge as a sustainable approach in wheat breeding. Mutation breeding, particularly through physical mutagens such as γ-rays, offers an economically viable strategy for creating novel genetic resources capable of combating a wide spectrum of biotic and abiotic stresses.

We employed the high throughput DArTseq™ technology to assess the genetic diversity and functional annotation of 33 newly developed wheat mutant lines compared to their wild type. A total of 157,608 presence-absence variants (PAVs) were identified distributed across all wheat sub-genomes. The maximum number of PAVs were detected on Chr 7D (2877) followed by Chr 7B (2711), Chr 2B (2658), and Chr 3B (2622). Across mutant lines, the largest number of variants were identified in mutant line Pb-M-2061 (23,643) and Pb-M-59 (22,381). Hierarchical clustering was performed, and mutant lines were grouped into three main clusters.

Furthermore, out of 7,910 PAVs consistently identified over replicates, 3,252 were those that were present in mutant lines but were absent in the wild type. Among these, 1,480 were found in mutant line Pb-M-1027 and 656 in line Pb-M-1323. These PAVs were further characterized within the wheat transcriptome. Out of the 3,252 PAVs specific to mutants, 1,238 were found in the wheat transcriptome that contained 152 characterized and 1,196 uncharacterized genes.

Clusters of orthologous genes (COGs) and Gene ontology (GO) terms associated with PAV-containing genes were identified that showed involvement in various pathways, including disease resistance, cellular processes, signaling, storage and metabolism. Mutant lines Pb-M-1027, Pb-M-2302, and Pb-M-1323 exhibited a diverse array of beneficial traits including disease resistance, increased grain yield, drought tolerance, heat tolerance, larger grain size, improved photosynthetic efficiency, high harvest index, cold tolerance, and high chlorophyll content.

Other lines, including Pb-M-1575, Pb-M-1946, Pb-M-196, Pb-M-2517, Pb-M-2260, and Pb-M-1530, also displayed a wide range of valuable traits. Some traits were specific to certain mutant lines, including disease resistance to a specific disease, grain characteristics, and environmental stress tolerance.

Many traits were shared among multiple mutant lines, indicating the potential for incorporating these traits into breeding programs. These findings provide valuable insights into the genetic diversity, functional annotation, and trait associations of wheat mutant lines, offering significant potential for wheat improvement.

#553

Silicon fertilisation: wheat grain yield and soil organic carbon improvement—hitting two targets with one arrow

Omid Alasti[1], Waseem Ashfaq[1], Razlin Azman Halimi[1], Alexis Pang[1], Dorin Gupta[1]

[1] School of Agriculture, Food and Ecosystem Sciences, Faculty of Science, The University of Melbourne, Melbourne, Australia

Silicon (Si), an abundant element in the Earth's crust, has the potential to play a crucial role in sustainable agriculture by enhancing crop production and stress resistance. Maintaining or increasing soil organic carbon (SOC) levels in agricultural soils is challenging due to conventional practices that deplete SOC, leading to soil degradation and diminished fertility.

This study aimed to evaluate the impact of silicon fertiliser on grain yield and post-harvest SOC of the spring wheat variety 'Scepter' across three locations—in Victoria, Australia, over the 2022 and 2023 growing seasons, in different cultivated areas, under rainfed conditions. The experimental design was a randomised complete block with four replicates for each foliar Silicon (FS) treated and control plots in 2022, and eight replicates for each FS, Soil Silicon amendments (SA) treated, and control plots in 2023. In 2022, wheat crop at three locations experienced different degrees of water logging stress at critical growth stages due to heavy and continuous rainfall.

FS-treated plots saw a higher though non-significant difference in mean grain yield compared to controls (4.91 t/ha) at Dookie trial site. The positive trend was observed in FS-treated plots at 5.43% (p-value= 0.051) greater than controls (3.98 t/ha) at Birchip cropping trial site in 2022. However, at Riverine Plains trial site we found a significantly higher yield of 29.77% ($p<0.01$) in FS-treated plots compared to controls (2.41 t/ha). In the year 2023, which was a normal cropping year in case of climate conditions, the comparison of grain yields between FS-treated or SA-treated plots showed statistically similar yields compared to control plots at Dookie (7.01 t/ha), Birchip (5.99 t/ha) and Riverine Plains (3.34 t/ha) trial sites.

Soil organic carbon levels in FS plots at Dookie trial site depicted a significant 12.24% increase ($p<0.01$) compared to the control plot in 2023. SA-treated plots at Dookie trial site in 2023 exhibited a significant 23.72% increase in SOC% ($p<0.01$). At Birchip cropping trial site, FS-treated plots demonstrated a significant 13.19% increase ($p<0.01$) in SOC measurable, and SA-treated plots had a significant 11.28% increase ($p<0.05$). The significant differences in grain yield between the two years of this study, concerned with waterlogging stress in 2022, highlight silicon's positive impact under stress conditions compared to non-stressed conditions.

Additionally, the enhancement of SOC indicates silicon's potential role in promoting soil health in short-term applications. These findings support the use of silicon fertilisers as a sustainable agricultural practice to mitigate soil degradation and enhance stress resilience.

However, the observed positive trend in SOC of this study could be attributed to the increase in particulate organic carbon most likely due to recent crop growth in the short-term. Hence, this came into consideration whether the observed increase in OC due to applied silicon fertiliser is a one-time event or a consistent trend of increasing OC levels.

Understanding the full picture requires further research to explore the long-term effects and broader applications of silicon across different conditions.

Keywords: Silicon Treatment, Spring Wheat, Sustainable Agriculture, Victoria

#525

Integrating NDVI and precision agriculture into nitrogen and seeding

Ali Amamou[1]*, Oussama Hnizil[2], Aziz Baidani[2], Ilham Khlila[2], Abdelali Laamari[1], Nasserelhaq Nsarellah[1] and Rachid Moussadek[3]

[1]: National Institute of Agricultural Research, INRA Settat, Morocco
[2]: Faculty of Sciences and Techniques, Hassan First University of Settat, Morocco
[3]: International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

In Morocco's arid regions, cereals are grown under rainfed circumstances. They face many types of drought stress in these conditions. Furthermore, under such conditions, nitrogen fertilization represents a significant challenge for wheat, Morocco's most important cereal.

This study explores the interplay between nitrogen doses and seeding rates on wheat yield, biomass, and protein content, utilizing precision agricultural tools such as the Normalized Difference Vegetation Index (NDVI), Soil Plant Analysis Development (SPAD), and canopy temperature (CT). Our results show that an intermediate nitrogen dose (60 kg/ha) combined with a moderate seed rate (400 seeds/m2) substantially enhances wheat yield by 22.95%, surpassing other combinations.

Additionally, reduced nitrogen levels significantly increase protein content, demonstrating wheat's adaptive mechanisms to enhance protein synthesis under nitrogen constraints. NDVI analysis underscores pronounced growth during the tillering phase with high nitrogen, highlighting the importance of early-stage nutrient management. SPAD measurements further reveal that early nitrogen applications significantly boost chlorophyll content, essential for vigorous early growth, while CT data indicates optimal nitrogen and seed rates can modulate plant stress responses effectively.

As crops mature, the predictive capacity of NDVI declines, indicating the need for adjusted nitrogen strategies. Collectively, these findings advocate for a refined management of nitrogen and seeding rates, integrating NDVI, SPAD, and CT assessments to not only boost yields but also advance sustainable agricultural practices, optimizing crop performance while minimizing environmental impacts.

#218

Epistatic interaction of the *OPRIII* genomic region regulates root development in wheat

Gilad Gabay[1], Junli Zhang[2], Tzion Fahima[3] and Jorge Dubcovsky[2,4]
[1]French Associates Institute for Agriculture and Biotechnology of Drylands, The Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede-Boker, Israel.
[2]Department of Plant Sciences, University of California, Davis, CA 95616, USA.
[3]Department of Evolutionary and Environmental Biology, Institute of Evolution, University of Haifa, Haifa 3498838, Israel.
[4]Howard Hughes Medical Institute, Chevy Chase, MD 20815, USA.

Gilad Gabay: ggabay@bgu.ac.il, Junli Zhang: psjzhang@ucdavis.edu, Tzion Fahima: tfahima@evo.haifa.ac.il, Jorge Dubcovsky: jdubcovsky@ucdavis.edu

Wheat is an essential crop for global food security and is well adapted to a wide variety of soils. However, new stresses imposed by climatic change require new tools to optimize root architectures for changing environments. We previously reported the identification of a cluster of monocot-specific *12-OXOPHYTODIENOATE REDUCTASE* genes from subfamily III (*OPRIII*) that modulate differences in wheat root architecture through the regulation of the jasmonic acid (JA) signaling pathway. However, the gene networks regulating different root architectures and their impact on grain yield under water-limited conditions remain poorly understood.

In this study, we characterize a new QTL for root architecture that interacts with the *OPRIII* genes. We found that the triplicated *OPRIII* locus present in the recombined wheat-rye chromosome 1RW is associated with short roots in Hahn but not in the Pavon background. In the F_2 progeny of a cross between Hahn-1RW and Pavon-1RW, we identified a significant QTL for root length on chromosome 4B (*Qrln.ucw-4B*).

We demonstrated a significant interaction between this QTL and 1RW using a second F_2 population from the cross Pavon-1RW x Hahn-1RS and a BC_4F_2 population segregating for both loci in the Hahn background. Using heterogeneous inbred families (HIFs) from a Pavon-RW x Hahn-RW F_5 line, we narrowed down the *Qrln.ucw-4B* to 6.37 Mb interval including 49 candidate genes.

We further investigated these genes and their interactions with the 1RW locus using multiple transcriptome analyses of wild-type and transgenic lines over-expressing *OPRIII*. These analyses identified a short list of candidate genes with potential interactions with *OPRIII* genes for the regulation of root development in wheat.

These genes include key regulators in the JA-signaling biosynthetic pathways and ethylene phytohormones. The *OPRIII* genes and their interactions provide a useful entry point to engineer root architecture in wheat and potentially other cereals.

#20

Boosting wheat proteins sustainably by annually rotating cereals with forage legumes

Robert J Harrison[1,2], **John G Howieson**[2], **Tom J Edwards**[1,2], **Emma J Steel**[2], **Chris M Poole**[2], **Ronald J Yates**[1,2]
[1] Department of Primary Industries and Regional Development (DPIRD), Western Australia
[2] Legume and Rhizobium Sciences (LRS), Sustainable Farming Systems, Food Futures Institute, Murdoch University, Perth, Western Australia

r.harrison@murdoch.edu.au

Wheat gluten is one of the most important proteins on the planet, but its end-use qualities are determined by the amount of nitrogen biosynthesised in the grain. Input-intense cereal production has a heavy reliance on nitrogen supplied from ammoniacal fertilisers. This practice can contribute up to 50% of the on-farm emissions of greenhouse gasses in agriculture.

Despite increasing rates of application of synthetic nitrogen to cereals, wheat grain protein levels, in developed nations, have been frequently failing to reach the premium grade required by the bread-making market. We hypothesized that wheat grain protein could be increased by growing the cereal on legume residues which are rich in biologically fixed N.

This approach was compared against growing wheat with fertiliser nitrogen in different cropping rotations in a dryland Mediterranean climate over 2-4 years, on different soil types. Wheat grown after both leguminous and non-leguminous treatments received low, medium and high rates of urea to indicate if forage legumes can provide sufficient nitrogen for sustainable wheat production.

At all sites and years studied we discovered that wheat grains produced following a year of forage legumes had significantly higher protein levels than when grown after non-leguminous crops in rotation. These results were achieved in combination with a reduction in on-farm emissions (by over 200 kg/ha of CO_2) without compromising yield, as indicated by emissions accounting.

In summary, incorporating suitable forage legumes in dryland farming systems enables wheat production with increased proteins and low emission intensity to promote both economic and environmental sustainability.

#331

Responses of long coleoptile wheat on ameliorated soils

Javid M[1], Blake A[1], Kupsch M[1], Wilkins R[1], Rebetzke G[2] and Davies S[1]

[1] Department of Primary Industries and Regional Development (DPIRD), Cnr Crooks Road and Great Eastern Highway, PO Box 432, Merredin WA 6415, Muhammad.Javid@dpird.wa.gov.au
[2] Commonwealth Scientific and Industrial Research Organisation (CSIRO) GPO Box 1700, Canberra, ACT 2601, Australia

Sandplain soils are important for wheat production but are often affected by numerous constraints including water repellence, subsoil acidity, compaction, and poor water and nutrient retention. Strategic deep tillage techniques such as deep ripping and rotary spading can address these issues, potentially boosting wheat yields by up to 35%.

However, achieving consistent seeding depth and preventing furrow infill after amelioration are crucial for successful crop establishment and maximising wheat productivity. Long coleoptile wheat carrying alternative *Rht18* dwarfing gene and characterised by an elongated protective sheath for the developing shoot, could mitigate these limitations. This hypothesis was evaluated in field experiments across Ogilvie, Merredin, and Yuna regions of Western Australia in 2022-23. A CSIRO developed long coleoptile line, Mace-18 was compared with Scepter, Mace (short/medium coleoptile) and Calibre (mid-long coleoptile).

Genotypes were sown shallow (3-4cm) and deep (10-12cm) on no-till, ripped (except Ogilvie), and rotary spaded soils. Coleoptile length (except Ogilvie), plant density, crop weed competition and grain yield responses were assessed. At Ogilvie in 2022, deep sown Mace establishment was reduced by 55% compared to a 22% reduction for long-coleoptile Mace-18. The poorer establishment of Mace resulted in poorer crop competition resulting in more grass weeds compared to the long coleoptile Mace-18.

Wheat grain yield recovery from deep sowing was excellent for Mace-18, Scepter and Calibre, except for the short coleoptile Mace, exhibiting 13% grain yield reduction. Soil amelioration improved grain yield by 15-26% across all sowing depths and genotypes. In 2023, as characterised, Mace-18 on deep sowing had 15-30% longer coleoptiles over the other genotypes demonstrating potential for overcoming deeper placement. In Merredin, and Yuna experiments, regardless of soil treatment, Mace-18 maintained higher plant density at deep sowing compared to the other genotypes highlighting its establishment advantage.

Overall, shallow sowing increased grain yield by 8-10% compared to deep sowing. In Merredin, across both sowing depths, Mace-18 maintained the same yield, while the other genotypes at deep sowing showed a yield reduction of 8-17% compared to shallow sowing.

Ripped and spaded soil treatments improved yield by 16% and 19%, respectively, regardless of sowing depth in Merredin, although these responses were not repeated in Yuna, likely due to a warm-dry finish to the season in 2023.

While ongoing research is crucial for optimising agronomic practices and varietal selection, the preliminary evidence overwhelmingly indicates that long coleoptile wheat holds significant promise for farmers managing ameliorated soils and sustainable production of wheat.

#214

Wheat yield prediction with optimized deep neural network using spectral vegetation indices

Lokeshwari M[1,2], Girish Kumar Jha[1*], Jyoti Kumari[3], Rajender Parsad[1], Sudhir Navathe[4], Yashavantha Kumar KJ[4], Sundeep Kumar[3], Gyanendra Pratap Singh[3], P V Varaprasad[5] and Arun Kumar Joshi[6]

[1]ICAR-Indian Agricultural Statistics Research Institute, Pusa, New Delhi-110012, India
[2]The Graduate School, ICAR-Indian Agricultural Research Institute, Pusa, New Delhi-110012, India
[3]ICAR-National Bureau of Plant Genetic Resources, Pusa, New Delhi-110012, India
[4]Agharkar Research Institute, Pune-411004, Maharashtra, India
[5]Department of Agronomy, Kansas State University, Kansas-66506, USA
[6] Borluag Institute for South Asia, CIMMYT, South Asia Office, NASC Complex, New Delhi-110012, India

[*]Presenting author (Email: girish.stat@gmail.com; girish.jha@icar.gov.in)

Crop improvement programs aim to develop high yielding varieties coupled with resistance to biotic and abiotic stresses and nutritional superiority. Grain yield is a complex trait governed by genotype, environment, and their interaction. Growing a large number of genotypes under multiple environments and measurement for grain yield and its components are tedious and resource consuming tasks.

Therefore, there is a great need for novel, cost-effective techniques to evaluate the performance of crops at field scale through indirect selection of easily scorable traits using artificial intelligence based algorithms on comprehensive data. Hand-held or vehicle-mounted active proximal sensing offers a non-destructive method for monitoring crops within fields by providing real-time spectral vegetation indices.

The study aims to incorporate such proximal sensing data into a deep learning architecture for field scale wheat yield prediction. The novelty of this study is to predict the wheat yield based on normalized difference vegetation indices (NDVIs), canopy temperatures (CTs) and plant height (PH) by employing deep neural network (DNN) optimized through genetic algorithm (GA). NDVIs and CTs data were collected during the growing season at different growth stages from tillering through senescence (ground cover, heading, anthesis, grain filling and maturity).

Models are developed on crop yield data using 70% of a total 3350 germplasm of wheat planted in two growing environments (irrigated and rainfed) and two different locations during the winter season 2020-21.
Yield data on 1005 germplasm of wheat are used as validation dataset to assess the performance of developed model on unseen data. The developed GA optimized DNN model is compared and found superior to different machine learning models such as random forest regression (RFR), least absolute shrinkage and selection operator (LASSO), and support vector regression (SVR) for wheat yield prediction.

Notably, when analyzing individual feature groups, NDVIs at five different growth stages of wheat provide adequate predictions (R^2 values ≥ 50%) of the yield under both environments. Additionally, the random forest method was employed to identify significant features among each feature group. This study introduces the application of a GA-optimized deep neural network, leveraging handheld or vehicle-mounted proximal sensing data for predicting wheat yield at field scale.

This innovative approach promises to significantly benefit the breeding community, providing a powerful and efficient tool for the pre-harvest evaluation of a large volume of germplasm, marking a major advancement in agricultural practices.

#259

A survey of commercially-available crop management technologies for grain production

Sayma Shammi[1,*], Michael Scobie[2], Justine Bailie[2], Corey Plant[1]

[1] Institute for Resilient Regions (IRR), University of Southern Queensland, Toowoomba 4350, Queensland
[2] School of Agriculture and Environmental Science, University of Southern Queensland, Toowoomba 4350, Queensland

sayma.shammi@unisq.edu.au, michael.scobie@unisq.edu.au, justine.baillie@unisq.edu.au, corey.plant@unisq.edu.au

The demand for cereal grains is increasing with population growth and food security concerns. There is an urgent need to adopt sustainable agricultural practices that increase yields. To improve industry productivity, sustainability, and resilience, adopting technological advancements in the grain sector is crucial.

The future of the grain sector depends on transforming the way we farm, equipping grain farmers with the tools and capabilities to adapt, reorganise, and transition in response to various challenges, such as extreme seasonal events like frost and drought, pest incidence, disease risks, and soil moisture management. As Australia is emerging as a leading hub for Agriculture 4.0, the agricultural landscape is being revolutionised by the next generation of transformative technologies, with over 2500 agricultural technology (AgTech) and food technology enterprises across Australia.

However, certain technology adoption barriers exist. Farmers may resist new technologies because of their dependency on familiar methods that have proven effective, leading to reluctance to invest in new or unfamiliar solutions.

This survey aims to identify scope-ready products for remote sensing that offer leading-edge developments in digital agriculture and automation at a commercial "Smart Farm" scale. We developed a novel taxonomy to categorise the five major crop management practices in grain farming. We searched four electronic databases to identify existing scope-ready commercial AgTech products.

A search strategy was developed to ensure all the relevant commercial products and services are relevant to the crop management practices. In this survey, relevant products were profiled based on the proposed taxonomy to provide tangible solutions that improve yields and ensure the long-term sustainability and resilience of current farming practices. We discussed the techniques used in the crop management categories, i.e., data acquisition, data transmission, data storage, data analysis, and decision-making.

We also summarised the current challenges and discussed the opportunities to integrate and assess the practical and commercial feasibility of various technologies in large-scale cropping systems.
This survey identifies the breadth of commercially available technologies applicable in the grains industry and analyses the gap to identify areas not currently serviced.

#196

Seed production partnerships for sustainable wheat production and enhanced food security in hot, dry environments

Izzat S. A. Tahir[1,2], Yasir S. A. Gorafi[1,2,3], Amani A. M. Idris[1], Awad A. E. Elbashir[1], Abu Sefyan I. Saad[1], Hala M. Mustafa[1], Ashraf M. A. Elhashimi[1], Omer Elsheikh[1], Elfadil M. E. Elbashier[1], Hisashi Tsujimoto[2]

[1] Agricultural Research Corporation, P. O. Box 126, Wad Medani, Sudan
[2] Arid Land Research Center, Tottori University, Tottori 680-0001, Japan
[3] Graduate School of Agriculture, Kyoto University, 606-8502, Japan

In the era of food insecurity, securing quality seed is of paramount importance for sustainable production and delivery of the required quantity and quality of major food crops. Seeds are the basis of any good agricultural practice that intend to provide food security in the wake of climate change for an ever-growing population.

A sustainable and balanced strategy is needed to ensure continued seed production and delivery in an economically feasible manner. Based on a previous comprehensive seed system analysis, a strong public and private partnerships (PPPs) have been established between public and private seed producers. Variety maintenance, pre-basic and basic seed production of all commercial, heat stress-tolerant bread and durum wheat varieties were carried out within the activities of the project "Development of Climate Change Resilient Innovative Technologies for Sustainable Wheat Production in the Dry and Heat Prone Agro-Ecologies of Sudan and Sub-Saharan Africa".

Through strong PPPs, certified seeds of the heat-tolerant wheat varieties were produced in Sudan and disseminated to farmers in the project's innovation platform sites and beyond. The quantity of seed produced by the project in partnership with the public and private sectors increased from 41.7 tons in the first season (2019/2020) to 5411 tons in the 2021/2022 season, and to more than 13,600 tons in the 2022/23 season. In the first season, the project with its partners contributed only 0.12% to the total national seed production; however, the project share increased to 19% in the 2021/2022, and to more than 34% in the 2022/2023.

To support the dissemination of quality seeds among farmers, especially in remote rural areas, informal community-based seed production was also implemented. As an important element of sustainability, the capacity of all stakeholders involved in seed production was strengthened. Mainly due to the use of high-quality seeds, a direct comparison between participating farmers (PF) and non-participating farmers (NPF) in project activities in 2021/2022 showed a 35% increase in the productivity of PF compared to NPF.

The approach adopted here enabled the establishment of a strong partnership through which more than 90% of the project's seed was produced, while the seed produced directly by the project accounted for about 6%.

#184

Toward hybrid wheat production in Pakistan, Bangladesh and Ethiopia

Irfan Ahmad Baig[*1,], Hasneen Jahan[2], Tesfaye Solomon[3], Muhammad Ali Imran[1], Choudary Ihtasham Ali[1]

Rebecca Thistlethwaite[4], Richard Trethowan [4]
[1] Muhammad Nawaz Shareef University of Agriculture, Multan-Pakistan
[2] Bangladesh Agricultural University, Mymensingh- Bangladesh
[3] Ethiopian Institute of Agricultural Research
[4] The Plant Breeding Institute, The University of Sydney

The adoption of hybrid seeds in various crops has improved yields and reduced the yield gap across the farming classes in numerous developing countries. However, until recently, hybrid wheat has not been readily available to farmers. Hybrid wheat could reduce the impacts of poor food security, a consequence of low productivity, inefficient seed systems and a changing climate in many countries. Pakistan, Bangladesh and Ethiopia have large populations and are struggling to ensure basic food security for hundreds of millions of people.

The University of Sydney and partner institutions in the current ACIAR funded project used a new hybrid wheat technology (the BLA system). This initiative addresses bottlenecks in current seed systems by working with universities, government agencies and small local seed businesses to introduce locally bred hybrid wheat seeds, thereby improving seed availability, increasing wheat productivity, and enhancing food access. Nevertheless, the success of the initiative is largely dependent upon alignment of the research with existing socio-economic conditions and cultural values of large numbers of smallholder wheat growers in these countries. A survey-based study using mixed method approach has been conducted to analyze the factors affecting hybrid wheat seed adoption rate and consumer preferences. A scenario analysis was then used to study the policy level economic implications at different rates of adoption of hybrid wheat seed.

Data were collected from a diverse group of more than 500 respondents, representing different farming classes, regions and cultures. These data were analyzed using multiple techniques including sample mean comparison, multiple logistic regression, and Technical Efficiency Analysis (TE). Results showed that farmers who used hybrid seeds not only achieved higher yields and profits but also operated with greater technical efficiency. Significant factors influencing the adoption of hybrid wheat included land holding size, access to credit, existing asset base, access to market information, and availability of extension services.

Additionally, the DEA model showed that farmers who adopted hybrids had a 10% higher technical efficiency than those growing traditional cultivars. Analysis of consumer data showed that female wheat flour users prioritized taste and aroma of products, whereas male consumers prioritized price and access when purchasing flour.

In summary, the adoption of hybrid wheat seeds holds promise for addressing food security challenges, however, success will depend on careful alignment with local conditions and farmer needs.

#464

Cultivating climate-resilient wheat: assessing an international spring wheat collection across the Americas

Dr Priyanka Gupta[1], Mr Martin Lacroix[1], Dr Martine Jean[1], Mr Boucher St Amour Vincent-Thomas[1], Mr Marc Antoine Rheault[2], Dr Martin Quincke[3], Dr Mohan Kohli[4], Dr Igor Valerio[5], Ms Camila Turra[5], Dr Matthew Reynolds[6], Robert Beauchemin[7], Jules Beauchemin[7], Dr Brent McCallum[8], Dr Maria Antonia Henriquez[8], Dr Reem Aboukhaddour[9], Dr Francois Belzile[1], Dr Silvia B Rosa[2]

[1]Université Laval , , Canada, [2]Centre de Recherche sur les Grains (CÉROM), , Canada, [3]Instituto Nacional De Investigación Agropecuaria Uruguay (INIA), La Estanzuela, Uruguay, [4]Cámara Paraguaya de Exportadores y Comercializadores de Cereales y Oleaginosas (CAPECO), Asuncion, Paraguay, [5]OR Melhoramento De Sementes, Passo Fundo, Brazil, [6]International Maize and Wheat Improvement Center (CIMMYT), Ciudad de México, México, [7]Les Moulins de Soulanges, St-Polycarpe, Canada, [8]Morden Research and Development Centre, Agriculture and Agri-Food Canada, Morden, Canada, [9]Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada, Lethbridge, Canada

Climate change poses a significant threat to wheat production. Developing resilient wheat varieties with enhanced genetic variability is crucial to mitigating the constraints posed by changing climatic conditions. Our study focuses on identifying climate-resilient wheat cultivars from an International Spring Wheat Collection (ISWC) composed of 150 cultivars from diverse regions across North and South America, including East and West Canada, Argentina, Brazil, Chile, Mexico, Paraguay, the United States, and Uruguay. We evaluate the ISWC genomically and phenotypically at six distinct locations: East Canada (three locations), Brazil, Paraguay, and Uruguay.

The ISWC undergoes a comprehensive evaluation for both above and below-ground traits, such as crop ground cover, normalized difference vegetation index (NDVI), root system architecture, yield and its components, disease resistance, and baking quality. This provides crucial insights into genetic variability and adaptability. Biotic and abiotic factors were evaluated in separate trials (2022-2024), with fungicides used in abiotic experiments. Extreme climate variations occurred during the study years across locations.

Our phenotyping assessments have revealed significant genotype variation among wheat cultivars, with some demonstrating superior adaptability across various locations. Notable variability was observed in all analyzed traits. In 2022, the Paraguay trial, harshly affected by drought and frost, had the lowest grain yield mean of 1.4 ton/ha (55 kg/ha to 2.6 ton/ha), while the highest mean was in Brazil at 6.3 ton/ha (1.8 to 9.2 ton/ha). Similarly, kernel weights (19.8 to 51.9 g/thousand kernels) and test weights (51.9 to 84.9 kg/hectolitre) exhibited considerable ranges, highlighting genetic diversity. Disease reaction was assessed based on natural infection and artificial inoculation in disease nurseries (leaf rust, stripe rust, and Fusarium head blight).

The ISWC is being genotyped using markers associated with specific traits of interest, such as disease and pest resistance, phenology, and end-use quality. Genotyping-by-sequencing (GBS) has revealed significant genetic diversity, delineating eight sub-populations aligned with specific breeding programs or regions, informative of natural selections, and breeding efforts. Our ongoing GWAS analysis on multi-environmental data aims to pinpoint genetic loci associated with climate-resilient traits, emphasizing validation and reproducibility to ensure robust findings.

By integrating three years of data (2022 to 2024) with genotyping insights, we strive to comprehensively understand genotype-phenotype-environment relationships. This holistic approach empowers breeders to develop wheat varieties resilient to climate change, bolstering global food security amidst evolving environmental challenges.

Keywords: *wheat, climate-smart, adaptation, genotypic diversity, America*

#63

Breeding climate resilient cultivars in China, progress and challenges

Zhonghu He[1,2*], Yong Zhang [1], Jun Yan[3], Yonggui Xiao[1], Jindong Liu[1], Shuanghe Cao[1]

[1] Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China
[2] International Maize and Wheat Improvement Center (CIMMYT), c/o CAAS, Beijing 100081, China
[3] Cotton Research Institute, c/o CAAS, Anyang 455000, Henan Province, China

Email: z.he@cgiar.org or hezhonghu02@caas.cn

Chinese wheat production is facing great challenges as climate change becomes much significant across the country, thus development of climate-resilient cultivar is crucially important. This presentation will summarize breeding progress and challenges under climate change scenario in China. (1) Yield potential is significantly increased, largely due to the extended grain-filling period characterized with early heading by warming (around 7 days advanced in comparison with 1980) and basically unchanged maturity, but yield stability over seasons and locations are decreased by fluctuation of temperature and rainfall, and increased occurrence of various diseases. (2)

Unmanned aerial vehicle (UAV) and ground-based remote sensing platforms equipped with multi/hyperspectral imagery, are used to characterize variation of agronomic and physiological traits among major cultivars across various locations, and then used for genomic selection for tolerance to drought and high temperature. (3) High yield potential is achieved by selection of short stature with around 80 cm through the combination of Rht 1/Rht 2+Rht 8+Rht 24, compact plant type (small and erect leaves), more spikes, and big and stable grain size. Tolerance to high temperature during grain-filling stage is well achieved by selection of fast grain-filling rate, stay-green and normal maturity. Multi-locational testing is promoted to select for yield potential and broad adaptation.

New white kernel cultivars such as Zhongmai 578, with tolerance to pre-harvesting and to high temperature, in combination of high yield potential and excellent pan bread and noodle qualities, are developed.
Contract production of excellent quality cultivar is achieved to increase farmer income and competiveness of milling industry. Over 20% yield increase with 7-10 days early is achieved by planting winter wheat in traditional spring wheat area such as Gansu and Qinghai. However, much more efforts are needed to understand the mechanism and develop new cultivars with tolerance to temperature fluctuation before heading, and resistance to sharp eyespot and Fusarium crown rot.

Additionally, diversity of cropping system is needed to reduce the occurrence of various diseases.

#304

India’s wheat revolution: path to global leadership by 2047

Ramadas Sendhil[1,*], Pouchepparadjou A[2,**]

[1] Pondicherry University (A Central University), Kalapet, Puducherry 605014, India.
[2] PJN College of Agriculture and Research Institute, Karaikal 609603, India.
* sendhil.eco@pondiuni.ac.in
**pouchepparadjou@gmail.com

India is on the brink of economic powerhouse in global wheat production, poised to reach the top position by the year 2047, commemorating the 100th year of independence. A global leader's top priority is to ensure secured food and nutrition for the ever-growing population, and this has to be achieved amidst several production challenges at the forefront, including climate change. Estimates foresee a 60% increase from the existing yield levels to attain this monumental feat.

In this context, an attempt has been made to project the growth trajectory of wheat production in India (second largest wheat producer) and compare it with China (largest wheat producer), sourcing the data from the Food and Agriculture Organization (FAO) and the United States Department of Agriculture (USDA). Analysis of growth-instability in wheat production (Business-As-Usual scenario) for the period between 1961 and 2024 indicates a positive growth for both India (3.71% per annum) and China (3.12% per annum) with an instability index value estimated at 6.76 and 12.81, respectively.

A similar kind of trend has been noticed when the production values are normalized by considering three-year moving average. This analysis emphasized that India is the most reliable and stable wheat producer. However, to attain the set target and feed the burgeoning population by 2047, a visionary and multifaceted approach is needed in research and development, encompassing technological innovations and interventions, sustainable wheat farming practices, pluralistic extension supported with policy reforms.

To identify the comparative advantage in wheat production and for strategic planning, a SWOC (Strengths, Weaknesses, Opportunities and Challenges) analysis has been carried out. In conclusion, India’s revolution in wheat for economic supremacy embodies a mission-mode framework that integrates research-extension-development-policies to serve as a path for global leadership by 2047.

#344

Wheat biofortification: charting research advances, assessing multi-dimensional impact, and framing policy agenda

Ramadas Sendhil[1,]* Adeeth Cariappa AG[2], Palaniswami Ramasundaram[3], Vikas Gupta[4], Gopalareddy K[5], Om Prakash Gupta[4], Anuj Kumar[4], Satyavir Singh[4], and Gyanendra Pratap Singh[6]

[1] Pondicherry University (A Central University), Kalapet, Puducherry 605014, India.
[2] International Maize and Wheat Improvement Center (CIMMYT), Hyderabad 502324, India
[3] Indian Council of Agricultural Research, New Delhi 110012, India.
[4] ICAR-Indian Institute of Wheat and Barley Research, Karnal 132001, India.
[5] ICAR-Sugarcane Breeding Institute, Coimbatore 641007, India
[6] ICAR-National Bureau of Plant Genetic Resources, New Delhi 110012, India.

* sendhil.eco@pondiuni.ac.in

Globally, the practice of enhancing the nutrient content in staple crops i.e., biofortification is recognised as a promising approach to combat the persisting level of micronutrient deficiency, especially in developing and pro-poor economies. Wheat, being a primary staple foodgrain, has immense potential to address the malnutrition problem.

In this context, this study aims to track and chart the progress of research in wheat biofortification (through VosViewer visualization tool), assess the impact of agronomic and genetic efforts (via meta-analysis), and suggests policy framework for upscaling and outscaling biofortified wheat varieties. Scopus database was used to retrieve the articles using the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) charter. The processed data from the retrieved articles was subjected to meta-analysis and bibliometric network charts to track respectively the multi-dimensional impact and research progress of biofortification.

Further, the popular 'economic surplus' model has been applied to quantify the economic impact of adopting a biofortified wheat variety in India. The bibliometric analysis highlights that emerging economies like India, Pakistan, and China are at the forefront of research on wheat biofortification, closely followed by Turkey, Australia, the United Kingdom, and the United States.

Meta-analysis indicate that agronomic interventions resulted in a remarkable increase of 1.60 standard deviation (SD) in 'Zinc' (Zn) and 1.70 SD increase in 'Iron' (Fe) concentration. Genetic interventions resulted in a substantial 74% and 79% increase in Zn and Fe levels, respectively.

It has been estimated that India's first biofortified wheat variety (WB 02) is cultivated approximately in 144,256 hectares since its release, generated an estimated economic surplus of INR 50.54 million (at constant 2011-12 prices). Overall, the effects of biofortification were substantial in Asia and African regions, grappling with micronutrient deficiencies and hidden hunger.

In the Indian context, genetic biofortification emerged to be impactful in comparison to agronomic biofortification, registering nearly three times higher efficacy than the latter. In addition, genetic biofortification is regarded as cost-effective and sustainable approach. This study advocates for policy framework to strengthen the value chain on wheat biofortification, and also to upscale and outscale the adoption of biofortified wheat varieties, thereby addressing hidden hunger and enhancing the nutritional outcomes.

#111

Contrasting impacts of pre-harvest field sprouting on the functionality of bread and durum wheats

Kun Wang, Carly Isaak, and Bin Xiao Fu*
Grain Research Laboratory, Canadian Grain Commission, 1404 – 303 Main Street, Winnipeg, MB, Canada R3C 3G7
*binxiao.fu@grainscanada.gc.ca

Pre-harvest sprouting is the undesirable germination of mature wheat kernels when wet conditions persist just prior to or during the harvest, resulting in quality loss due to the production of degradative enzymes. This study was conducted to investigate the impact of field sprouting on wheat end-use functionality of Canada Western Red Spring (CWRS) and Canada Western Amber Durum (CWAD), the two premium wheat classes grown in Western Canada for bread and pasta production, respectively.

Selected harvest samples submitted from the producers were composited based on their FN to generate multiple bands varying in Falling Number (FN) from 60 to above 350 s with 50 s increments. All composites were targeted for a constant wheat protein of 13.5%. The two wheat classes followed a similar power law model established from the relationship between α-amylase activity and pasting viscosity as measured by FN test and amylograph. With increasing level of sprouting damage or falling FN, however, CWRS and CWAD responded very differently in terms of changes in milling performance, dough properties and final product quality. The decrease of FN from 450 to 70 s for CWAD did not significantly alter durum semolina yield (67.0 ± 0.2%), ash content (0.73 ± 0.02%), gluten index (87 ± 2%), yellow pigment content (9.3 ± 0.4 ppm), and semolina colour, alveograph parameters and pasta firmness (628 ± 23g).

In contrast, the test weight and flour yield of the CWRS composites decreased gradually with the decrease of FN until ~150 sec., then dropped drastically once the FN fell below 100 sec. The gluten strength weakened progressively with the decrease of FN in each of the CWRS composites. The deterioration in dough handling properties and bread-making quality was very evident for CWRS, but the impact on pasta quality was limited for the CWAD composites. Unlike pasta-making, which involves low water absorption, short mixing time and high-temperature drying, the combination of high water absorption, greater starch damage and long fermentation in the bread-making process facilitate the detrimental effects of excessive α-amylase.

As reflected by milling performance, the endosperm of durum wheat and the starch and gluten proteins therein appear to be much less damaged during field sprouting when compared with bread wheat, likely because of the denser and harder kernel properties of durum wheat.

#84

Breeding of health-promoting wheat: phenotyping enhancement, germplasm mining, and molecular marker utilization

Wenfei Tian[1,2], Zhonghu He[1,2], Yan Zhang[1], Lei Zhi[1], Xue Gong[1], Tiantian Chen[1], Maria Itria Ibba[3], Velu Govindan[3], Awais Rasheed[2,4]

[1] Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 10081, China
[2] International Maize and Wheat Improvement Center (CIMMYT) China office, Beijing 10081, China
[3] International Maize and Wheat Improvement Center (CIMMYT), Texcoco 56130, Mexico
[4] Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan

Epidemiological studies have demonstrated the critical role of staple food on human health. Arabinoxylans (AXs), ferulic acid and alkylrescinols are health-promoting components in wheat. This presentation will summarize our recent progress on development and utilization of health-promoting wheat varieties for improved human health in China, CIMMYT and Pakistan. (1) We have developed enhanced phenotyping methods for analyzing phenolic acids and alkylrescinols, and successfully adapted the CIMMYT method for assessing AXs.

These eco-friendly methods have significantly increased analytical throughput by 3-5 times while reducing costs by 50%. (2) Our evaluation of the health-promoting qualities of diverse wheat lines, including 324 Chinese wheat varieties, 200 Pakistan wheat varieties, and 267 lines from CIMMYT's 53th IBWSN across multiple environments, has broadened our understanding of potential parental lines for enhancing health-promoting qualities. Notably, we found Taishan 1 and Shan 715 had higher AXs content than Yumai 34, the benchmark variety for AXs content reported previously. In spring wheat lines from China, a series of "Jinqiang" wheat lines, whose pedigree could be traced to "Wildcat" of Canada, demonstrated comparable AXs profile to Yumai 34.

In addition, we identified 16 lines from CIMMYT with high ferulic acid content and thousand kernel weight. (3) Through linkage mapping analysis of RIL population derived from Zhongmai 578/Jimai 22, we pinpointed 1 stable QTL of AXs, 3 stable QTL for ferulic acid and 4 stable QTL for alkylrescinols. KASP markers associated with these QTLs have been successfully developed and validated. The combined use of these markers in marker-assisted selection led to the development of CA17169 (now registered as Zhongmai 39), exhibiting improved health-promoting and bread-making qualities. Additionally, we developed variety-specific processing technique of Zhongmai 578, an elite variety with exceptional bread-making quality.

The pearled flour demonstrated 30% improvement in loaf volume compared to conventional whole meal flour, with maximized preservation of AXs, ferulic acid and alkylrescinols. Our study also found a significantly positive correlation between health-promoting quality of whole meal and refined flour, indicating that refined flour could also provide certain amount of ferulic acid and AXs.

Collectively, our findings highlight the importance of integrating knowledge from wheat genetics, food science, and human nutrition to enhance the intake of AXs and phenolics in wheat for improved human health. International collaboration on germplasm resources, phenotyping enhancements, and molecular marker development and application is crucial for advancing global nutrition and health security.

#394

Advance wheat quality management, procurement, preservation and supply in India

[1]Ratnesh Mishra,[1]Indrasheel

[1]Food Corporation of India, Varanasi, Uttar Pradesh, India.

mratnesh0407@gmail.com, indrasheel@gmail.com

Wheat (Triticum aestivum L.) belonging to one of the most diverse and substantial families, Poaceae, is the principal cereal crop for the majority of the world's population. This cereal is polyploidy in nature and domestically grown worldwide.

Wheat is the source of approximately half of the food calories consumed worldwide and is rich in proteins (gluten), minerals (Cu, Mg, Zn, P, and Fe), vitamins (B-group and E), riboflavin, niacin, thiamine, and dietary fibre. Wheat seed-storage proteins represent an important source of food and energy and play a major role in the determination of bread-making quality.

The two groups of wheat grain proteins, i.e., gliadins and glutenins, have been widely studied using SDS-PAGE and other techniques. Sustainable production with little input of chemicals along with high nutritional quality for its precise ultimate uses in the human diet are major focus areas for wheat improvement.

#127

Machine learning feature selection improves single and multi-trait genomic prediction of wheat quality traits

Amidou N'Diaye[1*], Teketel Haile, Curtis J. Pozniak[1]

[1]Department of Plant Sciences and Crop Development Centre, University of Saskatchewan, Saskatoon, SK Canada, S7N 5A8

email: amidou.ndiaye@usask.ca

Wheat is the most widely cultivated crop in the world and supplies 20% of food calories and protein to the world's population. Wheat breeders have been striving to improve the quality standards of the food industry by screening 1000's of breeding lines each year. However, the direct measurement of various end-use quality traits such as milling qualities is daunting and requires a large quantity of grain, traits-specific instruments, and sample throughput is low, all of which limits the screening process.

Advancements in genotyping technologies and the development of new computational approaches has spearheaded advancements in genomic selection (GS) strategies for the prediction of quality attributes with high accuracy. To optimize GS strategies, the selection of informative markers can be an effective strategy to reduce the number of markers and genotyping costs for the practical implementation of GS in wheat breeding.

While several studies have described the impacts of marker density on GS, none of them have addressed strategies to select the most predictive makers to maximize prediction accuracy. In the present study, we used feature selection (FS), a machine learning-based technique, to extract the most informative markers for implementing GS prediction models for end-use quality.

Three independent populations were genotyped with high-throughput SNP arrays and evaluated for various agronomic and quality traits, including yield, protein content, gluten index, dough tenacity, extensibility, and strength. Using FS, we substantially reduced the number of informative markers for all the traits in all populations.

Selecting only the most informative markers resulted in a substantial increase in prediction accuracy for all traits, as compared to using all available markers.For example, using 252 markers improved the prediction accuracy of gluten index by up to 42% in one population, while the improvement in gluten index prediction accuracy reached 164% with only 136 in a second population.

Because plant breeders usually evaluate the performance of breeding lines based on multiple traits, we also applied FS to multi-trait genomic prediction. Our approach gave similar or higher accuracies than the traditional multi-trait model which relied on all available markers, demonstrating the effectiveness of FS for both single- and multi-trait genomic prediction.

Thus, our study presents a novel FS strategy to effectively reduce marker density while maximizing trait prediction accuracy in breeding programs.

#56

Identification of *XAT-7A1*, A multicopy tandem gene responsible for carotenoid esterification in durum wheat

Rodríguez-Suárez C[1], Requena-Ramírez MD[1], Hornero-Méndez D[2]; Atienza SG[1]

[1] Institute for Sustainable Agriculture, CSIC, Avda. Menéndez Pidal s/n, E-14004, Córdoba, Spain
[2] Department of Food Phytochemistry, Instituto de la Grasa, CSIC. Campus Universidad Pablo de Olavide, Edificio 46. Ctra. de Utrera, Km 1, E-41013, Sevilla, Spain

Yellow pigment content, mainly due to the accumulation of carotenoids, is a quality trait in durum wheat grain as it confers the bright yellow color to pasta preferred by consumers. Also, carotenoids are essential nutrients exerting important biological functions in human health. Consequently, biofortification strategies have been developed in many crops to increase carotenoid content. In this context, carotenoid esterification is emerging as a new breeding target for wheat biofortification, as carotenoid esters have been found to promote both carotenoid accumulation and stability. Until recently, no carotenoid esters have been identified in significant proportions in durum wheat grains, and interspecific breeding programs have been started to transfer esterification ability from common wheat and *Hordeum chilense*.

In this work, *XAT-7A1* is identified as the gene responsible for carotenoid esterification in durum wheat. Sequencing, copy number variation and mapping results show that *XAT-7A1* is organized as tandem or proximal GDSL esterase/lipase copies in chromosome 7A. Three *XAT-7A1* haplotypes are described: Type 1, associated with high levels of carotenoid esters production (diesters and monoesters) and high expression in grain development; Type 2, present in landraces with low levels of carotenoid esters (monoesters) or no esters; and Type 3, copies without the signal peptide, resulting in zero-ester phenotypes.

The identification of *XAT-7A1* is a necessary step to make the carotenoid esterification ability available for durum and bread wheat breeding, which should be focused on the Type 1 *XAT-7A1* haplotype, and may be assessed as a single gene since *XAT-7A1* copies are tightly linked showing no recombination in the mapping experiments.

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#72

Emerging challenges in advance wheat quality management, procurement, preservation and end use supply in humid subtropical region

[1]Indrasheel, [1]Prem Kishan

[1]Food Corporation of India, Varanasi, Uttar Pradesh, India

indrasheel@gmail.com, **premkishan4u@gmail.com**

Wheat (*Triticum* spp.) was one of the first domesticated food crops, and for eight thousand years, it has been the basic food staple of the major civilizations of Europe, West Asia and North Africa. Currently, wheat exhibits large genetic diversity with over 25,000 types or cultivars, which are adapted to a wide range of temperate environments. FAO estimated that the world wheat production for year 2024 was approximately 797 million tons. Wheat grain can be processed into flour, semolina and other products that form the basic ingredients of many foods worldwide (e.g. bread, cookies, pastries, pasta, noodles, couscous, etc.).

These foods provide about 20% of the calories and protein source for a large portion of the world’s population. In densely populated countries, such as India or Pakistan, wheat is an important source of calories and proteins, and its consumption will probably increase in other countries such as Bangladesh due to the adoption of a “western lifestyle”.

Therefore, global wheat production needs to increase in the upcoming decades to cover the rising demand for this grain. here is also a range of pathogenic fungi and viruses that cause various leaf and root diseases in wheat. Disease control strategies are underway to limit the damage to the wheat crop. Furthermore, soil moisture level, the depth of seed plantation, PH control for fungal growth reduction.

Methodology: ***Study Area***: Varanasi district, Uttar Pradesh, India

FCI functioning in the Varanasi Mandal District: We annually receive 4300 MT of wheat local purchase of while 100000 MT/year of wheat distribution in Varanasi Region through PDS (Public Distribution System). So, rest of 96000 MT wheat is inward from outside of surplus rice state (Punjab & Haryana) through Rake.

Challenges:

1. Mycotoxins and Aflatoxins.
2. Poor soil fertility due to insufficient/overuse of fertilizers.
3. Soil Erosion, drought, flood, flash floods and water logging further destroy the crops to a great extent.
4. Resistance development in the insect, pest and weeds due to the prolonged use of same quality of fertilizers.

Result:

Name of the chemical	Dilution	Doses with diluted solution	Periodicity	Remarks
Malathion 50% EC	1:100	3L/100 m²	15 days (21 days in winter)	
Deltamethrin 25% WP	40 gms/L	3L / 100 m²	90 days (3 months)	
Aluminium Phosphide	9g/MT	....	Applied on insect spreading	

1. This is the overall data for pest management in FCI but now these days' insects show resistance against chemical treatment. So, there is need of design different option for pest management.
2. Overall, the cultivation area available to meet the demands of dense population like Varanasi is invariably low.

Discussion

1. We ensure that the supplies should reach to all beneficiaries of the concerned district.
2. A biometric scan is performed upon handing over the supplies to the beneficiary to make sure right person has received the right amount.
3. We will try to maintain the nutritional aspects of the product through fortification with essentials nutritional supplements like Folic Acid (10,000ug/kg), Cyanocobalamin (1000ug/kg) and Iron (3525mg/kg) to prevent the nutritional deficiencies.
4. On storage and preservation of wheat, control insects and pests with various methods like spray fumigation, rodent control and insect trapper for maintaining nutritional quality of wheat.
5. Maintain moisture with the help of scientific godown structure. Aeration to protect unwanted growth of fungi which causes mycotoxins and aflatoxins.

Way Forward

1. To design environment friendly, less-toxic and improved varieties of biofertilizers so that there is limited or no resistance development in the insects and pests.
2. Educate farmers on large-scale to procure better produces.

#337

Dissection of gluten gene loci with genome assemblies developed using HiFi long-read sequencing technology

Daowen Wang[1,*], Yuxin Yang[1], Kunpu Zhang[1], Zhiyong Wang[1], Guangwei Li[1]

[1] State Key Laboratory of Wheat and Maize Crop Science, College of Agronomy, and Center for Crop Genome Engineering, Henan Agricultural University, Zhengzhou 450002, China

Email: dwwang@henau.edu.cn

Gluten proteins, consisted of mainly two types of high-molecular-weight glutenin subunits, three types of low-molecular-weight glutenin subunits (LMW-GSs), and four types of gliadins, are major determinant of wheat end-use quality. Variations in the amount and composition of gluten proteins can largely shape the end uses of different wheat varieties.

Accurate identification of gluten proteins and their encoding genes is essential for efficient improvement wheat end-use quality. However, the gluten gene loci in wheat and close relatives are highly complex with multiple paralogs in each, especially those specifying LMW-GSs and gliadins. Moreover, gluten genes have strong allelic variations, further complicating the resolving of gluten proteins in different cultivars.

The advent of genomics has raised the possibility of resolving gluten genes and their complex loci at whole genome level. But it has proved difficult to accurately assemble these loci in the genome assemblies constructed using short-read sequencing technologies, likely due to the presence of multiple homologs with very high sequence similarities.

Therefore, we have constructed the genome assemblies of two elite common wheat cultivars (Xiaoyan 81 and Zhou8425B) by integratively using the latest HiFi long-read and Hi-C sequencing technologies. These highly contiguous and accurate genome assemblies have substantially improved the ability to correctly resolve gluten gene loci at whole genome level. The insights and resources generated in our research may enhance the manipulation of gluten genes for improving wheat-end use quality through genomics-assisted breeding in the future.

#220

Systemic identification of yield regulators via integration of multi-omics in wheat

Xuelei Lin[†], Yongxin Xu[†], Dongzhi Wang[†], Long Zhao[†], Jinchao Chen[†], Zhaoheng Zhang[†], Jun Xiao*

Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China

*Corresponding author: jxiao@genetics.ac.cn

[†]These authors contributed equally to this work

Bread wheat is an essential crop worldwide, supplying over 20% of the world's daily caloric and protein needs. The grain yield of wheat largely hinges on the architecture of the inflorescence and developmental process of endosperm. To unveil regulatory mechanisms governing the spike and grain development, we conducted transcriptome and epigenome profiling across key developmental stages of spike and endosperm. Our findings unveil that chromatin accessibility, H3K27ac, and H3K27me3 collectively regulate flowering transition, spikelet development, starch and seed storage protein (SSP) genes with varying impact.

Leveraging time-series RNA-seq and ATAC-seq data, we constructed a hierarchical transcriptional regulatory network (TRN) governing spike formation, starch biosynthesis, and SSP accumulation. Integration of the TRN with genome-wide association studies (GWAS), population transcriptome and phenotypic analyses of TILLING mutants led to the identification of 36 and 42 high-confidence novel regulators for spike and grain development, respectively.

Among these regulators, TaMYB30-A1 emerged as a key player downstream of WFZP, regulating fertile spikelet number. Notably, the superior haplotype of *TaMYB30-A1*, characterized by a C allele at the WFZP binding site, has been enriched in wheat breeding programs in China, contributing to enhanced agronomic traits. Additionally, TaABI3-A1 was found to modulate SSP accumulation while suppressing starch biosynthesis through transcriptional regulation.

Importantly, we established a free and open access Wheat Spike Multi-Omic Database (http://39.98.48.156:8800/#/). Our study not only identifies novel regulators but also offers an effective strategy for dissecting the genetic underpinnings of wheat yield traits, with practical value for wheat breeding.

#256

Photoperiod-1 allelism modifies the inflorescence transcriptome of bread wheat to alter spikelet architecture and flowering time

Adam Gauley[1,2] †, Marianna Pasquariello[1], Guilherme V. Yoshikawa[3], Abdul Kader Alabdullah[1], Sadiye Hayta[1], Mark A. Smedley[1], Laura E. Dixon[1,2], Scott A. Boden[1,3]

[1]Department of Crop Genetics, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, UK.
[2]Faculty of Biological Sciences, University of Leeds, Leeds, LS2 9JT, UK.
[3]School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Glen Osmond, SA, 5064, Australia.
† Present address: [3]Agri-Food and Business Institute, Belfast, BT6 9SH, UK.

Email: scott.boden@adelaide.edu.au

Photoperiod insensitivity has been selected by breeders to adapt crops to diverse environments and farming practices. In wheat, photoperiod insensitivity is contributed by constitutive alleles of *Photoperiod-1* (*Ppd-1*) that relieve the requirement of long daylengths to flower; however, these alleles also limit yield by reducing the number and fertility of grain-producing florets through processes that are poorly understood.

Here, we investigated the influence of *Ppd-1* on genes that control flowering time and inflorescence development using near-isogenic lines (NILs) that contain either photoperiod insensitive (*Ppd-D1a*) or null (*ppd-1*) alleles of *Ppd-1*.

By analysing the influence of *Ppd-1* in field-grown plants at stages when spikelet number is determined and floret development initiates, we found that *Ppd-1* regulates flowering and inflorescence development by determining the seasonal progression of gene expression. We report that *Ppd-1* influences the stage-specific expression of genes with roles in auxin signalling, meristem identity, and protein turnover, and analysis of differentially expressed transcripts discovered bZIP and ALOG transcription factors that regulate flowering time and spikelet architecture.

Intriguingly, the ALOG transcription factor shares a conserved role in barley, indicating it plays a central role in the formation of unbranched spike inflorescences of the Triticeae. These findings enhance our understanding of genes and that regulate inflorescence development in wheat and introduce new targets for improving yield potential and flowering time.

#482

Targeting chloroplasts to improve salt tolerance in wheat

Nivethitha Baluchamy[1], Nishanth Baluvanahalli Manjunath[1], Neeta Lohani[1], Qiu Fang, Zhonghua Chen[1,2], Jayakumar Bose[1,2,*]

[1]School of Science, Western Sydney University, Hawkesbury Campus, NSW, Australia
[2]Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, NSW, Australia

* E-Mail: J.Bose@westernsydney.edu.au

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 examined the ability of leaf tissue to maintain its greenness and photosynthesis in a range of cereals containing A, B, D and R genomes (*Avena sativa, Hordeum vulgare, Aegilos tauschii, Triticum monococum*, *T. durum*, three varieties of *T. aestivum*, *Secale cereale,* and a hybrid between *T.aestivum* and *S. cereale*) by exposing whole plants and excised leaves to 150mM and 50 mM of NaCl, respectively.

We measured chlorophyll content, photosynthetic parameters, dead leaf percentage, leaf ion (Na and K) concentrations, and Na distribution within the mesophyll cell compartments (chloroplasts, vacuole and cytosol) using Na-selective CoRoNaGreen dye. Our findings suggested that enhanced photosynthesis and the stay-green trait of wheat under salt stress are linked to the inheritance of the D genome.

Subsequently, we tested several wheat cultivars and landraces containing the D genome for enhanced tissue tolerance. Our results showed that there was a striking difference in leaf tissue tolerance and greenness maintenance among different cultivars of *T. aestivum* despite having the D genome.

#471

Wheat yield potential can be maximised by increasing r:fr light conditions at critical developmental stages

M. Fernanda Dreccer[1,*], Alec B. Zwart[2], Ralf-Christian Schmidt[3], Anthony G. Condon[2], Mary A. Awasi[1], Terry J. Grant[1], Alexander Galle[3], Stephane Bourot[3], Claus Frohberg[3]

[1] CSIRO Agriculture and Food, Queensland Bioscience Precinct, 306 Carmody Road, Saint Lucia, QLD 4067, Australia
[2] CSIRO Agriculture and Food, Building 101, Clunies Ross Street, Black Mountain, ACT 2600, Australia
[3] BASF Innovation Center Gent, BASF Belgium Coordination Center CommV, Technologiepark-Zwijnaarde 101, 9052 Gent, Belgium

[*]fernanda.dreccer@csiro.au

Sensing of neighbours via the Red to Far-Red light ratio (R:FR) environment may exert a cap to yield potential in wheat. The effects of an increased R:FR inside the canopy were studied in dense wheat mini canopies grown in controlled environments by lowering FR. To distinguish between effects exerted by light sensing and assimilate supply, the treatments were complemented with elevated CO_2, applied between different developmental timepoints to specifically impact tillering, spike growth, floret fertility and grain filling in different combinations.

The yield response to high R:FR was strongly dependent on the developmental stage in all three cultivars and pivoted between positive if applied after the start of stem elongation, and negative or null if applied before. Yield gains of up to 70% and 120% were observed respectively in two cultivars, associated with a higher number of tiller spikes and grains per spike in the main shoot, underpinned by the expression of an active N metabolism.

The response to the combination of high R:FR and elevated CO_2 or CO_2 alone were cultivar dependent. Taken together, our results suggest that R:FR exerts a significant control on yield potential in wheat and achieving a high R:FR from stem elongation to maturity is a promising lever towards a significant increase in grain yield.

#499

Accelerating access to high value wheat germplasm through the australian grains genebank strategic partnership

Sally Norton[1], Katherine Baum[1], Nicole Sawyer[1], Kerrie Forrest[2], Josquin Tibbits[2], Gabriel Keeble-Gagnere[2], Matthew Hayden[2]

[1] Australian Grains Genebank, Agriculture Victoria, Department of Energy, Environment and Climate Action (DEECA), Horsham, Victoria, Australia
[2] AgriBio, Agriculture Victoria, DEECA, Bundoora, Victoria, Australia

Email sally.norton@agriculture.vic.gov.au

The Australian Grains Genebank (AGG) safeguards the plant genetic resources (PGRs) that underpin agricultural grain crop production for Australia. It is custodian to one of the largest and most diverse collections of temperate and tropical grain crop species globally including cultivated, landrace and wild relative species. The AGG has the mandate to acquire, conserve, maintain and distribute PGRs to plant breeders and researchers across Australia, and operates in accordance with Australia's obligation under the International Treaty on Plant Genetic Resources for Food and Agriculture.

The AGG is a Strategic Partnership between the Grains Research and Development Corporation (GRDC) and Agriculture Victoria. The application of genomic and digital innovation is being used to unlock the genetic potential of germplasm conserved within the AGG to enable more effective, efficient and cost sustainable genebank management and increased accessibility to industry.

The AGG has implemented new policies and procedures across acquisition, distribution and genebank management that have accelerated access and increased the utility of germplasm. These policies and practice changes have integrated genomic and passport data, and developed diversity subsets to ensure only the most valuable PGRs are acquired, conserved, maintained and made accessible to genebank users to facilitate and accelerate the development of profitable, climate resilient grain crops.

Here we describe the Policy changes, germplasm and genomic data accessibility and explore how they will facilitate widespread access to PGRs for crop improvement and the benefits that will accrue to industry from these changes. The AGG is a vital asset for the agricultural sector, underpinning the development of more resilient and high-yielding grain crops, which are essential for food security and sustainable agriculture and the profitability of the Australian grains industry.

#486

Hybrid wheat initiation in Bangladesh: an assessment of adaptation to enhance wheat productivity

Abul Awlad Khan[1*], Md Abdul Hakim[1], M Mahbubur Rahman[1], Golam Faruq[1], Rebecca Thistlethwaite[2], Richard Trethowan[2]

[1]Bangladesh Wheat and Maize Research Institute (BWMRI), Nashipur, Dinajpur-5200, Bangladesh. [2]The University of Sydney, NSW 2006, Australia
*aakhanwrc@gmail.com

A set of 101 exotic F_1 wheat hybrids developed by a novel and practical Blue Aleurone (BLA) system at The University of Sydney were evaluated under Bangladesh Wheat and Maize Research Institute, Dinajpur and Rajshahi during 2022-2023 cropping season applying standard production technology and then produced F_1 wheat hybrids from the crosses between BLA derived $[2n = 42(msms) + 4E]$ male sterile lines $[2n = 42(msms)]$ and high yielding native cultivars with the objectives to release hybrid wheat variety suitable for Bangladesh condition. Obtained results revealed that 10 F_1 hybrids gave 6-19% increased yield over the best check variety, BARI Gom 33 at Dinajpur and up to 22% yield over another check, BARI Gom 32 at Rajshahi conditions.

Despite, taking the overall views into account, three exotic F_1 wheat hybrids, PBI20NOW-F1-142, PBI18N-F1-47 and PBI20N-F1-18 produced 18, 19 and 22% increased yield respectively over the best check variety. Therefore, regarding yield potential, these three hybrids are comparable to superior pure line wheat varieties. Concurrently hybrid seeds were produced from thirty cross combinations where six high yielding local varieties were crossed with five BLA derived male sterile lines for resilience to local environment.

After field performance assessment twelve F_1 wheat hybrids were selected for evaluation during 2023-2024 cropping season at Bangladesh Wheat and Maize Research Institute, Dinajpur and the highest grain yield was obtained from the cross BLA 2 (R26 3-1) × BARI Gom 33 followed by BLA 3 (PBI13 C030- SSD1C-SSD-SSD-11C-2N) × BWMRI Gom 3, with yield increase 26% and 18%, respectively over the best check variety. These two hybrids were resistant to leaf rust disease and *Bipolaris* leaf blight disease moreover, had medium stature, reasonable grain size and number of grains/spike with early phonological property, which are considered as acceptable characteristics of a highly adapted and high yield potential wheat hybrid under Bangladesh condition.

Since the methodical findings are the first sight of hybrid wheat research in Bangladesh, further authentication trial along with required quantities hybrid seed production is indispensable for the selected F_1 wheat hybrids, eventually to promote hybrid wheat varieties cultivation by the farmers of Bangladesh.

#90

Progress in understanding the biology of Magnaporthe oryzae pathotype triticum in Bangladesh

Krishna Kanta Roy[1*], Muhammad Rezaul Kabir[1], Kishowar E Mustarin[1], Golam Faruq[1], Xinyao He[2], Aakash Chawade[3], Dave Hodson[4], Pawan Kumar Singh[2]

[1]Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur-5200, Bangladesh;
[2]International Maize and Wheat Improvement Center (CIMMYT), El Batan, Texcoco, Mexico;
[3]Swedish University of Agricultural Sciences (SLU), Alnarp, Sweden;
[4]International Maize and Wheat Improvement Center (CIMMYT), Nepal.

***E-mail:** rkrishnaroy666@gmail.com (corresponding author- Roy)

The emerging wheat blast disease caused by the fungus *Magnaporthe oryzae* pathotype *Triticum* (MoT) is becoming a serious constraint to global wheat production. The disease was initially detected in Brazil in 1985, and later observed in several South American countries as well as in South Asia (2016) and Southern Africa (2018). This emerging and expanding disease urgently needs intensive research on different aspects of pathogen biology to mitigate the threat of wheat blast. Several strategic research activities on wheat blast pathology were undertaken by Bangladesh Wheat and Maize Research Institute (BWMRI) in the last 8 years (2017 to 2024).

These include study of pathogen biology, field survey and monitoring, long-term preservation of isolates, disease epidemiology, fungal virulence, identification of alternative hosts and cross infection, prevalence and survivability of MoT on wheat seeds/straw, infection assays, fungal morphology, molecular identification, and genetic diversity of MoT. Our results show that wheat blast spread from 8 to 29 districts in Bangladesh within the period of 2016 to 2024. To date, around 400 isolates of MoT have been isolated from wheat and other hosts, and all the isolates are preserved in deep freezers. Conditions have been optimized to develop pure cultures of the pathogen, namely: temperature (29^{o}C), light regime (continuous fluorescence light), media (OMA/PDA), and pH (6.5-7.0).

Our study revealed that high temperature (27-29^{o}C) and high humidity (>80%) along with high leaf wetness (>24hrs) is conducive for wheat blast outbreak under field conditions. Several grass weeds (*Eleusine, Digitaria, Panicum, Pennisetum* etc.) were identified as suspected alternative hosts for the disease. Survival rates on seed and wheat straw were also tested. Survival on wheat seeds was 1.5-2 years and on straw 2-3 months. Seed could serve as sources of infection for future seasons.

Regarding seed to seedling transmission, MoT can move from the coleoptiles, or even sometimes from the secondary roots to the first leaves. Cross infection studies demonstrated that MoT can infect some other cereal crops (durum, triticale, barley, and oat) and at least two grass species (*Eleusine* and *Pennisetum*) under laboratory conditions.

Electron microscopy studies indicated that MoT can infect and colonize the rachis within 72hrs after infection. Morphological and molecular characterization have been completed for around 290 isolates. Morphological and molecular variations among the isolates were observed over space and time. Results from these research activities at BWMRI in Bangladesh have resulted in increased understanding, and subsequent management strategies needed for MoT.

#206

Modelling gene-trait-yield relationships in wheat

Enli Wang[1,*], Hamish Brown[2], Greg Rebetzke[1], Zhigan Zhao[1,3], Bangyou Zheng[4], Neil Huth[5], James Hunt[6], John Kirkegaard[1], Maxwell Bloomfield[7], Corinne Celestina[6], Jess Hyles[1], Kenton Porker[8], Felicity Harris[9], Ben Biddulph[10], Ben Trevaskis[1]

[1] CSIRO Agriculture and Food, Black Mountain, ACT, Australia
[2] The New Zealand Institute for Plant and Food Research Limited, Christchurch, New Zealand
[3] China Agricultural University, Beijing 100094, China
[4] CSIRO Agriculture and Food, St. Lucia, QLD, Australia
[5] CSIRO Agriculture and Food, Toowoomba, QLD, Australia
[6] School of Agriculture, Food and Ecosystem Sciences, University of Melbourne, Melbourne, Australia
[7] Department of Animal Plant and Soil Sciences, La Trobe University, Bundoora, VIC, Australia
[8] CSIRO Agriculture and Food, Urrbrae, SA, Australia
[9] School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia
[10] Department of Primary Industries and Regional Development, East Perth, WA, Australia

Wheat growth and grain yield is strongly modulated by the complex genotype (G) by environment (E) and management (M) (GxExM) interactions. Process-based crop modelling has been considered as an effective means to disentangle the GxExM interactions and assist in developing breeding and management synergies to increase crop performance for target environments. However, most current crop models lack the ability to establish the gene-trait and trait yield relationships. They cannot effectively capture the genetic control of physiological traits and often assume uniformity of many important traits (e.g. leaf size, tillering, resource use efficiency and partition of resources to different organs) across genotypes, limiting their applications to evaluate the impact of elite traits of modern cultivars to optimize GxExM.

We present our recent efforts in modelling gene-trait-yield relationships in wheat. We incorporated the newest understanding in genetics to develop a molecular-physiology model to simulate wheat flowering time, which enabled simplification of phenotyping of phenology and prediction of flowering time and grain yield of new wheat cultivars from genotyping data and at time of release. In addition, we developed gene-based approaches for simulation of leaf development and grain yield of wheat with the new genetic traits of early vigour and long coleoptiles.

We demonstrate how incorporation of genetic understanding and data into farming systems modelling can enable gene-trait-yield simulations across environments to assist in the design of ideotypes and management strategies to optimise GxExM for increased productivity and resilience of wheat under climate change.

#467

Industry-led digital tools to unlock the genetic potential of the australian grains genebank

Yu K[1], Isdale D[1], Chisanga D[1], Mahdi R[1], Forrest KL[1], Wong D[1], May M[2], Tibbits J[1,3], Norton S[2], Keeble-Gagnère G[1], Hayden MJ[1,3*]

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

The genomic characterization of entire genebank collections around the world is creating a vast amount of data for plant genetic resources (PGRs) including wheat. To fully unlock their potential for crop genetic improvement, it is important to make PGR data accessible in ways that facilitate their use in breeding and research.

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 centre. A key part of its program is to develop digital tools to enable the Australian grains industry to unlock the genetic potential of PGRs conserved within the AGG. This is being achieved through an industry driven co-design, co-develop, co-deliver model, where industry input is used to define use-cases that describe how they want to interrogate PGR data to facilitate the selection of PGRs to address research and breeding questions.

Here, we provide examples of digital tools and functionality currently developed to facilitate industry use of PGRs in breeding and research. *Lamington*, a Shiny application implementing CoreHunter3, was developed to simplify the selection of PGR core sets based on user-defined criteria. *Pretzel*, an interactive webtool for visualising multidimensional datasets from the scale of the nucleotide to pangenome, provides functionality that enables PGR genotype data to be seamlessly linked to research and breeding knowledge.

The digital tools being developed aim to provide genebank users without specialised bioinformatics skills with access to PGR data in formats that will facilitate their selection and optimal use in breeding and research.

#518

Understanding the potential of nitrogen use efficiency genes in wheat using CRISPR/CAS9

Hitashi Aggarwal[1], Yong Han[1,2], Sina Nouraei[1], Vanika Garg[1], Tristan Coram[3], Wujun Ma[1,4], Michael GK Jones[1] and Rajeev K Varshney[1,*]

[1]Centre for Crop & Food Innovation, WA State Agricultural Biotechnology Centre, Food Futures Institute, Murdoch University, Murdoch, WA 6150, Australia
[2]Department of Primary Industries and Regional Development, South Perth 6151, Australia
[3]Australian Grain Technologies, Waite Campus, Wine Innovation Central Building, Urrbrae, SA 5064, Australia
[4]College of Agronomy, Qingdao Agriculture University, Qingdao 266109, China

*Correspondence: rajeev.varshney@murdoch.edu.au

The global demand for food is rapidly increasing, necessitating advancements in crop productivity and sustainability. Wheat, a crucial staple food crop, plays a pivotal role in meeting this escalating demand. One of the key traits influencing wheat yield and environmental impact is Nitrogen Use Efficiency (NUE).

Addressing NUE directly poses a substantial challenge, given its dependence on both grain yield and protein content, which exhibit a negative correlation with each other. Moreover, the excessive use of fertilizers contributes to greenhouse gas emissions, underscoring the imperative need for improvement in NUE. CRISPR/Cas9 technology offers a promising method for validating NUE genes. This improvement is crucial not only for achieving enhanced crop yields but also for maintaining environmental stability. The majority of trait mapping studies aimed at identifying Quantitative Trait Loci (QTLs) governing NUE-related genes were conducted in non-Australian environments.

This results in a scarcity of resources for understanding the NUE genes, specifically in Australia. However, two major studies have been conducted on Australian wheat populations, generating valuable data on NUE QTLs and identifying specific regions governing NUE genes.

In this project the candidate NUE genes identified through these QTL studies will be validated using CRISPR/Cas9 approach. The ultimate goal of this study is to improve nitrogen utilization in wheat, significantly boosting crop productivity and reducing environmental impact.

#451

Innovar project highlights how to use genomics to characterize durum wheat varieties' breeding value

Matteo Bozzoli[1], Cristian Forestan[1], Martina Bruschi[1], Sandra Stefanelli[1], Francesco De Sario[1], Alessia Confortini[1], Anna Giulini[2], Tommaso Bardelli[2], Elena Novarina[2], Matteo Ruggeri[3], Davide Meriggi[3], Valentina Manstretta[3], Edoardo Bartoccetti[4], Sripada Udupa[5], Preben Klarskov Hansen[6], Shun Sakuma[7], Manar Makhoul[8], Rod Snowdon[8], Thorsten Schnurbusch[9], Humberto Fanelli Carvalho[10], Julio Isidro y Sánchez[10], Fiona Doohan[11], Lisa Black[12], Claudio Ratti[1], Roberto Tuberosa[1], Marco Maccaferri[1]

EU H2020 FP7 INNOVAR project aims to augment existing wheat CPVO Distinctness-Uniformity-Stability (DUS) and Value for Cultivation and Use (VCU) protocols to include a combination of traditional phenotyping and innovative genomics and phenomics techniques. INNOVAR developed two panels of durum and bread wheat representative of the European germplasm (250 and 280 cultivars, respectively). Additionally, UNIBO and international partners assembled the Svevo Durum wheat reference genome and a comprehensive Global Durum Genomic Resources including: the Tetraploid Global Collection (TGC: 1,856 tetraploids) and the Global Durum Panel (GDP: 1,033 varieties worldwide).

All resources were genotyped with the Illumina 90K wheat SNP Array and characterized under a network of field trials for: (i) 52 DUS agronomic traits, (ii) phenology, agronomic, yield and quality traits, (iii) 20 vegetation indexes obtained by UAV drone flights at eight key developmental stages, (iv) response to wheat diseases including Soil Borne Cereal Mosaic Virus (SBCMV) and yellow rust (YR). Haplotype genome wide association analysis on DUS traits revealed strong signals for all traits. We report about two valuable loci, considered as case-studies for DUS and VCU, responsible for grain yield and resistance to SBCMV. In wheat, grain yield is positively associated with grain number per inflorescence.

We identified *Grain Number Increase-2* (*GNI-2*) on chr. 2A (R^2=38%), a paralogue of *GNI-1*, as a main determinant of grain number increase in the analysed durum panels. We fine mapped it with 11 KASP® markers to a 3.9 Mb interval. We demonstrated that alleles showing suppressed or reduced expression at both *GNI-2* and *GNI-1* increase grain yield in both durum and bread wheat. As to SBCMV resistance, a major QTL on chr. 2B named *QSbm.ubo-2BS=Sbm2* (R^2=60%) was identified and fine mapped to a region of 1.5 Mb. We designed 21 KASP® markers defining resistant/susceptible haplotypes to SBCMV.

The resistant haplotype spread in both North American, French and Northern Italian germplasm while highly susceptible alleles were identified in Mediterranean region and CIMMYT germplasm. The gene content of the locus showed a clear enrichment for disease-response related genes.

The development of informative KASP markers and candidate gene identification will augment the information accuracy for variety selection in breeding programs and European CPVO registration protocols. The Global Durum Genomic Resources provides unprecedented opportunities to exploit the tetraploid diversity.

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Affiliations

[1]Alma Mater Studiorum – Università di Bologna, Department of Agricultural and Food Sciences and Technologies (DISTAL), viale Giuseppe Fanin 40-50, 40127 Bologna, Italy, [2]Research Centre for Plant Protection and Certification, Via Venezian 22, 20133, [3]Horta, Via Sant'Alberto 327 - 48123 Ravenna (RA), Italy, [4]Salt & Lemon Srl - Piazza Mascagni 11, 10015 Ivrea (TO), [5]International Center for Agricultural Research in the DryAreas (ICARDA) Rabat 10112, Morocco, [6]Department of Crop Protection, Research Centre Flakkebjerg, Danish Institute of Agricultural Sciences, 4200 Slagelse, Denmark. [7]Agrogenomics Research Center, National Institute of Agrobiological Sciences, 305-8602 Tsukuba, Japan, [8]Department of Plant Breeding, Giessen University, Heinrich-Buff-Ring 26, D-35392 Gießen, Germany, [9]Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, [10]Centro de Biotecnología y Genómica de Plantas UPM – INIA Parque Científico y Tecnológico de la U.P.M. Campus de Montegancedo Autopista M-40, Km 38 - 28223 Pozuelo de Alarcón, Madrid, Spain, [11]UCD School of Biology and Environmental Science, UCD Earth Institute and UCD Institute of Food and Health, University College Dublin, D04 V1W8 Dublin, Ireland, [12]AFBI, Plant Testing Station, Crossnacreevy, Belfast BT6 9SH, United Kingdom

Emails: matteo.bozzoli2@unibo.it , cristian.forestan@unibo.it , martinabruschi86@gmail.com , sandra.stefanelli@unibo.it , francesco.desario3@unibo.it , alessia.confortini2@unibo.it , annapiamaria.giulini@crea.gov.it , tommaso.bardelli@crea.gov.it , elena.novarina@crea.gov.it , m.ruggeri@horta-srl.com , d.meriggi@horta-srl.com , v.manstretta@horta-srl.com , edoardo.bartoccetti@saltlemon.it , s.udupa@cgiar.org , pkh@tystofte.dk , ssakuma@tottori-u.ac.jp , manar.makhoul@agrar.uni-giessen.de , rod.snowdon@agrar.uni-giessen.de , schnurbusch@ipk-gatersleben.de , humberto.fanelli@gmail.com , j.isidro@upm.es , fiona.doohan@ucd.ie , lisa.black@afbini.gov.uk , claudio.ratti@unibo.it , roberto.tubersa@unibo.it , marco.maccaferri@unibo.it

#415

Modelling plant-pathogen-interactions across different environments (RUSTHEALTH)

Claudia Breitkreuz[1], Tim Kloppe[2], Kerstin Flath[2], Philipp Schulz[2], Jochen Reif[3], Efraim Contreras Estrada[3], Andreas Stahl[1], Albrecht Serfling[1]

[1]Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Erwin Baur Straße 27, 06484 Quedlinburg, Germany
[2]Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Field Crops and Grassland, Stahnsdorfer Damm 81, 14532 Kleinmachnow, Germany
[3]Breeding Research Department, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Seeland, Germany

claudia.breitkreuz@julius-kuehn.de ; tim.kloppe@julius-kuehn.de ; kerstin.flath@julius-kuehn.de; philipp.schulz@julius-kuehn.de ; reif@ipk-gatersleben.de ; contreras@ipk-gatersleben.de ; andreas.stahl@julius-kuehn.de ; albrecht.serfling@julius-kuehn.de

With their broad host spectrum, rusts (Pucciniales) play a decisive role as obligate biotrophic fungi in agriculture. In wheat production in particular, yellow rust (*Puccinia striiformis*), leaf rust (*Puccinia triticina*) and, in future, stem rust (*Puccinia graminis*) due to climate change, cause considerable yield losses.

To reduce the infection level and at the same time avoid extensive use of fungicides, breeders invest massive effort in the development of wheat varieties with respective resistances. Despite, vertical resistance break downs occur regularly as a consequence of adaption of the pathogen, but also as a lack of knowledge in temporal and spatial occurrences of rust races with distinct virulence/avirulence pattern.

In the project RustHealth, we aim to address specifically this lack of knowledge by investigating plant-pathogen-interactions at 13 different locations across Germany in three consecutive years. In the first year, infection by stripe and leaf rust is documented for in total 200 wheat varieties selected from German elite cultivars and genebank accessions. These field trials are complemented with infection incidences of different rust races, e.g. Warrior(-) for stripe rust and 77 WxR for leaf rust, measured by the Macrobot platform under controlled conditions.

The comparison of controlled and field conditions at the 13 different locations will indicate environment dependent effects on infection patterns. In parallel, leaves from infected plants are collected to investigate spatial patterns of rust race occurrences. Overall, these datasets will be compiled to model genotype rust x genotype wheat x environment relationships.

In contrast to stripe and leaf rust, stem rust is artificially inoculated to check for unknown resistance loci in plant genetic resources. Overall, this project contributes to a better understanding of plant-pathogen-interactions strengthening breeding efforts by the development of markers, breeding material and knowledge about the biochemical background of resistances against rusts in wheat.

#279

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

Oscar Carey-Fung[1], Jesse T. Beasley[1], Roger Hellens[2], Alexander A. T. Johnson[1]
[1]School of BioSciences, The University of Melbourne, Melbourne, Australia
[2]GenXtraits, California, USA

ofung@student.unimelb.edu.au

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

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

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

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

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

#125

The roles of root exudates in root-soil interactions in monocots (Wheat) and dicots (Arabidopsis)

Emily Carr[1], Claire Grierson[1], Keith Edwards[1], Malcolm Hawkesford[2], Xiaoxian Zhang[2], Hannah Cooper[3], Richard Whalley[2], Sacha Mooney[3]

[1] School of Biological Sciences, 24 Tyndall Avenue, University of Bristol, Bristol, United Kingdom, BS8 1TQ
[2] Rothamsted Research, West Common, Harpenden, United Kingdom, AL5 2JQ
[3] Life Sciences Building, East Drive, University of Nottingham, Nottingham, United Kingdom, NG7 2TQ

ec17981@bristol.ac.uk; claire.grierson@bristol.ac.uk; k.j.edwards@bristol.ac.uk; malcolm.hawkesford@rothamsted.ac.uk; xiaoxian.zhang@rothamsted.ac.uk; hannah.cooper@nottingham.ac.uk; richard.whalley@rothamsted.ac.uk; sacha.mooney@nottingham.ac.uk

The Global population is increasing rapidly, and the yield of staple crops like wheat must be increased to feed everyone. Despite this, recent peer reviewed research indicates that an annual total of nine million tonnes of wheat are lost globally to soil erosion – equivalent to total UK wheat production in 2020. Declining crop yields are traditionally increased by agricultural intensification; however, this causes soil degradation and desertification, which exacerbates the issues facing food production.

Therefore, reducing the loss of arable land is critical for the future success of agriculture. One potential solution is root exudates, which aggregate soil particles and therefore improve soil structure.

There is much to understand about root exudates and the pathways for their release, but the two candidate genes *xyloglucan endotransglucosylase 23* (*XTH23*) and *ABC2 homolog 6* (*ATH6*) have been proposed to affect exudate compositions. We are using Arabidopsis mutants in these genes for uprooting and soil binding experiments to compare their adhesive properties.

To investigate whether these genes have similar functions in monocots and dicots, the results from these experiments will be compared to similar results from wheat mutant experiments. This has required the creation of wheat *ATH6* mutants using TILLING lines. CRISPR technology has also been utilised to create *XTH23* wheat mutants.

The CRISPR process was completed in house, using golden gate cloning to make the constructs that were introduced to the wheat genome though biolistic transformation. Subsequent genotyping has successfully identified CRISPR mutants lacking the majority of their coding sequences which can be taken forward for exudate analysis.

This has required the development of a new assay for quick and facile wheat root exudate collection as well as a new uprooting assay to help measure root-soil cohesion in wheat.

#363

Unveiling the mechanism of the sr62 wheat tandem kinase

Renjie Chen[1], Jian Chen[2], Megan A. Outram[2], Oliver R. Powell[1], Karthick Gajendiran[1], Yan L. Wang[1], Guotai Yu[1], Stefan A. Arold, Peter N. Dodds[2], Brande B. H. Wulff[1]

[1]King Abdullah University of Science and Technology, Biological and Environmental Science and Engineering Division, Thuwal, Saudi Arabia
[2]CSIRO Agriculture and Food; Canberra, Australian Capital Territory, Australia.

renjie.chen@kaust.edu.sa

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

Additionally, we cloned the corresponding fungal AvrSr62 effector from stem rust isolate Pgt21-0 and showed its interaction with Sr62$^{\mathrm{TK}}$. Alphafold predictions suggest that a β-finger in the N-terminal kinase domain of Sr62$^{\mathrm{TK}}$ contributes to Sr62$^{\mathrm{TK}}$ homodimerization and AvrSr62 binding. We found that different orthologous NLR genes from wheat can support the Sr62$^{\mathrm{TK}}$-mediated immune response, both in wheat protoplasts and in *N. benthamiana* leaves, upon detection of the AvrSr62 effector.

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

#102

From drones to satellites: biophysics-informed machine learning provides remote estimation of dynamic biomass across scales

Qiaomin Chen[1,†,*], Zhi Chen[2,‡], Pengcheng Hu[3], Bangyou Zheng[4], Daniel T.L. Smith[1], Javier Fernandez[1], Ismail I. Garba[1], Scott C. Chapman[1,*]

[1]School of Agriculture and Food Sustainability, The University of Queensland, Queensland 4067, Australia. [2]School of Electrical Engineering and Computer Science, The University of Queensland, Queensland 4067, Australia. [3]CSIRO Agriculture & Food, GPO Box 1700 Canberra, ACT, 2601, Australia. [4]CSIRO Agriculture & Food, Queensland Biosciences Precinct, Queensland 4067, Australia

Emails: qiaomin.chen@uq.edu.au or scott.chapman@uq.edu.au

Improving crop productivity with a low environmental impact is highly needed to fulfill a sustainable food demand in face of increasing population and irreversible climate changes. Dynamic biomass prediction is critical for crop growth monitoring and subsequent crop production management. Although methods for dynamic biomass prediction have been progressing from descriptive model to mechanism model and process-based model, these models are unsuitable for large-scale and wide-range applications due to parameterization issues.

Developments in remote sensing and machine learning technologies lead to data-driven models that overcome difficulties related to parameterization, but extending applications of these models in new environments are usually restricted to training data collected from limited environments. To address these challenges, here we present a biophysics-informed machine learning framework model that incorporates biophysical information into training data and learning process.

The resulting framework model provides plausible and reliable prediction of wheat biomass dynamics from sowing to harvest at different spatial scales using daily weather data and limited spectral data at corresponding spatial resolution during crop growth season.

Our proposed model provides plausible prediction of dynamic biomass from near weekly frequency of spectral observations combined with daily weather data on independent simulation datasets, with seasonal relative mean absolute error (RMAE) within 20% and of ~7% on average for more than 250 thousand scenarios. While validating on experimental datasets, it offers a prediction accuracy with seasonal RMAE of 20-26% for trial-level biomass and of 12-19% for plot-level biomass.

This model offers an effective and efficient manner for multiscale dynamic biomass prediction, which not only bridges agricultural remote sensing and plant high-throughput phenotyping but also demonstrates the potentials of combining crop modelling and machine learning for crop growth monitoring.

#281

Uncovering new sources of resistance against Chilean stripe rust races: genome-wide association mapping analysis in a common wheat panel.

Nicolas Cobo[1,*], Dalma Castillo[2], Ivan Matus[2], Paula Silva[3]

[1] Facultad de Ciencias Agropecuarias y Medioambiente, Universidad de La Frontera. Temuco, Chile. [2] Instituto de Investigaciones Agropecuarias, INIA Quilamapu. Chillán, Chile. [3] Instituto Nacional de Investigación Agropecuaria (INIA), La Estanzuela. Colonia, Uruguay.

In Chile, stripe rust caused by Puccinia striiformis f. sp. tritici (Pst) has been the main disease threatening wheat production since 2018. During the last two decades new races of Pst with increased aggressiveness, broader virulence profiles, and tolerance to high temperatures have spread worldwide, producing devastating epidemics in both common and durum wheat.

Pst pathotypes isolated in Chile are among the most aggressive identified worldwide and can produce total yield loss if not controlled on time. The rapid erosion of Pst resistance genes caused by the appearance of new races of the pathogen, the lack of knowledge about the genetic basis of resistance to local races, and the rapid loss of resistance in Chilean commercial varieties during recent years, evidence the urgent need of finding new sources of resistance effective against local races.

To detect effective resistance loci that can be efficiently deployed in breeding programs, an association mapping panel of 180 common wheat cultivars and breeding lines from Chile, Uruguay and Mexico was evaluated for both adult-plant and seedling stage resistance to Pst. Field evaluations were conducted in five environments in Chile (2 locations in 2022-23 and 3 locations in 2023-24 seasons), while seedling stage screening was conducted for three Pst genetic groups (PstS7, PstS10, PstS13) at INIA-Uruguay.

Genotyping with the Illumina® iSelect® 25K Infinium® Single Nucleotide Polymorphism (SNP) genotyping array yielded 11,555 polymorphic markers after QC analyses (missing data $< 10\%$ and MAF > 0.05). Undergoing genome-wide association analyses have identified several significant QTL with some of them being consistent across multiple environments and explaining up to ~50% of the trait phenotypic variance for infection type and severity.

Our preliminary results revealed a high phenotypic variation for resistance to Chilean stripe rust races in the panel studied, and represent a step towards the diversification of current resistance genes deployed in local varieties. In addition, molecular markers (i.e., KASP assays) will be developed for SNPs associated with stripe rust resistance, providing a valuable tool for wheat breeding programs to facilitate their rapid adoption.

#476

Using environmental covariates to improve genotype by environment interaction modelling in genomic prediction models in wheat

Rishap Dhakal[1], Guillermo Sniadower[2], Paula Silva[3], Betina Lado[2], Martin Quincke[1], Pablo González Barrios[2], Lucia Gutiérrez[1]

[1] Department of Plant and Agroecosystem Sciences, University of Wisconsin-Madison, 1575 Linden Drive, Madison, WI 53706, USA. [2] Facultad de Agronomía, Universidad de la República, Av. Garzón, 780, Montevideo 12900, Uruguay. [3] Instituto Nacional de Investigación Agropecuaria (INIA), 70000 Colonia, Uruguay

Differential performance and changes in the rank of genotypes across environments make selecting the best genotypes challenging in both Multi-Environment Trials (MET) and Target Population of Environments (TPE). Therefore, it is relevant and crucial to study the causality behind rank changes, known as genotype by environment interaction (GEI). With the integration of genomic selection in wheat breeding schemes, the need to develop genomic prediction models that incorporate GEI for better selection became apparent. Observed variance-covariance structures fitted in mixed model (GBLUP GxE) settings have been proposed.

However, such strategies are limited in their ability to predict beyond the tested environment boundaries. In contrast, the idea of using environmental covariates (EC) either in variance-covariance structure (GBLUP GxEC) or random regression model (RRM) has gained more attention due to its ability to better connect genotype to phenotype and its advantage in predicting genotype performance in untested environments.

Our main goal is to develop an improved genomic prediction model that integrates GEI and EC. We hypothesize that using EC will provide better characterization of environments and enhance prediction power for both tested and untested environments. In addition, using RRM will enable better characterization of genotypes. We are using 11 years grain yield dataset (2010-2020) from 4 locations in the National Wheat Breeding Program (WBP) of Uruguay.

We will fit different genomic prediction models, including GBLUP, GBLUP GxE, GBLUP GxEC, and RRM, and test their predictive ability using different cross-validation schemes (CV1, CV2, and CV0). The best selected model could be utilized in the breeding program for selecting candidates in wheat breeding pipeline considering GEI.

Along with that, RRM fitted with EC could help us for selection of adapted materials in a climate change context.

#254

Unveiling floral biology for enhanced hybrid wheat production

Samira El Hanafi[1] and Jochen C. Reif[1]

[1] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Seeland, Germany

Emails hanafi@ipk-gatersleben.de; reif@ipk-gatersleben.de

The development of hybrid wheat varieties holds significant promise for enhancing agricultural productivity. Central to this endeavor is a comprehensive understanding of floral biology, which underpins successful hybridization. However, the main bottlenecks are the sluggish selection of complementary parent lines and the high costs of hybrid seed production.

To elucidate the key aspects of floral biology relevant to hybrid wheat production, we tested hybrid seed set of diverse central European elite material using chemical hybridization agent (CHA) and cytoplasm male sterility (CMS) systems pollinated with either elites or plant genetic resources (PGR) in multi-environmental field experiments.

The results showed that female tester lines significantly impacted seed set, with environmental factors playing a prominent role. The observed variation in female effects is partially attributed to inherent differences in pollen-receptivity qualities and overall line performance per se. In contrast, the variability associated with the male tester lines was significantly lower than that observed with the female tester lines.

In the other hand, our findings revealed that elite male lines showed 1.6-fold better seed set compared to plant genetic resources. Nevertheless, male lines substantially reduce adverse effects of PGRs when tested in a hybrid background. The use of CHA-based approach demonstrated a notable advantage over CMS-based approach, resulting in a 1.7-fold improvement in seed set compared to the latter.

This improvement is likely attributed to the full restoration of fertility in the produced hybrids using CHA. However, additional investigations are warranted to validate this observation, emphasizing the importance of deepening our understanding of the genetics underlying female receptivity in hybrid wheat production to optimize seed set efficiency and advance hybrid breeding strategies.

#329

Unveiling diversity and adaptation: hulled wheat for mediterranean climate

Aviya Fadida-Myers[1,2] Dana Fuerst[3], Aviv Tzuberi[1], Shailesh Yadav[1], Kamal Nashef[1], Rajib Roychowdhury[1], David Maleda[1], Marina Bizerman[1], Carolina Paola Sansaloni[4], Maria Itria Ibba[4], David Bonfil[5], Sariel Hübner[3] and Roi Ben-David[1*]

[1] Department of Vegetables and Field Crop, Institute of Plant Sciences, Agricultural Research Organization (ARO) - The Volcani Center, Rishon LeZion 7505101, Israel. [2] The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 7610001, Israel. [3] Galilee Research Institute (Migal), Tel-Hai Academic College, Upper Galilee 12210, Israel. [4] Genetic Resource Program, International Maize and Wheat Improvement Center (CIMMYT), Carretera México-Veracruz Km. 45, El Batán, Texcoco, C.P., 56237, Mexico. [5] Field Crops and Natural Resources Department, Agricultural Research Organization, Gilat Research Center, Israel.

Email: Aviya.fadida@mail.huji.ac.il

Health-food market trends promote new commercial interest in ancient wheat such as emmer (Triticum turgidum dicoccum) and spelt (T. aestivum spelta). Both crops enjoy growing demand from consumers and farmers as high-value premium grain crops. Previous studies in our lab on diverse collections of emmer (n=121) and spelt (n=150) have shown that early phenology is a key adaptive factor for both crops under the Mediterranean environment.

In such environments, late phenology genotypes are exposed to prolonged and severe terminal drought before or right after anthesis, resulting in significant reductions in grain yield penalties. To study the agronomic and grain quality potential of spelt and emmer in the Mediterranean region, we have initiated two comparative studies: (1) GxExM field study: this study includes representative accessions (n=2) of each of the following crops (emmer, durum, spelt, and bread wheat) (G) across three environments (E).

Two agronomic aspects were investigated: irrigation (with and without supplementary irrigation) and field stand (70 vs 140 kg seeds/ha) (M); (2) Grain shape association study: a large collection (n=507) of emmer, durum, spelt, and bread wheat was genotyped by DArTseq and characterized for grain shape and grain quality (e.g., gluten content and quality, Fe, Zn content, SDS etc.) across three environments in order to assess genomic and phenotypic associations.

Analysis of grain yield using AMMI has highlighted the low yields of late phenology spelt, especially under water-limited conditions. As expected, lower field stand resulted in lower grain yield, however, this negative response was significantly higher in both modern wheats with a reduction of ~30%. Emmer showed relatively high stability across different environments and management practices, including water deficit. However, emmer and spelt yielded about 60% to 30% of durum and bread wheat, respectively, indicating the clear need for breeding and selecting for early prototypes.

In the association study, we observed significant differences in grain shape between the four wheat groups, particularly in grain roundness, a parameter associated with higher flour extraction rate. Complementary quality analysis of this germplasm is currently being conducted in collaboration with the CIMMYT quality lab.

Once finalized, this analysis will enable us to associate grain shape and grain quality with SNP variation across wheat genome. Applying this knowledge via targeted breeding aimed at developing early-flowering emmer and spelt varieties with high grain quality, better adapted to Mediterranean environment, is also being discussed in the context of diversifying agro-systems and enhancing farmer's profits.

#493

Environment characterisation of wheat national variety trials in Australia

Javier A. Fernandez[1,*], Vivi Arief[1], Pengcheng Hu[1,2], Bangyou Zheng[2], Carla Gho[1], Jip Ramakers[3], Martin Boer[3], Fred van Eeuwijk[3], and Scott Chapman[1]

[1] The University of Queensland, St Lucia, Brisbane, QLD 4067, Australia. [2] CSIRO, St Lucia, Brisbane, QLD 4072, Australia. [3] Wageningen University of Research, Wageningen, Gelderland 6708PB, the Netherlands.

Correspondence: j.fernandez@uq.edu.au

To assist growers and industry in varietal decision-making, variety performance trials are conducted to evaluate the yielding ability of commercially available cultivars across different growing conditions. With more than a hundred wheat (Triticum aestivum L.) trials each year, the National Variety Trials (NVT) program in Australia encompasses diverse environmental conditions producing large genotype-by-environment (G×E) variability.

In this study, we aimed to classify the seasonal environmental patterns in NVT into few interpretable groups or environment types (ET) as a potential useful strategy for describing such G×E variability in Australia. We compared approaches for such classification that vary in which way the environmental information were taken into account: static or functional indices.

The former considers environmental effects via temporally static or aggregated data by phenological phases of the crop, while the latter combines functional environmental indices to capture the dynamic nature of G×E over the season via continuous time series data. When the resulting ET were included in mixed model analyses, both the ET and G×ET terms were consistently significant.

However, G×ET accounted for larger proportion of variance when ET were defined based on static indices. ET derived from this method were notoriously different in three main characteristics: water, nitrogen, and cold stresses. Across 2015 to 2021 wheat NVT, the most frequent environment type was associated with low water and nitrogen availability, while those with optimal conditions were considerably less frequent.

Our study provides insights into the value of incorporating advanced environmental characterisation techniques in the classification of Australian wheat variety trials. These findings can inform breeders, agronomists, and growers in tailoring strategies to address climate variability challenges and enhance wheat yield in diverse environmental conditions.

#141

Economic analysis of genetic resistance in cropping systems: a case study of Australian wheat-rust systems

Abebayehu Geffersa[1], Susie Sprague[1] and Luke Barrett[1]

[1] Commonwealth Scientific and Industrial Research Organisation (CSIRO) Agriculture and Food, GPO Box 1700, Canberra, ACT 2601, Australia

Email: abebayehu.geffersa@csiro.au

Genetic resistance provides an efficient means of controlling rust diseases of wheat. However, pathogen evolutionary adaptation, leading to the breakdown of resistance, is common and can cause damaging epidemics. This requires farmers to use higher-input methods for disease control, and ongoing investment in research and development associated with the breeding of wheat varieties with new forms of effective resistance.

However, there has been relatively little effort to quantify the overall economic benefits that farmers receive from effective resistance. This makes it difficult to accurately articulate to farmers the advantages of adopting new resistant varieties as opposed to using alternative control methods (e.g. fungicides), or for breeders and other stakeholders to understand the value associated with the development of new resistant germplasm.

In this paper, we examine the economic benefits of wheat genetic resistance to rust disease in Australian cropping systems. We start by introducing a flexible economic framework to quantify the benefits of resistance. Leveraging empirical data for 2021 cropping season—over 5,000 observations from the Australian wheat-rust systems—we (i) examine how rust-resistant wheat cultivars are adopted and replaced; and (ii) quantify the economic benefits of wheat-rust resistance in terms of input-savings.

Our findings show that the use of resistant wheat cultivars reduces the fungicide application (% of farms applying fungicide) and intensity (frequency of applications of fungicide). Specifically, we observed a reduction of 12.4% in application and 0.117 in intensity. Extending our analysis, we demonstrate that the input-saving effect of cultivar resistance can lead to an average cost saving of AU$7.98 per hectare across all production zones.

A significant reduction in reliance on fungicides also has the potential to promote sustainable disease management, especially in low-input cropping systems. However, such benefits can only be fully realized if significant effort is put into identifying effective incentives for the adoption and uptake of resistance deployment strategies to increase resistance durability.

#361

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

David Gilbert[1], Nicolas Trenk[1], Alberto Prieto[2], Clare Moscrop[2], Andrew Steed[1], Rose Mcnelly[1], Alba Pacheco-Moreno[1], Tom O'hara[1], Kumar Gaurav[1], Kara Boyd[1], Jitender Cheema[1], Marianna Pasquariello[1], David Seung[1], Paul Nicholson[1], Ruth Bryant[2], Sanu Arora[1*]

[1]John Innes Centre, Norwich Research Park, Norwich, UK. [2]RAGT Seeds Ltd[2], Cambridge, UK

david.gilbert@jic.ac.uk and sanu.arora@jic.ac.uk

Eyespot, caused by the fungal pathogens Oculimacula yallundae and Oculimacula acuformis, is a historically important disease of wheat and other cereals. To date, limited resistance loci have been deployed in wheat, with breeding largely focusing upon the usage of a single R gene, Pch1. However, whilst Pch1 has shown an enduring efficacy against both eyespot causative pathogens, dependency upon a single or handful of resistance sources is a strategy that has proven persistently unwise.

With numerous examples of R gene breakdown often associated with single gene deployment. Perhaps worryingly, efforts to identify further sources of resistance amongst wheat landraces and cultivars has failed to yield any new major effect loci. Indeed, whilst Pch2 (and a separate QTL, QPch.jic-5A) were identified in French wheat cultivar Capelle deprez, both Pch1 and Pch3 were derived or detected in wheat wild relatives Aegilops ventricosa and Dasypyrum villosum respectively.

In our research, through a collaborative project with an industrial partner, RAGT, we have focused on identifying the causative sources of resistance to O. yallundae infection that has been reported in Ae. tauschii. Utilising historical and newly generated data combined with k-mer based association genetics we have uncovered an NLR gene on chromosome 1D that provides protection against O. yallundae in addition to a new QTL detected on chromosome 3D.

We have provisionally designated it as Pch4 and have shown that resistance segregates in an additive dominant manner and conveys the same level of resistance when transferred into wheat. Pch4 provides an equivalent resistance in wheat as Pch1 and will therefore be useful for introducing into breeding programs. Excitingly, we show that whilst this gene is the dominant source of resistance in Ae. tauschii, there are other unknown sources available.

To our knowledge this is the first example of an R gene cloned from Ae. tauschii that conveys protection against a soil borne pathogen, and therefore opens new avenues for future research.

#542

Limitations of phenomic prediction for evaluating wheat stem sawfly resistance in wheat

Sydney Graham*[1], Katherine Frels[1]

[1]University of Nebraska – Lincoln (UNL), Department of Agronomy & Horticulture, Lincoln, USA 68583

*sgraham12@huskers.unl.edu, kfrels2@unl.edu

The expanding region of the Wheat Stem Sawfly (WSS) threatens wheat in the Great Plains region of the United States. Although increased stem solidness improves resistance to WSS, developing solid-stemmed cultivars requires time-consuming and destructive phenotyping methods.

To expedite development of WSS resistant cultivars a high-throughput phenotyping method for evaluating stem solidness and WSS infestation is needed. Therefore, we aimed to assess the potential of phenomic prediction with uncrewed aerial systems (UAS) to predict stem solidness, WSS infestation, and yield in wheat.

Multispectral and red-green-blue UAS data was collected at several time points at two naturally infested locations in Western Nebraska from 2022-2023. The UAS measurements were used to calculate spectral reflectance indices, which were compared with yield, plant height, stem solidness, and WSS infestation. Linear and ridge regression models were then trained to use spectral indices to predict yield and WSS infestation. We found plant height and stem solidness were significantly negatively correlated ($R^2 = -0.36$) and stem solidness did not affect yield ($R^2 = 0.02$).

At flowering, WSS infestation was significantly correlated to the green band ($R^2 = 0.36$), and during grain fill, stem solidness was significantly correlated with several indices. For WSS, the prediction accuracies were -0.44 for ridge regression and 0.06 for linear regression. Despite the significant correlations, our ability to predict WSS resistance was low, and we did not find a viable high-throughput phenotyping system for WSS.

Plant breeders will continue progressing with labor-intensive WSS screening methods while searching for an improved phenotyping system.

#132

Short-season wheat for winter sowing in Australia: is the 100 day concept feasible?

Timothy Green[1, *], Dr Juan Sergio Moroni[1], Dr Felicity Harris[1], Dr Jim Pratley[1], Dr Daniel Mullan[2], Dr Greg Rebtzke[3]

[1]Gulbali Institute, School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga, 2678, NSW, Australia. [2] InterGrain, Bibra Lake, 6163 WA, Australia. [3]CSIRO Agriculture and Food, 2601, ACT, Australia

A recent focus of wheat breeders in Australia has been on developing longer maturity varieties. These phenologically slower varieties can be sown earlier in the year to harvest moisture over a longer growing season and still flower in the optimum flowering window.

However, a changing global climate is shifting rainfall out of autumn, when it is required for sowing and enabling good establishment, and into early winter. This shift in the autumn break is forcing more growers to sow dry in order to cover increasingly larger sowing operations and resulting in poor emergence and subsequent grain yields.

Furthermore, herbicide resistant weed populations are increasing globally, requiring more time to control weeds between seasons with double-knock herbicide applications and other alternate strategies. The proposed 100-day wheat ideotype would be sown mid-winter, and then grow and develop rapidly to enable optimum timing of flowering and profitable yields.

Broadscale field experiments across multiple years at Merredin, Yanco, Wagga Wagga, and Narrabri have evaluated over 100 pre-breeding, highly early vigorous wheat lines. Experiments conducted at Wagga Wagga in 2021 and 2022 experienced wetter than normal conditions with excellent rainfall, and minimal harmful frosts compared to an average season.

In these conditions when sown mid-winter, some pre-breeding lines yielded significantly higher than current commercial lines. The traits these lines possess will be presented as examples of an ideal ideotype of a late-sown short-season wheat for Australia. Such an ideotype will provide breeding companies with a list of identified plant traits for introgression into a commercially viable wheat variety.

#408

The 4t and 7t introgressions from amblyopyrum muticum and the 5au introgression from triticum urartu increases grain zinc and iron concentrations in hexaploid wheat backgrounds.

Veronica F. Guwela[1, 2, 3], Moses F. Maliro[3], Martin R. Broadley[1, 2], Malcolm J. Hawkesford[2], James M. Bokosi[3], Surbhi Grewal[1] Benedict Coombes[4], Anthony Hall[4], Caiyun Yang[1], Mike Banda[3], Lolita Wilson[1], Julie King[1]*

[1] School of Biosciences, University of Nottingham, Loughborough LE12 5RD, United Kingdom1. [2] Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, United Kingdom2. [3] Lilongwe University of Agriculture and Natural Resources, P.O. Box 219, Lilongwe, Malawi3. [4] Earlham Institute, Norwich Research Park, Norwich, NR4 7YZ, United Kingdom4.

Email: v.guwela@gmail.com julieking@nottingham.ac.uk

Micronutrient deficiencies (MNDs) particularly zinc (Zn) and iron (Fe) remain widespread in sub-Saharan Africa (SSA) due to low dietary intake. Wheat is an important source of energy globally, although cultivated wheat is inherently low in grain micronutrient concentrations.

Hexaploid wheat/Am. muticum and wheat/T. urartu BC1F3 introgression lines, developed by crossing three Malawian wheat varieties (Kenya nyati, Nduna and Kadzibonga) with DH-348 (wheat/Am. muticum) and DH-254 (wheat/T. urartu), were phenotyped for grain Zn and Fe, and associated agronomic traits in Zn-deficient soils in Malawi.

Whole genome sequencing, Chromosome specific KASP genotyping and Genomic in-situ hybridization revealed that some introgression lines carried either a combination of the 4T and 7T Am. muticum introgressions on wheat chromosome 4D and 7A respectively, or a 4T and 7T segment only. Introgression lines with the T. urartu segments had the 5Au segment recombined with wheat chromosome 5A.

Field phenotyping results showed that 98% (47) of the BC1F3 introgression lines had higher Zn above the checks Paragon, Chinese Spring, Kadzibonga, Kenya Nyati and Nduna. 23% (11) of the introgression lines showed a combination of high yields and an increase in grain Zn by 16-30 mg kg -1 above Nduna and Kadzibonga, and 11-25 mg kg -1 above Kenya nyati, Paragon and Chinese Spring.

Among the 23%, 64% (7) also showed 8-12 mg kg -1 improvement in grain Fe compared to Nduna and Kenya nyati. Grain Zn concentrations showed a significant positive correlation with grain Fe, whilst grain Zn and Fe negatively and significantly correlated with TKW and grain yield.

Use of wheat wild relatives in breeding programs has potential to increase mineral nutrient density in wheat grains.

#287

Pan-genome facilitated discovery of dominant north American stripe rust lineage's origin in somatic hybridization

Samuel Holden[1,2], Meng Li[1], Mehrdad Abbasi[1], Ramandeep Bamrah[1], Sang Hu Kim[3], Sean Formby[3], Sean Walkowiak[4], Guus Bakkeren[3], Gurcharn S. Brar[2]

[1] Faculty of Land and Food Systems, The University of British Columbia, Vancouver, BC V6T 1Z4, Canada. [2] Faculty of Agricultural, Life and Environmental Science, University of Alberta, Edmonton, AB T6G 2P5, Canada. [3] Agriculture and Agri-Food Canada, Summerland Research Center, BC, V0H 1Z0, Canada. [4] Canada Grain Commission, Grain Research Laboratory, MB, R3C 3G8, Canada.

Samuel.holden@ubc.ca, meng.li@botany.ubc.ca, Mehrdad.abbasi@ubc.ca, david.kim2@agr.gc.ca, sean.formby@gmail.com, sean.walkowiak@grainscanada.gc.ca, guus.bakkeren@agr.gc.ca, gurcharn.brar@ualberta.ca.

Biotic stressors such as pathogens can jeopardize global food security. When considering the wheat rust diseases (stripe, stem, and leaf rust), the largest threat to production comes from the emergence of new, highly virulent pathogen strains as exemplified by the 2015 'Warrior' epidemic in Europe, and the ongoing spread of Ug99 across Africa.

We take advantage of recent advances in sequencing technology and field pathogenomics which have made it viable to monitor the population dynamics of wheat rusts through sampling and genotyping field populations to assemble the genomes of over 20 isolates of Puccinia striiforimis f. sp. tritici (Pst, the causal pathogen of wheat stripe rust), including PacBio HiFi and HiC assembly of four fully phased genomes which account for each of the two haploid nuclei (the dikaryons) present in a rust colony; developing the first field pan-genome of Pst.

By comparing representatives of the major lineages present in North America, we used these data to demonstrate that the Pst18 lineage which has become dominant in North America, supplanting the older PstS1 lineage shares a single haploid nuclear genome with PstS1; indicating that the two are related through somatic hybridization with a third, unknown, lineage.

To our knowledge this marks the third conclusive demonstration of lineage emergence via somatic hybridization in the wheat rusts and the first in Pst specifically. Coupled with the mixed evidence to support ongoing sexual recombination in Pst this result has powerful implications for the emergence of novel lineages worldwide, and underscores the importance of monitoring field populations for the introduction of genetic novelty which may accompany the emergence of totally new pathogen races.

#322

Improving fungal effector prediction and enabling cultivar selection by integrating disease phenotyping and pathogen pan-genomics

[1]Mohitul Hossain, [1]Kristina Gagalova, [1]Naomi Gray, [1]Pavel Misiun, [1]Fiona Kamphuis, [1]Lukas Hunziker, [1]Angela Williams, [1]Eiko Furuki, [1]Huyen Phan, [1]James Hane

[1]Centre for Crop & Disease Management, School of Molecular & Life Sciences, Curtin University, Perth, Australia.

Emails: Mohitul Hossain - mdmohitul.hossain@postgrad.curtin.edu.au and James Hane - james.hane@curtin.edu.au

Pathogen-secreted effectors mediate fungal crop diseases and accurate identification of effectors can help develop disease resistance cultivars. However, in fungi, accurately predicting effectors is complicated because they generally lack sequence conservation.

In the last decade, predictive tools based on the physicochemical properties of effectors have significantly improved in accuracy, but a gap remains between the number of predictions and what can be validated experimentally, so obtaining a reliably reduced candidate list is important. Effector prediction tools have not yet incorporated disease phenotype data that may identify candidates directly associated with host-specific disease outcomes.

Here we propose a method for further refinement of effector candidates - “EffectorFisher” – which mines low-cost pan-genome survey data and integrates disease phenotyping with established bioinformatic methods. EffectorFisher was benchmarked on the Parastagonospora nodorum-wheat pathosystem, for which there are 5 known effector loci (ToxA, Tox1, Tox3, Tox5, Tox267). EffectorFisher significantly improved effector prediction, demonstrated by improved ranking of known effectors by 2 to 13 times compared to previous methods and also reduced total candidate numbers by ~3.5-fold.

To further investigate potential applications of EffectorFisher, a minimum-viable experimental design was assessed with simulated datasets with reduced numbers of pathogen isolates (20-100) and/or phenotyped cultivars (2-10), which generated comparable results for known effectors for upwards of approximately ~40 isolates and ~4 cultivars.

Preliminary application of EffectorFisher to the Zymoseptoria tritici-wheat interaction (versus 6 known effectors) has yielded a similar trend of improved known effector ranking and candidate reduction. Furthermore, numerous past pan-genomic surveys of fungal pathogens typically lack quantitative phenotyping panels, yet have recorded basic cultivar metadata.

To make use of such legacy datasets, we incorporated non-quantitative cultivar data into EffectorFisher and demonstrated similar outcomes, which allows for its broader application.

EffectorFisher generates isolate-specific effector isoform profiles, which can be used to predict which effector (or candidate) isoforms confer higher or lower virulence against specific cultivars. Our new capability for effector isoform profiling has led to new development of methods to match surveyed isolate-specific isoform fingerprints to disease phenotype and yield databases, with a goal of optimal paddock-specific selection of cultivars for both yield and disease resistance.

Overall, EffectorFisher demonstrates that incorporating phenotypic data can significantly improve the effector prediction process and has led to the development of new capabilities for management of biotic stress and cultivar selection. So far EffectorFisher has been applied to two wheat pathogens - a necrotroph and hemibiotroph - but is broadly applicable to many other patho-systems.

#498

Pathogen (P. striiformis) genetic diversity in European wheat responses studied in Pakistan's Himalayan region

Aamir Iqbal[1]*, Zia ur Rehman[1], Muhammad Rameez Khan[1], Shahid Ulllah Khan[1], Muhammad Arif[1], Safi Ullah[1], Monsif Ur Rehman[2], and Sajid Ali[1,2]*

[1]Department of Agriculture, Plant Breeding & Genetics, Hazara University Mansehra, Pakistan.

*Corresponding author: aamiriqbal890@aup.edu.pk

This study involved 30 European (EU) wheat lines and encompassed multiple locations, spanning a total of 25 environments (location x year). The diversity of yellow rust disease resistance was also assessed using molecular markers (11 Yr markers). The study also assessed the existence of diverse and recombinant Yr (P. striiformis) pathogen genetic lineage on European (EU) wheat lines prevalent in the studied.

The results indicated a steady progression of the disease pressure across the scoring at locations during the study period. Considering the impact of different years, the disease incidence was highest in 2018 and lowest in 2022. For much of the EU wheat lines, the disease incidence ranged from 20% to 60% during the 2018-2019 seasons. In contrast, the overall disease incidence was very low, ranging from 0% to 20%, at all locations during the 2020-2021 season.

Among the tested EU wheat lines, none exhibited complete resistance across all tested locations (ACI=0). This suggests the presence of major gene-based resistance or possibly a combination of several minor genes, as opposed to partial resistance. The diversity of EU wheat lines based on molecular markers was evaluated using a set of 11 molecular markers linked to yellow rust (Yr) genes.

The analysis revealed that the Yr5 markers was the most common, detected in 89% of the EU wheat lines, followed by Yr34 (detected in 68%), and Yr6 (detected in 21%) of the tested EU wheat lines while none of the EU wheat lines showed the presence of Yr1, Yr7, Yr9, and Yr36. This highlights the presence and absence of diverse yellow rust disease resistance in EU wheat lines, making them a valuable resource for enhancing the resistance level in Pakistani (PK) germplasm.

The result also showed that the population was relatively divergent compared to PK germplasm. An overall selection was observed in the isolates collected from EU or PK germplasm whereas this impact was absent within the germplasm for wheat lines, which means that the overall germplasm had impact on the pathogen structure, but within each germplasm the wheat lines are equally infected by different pathogen lineages. Additionally, different pathogen genetic lineages attacked the EU wheat lines and none of these EU wheat lines were attacked by a specific pathogen lineage across the locations.

This information on the response of EU wheat lines not only provides valuable insights for utilizing them as a source of novel resistance but also aids in predicting their performance against potential future invasive strains from the Himalayan region. The deployment of EU wheat lines and their crossing with PK germplasm would contribute strongly to reduce the disease burden on wheat under the Pakistani conditions.

#96

Exploring basal spikelet infertility through spatial transcriptomics

Maximillian Jones[1], Katie Long[1], Anna Elisabeth Backhaus[1,2], Nikolai Adamski[1], Ashleigh Lister[3], Jun Xiao[4], Cristobal Uauy[1]

[1] John Innes Centre, Norwich, United Kingdom
[2] International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco
[3] Earlham Institute, Norwich, United Kingdom
[4] Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China

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

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

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

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

We have also begun exploring spike development with fully spatial transcriptomics. Our semi-spatial dataset was used to inform selection of a 300-gene panel for VizGen MERFISH, a highly multiplexed form of fluorescence in-situ hybridisation providing subcellular resolution of single transcripts. MERFISH transcript counts correlate well with expression values from the semi-spatial RNA-seq ($\rho=0.67\pm0.04$ SD), indicating that the data can be used quantitatively as well as qualitatively. In addition to validating findings from our semi-spatial study, the data is proving useful for generating new hypotheses on the genetics of spike development and basal spikelet infertility.

#339

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

Katharina Jung[1,*], Reiko Akiyama[1], Jilu Nie[2], Naoto Hamaya[1], Masahiro Kishii[3], Naeela Qureshi[4], Sridhar Bhavani[5], Thomas Wicker[6], Beat Keller[6], Shuhei Nasuda[2,*], Kentaro K. Shimizu[1,7,*]

[1] Department of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland
[2] Graduate School of Agriculture, Kyoto University, Japan
[3] Japan International Research Center for Agricultural Sciences (JIRCAS), Japan
[4] International Maize and Wheat Improvement Center (CIMMYT), Mexico
[5] International Maize and Wheat Improvement Center (CIMMYT), Kenya
[6] Department of Plant and Microbial Biology, University of Zurich, Switzerland
[7] Kihara Institute for Biological Research, Yokohama City University, Japan

*Corresponding authors: katharina.jung@ieu.uzh.ch; nasuda.shuhei.5z@kyoto-u.ac.jp; kentaro.shimizu@uzh.ch

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

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

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

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

The detection of two well-known rust resistance genes (Lr34/Yr18 and Lr67/Yr46) validates our results and their respective origins in China and Pakistan. Additionally, the identification of two potentially novel resistance genes on chromosome 3D and 5B presents promising candidates for integration into breeding programs.

By utilizing diverse wheat varieties in the NAM population originating from key geographic regions, we have gained valuable insights into the geographic distribution and evolution of yellow rust resistance.

#537

Developing superior lines with enhanced NUE using marker-assisted backcrossing

Tarkjot Kaur[1], Sina Nouraei[1], Rakshith S R Gowda[1], Vanika Garg[1], Kefei Chen[2], Darshan Sharma[3], Dion Bennett[4], Rajeev K Varshney[1*]

[1]Centre for Crop and Food Innovation, WA State Agricultural Biotechnology Centre, Food Futures Institute, Murdoch University, Murdoch, WA 6150, Australia. [2] Curtin Biometry and Agriculture Data Analytics, Molecular and Life Sciences, Curtin University, Bentley, WA 6102, Australia. [3] Department of Primary Industries and Regional Development, South Perth, WA 6151, Australia. [4] Australian Grain Technologies, Northam, WA 6401, Australia.

*Correspondence: rajeev.varshney@murdoch.edu.au

Wheat (Triticum aestivum L.) is one of the most important crops in the world, but the grain quality and quantity is heavily reliant on applied nitrogen. Wheat utilizes only about a third of the applied nitrogen, making it one of the most nitrogen-inefficient crops.

Australian wheat industry has been suffering from low nitrogen use efficiency (NUE) and nitrogen dilution effect, leading to lower grain protein content (GPC). Since GPC is the prime decider of market price in national and international markets, optimizing NUE is paramount for enhancing the productivity and profitability of the farmers while also alleviating the negative impacts of fertilizers on the environment.

A previous study assessed the genetic basis of 22 traits contributing to enhanced NUE using 6 doubled haploid (DH) populations. Based on phenotypic screening across four locations in Australia, the study identified 14 quantitative trait locus (QTL) groups on 10 different chromosomes. Through reanalysis of the QTLs using the combined consensus map of the six DH populations, six major effect QTL clusters have been identified for 3 key traits namely GPC, grain yield (GY) and NUE, accounting for 4.36 – 22.89% of the phenotypic variance.

Out of the 6 clusters, one cluster on chromosome 2B has been identified to be associated with all the three traits across two locations while accounting for 14.09 – 22.89% of the phenotypic variance. Our study aims to introgress this QTL cluster into 2-3 leading Australian varieties/lines to develop lines with higher NUE and GPC without any compromise on yield.

The parent of the DH contributing to the superior alleles of the QTL cluster and specific DH lines will be used to perform strategic crosses with target Australian genotypes for marker-assisted backcrossing (MABC) in controlled conditions followed by rapid generation advancement.

The resultant lines will be screened on the basis of background recovery of the genomes of the target Australian genotypes to obtain a subset of high NUE introgression lines, which can be advanced for multi-location trials across different Australian wheat production environments.

This study will not only provide markers associated with NUE-related traits but also the superior NUE lines for global wheat improvement.

#158

Assessing leaf rust resistance in wheat under elevated CO2: Insights from greenhouse and field studies

Jasper Krößmann[1], Lars Kretschmer[2], Lisa Waßmann[3], Bernd Rodemann[3], Lorenz Kottmann[2] Albrecht Serfling[1] and Andreas Stahl[1]

[1] Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Resistance Research and Stress Tolerance, Quedlinburg, Germany. [2] Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Crop and Soil Science, Braunschweig, Germany. [3] Julius Kühn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Crops and Grassland, Braunschweig, Germany.

Common wheat (Triticum aestivum L.) stands as a cornerstone of global human nutrition, yet its vulnerability to leaf rust infections, instigated by Puccinia triticina, poses a substantial threat, potentially resulting in significant yield reductions. To safeguard yields, the development and cultivation of resistant varieties emerge as the most efficacious and environmentally sound strategy.

The atmospheric CO2 concentration has almost doubled since the pre-industrial time and it can be firmly assumed, that the CO2 concentration will continue to increase in the years to come. Despite numerous studies examining the impacts of elevated atmospheric CO2 concentrations, a comprehensive investigation into its effects on infection and resistance behaviour across a diverse range of wheat varieties remains notably absent from the literature.

In the WheatFACE project, we aim to deepen our understanding of how elevated CO2 levels affect resistance mechanisms in winter wheat. In particular, we are investigating whether elevated CO2 levels increase susceptibility due to increased biomass growth from CO2 fertilisation or, conversely, reduce fungal invasion through mechanisms such as increased stomatal closure or enhanced plant resistance.

To investigate these dynamics, we exposed two different European winter wheat populations, each comprising around 200 genotypes, to different CO2 concentrations (420 ppm vs. 800 ppm) while infecting them with two particularly aggressive leaf rust isolates. The evaluation of leaf rust infestation includes both manual assessment and digital quantification of the infested leaf area using high-throughput technology. The resulting findings provide information on the influence of elevated CO2 on the severity and extent of leaf rust infection in wheat.

In addition, in a two-year field trial, we tested twelve European winter wheat varieties with different resistance behaviour to leaf rust at different CO2 concentrations under field conditions using a FACE (Free Air Carbon Dioxide Enrichment) system under field conditions. Data to date emphasise a remarkably high repeatability (h2: 0.6 to 0.9) between the respective experiments. This provides unprecedented insights into the interaction of CO2 and leaf rust infection and lays the foundation for further genetic studies.

#273

Enhancing wheat yield potential: integrating genomic and phenomic approaches for biomass partitioning optimisation

Sudip Kunwar[1*], Dr. Md. Ali Babar[2], Jordan Mcbreen[2], Naeem Khan[2], Janam Acharya[2], Samuel Adewale[1], Vitor Cunha[3], Yiannis Ampatzidis[3]

[1]Plant Breeding Graduate Program, UF/IFAS, [2]Department of Agronomy, UF/IFAS, [3]Department of Agricultural and Biological Engineering, UF/IFAS

Phenotyping a large set of germplasms presents a substantial hurdle in extensive plant breeding programs. Although genomic prediction offers a contemporary solution by leveraging genotypic data to predict intricate traits, it lacks site-specific details and can also be expensive. Remote sensing data emerges as a potential solution, providing detailed in-season data that indirectly incorporates environmental impacts on genotypes. The stagnation in enhancing the harvest index (HI) in wheat since the 1990s primarily arises from the labor-intensive nature of its assessments. This has impeded the potential advancements in wheat genetic yield. Traits such as spike partitioning index (SPI) and fruiting efficiency (FE) have surfaced as crucial factors associated with HI.

This study aims to develop efficient phenotyping tools for assessing HI and related traits, integrating genomics, and phenomics data from unmanned aerial vehicle (UAV) sensors. Conducted in Citra, Florida, from 2022 to 2024, the prediction model training population encompassed 160, 306, and 306 elite facultative soft wheat lines, respectively.

Furthermore, a separate validation study in 2024 utilized genetically related yet distinct lines to the training set, developed from the University of Florida's world food crop breeding program. These lines underwent comprehensive trait characterization, encompassing HI, grain yield, grain number, SPI, FE, thousand-grain weight, and plant height, alongside UAV-based hyperspectral, multispectral, and thermal data acquisition. Genotyping utilizing the Genotype by Sequence (GBS) method facilitated the development of SNP markers.

The findings revealed substantial genetic diversity among genotypes across all traits, with significant correlations observed between manual data collection and UAV-based remote sensing data. The single kernel phenomics model exhibited superior performance over the genomics model for most traits, although the model combining both genomic and sensor fusion data (G+H+W) demonstrated the highest efficiency.

In conclusion, this study highlights the utility of UAV-based remote sensing data, either independently or to enhance the predictive ability of genomic prediction model. These advancements are instrumental in estimating the harvest index (HI) and associated biomass partitioning traits in large scale wheat breeding program, ultimately facilitating accelerated genetic yield improvements.

#61

Dissecting the Australian wheat powdery mildew pathotype

Ancy Kuthokathan Tony[1], Simon Ellwood[1], Huyen Phan[1], Daniel Mullan[2], Fran Lopez Ruiz[1], Benjamin J. Saunders[3], and Kar-Chun Tan[1]

[1]Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University. [2]InterGrain Pty Ltd. [3] School of Molecular and Life Sciences, Curtin University, Bentley, Western Australia, Australia

ancy.kuthokat@postgrad.curtin.edu.au

Wheat powdery mildew (WPM) is caused by the obligate biotrophic fungus, Blumeria graminis f. sp. tritici (Bgt). WPM is responsible for AU$18 million dollar in yield loss annum. Current strategies used to minimise the impact of WPM includes crop rotation, chemical control and genetic resistance.

Fungicide-resistant WPM have been reported across Australia therefore, genetic resistance is an effective and sustainable solution to control WPM. This requires information on current pathotypes, unfortunately, no proper studies have been reported on the pathotype and population structure of Australian Bgt. We collected 16 Bgt isolates from across Australia from 2020 to 2022.

We determined the level of virulence of these Bgt isolates using a diverse wheat panel consisting of varieties with defined WPM resistance (R) genes. As a result, we observed multiple R genes that can be deployed into Australian commercial varieties for effective resistance to WPM.

In addition, non-metric multidimensional scaling of virulence scores demonstrated evidence of distinct Bgt pathotypes present in Australia, revealing Western Australian isolates as significantly more aggressive than Eastern Australian isolates.

This data will be communicated to the cereal industry to enable effective deployment of the resources to improve profit through increased yield and grain quality. We also anticipate that the use of effective genetic resistance will reduce the demand for fungicides and thus increase the life span of a fungicide for future use. This will fill the voids between the researchers and the growers.

#306

Mining rust resistance in salt tolerant wheat germplasm

Charu Lata[1]*, Pramod Prasad[1], O.P. Gangwar[1*], Jayanth Kallugudi[1], Subodh Kumar[1] and Gyanendra Singh[2]

[1]ICAR-IIWBR, Regional Station, Flowerdale, Shimla, India. [2]ICAR-Central Soil Salinity Research Institute Karnal, India.

*Charu.sharma@icar.gov.in

Wheat (Triticum aestivum L.) is the second most important food crop globally, supports food security for millions. The sustained limitations on global wheat production persist due to the onset of various biotic and abiotic factors. Rust, caused by three distinct species of fungal genus Puccinia, stands as a significant biotic threat. Concurrently, increasing prevalence of salinity on a global scale emerges as a significant abiotic stressor, resulting in decreased wheat production across key wheat-growing regions worldwide.

In this piece of work, 41 salt-tolerant wheat lines were screened for rust resistance at seedling as well adult plant stages. The presence of rust resistance genes in these varieties were evaluated using gene matching technique in seedling stage (SRT) and through robust molecular markers linked to resistance gene as well as these lines were also screened for adult plant stage. The molecular studies confirmed presence of Yr2 gene in twenty three lines and gene Yr9 in four wheat varieties in association with Sr31 through SRT and molecular markers.

The presence of Lr24/Sr24 genes was identified in HD 2851 and KRL 2029 wheat genotypes and four wheat lines (KRL 283, KRL 2013, KRL 2017, and KRL 2029) were found to carry the Lr26 gene. With the exception of KRL2013, none of the wheat lines displayed absolute resistance to all Pst races used in this study. Additionally, Lr13 gene was found in a maximum of twenty-four wheat lines, with varying reaction responses to different leaf rust pathotypes.

The stem rust resistance gene Sr11 was identified in twelve wheat genotypes followed by Sr28, in ten wheat genotypes and Sr31 found in three wheat lines. Sr24 showed resistance towards all tested Pgt races, postulated in two wheat varieties HD 2851 and KRL 2029. Lr68 an effective race non-specific APR gene identified in fifteen wheat lines with the help of CsGs-STS marker.

Presence of Yr9/Lr26/Sr31 was confirmed using the SCSS30.2 marker, which produced a distinct 550bp band in five wheat genotypes. Although many salt-tolerant wheat lines were susceptible to yellow rust during the seedling stage, a few lines showed APR during 2020 and 2021 despite their susceptibility at the seedling stage.

Three lines (KRL 213, KRL219 and KRL 238) showed complete resistance in adult plant stage for leaf rust. The research yielded valuable scientific knowledge about the genetic foundation of rust resistance in salt-tolerant wheat varieties, enhancing our comprehension of wheat breeding approaches aimed at combating rust resistance.

#289

Marker-assisted gene pyramiding for improving fungal disease resistances in Canadian pre-breeding wheat cultivars.

Meng Li*[1], Vincent Fetterley*[1], Justin Chan*[1], Zoe Marshall*[1] and Gurcharn Brar[1,2]
*These authors have contributed equally.

[1]Faculty of Land and Food Systems, The University of British Columbia, 2357 Main Mall, Vancouver, BC, Canada, V6T 1Z4. [2]Faculty of Agricultural, Life and Environmental Science, University of Alberta, Edmonton, AB T6G 2P5, Canada.

Emails: justinc8@student.ubc.ca; vfett@mail.ubc.ca; meng.li@botany.ubc.ca; gurcharn@ualberta.ca

Canada is one of the world’s leading producers of wheat and has exported over 21 tons of wheat annually since 2017. Wheat plays an important part in the Canadian economy, but several fungal diseases threaten Canada’s wheat production. Fusarium head blight (FHB), stripe rust (YR), leaf rust (LR), and stem rust (SR) can all cause significant yield losses in susceptible wheat cultivars under optimal environmental conditions, which can result in significant economic losses.

Therefore, the Prairie Recommending Committee for Wheat, Rye and Triticale (PRCWRT) classifies theses fungal diseases as priority one, for which new wheat varieties need to have a moderate level of genetic resistance. Genetic resistance is a cost-efficient and sustainable approach for disease management by lowering the need for intensive fungicide application in crop fields.

The presence of multiple resistance genes in the same genotype improves the efficacy and durability of the resistance. Now, we crossed over 10 Canada Western Red Spring (CWRS) wheat lines containing different alleles of resistance genes including YR, LR, SR and FHB-resistant genes and then employed the Kompetitive allele specific PCR (KASP) genotyping assay to identify a single, homozygous wheat line stacking multiple disease resistance genes.

Contrary to conventional selective breeding which requires significant investments of labour and time, the KASP assay allows for the time and cost-effective selection of individuals carrying multiple homozygous disease-resistant gene alleles.

The ultimate goal of this study is to generate pre-breeding wheat lines harboring multiple wheat disease resistance genes. These lines will be readily used as novel sources by Canadian wheat breeders and will facilitate the introgression of numerous fungal resistance genes in CMRS wheat cultivars.

#200

Quality and agronomic trait analyses of pyramids composed of wheat genes ngli-d2, sec-1s and 1dx5+1dy10

Qier Liu[1], Zhimu Bu[1], Haixia Yu[1], Dewei Kong [1], Yanbing Huo [1], Xinyu Ma [1], Hui Chong [1], Xin Guan [1],Daxin Liu [1], Kexin Fan [1], Min Yan [1],Wujun Ma[2]*, Jiansheng Chen[1]*

[1]National Key Laboratory of Wheat Improvement, College of Agronomy, Shandong Agricultural University, Tai'an, Shandong 271018, China. [2] College of Agronomy, Qingdao Agricultural University, Chengyang District, Qingdao 266109, China.

zhimubu@163.com (Z.B.); Qier Liu: 15380334639@163.com (Q.L.); yuhaixia66@163.com (H.Y.); 13793022203@163.com (D.K.); m18854801061_1@163.com (Y.H.); mxyv1998@163.com (X.M.); ch18263898681@163.com (H.C.); xinner94@163.com (X.G.); 15053810615@163.com (D.L.); 18554211273@163.com (K.F.); 13653305680@139.com (M.Y.)

*Correspondence: w.ma@murdoch.edu.au (W.M.); jshch@sdau.edu.cn (J.C.); Tel.: +86-532-58957052 (W.M.); +86-538-8249236 (J.C.)

(Due to rising living standards, improving wheat's quality traits by adjusting its storage protein genes is essential. The introduction or locus deletion of high molecular weight subunits could provide new options for improving wheat quality and food safety. In this study, digenic and trigenic wheat lines were identified, in which the 1Dx5+1Dy10 subunit, and NGli-D2 and Sec-1S genes were successfully polymerized to determine the role of gene pyramiding in wheat quality.

In addition, the effects of ω-rye alkaloids during 1BL/1RS translocation on quality were eliminated by introducing and utilizing 1Dx5+1Dy10 subunits through gene pyramiding. Additionally, the content of alcohol-soluble proteins was reduced, the Glu/Gli ratio was increased and high-quality wheat lines were obtained. The sedimentation values and mixograph parameters of the gene pyramids were significantly increased under different genetic backgrounds.

Therefore, genes the 1Dx5+1Dy10, Sec-1S and NGli-D2 have a pyramiding effect that enhances the elasticity of dough. The modified gene pyramids had a superior protein composition compared to the wild type. The Glu/Gli ratios were higher in the type I digenic line and trigenic lines that contained the NGli-D2 locus compared to the type II digenic line without the NGli-D2 locus.

In comparison to the wild type, both the type II digenic line and trigenic lines had significantly higher levels of unextractable polymeric protein (UPP%) and Glu/Gli ratios. In addition, the celiac disease (CD) epitopes' level of the gene pyramids significantly decreased. The strategy and information reported in this study could be very useful for improving wheat processing quality and reducing wheat CD epitopes.)

#187

Hybrid wheat: parental allele donation effects on baking quality

James Milson[1,2], Buddhini Kiriwaththuduwa[1], Marco Catoni[2], Nicholas Bird[3], Philippa Borrill[1]

1. John Innes Centre, Norwich Research Park, Norwich NR4 7UH, UK. 2. School of Biosciences, University of Birmingham B15 2TT, UK. 3. KWS UK Ltd, 56 Church Street, Thriplow, Royston SG8 7RE, UK.

Email: James.Milson@jic.ac.uk

Hybridisation has been shown to greatly improve crops such as maize and rice, yet despite there always being an interest in hybrid wheat production has lagged behind due to high production costs. Now with molecular advances the deployment of a system based F1 production is coming closer, this means there is now an additional focus on understanding the baking quality in hybrid wheat. Baking quality is influenced by multiple loci comprised of multiple alleles.

While effects have been well studied in inbred lines, the interaction between alleles within hybrids is not known, especially in a heterozygous state. We have tested the baking quality of 257 single-cross F1 hybrids made from 120 female and 67 male inbred parental varieties, grown over three consecutive years of field trials. From this we genotyped the parental varieties and predicted hybrid genotypes for known quality associated alleles of the Glu-1 loci which predominantly influences dough rheological traits in inbred lines.

Hybrids with homozygous alleles follow previously reported trends in the baking quality, with high-molecular weight glutenin subunit alleles influencing the largest number of measures of baking quality. Interestingly, when alleles were in a heterozygous state, we found that variations in the baking quality phenotype can depend on the parental donor with either the maternally or paternally inherited alleles having a larger effect on certain traits, i.e. the heterozygous group tends to follow the trend of the homozygous group for a parental allele.

However, some traits do not show a parental bias. We hypothesised that parental bias of heterozygous genotypes could be due to differences in the expression levels of the parental donations, as has been previously reported in other hybrid crops such as maize. We used allele-specific qPCR primers to test the relative expression of alleles donated by different parents.

We found that despite there being differences in the phenotype depending on which parent donated which allele this is not reflected in differences in the expression of the alleles, in either F1 or F2 reciprocal hybrids. This work is aiming to guide hybrid wheat breeding efforts by determining the quality traits necessary in each parental component to efficiently produce high quality hybrid wheat.

#51

Transcriptomic analysis revealed compensatory pathogenicity mechanisms expressed in parastagonospora nodorum lacking major necrotrophic effectors

Shota Morikawa, Chala Turo, Kasia Clarke, Darcy Jones and Kar-Chun Tan

Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Perth, Australia

The fungus Parastagonospora nodorum causes septoria nodorum blotch (SNB) of wheat by secreting a suite of proteinaceous necrotrophic effectors (NEs) to induce tissue necrosis upon infection. Tox effectors only induce necrosis/chlorosis on wheat cultivars that possess matching dominant susceptibility genes (Snn).

It has been demonstrated that multiple NE-Snn interactions dictate the outcome of SNB through additive but also epistatic interactions. In this study, we have generated a P. nodorum mutant (toxa13) that lacked major NE genes; SnToxA, SnTox1 and SnTox3. Surprisingly, the virulence of P. nodorum toxa13 is comparable to the wildtype on modern bread wheats despite ablating three NE-Snn interactions.

This suggests that other functionally redundant pathogenicity mechanisms compensate for the loss of the three major effectors. A comparative RNA-Seq study revealed that the NE gene SnTox267 and two phytotoxic secondary metabolite (SM) gene clusters were highly up-regulated in toxa13 in-planta.

Furthermore, several candidate NE genes, uncharacterised SM gene clusters and signal transduction genes were also found to be up-regulated and may contribute to maintaining the virulence of toxa13. Characterisation of these genes for their role in the sustained virulence of P. nodorum toxa13 will be discussed.

#504

Genome-wide characterization, evolution, genomic organisation and expression patterns of cct gene family in durum wheat

Nida Mushtaq [1,*], Cristian Forestan[2], Pasquale De Vita[3], Salvatore Esposito [3,4], Nicola Pecchioni [3,1]

[1] Dipartimento di Scienze della Vita, UNIMORE - Università di Modena e Reggio Emilia, Reggio Emilia, Italy. [2] Department of Agricultural and Food Sciences, University of Bologna, Bologna, Italy. [3] Centro di Ricerca Cerealicoltura e Colture Industriali, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economica Agraria (CREA), Foggia, Italy. [4] National Research Council of Italy, Institute of Biosciences and BioResources, Research Division Portici (CNR-IBBR), Portici, Italy.

320383@studenti.unimore.it, cristian.forestan@unibo.it, pasquale.devita@crea.gov.it, salvatore.esposito@crea.gov.it, nicola.pecchioni@crea.gov.it

The CCT (CO, COL, and TOC1) domain-containing gene family functions as a pivotal regulator in fundamental biological processes, encompassing the intricate regulation of flowering, the synchronization of circadian rhythms, and defense against abiotic stresses. In particular, the CCT gene family includes two key players, VERNALIZATION2 (VRN2) and PHOTOPERIOD1 (PPD1), indispensable for governing the timing of flowering in response to environmental cues.

However, to date, no comprehensive genome-wide analysis of the CCT gene family in durum wheat has been conducted. Hence, using the recent high-quality Svevo reference, we identified 81 TtCCT candidate genes unevenly distributed across 13 chromosomes, except chromosome 3A, lacking any candidate. Phylogenetic analysis delineated four distinct subfamilies, namely, COL (34), CMF (29), PRR (10), and ZIM (8).

The exon-intron distribution and protein motifs demonstrated remarkably similar compositions within the same subfamily. Twelve TtCCTs, all belonging to the CMF subfamily arose from tandem duplications, while thirty-two TtCCTs underwent whole-genome duplications (WGD)/segmental events, leading to three duplicated gene pairs within the CMF subfamily, ten in COL, two in ZIM and one in PRR.

Despite the number of duplicated TtCCTs, the Ka/Ks ratio indicated that they were under purifying selection, confirming the important role of this gene family. Furthermore, the promoter survey highlighted cis-regulatory elements (CREs) involved in different mechanisms including phytohormone signaling, plant development, and abiotic/biotic stress responses, suggesting that TtCCTs might be involved in various plant processes.

Using RNAseq data from 30 samples representing different tissues and developmental stages, two clusters (I and II) were identified. Cluster I peaked during the spike development and pistil morphogenesis, whereas Cluster II was induced during (early) seed development. Interestingly, among the putative TtCCT targets predicted through the gene regulatory network (GRN) approach, two common genes interconnecting clusters I and II were identified as potential switchers, probably enabling the activation of Cluster II through the targets regulated by Cluster I.

This underscores the fine-tuning regulation of durum wheat development stages, where TtCCT genes might be involved. Our findings offer an in-depth insight into the functions of CCT genes in durum wheat, hypothesize their molecular mechanisms, and establish a foundation for further functional characterization of CCT genes in durum wheat.

#335

Population structure of aegilops umbellulata zhuk. Shown by genome-wide genotype data analysis

In Son[1*], Nozomi Kasazumi[1], Moeko Okada[2], Kentaro Yoshida[3], Yoshihiro Matsuoka[1]

[1] Graduate School of Agricultural Science, Kobe University. [2] Graduate School of Science and Technology, Niigata University. [3] Graduate School of Agriculture, Kyoto University

Email: 211a515a@stu.kobe-u.ac.jp

Wild wheat Aegilops umbellulata Zhuk. (2n=2x=14, UU genome) is a valuable genetic resource for breeding of bread wheat (Triticum aestivum L. subsp. aestivum), which is one of the world's most important food sources. The natural distribution of the Ae. umbellulata ranges from the Aegean islands, Anatolia, and the Middle East to Transcaucasus.

This species exhibits a wide genetic and phenotypic variations. Understanding the basis of these variations may provide valuable insights into the effective utilization of Ae. umbellulata in improving modern bread wheat varieties. As a step toward this goal, we analyzed the population structure of 199 accessions of Ae. umbellulata that largely cover the species natural range. All accessions were provided by the National BioResource Project-Wheat (Kyoto University, Japan).

The GRAS-Di® sequencing reads were aligned to a chromosome-scale genome assembly of Ae. umbellulata (Abrouk et al., 2023, Sci Data 10: 739), and then 12,240 pruned single nucleotide polymorphisms (SNPs) were obtained. Population structure analysis based on the SNP genotypes showed that Ae. umbellulata has two genetically distinct lineages, namely UmbL1 and UmbL2.

The UmbL1 accessions spread in the region ranging from Turkey to northern Iraq, while the UmbL2 accessions spread in the western part of the Anatolian Peninsula, including the Aegean islands and Cyprus. Furthermore, the UmbL1 lineage was loosely divided into two sublineages: UmbL1e found in northern Iraq and UmbL1w found in central Turkey.

Within UmbL1, two groups of accessions with mixed genetic ancestries were found: one consisting of accessions with high percentages of the UmbL1e and UmbL1w genetic ancestries, and the other consisting of accessions with high percentages of the UmbL1w and UmbL2 genetic ancestries.

These groups, collectively named UmbL1x, might have originated through inter-lineage/sublineage hybridizations. Importantly, the population structure found in the present study was consistent with the one found in our previous study, which used an RNA-seq-based genotyping approach (Son et al. 2024, Sci Rep 14: 7437). These lineages/sublineages were not completely distinguished by morphology.

However, lineage-specific trends were observed in spike morphology: UmbL1w accessions have relatively long awns, and UmbL2 accessions have relatively short inter-spikelet rachis lengths.

#479

One step closer to a commercial-scale hybrid wheat breeding through synthetic rf3 protein prospects

Gilang Bintang Fajar Suhono[1,2], Joanna Melonek[3], Ian Small[1,2]

[1] ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia, Crawley, WA, 6009, Australia. [2] School of Molecular Sciences, The University of Western Australia, Crawley, WA, 6009, Australia. [3] Division of Plant Sciences, Research School of Biology, The Australian National University, Canberra, ACT, 2601, Australia.

Email: gilangbintangfajar.suhono@research.uwa.edu.au

Hybrid breeding based on cytoplasmic male sterility (CMS) utilises a mitochondrial gene that induces pollen abortion and prevents self-pollination. Pollen production in the F1 generation is restored by Restorer-of-fertility (Rf) genes, which suppress the CMS phenotype.

A well-functioning CMS-Rf system in bread wheat (Triticum aestivum) would enable large-scale hybrid seed production at a low cost. Recently, the CMS-causing mitochondrial gene in wheat carrying Triticum timopheevii CMS has been identified as orf279, and the sequences of two Rf genes (Rf1 and Rf3) have been cloned1. Rf1 and Rf3 proteins, which belong to the family of pentatricopeptide repeat (PPR) proteins, were shown to bind to the orf279 transcript following the co-called ‘PPR-code’ and induce its cleavage.

However, the suppression of orf279 RNA by both Rf proteins differs in various wheat cultivars. In vitro analyses indicated that the RNA-binding affinity of Rf1 and Rf3 proteins to the RNA target is weaker than other well-characterised PPR proteins.

I hypothesised that the poor RNA binding of natural Rf proteins could be the direct cause of inconsistent levels of fertility restoration. I proposed synthetic Rf proteins as a novel tool to improve the suppression of orf279 through stronger binding affinity. In vitro RNA-binding assays showed that designer synthetic Rf3 proteins bind their RNA targets at least fifty times stronger than the natural ones.

The best candidate synthetic Rf3 gene has been transformed to Fielder*CMS wheat and future in planta RNA cleavage analyses will determine whether the stronger binding directly translates into more consistent cleavage of the CMS gene in wheat mitochondria and better fertility restoration in the F1 generation for commercial scale hybrid wheat breeding program.

Reference:

1. 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 and Small I (2021) The genetic basis of cytoplasmic male sterility and fertility restoration in wheat. Nature Communications. 12 (1), 1036.However, lineage-specific trends were observed in spike morphology: UmbL1w accessions have relatively long awns, and UmbL2 accessions have relatively short inter-spikelet rachis lengths.

#251

Sterility of basal spikelets in wheat. Predetermined fate or a matter of resources?

Santiago Tamagno[1,*], Constanza S. Carrera[1], Sofia I. Marchese[1], Roxana Savin[1], Gustavo A. Slafer[1,2]

[1] Department of Agricultural and Forest Sciences and Engineering, University of Lleida - AGROTECNIO-CERCA Center, Av. Rovira Roure 191, 25198 Lleida, Spain. [2] ICREA, Catalonian Institution for Research and Advanced Studies, Spain.

*Corresponding author: santiago.tamagno@udl.cat

Wheat (Triticum aestivum L.) spikes commonly present a lanceolate shape depicting the maximum fertility in central spikelets, whereas basal ones are typically sterile or rudimentary, not achieving full development. This is frequently observed under agronomic growing conditions, leading to the assumption that basal spikelets are constitutively sterile.

Evidence from studies impairing growth during stem elongation shows that spike floret survival drastically decreases, suggesting that assimilates supply to the spike plays a key role in spikelet fertility. However, it is not known whether high resource availability per plant could prevent floret mortality in basal spikelets.

We present data from two field experiments assessing spike fertility in the four most basal spikelets of the spike from the main shoot and tillers. Experiments comprised a collection of genotypes with different years of release and another with different tillering habits under contrasting sowing densities (agronomic densities vs. isolated plants) to generate a condition of high assimilate supply to the spike. As expected, under agronomic conditions, most basal position was sterile in 12 out of 14 cases and all cases in the tillers.

However, in the isolated condition in each experiment, sterility in basal spikelets was absent and less frequent in spikes from main shoots and tillers. Modern genotypes had a higher number of grains per spikelet than their older counterparts and low tillering habit genotypes showed the least response in spikelet fertility.

Regardless of the growing condition and experiment, the fertility of spikelets in the spike showed an acropetal gradient depicting the typical lanceolate shape, suggesting a constitutive trait in wheat spikes. Yet, fertility in basal spikelets does not seem to be a constitutive trait, as they became fertile when resources were increased. Further understanding of fertility in these positions will be instrumental in increasing grains per spike and wheat potential yield.

#413

Impact of breeding and environmental gradient on the genetic makeup of wheat populations

Zerihun Tadesse[1,2*], Emma Mace[2], David Jordan[2], Kai Voss-Fels[3], Alemayehu Assefa[4], Lee Hickey[2]

[1]International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico. [2]Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Australia. [3]Hochschule Geisenheim University, Geisenheim, Germany. [4]Ethiopian Institute of Agricultural Research, Addis Ababa, Ethiopia

*E-mail: z.tarekegn@cgiar.org

The impact of breeding period and geographical origin on the genetic makeup of wheat populations is a subject of considerable interest in agricultural genetics. In this study, the diversity panel included 908 genotypes sourced from the N.I. Vavilov Institute of Plant Genetic Resources, Consultative Group on International Agricultural Research (CGIAR), and national wheat research programs were investigated using 19,070 polymorphic DArTseq SNP markers to explore the genetic diversity, population structure, and selection signatures.

The population structure analysis divided the study panel into five subpopulations, revealing clear groupings based on breeding periods and environmental adaptation. Principal component analysis (PCA) further confirmed the clustering pattern between modern (clusters 1 and 2) and historical (clusters 4 and 5) wheat populations.

Cluster 3 was intermediate and comprised of genotypes from all breeding improvement periods. Regression analysis between the PCA clustering pattern and year of release revealed a correlation of 0.72. Additionally, clusters 4 and 5 were distinctly grouped based on environmental gradients, with a regression coefficient of 0.30 between PCA and the altitude of the source country of the genotypes. Genotypes in Cluster 4 were primarily sourced from drier regions, such as India and Pakistan, whereas those in Cluster 5 originated from cooler regions, including Russia, Armenia, Ukraine, and China.

Pairwise population differentiation statistics (FST) indicated significant genetic differentiation between modern and historical wheat populations (FST range: 0.034 - 0.190). Linkage disequilibrium (LD) decay revealed a slower LD decay in modern wheat compared to historical (LD decay: 1.53 Mb vs. 0.97 Mb with r2=0.23), which is one of the possible influences of modern breeding. This difference was clearly reflected in the haplotype block analysis, which showed significant variations in the number and size of the blocks between the two populations.

Using eigenGWAS approaches, 186 haplotype block regions were identified under selection, and some of the genomic regions were in proximity to known adaptation, quality, and disease resistance genes. Overall, this study highlights the impact of breeding period and environmental gradient on wheat genetic diversity. Furthermore, exploring landraces based on beneficial haplotype blocks will help enrich the genetic diversity of modern wheat populations in response to changing environmental conditions.

#277

Investigating the TaHRZ gene family for iron biofortification and abiotic stress tolerance in bread wheat

Lily Tarry-Smith[1], Oscar Carey-Fung[1], Jesse T. Beasley[1], Alexander A. T. Johnson[1]

[1]School of BioSciences, The University of Melbourne, Parkville, Australia

ltarrysmith@student.unimelb.edu.au

Crops adapt to low iron (Fe) soils through a complex network of transcription factors that regulate Fe uptake, translocation, and storage. The Hemerythrin motif-containing Really Interesting New Gene (RING)- and Zinc finger protein (HRZ) is a master negative regulator within the plant Fe homeostasis network and knockdown of OsHRZ expression increases tolerance to Fe deficiency and grain Fe concentrations in rice (Oryza sativa L.). This study describes the identification, annotation, and characterisation of six TaHRZ1 and TaHRZ2 homoeologs in the bread wheat (Triticum aestivum L.) genome1. In silico phylogenetic and protein analyses demonstrated that TaHRZ proteins possess Fe binding and polyubiquitination activity, and segregate into two groups: TaHRZ1 and TaHRZ2.

Reverse transcription PCR analyses revealed unique tissue expression profiles for each TaHRZ homoeolog, and rapid upregulation of all TaHRZs under Fe deficiency, suggesting the TaHRZs function as master negative regulators of wheat Fe homeostasis. We are now using gene editing to knock-out individual TaHRZ homoeologs in bread wheat and assess the effect on grain Fe concentration and plant tolerance to Fe deficiency. Together this research represents a culmination of novel wheat genomic resources and breeding techniques and highlights a novel and possibly transgene-free strategy for future wheat Fe biofortification.

1Carey-Fung O, Beasley JT, Johnson AAT. Annotation and Molecular Characterisation of the TaIRO3 and TaHRZ Iron Homeostasis Genes in Bread Wheat (Triticum aestivum L.). Genes (Basel). 2021 Apr 27;12(5):653.

#108

Generating stripe rust resilient wheat cultivars with haplotype-based selection and vavilov wheat genetic diversity

Jingyang Tong[1], Zerihun T. Tarekegn[1], Lee T. Hickey[1], Sambasivam K. Periyannan[1,2], Eric Dinglasan[1], Ben J. Hayes[1]

[1] Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, Queensland, Australia
[2] School of Agriculture and Environmental Science & Centre for Crop Health, University of Southern Queensland, Toowoomba, Queensland, Australia

Stripe rust (YR) is an economically important disease threatening wheat production globally. Resistance to YR is affected by environment and genotype by environment interaction (GEI) effects. We aimed to derive selective breeding strategies to achieve rapid improvement for YR resistance that is stable across environments. We investigated disease responses of YR in the Vavilov wheat diversity panel (n = 295) in 11 field experiments, including three in Australia and eight in Ethiopia during multiple years.

A Factor analytic (FA) model was used to fit to GEI of YR resistance across environments. Using the FA model, two breeding zones, Australia and Ethiopia, could be clearly defined based on the genetic correlations. Line-level selection was conducted with the overall performance (OP) and stability of YR resistance in environments, where OP was the genotype score for the first latent factor (F_1) expressed in trait units across environments and stability was the root-mean-square deviation (RMSD) from the regression line associated with latent variable F_1. To calculate GEBV for these two traits, 34,899 genome wide markers and a GBLUP model were used. These GEBV were compared with GEBV from a multi-trait GBLUP model, where each environment was fitted as a separate trait, and performance was the average GEBV across traits and stability was the standard deviation of GEBV across environments (traits).

Results showed that the GEBV from the FA approach and the multi-trait approach were in good agreement, having high correlations (Spearman $r = 0.49 – 0.68$). By adopting haplotype-based local GEBV, the haploblocks with the highest variances of haplotype effects were identified to be associated with OP and RMSD of YR resistance, respectively, in combined Australia and Ethiopia environments, and the haplotypes with favorable effects on both OP and RMSD were identified. Compared with truncation selection, longer-term and higher-ability genetic gain of both OP and RMSD could be achieved by optimal haplotype selection (OHS) to choose parental crosses based on a selection index (SI) of OP and RMSD.

Through simulation we also demonstrate that selections in Australia/ Ethiopia can improve OP and RMSD of YR resistance in the constant breeding zone (i.e. Australia/ Ethiopia), but not in changing breeding zones (i.e. Ethiopia/ Australia), although OP to some extend can be improved.

Parental selections with OHS and SI in combined environments were predicted to advance long-term genetic improvement for both average performance and stability of YR resistance across global breeding environments, including Australia and Ethiopia.

#167

Streamlining process-based wheat models: a framework for efficient data collection and parameterization

Luis Vargas-Rojas[1], Matthew Reynolds[2], Diane R. Wang[1*]

[1]Department of Agronomy, Purdue University, West Lafayette, IN, United States
[2]Wheat Physiology Group, International Maize and Wheat Improvement Center (CIMMYT), Texcoco, Mexico

* drwang@purdue.edu

Dynamic process-based plant models are computerized simulations of plant growth, development, and yield that use measurements of environmental factors and physiological processes as input data to generate predictions. However, using process-based models in wheat phenotyping programs still face challenges when parameterizing across multiple genotypes, such as (2) The need for large amounts of detailed datasets as parameters to run the simulations, many of which require destructive and disruptive sampling methods to obtain. (2)

The lack of standardized approaches needed to parameterize across extensive collections of genetically distinct, but often related, individuals that are typical of breeding populations. In this research, we developed a methodology for determining the most practical combination of remote sensing, proximal sensing, and direct measurement data to parametrize a process-based crop model for contrasting 14 wheat genotypes.

For the model parametrization, we collected a comprehensive dataset of remote, proximal, and direct measurements of a panel of wheat genotypes under three environments: well-watered, drought, and high temperature. Data were collected over two growing seasons from an experiment established at the CIMMYT's research station located in Northwestern Mexico. To facilitate data collection, we developed a framework that reduces the time needed for data collection and data processing.

Then, a decision tree approach was used to estimate key crop state variables from the available data sources, which were then used to parameterize the crop model using inversion methods. This methodology facilitated the integration of remote sensing and crop modeling to enhance wheat breeding programs and crop performance prediction.

#136

Domains of the transcription factor pnpf2 are essential for regulation of virulence factors in the phytopathogenic fungus P*arastagonospora Nodorum*

Callum Verdonk[1], Shota Morikawa[1], Evan John[1,2], Carl Mousley[3], Bernadette Henares[1], Kar-Chun Tan[1]

[1] Centre for Crop and Disease Management, School of Molecular and Life Sciences, Curtin University, Perth, Australia
[2] Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan
[3] Curtin Health Innovation Research Institute, Curtin Medical School, Curtin University, Perth, Australia

callum.verdonk@curtin.edu.au
kar-chun.tan@curtin.edu.au

Septoria nodorum blotch (SNB) is a devastating disease of wheat and is caused by the fungal pathogen *Parastagonospora nodorum*. The primary cause of SNB is proteinaceous effectors, which interact with wheat host sensitivity genes.

These effectors are controlled by DNA-binding regulatory proteins called transcription factors (TF), of which the Zn2Cys6 zinc-finger type is one of the most abundant in fungi. These zinc-finger-containing TFs possess a DNA-binding domain (DBD) and central “middle homology domain” (MHD). In *P. nodorum* the TF PnPf2 is a positive regulator of major necrotrophic effector genes required for host-specific virulence on wheat.

PnPf2 is a DBD-MHD protein with a disordered C-terminus tail which has low homology with its orthologs. We demonstrated that the DBD and MHD are essential for PnPf2 function but are not the main drivers of pathogenicity. Instead, only the disordered C-terminus tail is seemingly required for both effector activation, as well as disease symptoms on wheat.

These observations indicate a novel, uninvestigated mode of action by zinc-finger TFs, not currently characterised in other fungal pathogens. By understanding the structural and biological mechanisms of PnPf2, we can develop novel strategies to minimise the impact of effector-mediated diseases like SNB across Australia and worldwide.

#94

Characterising the gametocidal gene in a wheat-aegilops sharonensis introgression line

Nicola Walter[1], Cai-yun Yang[1], Duncan Scholefield[1], Stephen Ashling[1], Karafiatova Miroslava[2], Michael Wilson, Ian King[1], Julie King[1], Surbhi Grewal[1].

[1] Nottingham Wheat Research Centre, Plant and Crop Sciences, School of Biosciences, University of Nottingham, Sutton Bonington campus, Loughborough LE12 5RD.
[2] Institute of Experimental Botany ASCR, Centre of Structural and Functional Genomics, Slechtitelu 31, 779 00 Olomouc, Czech Republik
[3] School of Computer Science, University of Nottingham, Jubilee Campus, Wollaton Road, Nottingham, NG8 1BB

Nicola.walter@nottingham.ac.uk, Surbhi.grewal@nottingham.ac.uk

Wild relatives of bread wheat (*Triticum aestivum*) are often utilised to increase wheat's genetic diversity. For example, *Aegilops sharonensis* contains gene *Sr62* that confers stem rust resistance. However, some wild relatives including *Ae. sharonensis* contain gametocidal genes, also known as cuckoo chromosomes.

These selfish genes ensure preferential transmission to offspring through inducing chromosomal breakages in gametes that lack them, resulting in agronomically unstable lines with shrivelled seeds, unhealthy plants and significant yield reductions. Due to the selfish nature of these genes, they are extremely difficult to remove through traditional methods of breeding, preventing useful germplasm from being used in breeding programmes.

Using molecular markers and skim-sequencing we characterised a previously generated gametocidal gene (Gc2) containing translocation line, T4B-$4S^{sh}$. This line was shown to carry a 9Mb chromosome $4S^{sh}$ segment from *Ae. sharonensis* introgressed into chromosome 4B of wheat. We developed an F_1 population by crossing this line with wheat cv.

Chinese Spring and treatment of 4800 F_1 seeds with 0.3% and 0.4% ethyl methane sulfonate (EMS) resulted in 67 M_1 plants with potential loss-of-function in the *Gc2* locus. Ten progeny from each M_1 plant were genotyped to verify the normal segregation of the $4S^{sh}$ segment. Mendelian segregation was observed in two families, confirming mutations in the gametocidal locus. Two additional mutants demonstrated abnormal segregation patterns.

Chromosome flow sorting was used as a means of complexity reduction to isolate the recombinant chromosome 4B-$4S^{sh}$ prior to short-read sequencing of the wild-type T4B-$4S^{sh}$ and mutant lines. Sequencing data were analysed using the MutChromSeq pipeline to identify candidates in the introgressed segment for further downstream analyses and targeted mutagenesis.

Ultimately, this research has taken great steps towards identification of the long sought-after gametocidal gene, *Gc2.* Additionally, these mutant lines can be used within breeding programmes to remove *Gc2* from wheat-Ae. sharonensis introgression lines, allowing valuable germplasm with potentially advantageous traits to be released to breeders.

#144

Investigating wheat (T*riticum aestivum*) resistance mechanisms against russian wheat aphid (*Diuraphis noxia*) infestations

Ayesha Warnasooriya[1], Yong Han[2], Wei Xu[1*]

[1] Food Futures Institute, Murdoch University, 90 South St, Murdoch, WA 6150, Australia
[2] Agriculture and Food, Department of Primary Industries and Regional Development, South Perth, WA 6151, Australia

*Corresponding author: w.xu@murdoch.edu.au

Wheat stands as one of the primary and widely cultivated cereal crops, valued for its high nutritional content, richness, and adaptability as a staple food, playing a crucial role in ensuring global food security and nutritional stability. However, various abiotic and biotic stresses pose threats to global wheat production, with particular concern for the Russian wheat aphid (RWA, *Diuraphis noxia*), an invasive insect pest that causes significant risk to the industry.

Due to its rapid breeding, wide distribution, and ability to feed on a diverse range of host plants, RWA collectively poses a significant challenge to control. RWA was recently introduced to Australia, and subsequently, its distribution spread across all states, including major wheat-growing areas. Hence, it is essential to explore the key aspects involved in managing this pest. Development of RWA resistant wheat varieties is considered as the most efficient and sustainable approach to manage infestations of the RWA.

Furthermore, it serves as a cost-effective and eco-friendly strategy, integral to integrated pest management. Although different RWA-resistant genetic loci have been identified in various regions globally, the specific causal genes and corresponding mechanisms for resistance have not yet been determined or introduced into Australian commercial wheat varieties. Understanding the activation of defense mechanisms and signaling pathways triggered by RWA feeding in wheat plant tissues can aid in investigating molecular level responses and identifying resistance genes; however, this information remains relatively unexplored.

This project will first determine the biology, performance, and population dynamics of RWA in the local environment. Furthermore, the investigation of wheat plant metabolic responses and defense mechanisms against RWA attack will be conducted, with a focus on the molecular pathways responsible for RWA resistance in wheat.

The project will advance our knowledge of wheat resistance mechanisms to RWA and sustain food security by genetic improvement of RWA resistant in wheat.