FINAL PROGRAM IBGS 2024 ROSARIO ARGENTINA

OCTOBER 27 Sunday

18.00-21.00 **REGISTRATION AND WELCOME RECEPTION:**
Hotel Solans Presidente, Corrientes 919, Rosario, ARGENTINA

OCTOBER 28 Monday

08:00 - 8:30 **REGISTRATION AND POSTER SETUP**:
Bolsa de Comercio de Rosario, Paraguay 777, Rosario ARGENTINA

08:30 - 9:00 **OPENING SESSION**
Representatives of IBGS14 LOC, IBGS IOC, Schools of Agriculture of National University of Rosario and University of Buenos Aires, Argentina.

SESSION A: BARLEY GENOMICS *(Chair: Dr Ariel Castro)*

09:00-09:30 Keynote speaker: **Dr. Ping Yang**, *State Key Laboratory of Crop Gene Resources and Breeding Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (ICS, CAAS)*

09:30-10:30 Speaker conferences of 15´ exposition + 5´questions (4 speakers)

09:30-09:50 Speaker 1: **Shun Sakuma**
09:50-10:10 Speaker 2: **Ana Badea**
10:10-10:30 Speaker 3: **Marina Pupke Marone**
10:30-10:50 Speaker 4: **Dongying Gao**

10:50-11:00 **Coffee break**

SESSION B: MORPHOLOGY, PHENOLOGY, AND DEVELOPMENT (*Chair: Dr Santiago Alvarez Prado)*

11:10-11:40 Keynote speaker: **Dr Laura Rossini**, *Full professor, Università degli Studi di Milano*
Speaker conferences of 15´ exposition + 5´questions (3 speakers)

11:40-12:00 Speaker 1: **Guangqi Gao**
12:00-12:20 Speaker 2: **Volodymyr Radchuk**
12:20-12:40 Speaker 3: **Thorsten Schnurbusch**

12:40- 14:10 **Lunch break**

SESSION C: GENETICS AND BREEDING FOR YIELD AND ITS COMPONENTS
(Chair: Dr Euclydes Minella / Antonio Aguinaga)

14:10-14:50 Keynote speaker: **Dr Alessandro Tondelli,** *CREA - Research Centre for Genomics and Bioinformatics, Fiorenzuola d'Arda, Italy*
Speaker conferences of 15´ exposition + 5´questions (5 speakers)

14:50-15:10 Speaker 1: **Ana Heilman Morales**
15:10-15:30 Speaker 2: **Gustavo Slafer**
15:30-15:50 Speaker 3: **Liina Jakobson**
15:50-16:10 Speaker 4: **Matheus Siqueira**
16:10-16:30 Speaker 5: **Congcong Jiang**

16:30 - 18:00 **POSTER SESSION / Coffee & Snacks**

OCTOBER 29 Tuesday

08:30-09:00 **Poster setup**

SESSION D: RESOURCE USE EFFICIENCY *(Chair: Daniel Miralles)*

09:00-09:30 Keynote Speaker: **Dr Roxana Savin**, *Professor, University of Lleida & Agrotecnio*

Speaker conferences of 15´ exposition + 5´questions (2 speakers)

09:30-09:50 Speaker 1: **Shengming Yang**
09:50-10:10 Speaker 2: **Anna Guðrún Þórðardóttir**

10:10-10:30 **Coffee break**

SESSION E: BIOTIC STRESSES *(Chair: Silvia Pereyra INIA Uruguay)*

10:30-11:00 Keynote Speaker: **Dr Brian Steffenson,** *Department of Plant Pathology, University of Minnesota*

Speaker conferences of 15´ exposition + 5´questions (4 speakers)

11:00-11:20 Speaker 1: **Fluturë Novakazi**
11:20-11:40 Speaker 2: **Roger Wise**
11:40-12:00 Speaker 3: **Ben Ovenden**
12:00-12:20 Speaker 4: **Robert Brueggeman**

12:20-14:00 **Lunch break**

SESSION E: ABIOTIC STRESSES *(Chair: Silvia Pereyra INIA Uruguay)*

14:00-14:30 Keynote speaker: **Dr Ramamurthy Mahalingam**, *Leader at the USDA Cereal Crops Research Unit in Madsion, USA.*

Speaker conferences of 15´ exposition + 5´questions (3 speakers)

14:30-14:50 Speaker 1: **Jonathan Jacobs**
14:50-15:10 Speaker 2: **Brad Baxter**
15:10-15:30 Speaker 3: **Klaus Pillen**

15:30-15:50 **ICARDA: Dr. Miguel Sanchez**

15:50 - 16:50 **Flash & Dash presentations** *(Chair: Dr Gabriela Abeledo)*

Short oral presentation of Poster from students and postdocs Speakers of the selected posters will be invited by the LOC to be exposed in 2-3 minutes (15 presentations)

16:50 - 18:30 **POSTER SESSION II / Coffee & Snacks**

OCTOBER 30 Wednesday

08:00-08:30 **Poster Setup**

SESSION F: BARLEY BREEDING SUCCESS STORIES *(Chairs: Silvia German, Daniel Miralles)*

08:30-09:00 Keynote Speaker: **Dr David Moody**, *Intergrain. Perth, Australia*

09:00-10:00 Speakers: Barley Breeding success story in South America *(Antonio Aguinaga / Ariel Castro / Euclydes Minella)*

10:00-10:40 **Coffee break**

SESSION G: BARLEY END USE: FOOD, FEED, AND NOVEL PRODUCTS *(Chair: Daniel Vazquez)*

10:40-11:10 Keynote speaker: **Dr. Marta Izydorczy,** *Grain Research Laboratory, Canadian Grain Commission, and an adjunct professor at the Department of Food Science, University of Manitoba, Winnipeg, Canada*

Speaker conferences of 15´ exposition + 5´ questions (2 speakers)

11:10-11:30 Speaker 1: **Linda Legzdiņa**
11:30-11:50 Speaker 2**: Omvir Singh Ujjlain**

SESSION H: MALTING AND BREWING *(Chair Antonio Aguinaga)*

11:50-12:20 Keynote speaker: **Dr Bertram Sacher,** *Chair of Brewing and Beverage Technology, TUM School of Life Sciences, Technical University of Munich*

12:20-14:00 **Lunch break**

Speaker conferences of 15´ exposition + 5´questions (4 speakers)

14:00-14:20 Speaker 1: **Cameron Matthews**
14:20-14:40 Speaker 2: **Monika Kavanová**
14:40-15:00 Speaker 3: **Silvina Baraibar**
15:00-15:20 Speaker 4: **Roberto Benech-Arnold**

15:20-16:20 **Flash & Dash presentations** (*Chair Dr Gabriela Abeledo)*
Short oral presentation of Poster from students and postdocs Speakers of the selected posters will be invited by the LOC to be exposed in 2-3 minutes (15 presentations)

16:20-17:20 **POSTER SESSION III / Coffee & Snacks**

17:20-18:30 **EVENING WORKSHOP. Next venue for the 15th IBGS.**

19:30-22:30 **Farewell party: Dinner and Music Show**
Vista Río, Arturo Illia 1650, Rosario, Argentina

OCTOBER 31 Thursday

FIELD DAY: DEPARTURE OF BUSES 8:30 HOTEL SOLANS PRESIDENTE, Corrientes 919, Rosario, ARGENTINA

08:30-12:10	**FIELD DAY** *at Zavalla 30 Kms away from Rosario: Plots of barley genetic progress in Argentina, Uruguay and Brazil*
12:10	**LUNCH AT THE UNIVERSITY**
13:40	**CONGRESS CONCLUDES**

RETURN OF THE BUSES, HOTEL SOLANS PRESIDENTE, Corrientes 919, Rosario, ARGENTINA

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THEMATIC SESSIONS

01 Barley genomics

02 Morphology, phenology and development

03 Genetics and breeding for yield and its components

04 Resource use efficiency

05 Biotic and Abiotic stresses

06 Barley breeding success stories

07 Barley end use: food, feed and novel products

08 Malting and brewing

Genomics-assisted barley genetic studies

Ping Yang

State Key Laboratory of Crop Gene Resources and Breeding, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China; yangping@caas.cn.

Barley (*Hordeum vulgare*; 2n = 14) is a diploid species with a genome smaller than those of other members of the Triticeae tribe, making it an attractive model for genetic studies in Triticeae crops. Its large and highly repetitive 5.1 Gb genome is still a challenge for genetic studies. Fortunately, the advent and rapid evolution of high-throughput sequencing methodologies, coupled with the efforts of the International Barley Sequencing Consortium (IBSC), has led to the release of the first reference genome for barley cultivar Morex in 2017. The initial reference genome and its subsequent updated assembles, such as Morex V3, have been popularly used in barley research community. The pan-genomes of cultivated barley, wild barley (*Hordeum vulgare* ssp. *spontaneum*) and other *Hordeum* species, as well as Genebank genomics have further highlighted the essential role of genomic methodologies. By taking advantage of these established genomic resources and techniques, I would like to represent some of the work in my group, to demonstrate that genomics, in conjugation with mutagenesis and genome editing technologies, has revolutionized genetic studies and breeding in barley and beyond.

Keywords: GWAS; Map-based cloning; TILLING; Genome editing

Session: 01 Barley Genomics

Genomics-assisted barley genetic studies

Ping Yang

State Key Laboratory of Crop Gene Resources and Breeding, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China
*Correspondence: yangping@caas.cn.

Barley (*Hordeum vulgare* L.; 2n = 14) is a diploid species with a genome smaller than those of other members of the Triticeae tribe, making it an attractive model for genetic studies in Triticeae crops. However, the large and highly repetitive 5.1 Gb genome is still a challenge in genetic studies. Thanks for the rapidly-evolved high-throughput sequencing methodologies and decrease on sequencing as well as inputs of International Barley Genome Sequencing Consortium (IBSC), the first reference genome of barley cultivar “Morex” was released in 2017. This version of reference genome and following updated assembles (e.g. Morex V3) have been popularly used in barley research community. The pan-genomes of cultivated barley, wild barley (*Hordeum vulgare* ssp. *spontaneum*) and *Hordeum* species, as well as Genebank genomics have further highlighted vital role of genomic methodologies. By taking advantage of the established genomic resources and methods, here I would like to introduce some works in my group, in order to provide a hint that the genomics together with mutagenesis and genome editing technologies has sharped barley genetic studies and breeding.

Keywords: GWAS; BSA; Map-based cloning; TILLING; TILLING-by-sequencing (TBS); Genome editing

Poster display AND oral presentation / 01 – Barley genomics

Title: Annotation and exploitation of repetitive sequences for barley pan-genomics and improvement

Dongying Gao, Small Grains and Potato Germplasm Research Unit, USDA-ARS, Aberdeen, ID 83210, USA

The genomes of many important crops including barley contain large fractions of transposons. Once considered as 'junk DNA', these repeats are now known to play crucial roles in plant gene and genome evolution and have been used to develop molecular tools, such as gene-tagging and molecular markers. As many transposons can produce transcripts that may result in overestimating and incorrectly annotating functional genes. Thus, accurate transposon annotation is essential for all plant genome projects and related research. We developed a bioinformatics pipeline which has been used for identifying transposons in 19 plant genomes. In this study, I applied the pipeline to barley and developed a barley repeat database called Barley Repbase. Both Class I retrotransposons and Class II DNA transposons were detected. It is worth noting that I identified new terminal repeat retrotransposons in miniature (TRIMs) and endogenous pararetroviruses (EPRVs) which were not reported in other studies. I further searched against the full-length (FL) cDNA sequences and identified 71 unique transposon-related sequences. Among them, eight were structurally intact transposons. RT-PCR analysis indicated that all these transposons were expressed but they showed different transcription patterns in barley. I further compared their distributions across the barley pan-genomes and detected polymorphisms within cultivated barley genotypes and between wild and cultivated barley suggesting their recent transpositions. The polymorphic transposons were used to develop new molecular markers for assisting our barley breeding. The Barley Repbase was also used to screen other grass genomes including wild barley *Hordeum marinum* and rice, different transposon landscapes were detected. The Barley Repbase is a comprehensive repeat database and provides useful resources for barley pan-genomics, epigenomics and comparative genomics of the *Hordeum* genus. This database will be deposited in the GrainGenes website for public access, it is also available upon request.

Unraveling Genetic Diversity and Parental Lines Similarity in Malt Barley Breeding Population by KASP Marker: "Insights and Implications"

Endeshaw Tadesse1,2*, Kassahun Tesfaye3, Yohannes Fikadu4, Rebeka Gebretsadik5, Tesfahun Alemu4, Peer Wilde2
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ABSTRACT: Assessment of genetic diversity is an important component in conventional and marker-assisted breeding. Twenty-four genotypes (17 parents of 30 cross and 7 non-parental checks) were genotyped with 23 Kompetitive Allele Specific Polymerase Chain Reaction (KASP) markers at the German seed company KWS with the objectives, to: 1) assess the genetic diversity among parental lines and the similarity within the lines, 2) estimate Rogers Distances (RD) of parental line pairs and their correlation to the segregation variance within crosses (σ^2g). Data was analyzed using R software. Diversity analysis revealed an average RD between lines was 0.46 with a range of 0.32- 0.64. The dendrogram grouped the lines into three clusters. Importantly, the high malting European lines were grouped together with some of the ICARDA and Ethiopian lines implying some sort of gene flow. Correlations of RD to σ^2g ranged from 0.34 to - 0.19 and were not significantly deviating from zero, hence RD, proved to be not predictive for σ^2g. Parental lines homogeneity ranged from 71% to 100%. Heterogeneity, the opposite to homogeneity, within lines is hypothesized to originate from in-complete inbreeding in case of line Bekoji-1 x Grace and in-crossing events or technical admixture with other germplasm in case of the last-mentioned group of lines. When originating from technical mixture this should be considered as a wake-up call for the breeder to closely follow standard operation procedures for maintenance breeding.

Key Words: Genetic Diversity and Similarity, KASP marker, Parental line, Segregation Variance

Pan Genome Reveals the Genomic Signature of Australian Barley Varieties

Tianhua He[1], Viet Dang[1], Brett Chapman[1], Yong Jia[1], Haifei Hu[1], Penghao Wang[1], Xiao-Qi Zhang[1], Kenneth J. Chalmers[2], Camilla Beate Hill[1], Gabriel Keeble-Gagnere[3], Sharon Westcott[4], Yong Han[4], Josquin F Tibbits[3], Peter Langridge[2], Chengdao Li[1,4]

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Breeders' selections have played a significant role in shaping barley genomes and selecting genes and alleles for adaption to growth conditions. However, the genetic basis of adaptation to distinct agroclimatic conditions remains to be elucidated. Here we assembled the genomes of Australia's modern barley varieties Maximus, RGT Planet, Clipper, Stirling, Vlamingh, Laperouse, Buff and Yeti. With reference quality genomes and population-level re-sequencing data of modern barley varieties from Europe, Australia and North America, and field characterization on their phenology, we show that breeders have fundamentally transformed the genomic architecture and landscape of adaptive genes in Australian and North American barley varieties. Adapting to the Australian environment involves the selection and subsequent enrichment of pre-existing genetic variants within the European barley gene pool, but also later introduces non-European genotypes. Genes associated with photomorphogenesis, circadian rhythm and light response were under strong selection during the early breeding of European germplasm base to adapt to Australian environments. Modern Australian barley harbours dominant haplotypes conferring phenotypes. Our findings will inform the selection from germplasm pools and parental lines when establishing breeding populations to breed new varieties adapted to the novel environments.

Key words: Australia barley, Pan genome, Haplotype, Yield, Adaptation

Developing genome editing in barley as a tool for crop improvement: Challenges and opportunities

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Genome editing is a modern and disruptive technology that promises to greatly contribute to plant breeding. Barley represents the fourth most important cereal crop in terms of worldwide production, widely used for malting, and as a livestock feed, with a small proportion serving as human food. Developing genome editing in barley opens a big opportunity to accelerate breeding programs attending different aims of plant breeding, which include plant protection, nutrient use efficiency, biomass and grain production (yield), and nutritional quality among others, as it has already been shown in the scientific literature. CRISPR/Cas, currently the most popular genome editing technique, is considered a very precise tool to mutate target genes, deleting, inserting, or replacing specific nucleotides. It may be applied to elite germplasm, reducing the time needed to reach commercial varieties compared to other technologies. However, regulatory aspects require attention to get some markets with edited crops; Argentina, Brazil, and the United States are some countries that consider the varieties developed by genome editing as non-GMO, while European countries are more restrictive. Bioheuris is a startup company that combines synthetic biology and gene editing to develop herbicide-resistant crops. It works with two platforms; on the one hand, Heurik™ integrates rational design and directed evolution to identify mutations that confer herbicide tolerance, and on the other hand, Swap™ is the platform that introduces these mutations in crops using gene editing. The main advantages of this strategy are reducing development costs and time to market the varieties, particularly because it works with elite lines and edits more than one gene at a time. This scheme will prospectively be applied in barley to develop edited barley varieties with herbicide resistance.

06 – Barley breeding success stories

GRF4-GIF1 enable efficient transformation and genome editing in barley

Juan Debernardi, Mariana Padilla, Isabel A. del Blanco and David Tricoli
UC Davis

Genome editing allows precise DNA manipulation and holds great promise for a new green revolution. Yet, its potential for innovation is limited in many crops by low regeneration efficiencies and few transformable genotypes. In recent years, several molecular approaches have been developed that use morphogenetic genes to improve plant transformation. However, a common problem to those strategies is that the continuous expression of the morphogenetic genes can produce developmental defects and sterility.

We have recently reported that expression of a chimeric protein including the wheat transcription factor GROWTH-REGULATING FACTOR 4 (GRF4) and its cofactor GRF-INTERACTING FACTOR 1 (GIF1) dramatically increases regeneration efficiency of wheat transgenic plants, resulting in fertile plants. We observed increased regeneration efficiency in diverse recalcitrant wheat cultivars, triticale, maize, as well as the model rice cultivar Kitaake. By combining the GRF4-GIF1 and CRISPR-Cas9 technologies in a single vector, we can generate large numbers of edited wheat plants in a shorter time and in multiple commercial backgrounds.

We tested GRF4-GIF1 in barley transformation and genome editing. We observed that GRF4-GIF1 enhances transformation efficiency in the cultivar Golden Promise. Moreover, combining GRF4-GIF1 with a virulence helper plasmid improves transformation efficiency in UC Davis cultivars and Oregon State University breeding lines. Finally, we performed CRISPR-Cas9 editing experiments and successfully edited candidate genes for protein and b-glucan content in cultivars and breeding lines. Our results demonstrate that GRF-GIF chimera in conjunction with a virulence helper plasmid can improve the efficiency of gene-editing in barley cultivars.

Topics Session: Barley genomics

Integrating Genomics, Metabolomics, and Near-Infrared Spectra (NIR) for Predicting Yield and Malting Quality in Barley

Raffo, Miguel A.[1]; Sarup, Pernille Merete[2]; Jensen, Just[1]; Guo, Xiangyu[3]; Jensen, Jens D.[2]; Orabi, Jihad[2]; Jahoor, Ahmed[2]; Christensen, Ole F.[1]

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Significant attention has recently been given to the potential benefits of using multi-omics and chemometric technologies to improve plant breeding. Among the most remarkable is Phenomic Selection (PS), which relies on the use of near-infrared spectroscopy (NIR) to predict phenotypic values of breeding lines. The use of other technologies such as metabolomics has also shown potential for phenotypic prediction. In this study, we used a commercial barley breeding population phenotyped for grain yield, grain protein content, and five malting quality traits: extract yield, wort viscosity, wort color, filtering speed, and β-glucan, and we aimed to: (i) investigate genetic variation and heritability of metabolomic intensities and NIR wavelengths originating from leaf tissue and malted grain, respectively; (ii) investigate variance components and heritabilities for genetic models including genomic and metabolomics (GOBLUP-MI) or genomic and NIR wavelengths (GOBLUP-NIR); and (iii) evaluate the developed models for prediction of breeding values for the traits of interest. Importantly, our study differs from previous ones as we focused on utilizing NIR and metabolomic data for predicting genomic breeding values, while others have used it for phenotypic prediction. In total, 639 barley lines were genotyped using an iSelect9K-Illumina barley chip, and was recorded with 30,468 metabolomic intensities, and 141 NIR wavelengths. First, we found that a significant proportion of metabolomic intensities and NIR wavelengths had medium to high additive genetic variances and heritabilities. Second, we observed that both multi-omics models increased the proportion of estimated genetic variance for grain yield, protein, malt extract, and β-glucan compared to a purely genomic model (GBLUP). Finally, we assessed genomic and multi-omics models to predict breeding values in 5-fold and leave-one-breeding-cycle-out cross-validations, and we generally observed a similar accuracy between GBLUP and GOBLUP-MI, and a worse accuracy for GOBLUP-NIR. Despite this trend, GOBLUP-MI and GOBLUP-NIR enhanced predictive ability by 4.6 and 2.4 % for grain protein in leave-one-breeding-cycle-out and grain yield in 5-fold cross-validations, respectively, compared to a baseline GBLUP model, but differences were not significant (*P-value* > 0.01).

Mapping of barley *tweaky spike* locus and its relation to phytohormone content

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The discovery of radioactivity at the end of the nineteenth century played a key role in a series of historical landmarks that would lead to contemporary mutation breeding in crops. Mutation breeding has been commonly used to improve crop features regarding the seed size, fruit colour and flavour, that either cannot be found in nature or have been lost during evolution. During the pinnacle of mutational breeding a large collection of mutants, that have no direct economic value but are interesting as a source for genetic mapping and gene function identification, were created. The development of next-generation sequencing technologies also allows researchers to use cost-effective and less time-consuming methods to identify mutations.

A series of unique barley mutants with disturbed inflorescence development were created in the Vilnius University Department of Botany and Genetics. These mutants feature gradient inflorescence development and spikelet-free gaps in spikes, hyperdeveloped spike tips, and the most interesting feature is genome instability in the remote generations. Recently, we identified the candidate genes in the *tw* locus using whole genome sequencing of F_2 recombinant plants (from tw_2 × *WT* cross) possessing the *tw* phenotype, and the next step is candidate genes analysis in other allelic genotypes using Sanger sequencing, candidate gene validation in *WT* using CRISPR-cas9 system and exploration of changes in gene expression profile after the knock-out procedure.

This study also aimed to explore phytohormone association with *tw* mutation, by treating *tw* mutants and *WT* with exogenous phytohormones (gibberellin, cytokinin, melatonin and auxin). Four features of *tw* mutants were selected for phenotype analysis: spikeless gaps, the presence of an overdeveloped tip of the spike ("a crown"), lodicule transformations and variation in flower organ number. Only auxin had a statistically significant effect on all tested features of *tw* phenotype compared to control while other phytohormones altered mutant phenotype frequencies only in one or two features. This data suggests that the *tw* mutation mainly disrupts auxin physiology and mild effects of other phytohormones on *tw* phenotype might be a side effect of auxin gradient changes in *tw* barley spikes. The last step in auxin association analysis is to investigate auxin concentration along the spikes of *WT* and *tw* using high-performance thin-layer chromatography (HPTLC).

iTAG: Interactive laboratory exercises to explore genotype and phenotype using Oregon Wolfe barley

Roger P Wise[1,2*], *Gregory Fuerst*[1], *Nick Peters*[2], *Nancy Boury*[2], *Laurie McGhee*[3],

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**Presenting author: Roger.Wise@USDA.gov*

One of the basic concepts in biology is that an organism's physical traits are controlled by genes that are encoded in its DNA. "Inheritance of Traits and Genes" (iTAG) uses the morphogenetically diverse Oregon Wolfe Barley (OWB) population in a series of laboratory and classroom activities designed to connect visible traits (phenotype) to identifiable differences in the DNA sequence of genes. iTAG focuses on three traits to illustrate basic concepts in plant development, domestication, and disease resistance. Using the iTAG barley module, students observe the OWB spikes for seed color, two row vs. six row (encoded by *Vrs1*, a domestication trait), hooded vs. non-hooded (encoded by *BKn3* - a homoeotic mutation where the awn is replaced by a duplicate spikelet), and long awn vs. short awn (encoded by *Lks2,* which is epistatic to hooded). Lastly, the OWB population segregates for resistance or susceptibility to powdery mildew disease, due to different alleles of *Mildew locus a* (*Mla*). Participants learn basic molecular biology techniques of DNA extraction, polymerase chain reaction, gel electrophoresis, and document DNA polymorphisms among plants with different phenotypes. Instructors can then lead a discussion of how researchers associate genotype and phenotype. Thus, new researchers gain valuable experience in genetic history related to cellular pathways and developmental mutations; concepts critical to producing disease resistant crops and livestock, as well as human health. iTAG barley has been used successfully by nearly 50 instructors across the USA in >200 high school and community college biology classes from 2010 to 2023, impacting nearly 5000 students, of which one third were from underrepresented groups from urban to rural communities. The iTAG curriculum is freely available at: http://doi.org/10.1094/PHI-E-2023-09-0009

Supported in part by National Science Foundation - Plant Genome Research Program grant 13-39348, USDA-Agricultural Research Service project 3625-21000-067-00D and 5030-21220-068-000-D to RPW, USDA-National Institute of Food and Agriculture grant 2020-67013-31184 to RPW, NB, and NP, and Iowa Agriculture and Home Economics Experiment Station (IAHEES) Project 4208 to NP and NB.

Gene flow between wild and domesticated barley in Israel

Marina Pupke Marone[1], Yu Guo, Axel Himmelbach[1], Thorsten Schnurbusch[1], Zvi Peleg[2], Martin Mascher[1]

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Plant domestication has occurred very recently on a phylogenetic timescale. Hence, crop plants are often interfertile with their wild progenitors. In regions where wild and domesticated plants co-occur, hybridization can happen. If crop-wild geneflow – in either direction – confers fitness benefits, it can give rise to hybrid swarms that harbor, for instance, in a wild background beneficial alleles donated from a crop introgression. Barley (*Hordeum vulgare* L.) is an important crop species, whose domestication process has been intensely studied at the archeological, genetic, and molecular level. In past decades, botanists have observed "six-rowed wild barley", i.e. wild-growing barley showing three fertile florets per inflorescence node, which is otherwise a distinguishing feature of domesticated barley. Here, we report on the genomic analysis of six-rowed wild barleys from Israel. We conducted population genomic analyses using genotyping-by-sequencing data of 181 newly collected populations and their sympatric two-rowed counterparts. We observed that there was no clear genetic separation between two and six-rowed individuals from the same habitat. When comparing this dataset to a panel of 21,000 barley accessions, comprising both domesticated and wild barley, the samples collected in Israel, whether two-rowed or six-rowed, clustered together with other wild barleys. To investigate the allelic state of the *SIX ROWED SPIKE* (*Vrs1*) gene, we performed whole-genome sequencing of 55 samples. We found in six-rowed genotypes an extended haplotype (*vrs1.a1*) that predominates in domesticated barleys, but is rare in wild forms. This is consistent with gene flow from domesticated barley into wild populations and the preferential retention of a six-rowed (i.e. loss-of-function) allele of *Vrs1*. We speculate that pleiotropic effects of *Vrs1* on vegetative growth may confer advantages in a ruderal habitat.

Towards a bridging pangenome of barley: selection and sequencing of elite cultivars

Marina P. Marone[1], Erwang Chen[1], Axel Himmelbach[1], Yu Guo[1], Manuel Spannagl[2], Klaus Oldach[3], Julia Rudloff[4], Viktoria-Elisabeth Dohrendorf[5], Ahmed Jahoor[6], Martin Mascher[1], Nils Stein[1]

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The barley pangenome, featuring high-quality chromosome-scale genome assemblies, offers a wealth of information that can be used for precision breeding. In previous work, we assembled and annotated a total of 76 chromosome-scale barley genomes, comprising elite cultivars, landraces and wild accessions. We also obtained whole-genome sequencing (WGS) short-read data of 1315 genotypes. Now, our aim is to sequence more elite cultivars to explore structural variation (SV) and copy number variation (CNV) information for breeding. We selected cultivars that were genetically different (identity-by-state < 0.9) to the majority of the 76 genotypes. We used a comprehensive dataset with pedigree information to select the cultivars mostly used in breeding through history, as well as cultivars derived from old landraces from Central European countries. Then, the selected ones were sequenced with high-fidelity (HiFi) long-reads (PacBio Revio) and high-throughput chromosome conformation capture (Hi-C). We assembled 20 elite barley lines, including well-known varieties like Franka and Diamant. New large SVs were not observed. Moving forward, our plan involves sequencing approximately 150 diverse barley lines using PacBio Revio barcode sequencing (5x). We will use haplotype information from the WGS panel to maximize sequencing new regions. We aim to create a comprehensive pangenome integration dataset enriched with SV/CNV information, providing a robust foundation for precision barley breeding applications.

REFERENCE-LEVEL GENOME ASSEMBLIES AND COMPARATIVE ANALYSIS OF FIVE REPRESENTATIVE CANADIAN BARLEY CULTIVARS

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In this study, we present high-quality, reference-level genome assemblies for five representative, two-row Canadian barley cultivars, including two general-purpose cultivars CDC Austenson and Morrison, and three malting cultivars AAC Synergy, AAC Connect, and CDC Fraser. We combined Illumina paired-end reads, long mate-pair reads, PacBio reads, 10X Chromium linked read libraries, and chromosome conformation capture sequencing (Hi-C) data to meticulously construct chromosome-scale pseudomolecules. The TRITEX pipeline was used to assemble short reads, while Minimap2 and Miniasm were employed for the backbone assembly of PacBio reads. The quickmerge program was used to merge contigs generated from both TRITEX and Miniasm. For CDC Fraser, the assembly was completed by NRGene Technologies. The resulting genome sequences for these five cultivars, showcased genome sizes varying from 3.5 to 4.1 Gb, including unmapped scaffolds ranging from

102.9 to 536.87 Mb. Employing the benchmarking universal single-copy orthologous genes (BUSCO) analysis, our genome assemblies exhibited 89.3% (Morrison) to 98.32% (CDC Fraser) complete and single copy genes from the plant database (embryophyta_odb10). Dotplot comparisons of these five assemblies revealed a high level of chromosomal collinearity with two published barley reference genomes, Morex V3 (six-row) and Golden Promise V1 (two-row). Repeat sequence analysis utilizing RepeatMasker and the TREP repeat database, unveiled that transposable elements (TEs) constituted a substantial portion of the entire genomes, ranging from 78.17% to 81.92%. These findings mirrored the TE contents in Golden Promise V1 (80.61%) and Morex V3 (81.72%). The majority of TEs were LTR retrotransposons, with an average of 21.38% of the entire genomes for the *Ty1/Copia* superfamily and 48.21% of the entire genomes for the *Ty3/Gypsy* superfamily. To ensure an accurate annotation of protein-coding genes, we isolated mRNA (n=3) from five tissues of each cultivar, and generated a substantial dataset of 100-bp pair-end reads, ranging from 1,087.23 to 1,336.71 million reads per cultivar, totaling 107.49 to 132.49 Gb in size. Using the Braker3 pipeline, these high-coverage mRNA sequences, along with Orthodb genes, predicted 40,928–47,962 protein-coding genes for the five cultivars. Among these, 1,116–1,289 genes were predicted as resistance gene analogs (RGAs). Within these RGAs, 18 were caryopsis-specific, 17 coleoptile-specific, 19 inflorescence-specific, and 170 root-specific in different barley cultivars. The orthologous analysis of the five cultivars, along with 76 barley pangenome sequences, identified 10,667 orthogroups of single-copy genes. From the coding sequences of these single-copy genes, 230,256 single-nucleotide polymorphisms (SNPs) were identified, 111,081 of which were non-synonymous. Genome scan analysis demonstrated significant differences in genome regions between landrace/cultivars and wild genotypes, as well as between two- and six-row genotypes. Phylogenetic analysis further indicated that the five Canadian cultivars differed from other two- and six-row cultivars and wild genotypes.

These meticulously crafted reference-level assemblies, alongside the wealth of identified genes, offer significant potential to Canadian and international barley breeding programs. They also benefit the broader barley research community, catalyzing advancements in barley breeding and genetic research.

Note: Abstract submitted for consideration for oral or if not for poster presentation under Session: 01 – Barley genomics.

Modulation in the ratio of abscisic acid to gibberellin level underlies genetic variation of seed dormancy in barley

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Abscisic acid (ABA) and gibberellin (GA) are major regulators of seed dormancy, an adaptive trait tightly associated with the incidence of preharvest sprouting in cereal crops such as barley. The present study examined transcriptional regulation of ABA and GA metabolism genes and thereby changes in ABA and GA levels in seeds of barley genotypes exhibiting a range of dormancy phenotype. Our data revealed a very strong negative correlation between genetic variation in seed germination and embryonic ABA level (r=0.85), which is mediated via transcriptional modulation of the ABA biosynthesis, *HvNCED1*, and/or ABA catabolism, *HvCYP707A*, genes. Variation in seed germination exhibited a strong positive correlation with GA level (r=0.64), which is regulated via expression of genes involved in GA biosynthesis, *HvGA20ox2* and/or *HvGA3ox*s, and GA catabolism, *HvGA2ox3* and/or *HvGA3ox6*, genes. Modulation of the ABA and GA levels in the genotypes studied resulted in ABA to GA level ratio that exhibited a very strong negative correlation (r=0.84) with seed germination, indicating the significance of a shift in ABA/GA ratio in regulating genetic variation of dormancy in barley seeds. Our results overall show that transcriptional regulation of specific ABA and GA metabolism genes as one of the mechanisms underlying genetic variation in ABA to GA level ratio and therefore seed dormancy in barley.

Title: The mutant population of wild barley accelerates functional genomics

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Abstract
Wild barley (*Hordeum vulgare* ssp. *spontaneum*) is the primary gene pool of cultivated barley (*H. vulgare* ssp. *vulgare*) and hence a valuable genetic resource. Several wild barleys were used as genetic material for map-based cloning due to the presence of wild ancestral traits such as brittle rachis, strong seed dormancy, and two-rowed spike. These traits were controlled mainly by dominant functional alleles in the wild and recessive non-functional alleles in cultivated barley suggesting that wild barley leaves many intact genes for lesions. Here we have developed a mutant population from wild barley accession OUH602 to accelerate forward genetics. Plants were exposed to weak irradiation by cobalt 60 in the gamma field from the time of sowing to harvesting. This unique system is called chronic irradiation as opposed to acute irradiation commonly in plant mutagenesis. Grains from M1 plants (irradiated) were harvested manually and proceeded through the phenotypic screening of about 5,000 M2 plants and 50,000 M3 plants (as 10 plants derived from each M2 plant). In this study, we focused on inflorescence structure and found domestication-related mutants like six-rowed spikes and several novel plant architecture mutants. Causal genes were identified through a combination of traditional mapping approaches and rapid identification of the causal mutations using next-generation sequencing technology together with the OUH602 chromosome-scale assembly. Whole-genome shotgun sequences covering 5.7x genome were sufficient to detect the causal mutation. This wild barley mutant population is instrumental in improving barley and the discovery of novel genes and their mode of action.

Incorporating Generative AI Methods to Investigate Pan-Genome Associations across the Cereals

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The age of generative artificial intelligence (GenAI) is now upon us, and we are learning new ways to incorporate it into our daily life. The GrainGenes database has long housed information for the small grains
(since 1992) on topics such as genomes, genes, and traits, and has evolved incrementally with technological advances along the way. We are working on enhanced methods to access information and wish to determine if GenAI can serve as a useful tool to propel our knowledge further.

Early within the GenAI world (2023) there were constraints on the user with regard to required equipment, use fees, limited token-length access which reduced the parsed data volume, and the quality of training associated with the large language models (LLMs) then available. The ability to step-up these efforts have incrementally blossomed over 2024 with the availability of open-sourced tools with enhanced capabilities to provide a plethora of adaptive approaches to incorporate and analyze locally-sourced data collections. We have approached these studies on multiple fronts; through collections of research articles, building graphs based on database queries, and utilizing sequence annotations as paths for querying genome structure based on relevant genes associated with identifiable traits.

We have used collections of research papers (over 5000) resourced for a context-oriented topic of "cereal rust disease" to determine the extent for which it could deliver on problem solving. Specially crafted prompts provided interesting guides about the topic and were able to point to resources and aided expanded discussions to enhance information discovery. Such prompts have been able to direct attention to molecular markers, chromosome locations, dominant and recessive alleles, and some descriptions based on the context provided.

Identifying defined biological primary keys of the GrainGenes relational database schema, information was extracted and placed in a graph-based vector store to build relationships between germplasm, genes, and their semantic connotations. This is being looked at as a new approach toward constructing access to data or enhancing SQL queries for the complexities of questions which may be asked of a database.

Inter-specific crosses have played a role for bringing in new traits via gene introgression. The ability to survey cereals on a pan-genome scale may allow for new discoveries to aid this process. We have developed a capability of integrating the GFF3 files associated with genome descriptions as a roadmap for querying associated genes and traits using GenAI. Improvements in the high-throughput sequencing technologies have added greatly in recent times to the quality and depth of genome coverage and the breadth of species covered to gauge the diversity of cereal germplasm. Within many of the species represented there are highly studied germplasm and progenitors which will become crucial for better understanding the biology of these systems for developing crop improvement strategies. There are now over seventy pseudo-molecules available, or soon to be available, for wheat, barley, rye, and oat. Having these genomes available will allow us to survey relatedness between species in a pan-genome sense utilizing the annotated transcriptomes of high-quality reference genomes.

As this technology evolves, it is hopeful even further enhancements to GenAI will extend our capabilities. We plan to present the state of our findings and open discussions on how

such tools might be useful for our future.

Searching barley genetic resources for shoot architecture genes and alleles

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Shoot architecture traits are crucial for determining plant fitness and adaptability. Breeders exploit variations in these traits to develop crops with enhanced performance in response to evolving environmental conditions and agricultural practices. Ample genetic diversity for shoot architecture traits is available in barley germplasm collections. To further broaden the genetic diversity of barley, large-scale mutagenesis programs have created extensive mutant collections, taking advantage of its diploid genome. Our research leverages these genetic resources to identify and study genes and alleles that influence shoot architecture traits, such as tillering, culm morphology, and leaf size and orientation. In this talk, I will highlight our recent findings through selected case studies.

Session: 02-Morphology, phenology and development

Cloning and regulatory analysis of the *elongated outer glume 1* in barley

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In cereal plants glume is the outermost layer of each spikelet, in addition to the commonly four whorls of floral organs that mainly determined by MADS-box machinery. Studies in rice and wheat have identified some regulators of glume morphogenesis being transcription factors. From the previously established barley "HTX" mutagenesis population, several mutants similar to the "*Triple Bearded Mariout*" were identified. These mutants have the pair of glumes on each fertile spikelet elongated and expanded, with awn at the extension as long as that at the tip of lemma, therefore with two additional lemma-like organs in each spikelet. Through allelism test between this type of mutant and the previously identified barley *elongated outer glume* 1 (*eog1*) mutants *eog1.a* and *eog1.c*, these mutants were allelic to *eog1*. Combining gene mapping in segregation population and whole-genome resequencing of multiple mutants, the causal gene of *eog1* was cloned encoding a zinc-finger transcription factor. The EOG1 protein localizes in nucleus and protoplasmic membrane via transient expression system in either tobacco leaves or barley protoplasts. Bimolecular fluorescence complementation (BiFC) assay demonstrated that both wild-type and mutated proteins can form homo-dimers and hetero-dimers within the nucleus and cytomembrane. Transcriptional profiling showed that *eog1* predominantly expresses at the early stage (eg. W2.5-W3.5) along inflorescence development. Transcriptomic analysis between wild-type and *eog1* mutant of their young inflorescence at W3.5 stage revealed 788 differentially expressed genes (DEGs). They mainly functions such as hydrolase activity, transmembrane transporter activity, serine-type endopeptidase activity, and metal ion transport using functional annotation and GO enrichment analysis. KEGG enrichment showed that *eog1* interacts with the regulatory pathways such as MYB transcription factor pathway, EREBP transcription factor pathway, peroxidase pathway, and aquaporin pathway. Collectively, this study cloned the barley gene *eog1* controlling outer glume morphogenesis, laying a foundation for elucidating the molecular mechanism of barley glume morphogenesis in barley and beyond.

Keywords: Barley (*Hordeum vulgare* L.), *Elongated outer glume 1* (*eog1*); Allelic mutants; Gene mapping; Transcriptomic analysis

Session: 02 Morphology, phenology and development

Most Tibetan weedy barleys originated via recombination between *Btr1* and *Btr2* in domesticated barley

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Tibetan weedy barleys reside at the edges of qingke (hulless barley) fields in Tibet (Xizang). The spikes of these weedy barleys contain or lack a brittle rachis, with either two- or six-rowed spikes and either hulled or hulless grains at maturity. Although the brittle rachis trait of Tibetan weedy barleys is similar to that of wild barley (*Hordeum vulgare* ssp. *spontaneum* Thell.), these plants share genetic similarity with domesticated barley. The origin of Tibetan weedy barleys continues to be debated. Here, we show that most Tibetan weedy barleys originated from cross-pollinated hybridization of domesticated barleys, followed by hybrid self-pollination and recombination between *Non-brittle rachis 1* (*btr1*) and *2* (*btr2*). We discovered the specific genetic ancestry of these weedy barleys in South Asian accessions. Tibetan weedy barleys exhibit lower genetic diversity than wild and Chinese landraces/cultivars and share a close relationship with qingke, genetically differing from typical eastern and western barley populations. We classified Tibetan weedy barleys into two groups, brittle rachis (BR) and non-brittle rachis (NBR); these traits align with the haplotypes of the *btr1* and *btr2* genes. Whereas wild barleys carry haplotype combinations of *Btr1* and *Btr2*, each showing lower proportions in a population, the recombinant haplotype BTR2H8+BTR1H24 is predominant in the BR group. Haplotype block analysis based on whole-genome sequencing revealed two recombination breakpoints, which are present in 80.6% and 16.8% of BR accessions according to marker-assisted analysis. Hybridization events between wild and domesticated barley were rarely detected. These findings support the notion that Tibetan weedy barleys originated via recombination between *Btr1* and *Btr2* in domesticated barley.

Keywords: Tibetan weedy barley, *agriocrithon*, de-domestication, out-pollination, recombination, brittle rachis

Session: 02 Morphology, phenology and development

A novel type of malformed floral organs mutant in barley is conferred by loss-of-function mutations of the MADS-box gene *HvAGL6*

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The advanced model of floral morphogenesis is based largely on discoveries from Arabidopsis (*Arabidopsis thaliana*) and rice (*Oryza sativa*), but the regulatory machinery is less understood in Triticeae species. In this study, we identified a novel barley mutant with severely malformed floral organs (designated *mfo1*) from the "HTX" mutagenesis population. This type of mutant has its paleae, lodicules, and stamens all converted into lemma-like organs in each floret, resulting in multiple awns and the whole spike looks brushy. In the innermost whorl, the ovary maintains but weakly develops. Integrating bulked-segregant analysis, comparative whole-genome sequencing, and mutant TILLING, we identified and validated the causal gene of *mfo1*. Multiple allelic *mfo1* mutants were found to harbor loss-of-function mutations at the MADS-box gene *HvAGL6*, a key regulator in the "ABCDE model" of floral morphogenesis. Transcriptomic analysis comparing at young inflorescence of wild-type and *mfo1* plants revealed 380 differentially expressed genes (DEGs), which mainly involved in DNA-binding, protein dimerization, cell differentiation, and meristem determinacy. Pathway enrichment analysis highlighted significant associations between *HvAGL6* and a number of other MADS-box members, including those from the B-, C-, D-, and E-classes. These findings demonstrate that the regulatory model of floral morphogenesis is conserved across plant species and provide insights into the interactions between *HvAGL6* and other MADS-box regulators.

Keywords: Barley (*Hordeum vulgare*), floret, MADS, *AGL6*, TILLING

Early flowering gene *mat-b* encodes a leucine-rich repeat kinase

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Time of flowering is an important trait affecting crop yield. Between 1941 and 1988 more than 1200 early flowering barley mutants were isolated at the Swedish Seed Association. The mutants were generated by different mutagens. Allelism tests distributed early mutants to nine different loci: *mat-a, -b, -c, -d, -e, -f, -g, h* and *-i*. We identified the *mat-b* gene using bulked segregant analysis combined with whole genome sequencing. The gene HORVU.MOREX.r3.7HG0747230 is located on chromosome 7H at position 618787699-618791569 and has been annotated as a non-specific serine/threonine protein kinase. The protein has 21 leucin-rich repeats (LRR), which suggest that this protein is involved in protein-protein interactions. The LRR motive assigns this protein to a class of extracellular transmembrane receptors. Of 46 available *mat-b* mutants, we found mutations in 34, which represent 31 unique *mat-b* alleles. Mutations in all identified alleles result in defective protein. The mutations are distributed in DNA corresponding to all three domains of the protein; the LRR, the kinase domain and the transmembrane domain (TMD).

Variation in genes determining time of flowering is very important for the ability of plants to adapt to certain geographic regions. In times of global warming, this variation can be expected to become a more important breeding target if crop plants need to be grown and developed for different and new regions. We have been growing a small set of barley early flowering mutants (*mat-a.8*, *mat-b.7*, *mat-c.19*, *mat-e.18* and their mother cultivar Bonus) at four different locations (Sweden, Italy, Iceland and Russia). Mutants *mat-a.8* and *mat-e.18* are day-length neutral, whereas the earliness of *mat-b.7* and *mat-c.19* depends on day length. Depending on the ability of the mutants to respond to changes in day length, the mutants performed differently.

Molecular mechanisms of assimilate and hormone transfer in barley grains controlling endosperm filling

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Barley (*Hordeum vulgare*) grains accumulate large amounts of starch, proteins and lipids in their endosperm, the main storage organ in a grain. The carbohydrate and nitrogen sources deposited in the endosperm are provided by the maternal plant but the basis for their spatial allocation is obscure. Our research has discovered several interconnected mechanisms that control assimilate transfer between maternal seed parts and the endosperm in barley grains. Using ^{13}C-labelled sucrose, we monitored assimilate allocation in the developing barley grains *in vivo* by Magnetic Resonance Imaging (MRI) and discovered that the nucellar projection (NP) and endosperm transfer cells (ETC) are the primary tissues responsible for assimilate transfer within cereal grain. Continuous cell turnover and programmed cell death (PCD) occur on NP margins. Three much similar genes encoding Vacuolar Processing Enzyme, the protease responsible for PCD execution in plants, specifically expressed in NP with overlapping transcriptional profile. The simultaneous repression of these genes by RNA interference resulted in a disturbance to the wild-type progression of PCD, thereby compromising grain filling and realizing in lower-weighted grains due to decelerated accumulation of starch, proteins and lipids. MRI analysis revealed delayed sucrose transport into the developing endosperm and increased sucrose accumulation in maternal parts of a grain. This investigation has shown that PCD at maternal–filial borders of the barley grains serves to widen the rather narrow post-vascular assimilate route, thereby accelerating assimilate allocation.

Sugars Will Eventually be Exported Transporters (SWEETs) have been considered to facilitate the transport of sugars but may be also involved in the movement of phytohormones. Of 23 SWEET genes in barley, the *SWEET11b* transcription is restricted to the NP while the sucrose transporter 1 (*SUT1*) mRNA largely accumulates in ETC. A radiotracer assay revealed that expressing barley *SWEET11b* in *Xenopus* oocytes facilitated the bidirectional transfer of not only just sucrose and glucose, but also cytokinin. Barley plants harbouring a loss-of-function mutation of *SWEET11b* could not set viable grains, while the distribution of sucrose and cytokinin was altered in developing grains of plants in which the gene was knocked down. These data indicate that SWEET11b mediates the movement of both sucrose and cytokinin across the maternal–filial boundary. Decreasing *SWEET11b* expression in developing grains reduced grain size, sink strength and the contents of starch and protein. The control exerted by SWEET11b over sugars and cytokinins likely predetermines their synergy, resulting in adjustments to the grain's biochemistry and transcriptome.

Molecular mechanisms regulating carbohydrate and hormone allocation at the maternal-filial interface open new perspectives for improving the efficiency of the grain filling process in barley.

Map-based cloning the *Gpa1* gene regulating chloroplast biogenesis and tillering in barley

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Chloroplast biogenesis is critical for crop biomass and economic yield. However, chloroplast development is a very complicated process coordinated by cross-communication between the nucleus and plastids, and the underlying mechanisms have not been fully revealed. To explore the regulatory machinery for chloroplast biogenesis, we conducted map-based cloning of the *Grandpa* 1 (*Gpa1*) gene regulating chloroplast development in barley. The spontaneous mutation *gpa1.a* caused a variegation phenotype of the leaf, dwarfed growth, reduced grain yield, and increased tiller number. Genetic mapping anchored the *Gpa1* gene onto 2H within a gene cluster functionally related to photosynthesis or chloroplast differentiation. One gene (*HORVU.MOREX.r3.2HG0213170*) in the delimited region encodes a putative plastid terminal oxidase (PTOX) in thylakoid membranes, which is homologous to IMMUTANS (IM) of Arabidopsis. The *IM* gene is required for chloroplast biogenesis and maintenance of functional thylakoids in *Arabidopsis*. Using CRISPR technology and gene transformation, we functionally validated that the PTOX-encoding gene, *HORVU.MOREX.r3.2HG0213170*, is the causal gene of *Gpa1*. Gene expression and chemical analysis revealed that the carotenoid biosynthesis pathway is suppressed by the *gpa1* mutation, rendering mutants vulnerable to photobleaching. Our results showed that the over-tillering associated with the *gpa1* mutation was caused by the lower accumulation of carotenoid-derived strigolactones (SLs) in the mutant. The cloning of *Gpa1* not only improves our understanding of the molecular mechanisms underlying chloroplast biosynthesis but also indicates that the PTOX activity is conserved between monocots and dicots for the establishment of the photosynthesis factory.

SESSION THEMES

02 – Morphology, phenology, and development

Title: Light intensity accelerates barley development processes.

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The objective of this study was to investigate the impact of day length and light intensity on phyllochron and the induction of the reproductive stage in barley in two barley genotypes (INIA Arcadia and Norteña Carumbé) selected for their contrasting photoperiodic responses. The plants were cultivated in growth chambers at a constant temperature of 12 ºC and a relative humidity of 60%. Two photoperiods were defined: a short day (SD,12 hours of light - 12 hours of dark) and a long day (16 hours of light -8 hours of dark). The spectral quality of the LED luminaires used was identical, but four levels of light intensity were employed (LD, 1600, 1200, 400, 300 µmol photons m^{-2} s^{-1}). The combination of photoperiod and light intensity permitted the creation of high-energy (HE) and low-energy (LE) light environments during plant development . Number of leaves (NL) on the main stem of each plant was recorded on the Haun scale until the seventh leaf reached maturity. Degree-days (GDD) were calculated using a base temperature set at 0 °C. Phyllochron (GDD $leaf^{-1}$) was calculated as the inverse of the regression between NL and thermal time. On the shoot ápex, meristem development was recorded to calculate the switch to reproductive, recording the number of spikelet primordium (NSP) initiated once the plant had three leaves. Time at double ridge was calculated based on the linear regression between NSP and thermal time. Both genotypes exhibited a reduction in phyllochron in HE relative to LE. INIA Arcadia presented the same phyllochron in both SD and LD. In N. Carumbe, phyllochron in LD exhibited a lower duration than the SD one. In LEs, the phyllochron has a reduction under SD in both genotypes. We detected t a reduction in the thermal time to double ridge when the energy of the developmental light environment is increased. In HE, we observed a lower thermal time to double ridge in Arcadia under LDin comparison to SD. In N. Carumbe, however, the thermal time to double ridge is the same for both photoperiods. In LE the time to double ridge is identical for both photoperiods within each genotype. In this environment, under SD, N, Carumbe exhibits a higher thermal time to double ridge than Arcadia. However, under LD there is no difference between the genotypes.

Association between final leaf number, phyllochron and flowering time in barley under South American environmental conditions

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Genotype and environmental factors determine flowering time and its subphases, associated to changes in the final leaf number (FLN) and phyllochron. The number of leaf primordia on each steam is determined during the pre-inductive stage, at the beginning of development. The differentiated FLN as well as its rate of appearance (or its inverse, phyllochron) are closely associated with the duration of time to anthesis. In order to analyze these associations in malting barley under contrasting environments we studied 9 cultivars of contrasting phenology during two years and two sowing dates in field trials at Montevideo, Uruguay (34º51'S, 56º12'W): June 14, 2016; June 27, 2017; September 27, 2016; October 13, 2017. The main variation between years was in temperature: 2017´s experiments were approximately 2 °C warmer than 2016´s ones. Genotype x environment interaction (G x E) was detected for the time to anthesis (E-A), FLN and phyllochron. The phyllochron and the FLN were negatively and significantly correlated at early sowing dates but had no correlation at the late sowing dates. On the other hand, E-A was positively correlated with FLN and phyllochron at the late sowing date. In the early planting date, E-A was only correlated (positively) with FLN in the warmer year. Five cultivars had a longer time to anthesis in the higher temperature environment in the early planting date. In the warmer year, all genotypes developed more leaves in the early planting date and seven in the late planting date, compared with the cooler year. Under long photoperiod or rising temperatures, FLN is associated with the time to anthesis and could affect potential yield.

Identification of QTL associated to final leaf number, phyllochron and flowering time in barley in South American environments

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Combining the different phases of development with the appropriate environmental conditions is key for improving the adaptation of cereals to a given area, obtaining high quality grains and high yields. Knowledge about the genetic control of the time to anthesis (E-A) of the barley crop, the duration of its subphases and traits associated with development as final leaf number (FLN) and phyllochron can help to develop better adapted germplasm. Also, the differentiated FLN as well as its rate of appearance (or its inverse, the phyllochron), can determine the duration of time to anthesis. In order to advance in the understanding of the genetic factors determining these traits, we used a RIL population derived from the cross between two contrasting cultivars regarding phenology (INIA Ceibo and Norteña Carumbé). The population was genotyped with the Illumina 50K platform and a linkage map containing 1484 SNPs was developed. The population was phenotyped during two years at Montevideo, Uruguay (34º51'S, 56º12'W) at contrasting planting dates: June 14, 2016; June 27, 2017; September 27, 2016; and October 13, 2017. E-A, length of the subphases (emergence - Z21, Z21-Z31, Z31-anthesis), FLN and phyllochron were determined. Positive correlation was detected between E-A and FLN, with higher values at the late sowing date. The correlation between E-A and phyllochron was moderate in the two sowing dates of 2016. In addition, FLN correlated positively with Z21-Z31 in all environments. QTL effects detected for phenology, FLN and phyllochron in 2H, were mainly expressed in late environments. This QTL coincides in location with *Ppd-H1*, a photoperiod response gene. For E-A, Z21-Z31 and FLN, QTL effects were identified at the early sowing in 3H, coinciding with the location of *sdw1*, a semi-dwarfing gene with pleiotropic effects in phenology. In addition, two QTLs were detected in 6H, one with an effect on phyllochron and the other on FLN, in regions where there are no previous reports and without coincident effects on phenology. The QTLs detected for FLN and phyllochron in 6H in 2016 allow us to speculate with an independent control of these traits under short photoperiods season.

Relationship and genetic trends of adaptation of barley to the Icelandic environment

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The Icelandic growing season is challenging with risks of strong wind gusts and frosts. The photoperiod is long as well as the growing season compared to other subarctic regions, although the cumulative growing degree-days are lower due to low summer temperatures, frequent rains and lack of sunshine. The Icelandic barley breeding program has been focusing on breeding barley for earliness and other traits related to adaptation to the Icelandic environment which has resulted in the release of six cultivars, 3 two-row and 3 six-row. The Icelandic barley breeding program emphasized fast developing cultivars, to take advantage of the long photoperiod in spring and early summer, to mature at lower temperatures and straw strength to withstand strong wind gusts. The breeding program has achieved some success. The Icelandic barley population has been shown to head earlier than other Nordic varieties in controlled experiments and in Icelandic field trials. Lodging resistance has increased, as well as yield and grain size.
The Icelandic lines have been extensively crossed with Nordic varieties and to some extent to Scottish and Alaskan material. In this study we will use model-based clustering and principal component analysis to infer the extent of admixture with other material.
To do this, the Icelandic barley population will be supplemented with a reference panel. The reference panel will include, to the extent possible, ancestors in the Icelandic pedigree. Methods to construct local ancestries will be used to identify origins of haplotype segments in the Icelandic population. We will look for signatures of selection in the Icelandic population and estimate trends of genetic gain, relationship and admixture in the population and the genetic origin of adaptation to the Icelandic environment. By examining the trends in relationship and admixture, it is possible to identify whether the Icelandic breeding program has been depleting genetic variance in its germplasm and reintroducing it with foreign material. Discovering the origins of Icelandic barley, and relationship to other barley populations, is of historical interest and the results will potentially identify genomic regions or genes that have been under selection for adaptation to the extreme Icelandic environment.

Keywords: Nordic barley, population genetics, adaptation

An Early Life Story in the Shade—Turning Green while being Sheathed

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Photosynthesis is the basis for all energy sources essential to life. To date, studies of photosynthesis have mostly focused on the leaf as the primary tissue for the conversion of light into chemical energy. Importantly, plants similarly possess photosynthetically active chloroplasts in other tissues, such as flowers, fruits and seeds, that contribute to the final yield. For the *Triticeae* tribe, in barley (*Hordeum vulgare* L.) and wheat (*Triticum* spp.), it has been shown that the reproductive organs, e.g. awns and florets, can significantly contribute to final grain yield upon anthesis and grain filling stages. Although these organs develop chloroplasts at early pre-anthesis stages (i.e. during floral organ differentiation), whether these are photosynthetically active has not been fully investigated. The presence of functional and mature chloroplasts during immature inflorescence development varies among different grass species. For example, the developing inflorescence of *Pooideae* species (including wheat, barley, rye and *Brachypodium*), which are ecologically important in the cold season areas, show early immature inflorescence greening; while the more tropically based species, such as rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* L.), do not show this feature. For any organ to be autotrophic, it must have a fully developed photosynthetic machinery. Therefore, in our study, we conducted a series of experiments to decipher the photosynthetic capabilities of the immature barley inflorescence. In fact, the occurrence of any of these components, such as chlorophyll, chloroplasts, stomata and the key enzyme Rubisco, during immature spike growth and development allows us to determine the approximate stage (e.g. Waddington) at which these organs have a fully developed photosynthetic machinery. In addition, using LC-MS technique we attempted to detect the intermediate photosynthetic metabolites of the Calvin-Benson cycle, glycolysis and tricarboxylic acid (TCA) cycle indicating possible carbon fixation and metabolism. We also found that ambient light is able to penetrate the leaf sheaths to reach the developing inflorescence to initiate greening and chlorophyll biosynthesis. However, while preventing light from reaching the developing inflorescence during growth and development spikelet number and fertility were adversely affected, suggesting that light-induced greening of the immature spike is crucial for proper spikelet and grain development in barley. Our new insights from this study may therefore provide previously unrecognized light-inducible potentials of the immature spike to sustain autotrophic growth locally and independently from the rest of the plant.

Past and future trends in barley breeding for yield determination

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Plant breeding has led to significant improvements in crop performance over the last decades, as repeatedly assessed by analysis of historical data. Here we report on long-term trends in breeding progress for yield and yield-related traits in winter barley from a retrospective work based on almost 40 years of official variety trials in northern Italy, including an estimation of the effects due to environmental and genetic variability. Preliminary data from comparative trials to estimate genetic gain in a panel of varieties representing barley breeding for the southern European environment will also be reviewed. In addition, a meta-analysis of a field trial network of spring barley genotypes representing the diversity and history of European spring two-row barley breeding is presented. Changes in QTL frequencies over time revealed that early selection focused on phenotypes with high heritability, such as grain weight, and selection for grain yield occurred later, facilitated by reduced variance in other traits. Some of the detected genomic regions showed linkage in repulsion, suggesting targets for future breeding. Finally, future targets for further improvement are discussed, including increasing photosynthetic and water use efficiency and editing genes with a potential role in defining yield components.

GGE biplot Analysis of Genotype by Environmental Interaction, Principal Component Analysis and clustering of Barley genotypes (Hordeum vulgare L.) under Moisture Stress Areas of Ethiopia

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Abstract
GGE Biplot analysis illustrates the main effects of Genotype (G) and Genotype x Environmental Interaction (GEI). It is a graphical method used to analyze and interpret the interaction between genotype and environment in multi-environment trials (MET). A field experimental trial was conducted to evaluate 25 elite food barley genotypes and assess the effect of genotype and GEI on grain yield, aiming to identify high-yielding and stable genotypes under moisture-stress conditions. The trial was conducted in a 5x5 Triple Lattice Design across ten different environments from the 2019-2022 cropping seasons. Analyses included Analysis of Variance, GGE Biplot for Genotype by Environmental Interaction, correlation, Principal Component Analysis (PCA), and Hierarchical Cluster Analysis. Combined ANOVA results indicated highly significant($p \leqslant 0.001$) difference due to genotypes, main effects, environments and GE interaction among the studied genotypes for eight quantitative traits. Grain yield variation among genotypes ranged from 3.4t/ha to 4.7t/ha, with high-performing genotypes showing an 11.12% to 14.73% yield advantage over standard checks. Moderate to high broad sense heritability (H2) was observed for all traits. Based on GGE Biplot evaluation, the first mega-environment included test environments DH2, AS1, DH6, AN9, AL10, AS5, and DH8, with G2 and G9 showing the highest mean grain yield. The second mega-environment comprised AS3 and AS7, with G10 and G4 exhibiting high mean grain yield. Genotype G2 was identified as relatively ideal and stable across all environments, while G23, G10, and G4 were less stable. PCA revealed that 71% of gross variability was explained by the first three axes implies significant variation among the barley genotypes that suggests high opportunities for genetic improvement through selection, and four clusters were identified through hierarchical cluster analysis, with the 2nd and 3rd clusters potentially suitable for variety release and future barley breeding programs.

Keywords: Food barley, Genotype x Environment interaction, Grain yield, stability, PCA, cluster analysis

Characterization of *Sheathed Spike 2*, a regulatory gene of the peduncle length in barley

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Barley plant with short peduncle and sheathed spike can increase the number of spikes in area and protect young spikes from adverse weather. In cereal crops, the molecular mechanisms on peduncle elongation mainly relied on studies of rice mutants, and majority of the uncovered regulatory pathways associated to phytohormone metabolisms. Dissecting novel regulatory gene and their functional mechanism would extend our knowledge in plant architecture formation and provide candidates for genetic improvement. In this study, by taking use of the barley landrace "HTX" and its derivatives *sheathed spike* mutants M4950 and M4081 showing shortened peduncle, in conjugation with genetic analysis and whole genome sequencing, we cloned the recessive gene locus *ss2* that accounted for the *sheathed spike* phenotype. Through screening the “HTX” mutant library via TILLING technology, additional seven allelic mutants were obtained, all of which exhibited significantly shortened peduncle, confirming *ss2* as the determinant gene of sheathed spike phenotype. *SS2* is annotated to encode a membrane anchored silicon transporter, and this gene in wild-type plants has the transcriptional abundance in the rachis and peduncle at the heading stage. Both wild-type SS2 and mutant ss2 proteins were localized on the cytoplasmic membrane via the transient expression of fusion proteins in *Nicotiana Benthamiana* leaves. Measurement of the silicon content in the peduncle of near isogenic line NIL-*SS2* and NIL-*ss2* showed that the silicon content was significantly higher in the mutant than that in wild-type. The *ss2* mutant was able to complement the normal wild-type peduncle without Si supply in hydroponic culture experiment. Overall, our results highlight that *SS2* regulates peduncle length via suppressing the silicon accumulation, which provides a novel clue about the relationship between silicon content and peduncle development in barley.

Keywords: Barley (*Hordeum vulgare*), *sheathed spike 2*, peduncle, silicon, transporter

Barley Yield and Quality Traits Selection for Dryland and Irrigation Production

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Barley is one of the most important crops in the Northern Great Plains of USA. Breeding cultivars that have high and stable yield and end-user desirable qualities is important for growers and end-users for dryland and irrigated production, especially for malting industry. We evaluated 48 barley breeding lines and 2 check varieties under dryland (rainfed) and irrigated environments in 2022 in Sidney, Montana. The studies were conducted with randomized complete block design with 4 replications for each of the dryland and irrigated trials. Barley plant height, grain yield, grain protein, and plump numbers were measured. By plotting plant traits under irrigation against dryland in scatter plots, we can examine the performance of individual breeding lines and their response to the changes of environmental conditions. Yield is very sensitive to irrigation and all breeding lines had jump in yield from dryland to irrigated environment. Several breeding lines were identified to have the highest yields and performed much greater than nursery means at both dryland and irrigated environments. Protein concentration did not have a significant jump or decrease from dryland to irrigated environment, but great variations were observed among the breeding lines. A few breeding lines were identified to have significantly lower than trial mean protein concentrations under both dryland and irrigated environments, and two of them matched the lines selected for high yield in both environments. Separate plots for yield against protein at dryland and irrigated environments did not show clear correlations between yield and protein, indicating protein is controlled more by genetics than environment. Plump numbers of different breeding lines responded differently to environment. While most of the breeding lines showed greater than trial mean plump numbers in both environments and the top breeding lines showed little difference between dryland and irrigated environment, a small portion of the breeding lines had less than trial mean plump numbers under both environments and varied greatly from dryland to irrigation environment.

Presenting at: Genetics and Breeding for yield and its components Session

Are PPD-H1 effects on spike fertility of barley truly pleiotropic or they simply reflect the consequences of changing flowering time?

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Barley yield is strongly related to the number and fertility of spikes. Number of spikes per plant and number of fertile florets per spike are determined from jointing to flowering. Time to flowering is critical for adaptation and yield as it affects the fertility of both tillers and spikes. The PPD-H1 gene controls flowering time in barley, strongly influencing crop yield through improved adaptation. PPD-H1 was also attributed to impact spike fertility and yield. It is important to determine if these effects are truly pleiotropic or indirect. Recent findings evidenced that under extremely long photoperiods, spring barley near-isogenic lines for PPD-H1 tend to flower simultaneously, offering an opportunity to determine any genetic effects independently of flowering time. We examined the impact of PPD-H1 on spike fertility. Experiments were carried out in the field and in controlled conditions, with a factorial combination of (i) four near isogenic lines (NILs) combining alleles of both the photoperiod sensitivity gene PPD-H1 and the gene responsible for the red/far-red light photoreceptor PHYC, and (ii) two contrasting photoperiod conditions (12 and 24 h). NILs combined either the photoperiod-insensitive (ppd-H1) or -sensitive (Ppd-H1) alleles with either the late (PhyC-l) or the early (PhyC-e) forms of PHYC. The short photoperiod in the field was the natural and the extended was achieved with low-intensity lamps placed over the plots designated to have extremely long days. In the growth chambers, both photoperiods had the same daily radiation levels. In both experiments, the ppd-H1 allele increased spike fertility, regardless of the PHYC genetic background. This effect was mediated through allocating more resources to juvenile spikes and improving the efficiency for producing fertile florets. These effects of ppd-H1 were evident not only under 12 h photoperiod, but under the extremely long photoperiods as well. As in the latter condition NILs with ppd-H1 or Ppd-H1 alleles flowered simultaneously, we evidenced by the first time (as far as we are aware) that PPD-H1 has true pleiotropic effects on spike fertility, in addition to those that are related to lengthening time to flowering. These effects on spike fertility were mediated through those of photoperiod insensitivity on increasing the rates of survival of initiated floret primordia in line with the greater allocation of assimilates to the juvenile spike. Floret mapping revealed different patterns of fertility within the spike, with central spikelets consistently having fertile florets in both PPD-H1 NILs regardless of PHYC background and photoperiod. However, the sensitive Ppd-H1 line exhibited reduced fertility in distal spikelets compared to the insensitive ppd-H1 line, consistently across experiments. The ppd-H1 allele mainly influenced the less favoured distal florets and even induced more progress before floret mortality in apical spikelets.

Rapid Development of Naked Malting Barley through Targeted Mutagenesis

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Historically, covered (hulled) barley has been the preferred choice for malting, brewing, and distilling brewing due to the husk's role in protecting the embryo during harvest and its utility in filtering the wort post-saccharification. However, with advancements in technology such as centrifugal filtration, beer brewing with naked (hull-less) barley has become a viable alternative. Naked barley cultivars, primarily used for food, are rich in β-glucan and polyphenols, which have been considered detrimental in beer brewing. Therefore, the introduction of the naked trait into malting cultivars through cross-breeding is a time-consuming process due to the need to eliminate these undesirable traits. In this study, we aimed to rapidly develop naked barley germplasm suitable for malting by introducing targeted mutations into the hulled/naked gene (*NUD*) using the CRISPR/Cas9 method.

We utilized the doubled haploid line 'DH120366', derived from the cross between the covered malting barley cultivars 'Full Pint' and 'Golden Promise'. This line has shown high brewing suitability and transformation amenability (Hisano et al. 2017). We generated knockout barley mutations targeting the *NUD* gene via the *Agrobacterium*-mediated method and detected targeted mutations and transgenes by PCR. Upon investigating the sequence of the *NUD* gene in 16 transformed individuals, we confirmed chimeric mutations in one T_0 individual. The resulted T_1 grains exhibiting the naked trait were sown and seedlings showed two types of mutations (1 base insertion, 13 base deletion) in *NUD* gene. Furthermore, shotgun short-read sequencing analysis of the genomic DNA of the *nud* mutant lines and control lines detected the presence or absence of transgenes using the *k*-mer method. As a result, at least one mutant line was shown to have no remaining transgenes. Additionally, we cultivated the progeny in a controlled chamber and investigated the culm length, 1000-grain weight, and grain dormancy. We found no significant differences in these traits between the *nud* mutant and wild type 'DH120366'. This study demonstrates the feasibility of rapidly developing naked malting barley germplasm with high malting quality profile from a hulled line, altering only *NUD* gene, via genome editing technology.

Cytonuclear analysis of barley spike traits using a cytoplasm-aware population

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Crop wild relatives are a sought-after resource for the improvement of existing crop varieties and
for identifying loci underlying domestication. The population designs used can be broadly divided into diversity panels, bi- and multiparental populations, and diallel crosses, which all focus mainly on nuclear diversity. Here, we present the barley cytonuclear multi parent population (CMPP), which consists of 924 doubled haploid lines divided into 10 subfamilies,
each showcasing segregation of both nuclear and cytoplasmic wild alleles. Analysis of key spike traits collected in four field trials during 2022-2024 showed that the cytoplasmic origin affects all tested best linear unbiased predictions (BLUPs), and that specific wild cytoplasms also change
trait stability indices for thousand grain weight (TGW). Nuclear SNP data was scored using the
iSelect 50K platform and allowed us to exclude that trait differences between cytoplasmic groups are explained by nuclear drift. Using the marker data, the CMPP's design also allowed us to
efficiently scan for cytonuclear epistasis, resulting in 13 cytonuclear QTL (cnQTL), where the nuclear allele effect is conditioned by the cytoplasmic background. Juxtaposing the identified
cnQTL with marker-trait associations (MTAs) from classical GWAS analyses showed that these
loci are mostly missed with nuclear-only models, thereby highlighting the importance of cytoplasm-aware population designs. We also incorporated cytonuclear interaction information
into genomic prediction models, yielding a relative increase in cross-validation accuracy of up to
21.7 percent for Fruiting efficiency at maturity (FEm). Looking forward, cnQTL can present a starting point for the molecular dissection of cytonuclear interactions, while cytonuclear
epistasis-based genomic prediction models using populations such as the CMPP can potentially improve breeding outcomes.

How to Enhance QTL Detection in a newly established Barley Breeding Program: The Power of Multi-population GWAS

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Marker-assited breeding can offer an efficient shortcut for trait improvement in applied breeding programs. Statistical analyses such as genome-wide association studies (GWAS) play a pivotal role in identifying markers or quantitative trait loci (QTLs) associated with traits of interest. However, a significant constraint of GWAS power is the sample size, posing a challenge for newly established breeding populations with limited data accumulation. Here, we tackle this issue by combining data from a newly established 6-rowed winter (6RW) barley breeding population with datasets from well-established barley breeding programs that differ in their row types and/or seasonal growth habits. With a focus on the important agronomic trait, heading date, we apply single- and multi-population GWAS models and compare the findings. Using both a uni-variate (MP1) and multi-variate (MP2) model for multi-population GWAS, we explore the potential challenges of treating a trait scored in different populations as genetically identical and combining populations that differ remarkably in size. While single-population GWAS struggled to detected marker-trait associations within the 6RW population, the multi-population GWAS models significantly boosted the statistical power, resulting in the detection of a greater number of candidate QTLs. Furthermore, our study found that MP2 offered a notable advantage over MP1 by aligning with more realistic assumptions while detecting more robust marker-trait associations across populations. In summary, our study presents a statistically sound approach to enhance statistical power by combining data from distinct populations, thereby laying the groundwork for kick-starting genomics-based breeding in newly established breeding programs.

Impact of breeding on barley diversity and identification of selective sweeps by skim-sequencing

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Plant breeding programs aim to increase the frequency of desirable alleles while maintaining or enhancing genetic diversity within breeding populations. Understanding the evolution of this diversity is required to identify changes in genetic diversity and allele frequencies in specific genomic regions, thus facilitating informed breeding decisions. High-density genotyping tools now enable precise comparisons of genetic diversity and identification of genomic regions subjected to selection across chromosomes over different time periods. This study quantifies changes in genetic diversity throughout the history of barley (*Hordeum vulgare* L.) breeding in Uruguay. We analyzed commercial spring barley varieties and advanced breeding lines from different breeding periods, grouped according to their official evaluation or commercial release, covering over 20 genotypes per group from before 1980 to 2022. We genotyped 277 spring barley genotypes using skim-sequencing, identifying 148,000 SNPs used to analyze population structure, genetic distances, and allelic diversity indices. Our results reveal significant differences in genetic composition among groups from different breeding periods, which corresponds well with the history of the breeding programs and the varying importance of agronomical, disease resistance, and malting quality traits. The most recent period was the most genetically different. While no generalized change in genome-wide diversity over time was observed, specific regions, such as a large part of chromosome 5H, exhibited different allelic frequencies, and chromosome 1H showed lower diversity in the most recent genotypes. The increased frequency of a new haplotype on chromosome 5H and conserved regions on chromosome 1H align with findings from studies on recent European barley material, likely due to the high proportion of European-origin genotypes in recent breeding groups. Studying nucleotide diversity and allelic frequency changes in barley breeding programs helps inform breeding strategies, enabling the identification and introduction of specific genetic regions into elite breeding germplasm.

The HvDRR population – a platform for high resolution dissection of quantitative traits

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Understanding the factors contributing to yield and yield components has the potential to increase the gain of selection either by optimization of breeding programs or the exploitation of advanced breeding methods. We have developed the double round robin population of barley from crosses among 23 spring barley landraces and varieties with worldwide origin, which have been selected to maximize phenotypic and genotypic diversity. In this presentation, we will report about the genomic and phenotypic characteristics and especially diversity of the population that consists of about 4000 recombinant inbred lines from 45 bi-parental sub-populations. In addition, we will report for yield component traits details about their genetic inheritance and our approach to clone some of the underlying genes but also the power of the HvDRR population as calibration population for predictive breeding approaches.

Integrating phenomic and genomic information to enhance prediction of barley grain yield

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High prediction accuracy of the target traits is critical for achieving higher rates of genetic gain in plant breeding programs. Our research evaluates how prediction of grain yield could be enhanced using genomics and/or phenomics tools in barley breeding. High-throughput phenotyping (HTP) methods, such as drone-mounted multispectral cameras, could be used to collect vegetative indices and to the extent that those indices are genetically correlated with grain yield, they may be useful as secondary traits to improve prediction accuracy for grain yield in barley. Our results obtained from the evaluation of barley breeding trials (almost 2000 lines) over six environments suggest that the strongest genetic correlations between vegetative indices and grain yield is observed during the grain-filling stage, with NDRE showing the most consistent performance among the four vegetative indices evaluated. Incorporating vegetative indices in one-stage multi-trait pedigree and genomic prediction models provided only marginal improvement on the prediction ability and reliability of the grain yield breeding value, and the improvement only occurred when data were collected on both training and selection populations. The incorporation of marker data, however, always improved prediction ability and reliability of grain yield breeding value compared to pedigree predictions.

Elucidating Features of Spikelet Formation for enhanced Grain Yield in Barley

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Enhancing the yield potential and stability of small-grain cereals, such as wheat (*Triticum* sp.), rice (*Oryza sativa*), and barley (*Hordeum vulgare*), is a priority for global food security. Over the last several decades, plant breeders have increased grain yield mainly by increasing the number of grains produced in each inflorescence. This trait is determined by the number of spikelets per spike and the number of fertile florets per spikelet. Recent genetic and genomic advances in cereal grass species have identified the molecular determinants of grain number and facilitated the exchange of information across genera. Commonly, the birth of a floret proceeds through meristem initiation as well as floral organ identity specification and maintenance. During these processes, both endogenous and external cues can trigger a premature floral organ death, leading to reproductive failure. Recent advances in different cereal crops have identified both conserved and distinct regulators governing the birth of a floret. However, the molecular underpinnings of floral death in barley are just beginning to be understood. Due to its quantitative nature and environmental sensitivity, the degeneration of floral structures during pre-anthesis growth and development constitutes a complex, multilayered trait affecting final grain number. This trait appears to be highly predictable and heritable under standardized growth conditions, consistent with a developmentally programmed mechanism. To elucidate the molecular underpinnings of inflorescence pre-anthesis tip degeneration (PTD) in barley, we combined metabolomic, transcriptomic, and genetic approaches to show that barley inflorescence PTD is accompanied by sugar depletion, amino acid degradation, and abscisic acid responses involving transcriptional regulators of senescence, defense, and light signaling. Here, I will focus on the genetic basis of inflorescence architecture in barley, highlighting recent insights that have helped reduce the developmentally pre-programmed degeneration of floral organs. The accumulating information on inflorescence development can be harnessed to enhance grain yield potential of grain crops.

Evaluation of RGT Planet and Barke as Reference Genomes for Studying Genetic Diversity in a Barley Breeding Population

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Barley, a crop of global importance, has seen a revolution in research with recent advancements in genome sequencing. The accuracy and precision of genetic studies can be increased when the reference genome selected is appropriate for the breeding program under study. Here, we evaluate the suitability of different genomes, such as RGT Planet and Barke barley genomes, in order to provide crucial insights for their selection as reference genome. For this, we genotyped 864 genotypes of the advanced generation germplasm panel of the INIA Barley Breeding Programme. Twenty-five reference genotypes were sequenced at a 10X depth and 864 advanced breeding lines were genotyped using skim-sequencing. Our study evaluated the suitability of RGT Planet and Barke genomes for SNP calling of the skim-sequencing population to identify the best reference genome for the breeding panel. Using comparative genomics, we analyzed the quality of both assemblies, the percentage of similarity, and the differences between them. Additionally, we assessed SNP calling using both genomes on the same reference and advanced line population. This thorough analysis provide us with objective parameters for the selection of reference genome that more closely reflects the genetic variability present in the breeding panel.

Comparative analysis of selection responses using genomic prediction approaches and phenotypic selection on a barley elite population

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Genomic prediction is aimed to improve and accelerate the identification of superior genotypes, making it a promising tool for enhancing quantitative traits controlled by multiple genes. Although many studies have investigated the accuracy of genomic prediction, empirical comparisons with phenotypic selection are scarce, and even fewer studies have compared different genomic prediction strategies with phenotypic selection. Our ongoing study compares five genomic prediction approaches and phenotypic selection using a set of 1,400 individuals from a nested association mapping population of double haploid lines. These lines were obtained from crosses between modern European cultivars and local well-adapted germplasm, representing the typical germplasm used in South American breeding programs. The population was genotyped with the Illumina barley 50K iSelect chip, resulting in 6340 informative SNPs. A set of 150 genotypes was used to train a multi-trait model, including agronomic and malting quality traits (Bhatta *et al*., 2020). From the entire population, sub-samples were selected using different genomic prediction strategies and compared with one set selected by breeders based on phenotypic data. The selected material, together with a random sample from the original population were phenotyped in several field experiments for agronomic traits such as grain yield, plumpness, thousand-grain weight, and phenological traits (Z30, Z49, and Z90). The data is now being used to compare the effectiveness of the different selection strategies. We expect to find differences between the tested genomic prediction approaches, and that lines selected by those methods perform better than lines selected only using phenotype information.

Intraespecific variability for the relative location of the "sensivity windows" to temperature to predict pre-harvest sprouting behavior in moderns malting barleys

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Pre-harvest sprouting (PHS) is a common problem in malting barley crops in environments with precipitation towards the last stages of crop cycle. There are narrow thermal time windows within grain filling, known as "sensitivity windows" in which temperature modulates dormancy release rate after physiological maturity (PM), affecting crop susceptibility to PHS. In the first cultivars studied, the temperature sensitivity window was always located on the last stages of grain filling, close to PM. However, in the cultivars currently used, the relative location of the sensitivity windows varies considerably, being found at the beginning, middle or end of the grain filling period. In this work, the effect of temperature during grain filling on the dormancy release pattern of 8 malting barley cultivars widely used in Argentina was evaluated. Quilmes Carisma, Scrabble and Abi Balster were sown on different dates during 3 years (2015-2017). The cultivars Overture, Montoya, Charles and Yanara were also sown on different dates during the years 2021, 2022 and 2023, while the cultivar Andreia was sown in both periods (2015-2017 and 2021-2023). The aim of the different sowing dates was to generate thermal variability during grain filling. The grain filling period, from pollination to PM, was adjusted to a thermal time scale, which was then arbitrarily divided into bounded thermal time intervals. The mean air temperature within for each interval and for the whole filling period was calculated for the different sowing dates. Dormancy release pattern was followed by determining the germination index from PM to harvest. For all barley varieties, except Abi Balster, a significant ($p<0.001$) and positive correlation was determined between the germination index of grains with 10-20% moisture content and the mean temperature within one of the mentioned intervals. Then, simple temperature-based models for predicting crop PHS susceptibility were generated for each barley cultivar. Intraspecific variability in the relative location of the sensitivity window suggests that temperature may be affecting different physiological processes. Elucidating these processes and quantifying the effects of the temperature could contribute to improve the prediction models. Finally, models could be generated for those cultivars that share the relative location of the sensitivity window. On the other hand, this information can also be useful for malting barley breeding programs.

Automating the UAV Image Data Processing Workflow for Precision Agriculture Using Barley as a Model Crop

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The advancement in Unmanned Aerial Systems (UAS) technology is reshaping research at North Dakota State University (NDSU), particularly in precision agriculture, agronomy, and plant breeding. The barley breeding program is utilizing UAS technology to improve selection accuracy, reduce cycle time and ultimately accelerate genetic gain.

However, the integration of UAV data poses a challenge due to the substantial volume of generated data. While UAVs significantly improve data acquisition efficiency, the data processing stays burdened with manual tasks. Although many free and preoperatory software applications exist for converting raw image data into spectral bands and/or vegetation indexes, many of them are either specialized and demand users with an in-depth knowledge of coding or involve time-consuming manual plot identification.

Our goal is to develop innovative click and point analytic software solutions for researchers. Towards this end, we propose AGSkySight as an alternative to automate the entire UAV data pipeline workflow which includes image stitching, shapefile generation for plot identification using a machine learning model, and extraction of spectral bands and vegetation indexes at the plot level. Importantly, AgSkySight centralizes computing resources, alleviating users of the computational burden. Preliminary results indicate that the ongoing development of AgSkySight will significantly reduce the workload for plant breeders and agriculture researchers, leading to substantial cost and time savings.

This software enhances data processing efficiency and maximizes computing resources, ultimately advancing precision agriculture and plant breeding research at NDSU and in other public breeding programs.

Genomic Regions Associated with Grain Size and Weigt Traits in a Global Spring Barley (*Hordeum vulgare* L.) Collection.

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Barley (Hordeum vulgare L.) grain size is a significant factor in determining storage capacity during grain filling. Grain size is a complex quantitative trait that is easily influenced by environmental factors and directly affects yield and malt quality. In this study, our aim was to identify marker-trait associations related to grain size traits using a global spring barley collection genotyped with a 50K SNP chip.
Data on grain size traits were collected from a set of 275 barley accessions at two different sites over two consecutive years in Morocco. The barley lines exhibited a wide range of phenotypic variation. A broad-sense heritability of over 60% was obtained across all tested environments. Furthermore, highly significant correlations were observed among grain size and weight traits.
Population structure analyses showed four distinct subpopulations. Genome-wide association analysis (GWAS) using best linear unbiased estimators (BLUEs) unveiled 233 MTAs linked to thousand kernel weight, number of kernels per spike, weight of kernels per spike, and grain shape traits including length, width, perimeter, area, and circularity. 13 QTLs were identified across all environments. GWAS using row type as a cofactor identified 86 QTLs associated with grain size and weight traits, with 83 new MTAs on all chromosomes. Three stable genomic regions were located on chromosomes 1H (484.45 - 489.15 Mb), 2H (581 – 660.35 Mb), and 7H (496.43 – 532.13 Mb). Notably, these genomic regions harbor QTLs associated with multiple traits and are enriched with functional proteins involved in diverse cellular processes, including plant reproductive development and drought tolerance. Overall, the study provides essential information about significant markers and new QTLs associated with grain size and weight traits under Mediterranean dry environments.

Training population for a small genomic barley breeding program in an extreme environment

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The Icelandic barley breeding program has conducted field trials since the 1980s and is now introducing genomic prediction to the program. To train a model for genomic prediction, the historical field trial data were used. This is a challenge because the field trial data were not organized to collect phenotypes for a prediction model. Some of the tested individuals are unrelated to the breeding population, few individuals are available for genotyping, and the older data may not be informative for present performance. The goal of this study was to identify which part of the data should be used as a training population for a genomic selection program in Icelandic barley. To do this, we estimated predictive ability in the Icelandic barley population using different subsets of data. The phenotype data consisted of 7530 records of dry matter yield that were collected from 1987 to 2022. Genomic breeding values (GEBV) were predicted using a GBLUP model. To cross-validate the predictions, we used leave-one-out cross validation. We used the GBLUP model to obtain a corrected phenotype for the individual as the mean of the GEBV and residual. To obtain the corrected phenotype we used data from 2013-2022. The model was trained using both two row and six row barleys. The GBLUP model was run, with the phenotype of one individual set to missing and predicted using all other data. Predictive ability (PA) was then estimated as the correlation between $y*$ and GEBV. Additionally, the LR method was used to estimate relative accuracy, bias and dispersion. These correlations were computed for a subset of trials from 2015 to 2022. We did the cross validation using different subsets of data, starting with only the last year of trials and including one more year each time. This way we obtained estimates of PA using different amount of data. Predictive ability, relative accuracy, bias and dispersion indicated that field trial data from 2009-2022 should be used for genomic prediction for the Icelandic barley population. The prediction accuracy of the training population was validated using field trial data from 2023.

Modifying barley strigolactone pathway genes to improve weed suppression and yield

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Weeds cause up to 30% yield loss. Breeding crops with increased weed suppression can be a sustainable alternative to herbicides. Genetic modification of the strigolactone (SL) signaling pathway has shown promising results in increasing yield and altering plant architecture such as height and leaf area in rice. SLs are a group of phytohormones controlling developmental processes and stress responses in plants. There are over 10 genes involved in the biosynthesis, perception and downstream signaling of SLs.

Genes involved in the SL biosynthesis are *DWARF27* (*HvD27)*, *DWARF17 (HvD17)*, *DWARF10* (*HvD10)* and *HvMAX1*. SLs are primarily synthesized in roots, which was also supported by our qPCR results showing higher expression of *HvMAX1* in roots compared to other aboveground tissues. However, other biosynthesis genes *HvD27*, *HvD17* and *HvD10* were equally expressed in roots, leaves, and leaf sheaths. Transcripts of the SL receptor *HvD14* were also equally present in all studied tissue types. *HvD3,* which encodes F-box protein binding to the SL-receptor complex, had higher expression in roots than in aboveground tissues. *HvD53,* encoding the repressor protein of strigolactone signaling, was mostly expressed in leaves and leaf sheaths. Transcription factors involved in downstream signaling such as *HvMADS57* and *SQUAMOSA PROMOTER BINDING PROTEIN LIKE17 (HvSPL17)* showed specialization to roots, whereas *TEOSINTE BRANCHED1 (HvTB1*) did not show tissue-specificity. We also encountered significant varietal dependence of the SL gene expression pattern among barley varieties ‘Anni’, ‘Tuuli’, ‘Maali’, and ‘Golden Promise’.

Next, we targeted *HvD17, HvD14*, *HvD53* and *HvTB1* using CRISPR/Cas genome editing in barley. Barley varieties ’Anni’, ’Leeni’, ’Maali’, and ’Tuuli’ together with the standard variety ‘Golden Promise’ were tested for the suitability in Agrobacterium-mediated transformation and callus regeneration efficiency. Unfortunately, 'Anni', 'Tuuli' and 'Maali' were recalcitrant to Agrobacterium-mediated transformation, and ‘Leeni’ induced only very few callus-derived sprouts. Thus, ‘Golden Promise’ was used as the background genotype for further genome editing. We have isolated seven T0 regenerants for *HvD14*, 11 for *HvD17* and 30 for *HvTB1*. Among them, two mutant lines for HvD14 and three for HvD17 have been verified by sequencing.

Further research includes reverification of the newly created barley mutant lines. The newly created lines will be phenotyped for morphological traits such as tiller number, root architecture and plant height, agriculturally important traits such as yield, heading date and disease resistance as well as for their weed suppression ability in weed-crop competition study plots.

Phenotyping Barley Head Loss: New Insights and Approaches

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Abstract

Head loss in barley occurs when the peduncle breaks, and the head falls to the ground. The fallen heads will be excluded from harvest and have significant impact on yield. This problem is exacerbated by hot, dry winds and delayed harvests, particularly in western and southern Australia. Under severe conditions, head loss cause yield losses up to 50%. Head loss is a kind of stem failure associated with peduncle strength, flexibility, length, and diameter, but the precise influence of these traits remains unclear due to the challenges of measuring peduncle strength and significant interactions of genotype (G) × environment (E) in these characteristics. We aim to develop a reproducible phenotyping method to evaluate barley stem strength, facilitating the genetic dissection of chromosomal regions contributing to this trait. By creating methods that are independent of environmental conditions, we aim to provide a reliable tool for barley breeders. Field trials were conducted on 138 barley genotypes in Esperance, WA, Australia (2022 and 2023) and Gibson, VIC, Australia (2023). Our experimental setup utilized a randomized block design with paired plots. We developed a modified bending stress method using an Instron machine to evaluate peduncle strength by considering the center of mass and the natural pressure a spike head exerts on the peduncle, aiming to mimic field conditions as closely as possible. All samples were dried at 45°C for 48 hrs to reduce moisture content by 9-10%, normalizing environmental conditions. To validate our peduncle strength method, we manually counted the total number of tillers and heads retained from a 0.5m² quadrant to get head loss percentage. A simple correlation analysis revealed that force at break, recorded by Instron, was negatively correlated with head loss, while exposed peduncle length, plant height, peduncle length, internode length, peduncle diameter, and compressive displacement (CDB) were positively correlated; a linear mixed model (LMM) identified these traits, along with spike weight, as major contributors to head loss, collectively explaining 53% of the variance. Additionally, we evaluated lignin content in contrasting cultivars by examining head loss scores and values recorded for force at break. Higher lignin deposition in vascular bundles and adjacent cells was strongly correlated with head loss resistance. We are developing a predictive model using traits such as force at break, compressive displacement at break (CDB), peduncle length, and plant height. This model will enable breeders to select breeding lines for head loss resistance without relying on environmental conditions for head loss occurrence. By combining available sequence information with phenotypic data, we aim to uncover the mechanisms driving head loss. This comprehensive approach will ultimately lead to the development of more resilient barley cultivars.

Keywords: peduncle strength, instron, yield, lignin

Developing Automated Pipelines for Data Analytic Applications for Public Plant Breeding Programs

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With the advent of advanced technologies, North Dakota State University (NDSU) researchers are increasingly generating large datasets, necessitating streamlined tools for data analysis and interpretation. Our initiative focuses on developing and deploying innovative technologies to enhance data collection, experimental design, data analytics, and querying capabilities for breeders and agronomists. A notable case study is the barley breeding program at NDSU. By employing our tools, the program has enhanced its data analysis and experimental design processes, leading to more accurate selection decisions and improved crop resilience. The integration of our data pipelines for UAV image is beginning to be implemented for barley, which could significantly advance the way this breeding program addresses its breeding objectives. These tools are designed to enhance resilience in plant breeding and agronomy by accelerating decision-making processes and seamlessly integrating emerging technologies with traditional practices. We aim to meet the evolving demands of our public plant breeding programs at NDSU by putting technologies in the hands of the breeders.

Identification of a novel plant height QTL on chromosome 6H and its agronomic effects in field-grown spring barley (*Hordeum vulgare*)

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Barley (*Hordeum vulgare L.)* is well adapted to Australia's climate but can be prone to mechanical constraints such as lodging, head loss and straw strength issues, which negatively impact yield and crop profitability. Since the Green Revolution, many semi-dwarf genes such as *semidwarf 1* and *ari-e.GP* have been discovered and used in Australian barley breeding, but they can be associated with negative pleiotropic effects. A breeding line showing an ideal phenotype with intermediate height and erect plant architecture was used as a donor parent to develop two biparental mapping populations. From phenotyping the RILs (recombinant inbred lines) across 6 environments, a novel quantitative trait locus (QTL) on chromosome 6H was identified that is stable across multiple environments. This QTL is associated with reduced height, resistance to lodging and tolerance to head loss compared to wild-type, and it does not negatively impact yield nor have any maturity effects. *QTL6H.1* has similar height to *sdw1.d* and an additive effect in reducing height, internode length and spike count. Genes underlying the *QTL6H.1* are being investigated using a candidate approach. This height QTL will be a valuable addition to the breeders' semi-dwarf gene toolkit.

Keywords: Semi-dwarf, field, stem, barley, genetics, QTL

Barley yield physiology and traits to improve resource use or use efficiency

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Improving barley yield has long been a key focus in both breeding and crop management. To enhance yield, it is essential to understand the crop-physiological basis, as this helps identifying valuable traits and genes. These traits must be linked to improved resource use or increased efficiency in using resources. For the framework of this presentation, we firstly describe a relevant aspect of yield determination in barley and then focused on two case studies.

Yield is the result of the combination of two major components: the number of grains and their average weight. While both are important, evidence supports that yield improvements are more likely to come from increasing grain number. In barley, as with most grain crops, yield correlates more strongly with grain number than grain weight. Grain weight shows only a marginal response, if any, to changes in source strength, while photosynthesis appears downregulated during the effective period of grain filling. Additionally, water-soluble carbohydrates are frequently available to fill grains at physiological maturity. This makes it important to focus on factors that increase grain number when decide on parents for prospective crosses, as well as for selecting their progeny.

Grain number is determined by floret initiation and survival during the brief period of active growth of the juvenile spike, immediately before flowering. Floret survival is more critical than initiation in determining spike fertility, and it seems to be limited by resource availability. Therefore, genetic gains in grain number are more likely if traits/genes that enhance either crop growth during this critical period or the rate of floret development can be identified.

Regarding growth, most research has focused on photosynthesis at the organ level. In a comprehensive study aimed at identifying genetic material with superior biomass, we quantified the genetic variability in elite material for different morpho-physiological traits that influence biomass and yield. We observed significant variability in leaf characteristics and net photosynthesis measured at anthesis. However, these differences did not appear to impact biomass. Additionally, no relationship was found between the biomass produced at heading and the radiation intercepted by the genotypes. Instead, differences in radiation use efficiency (RUE) accounted for the variation in biomass production among genotypes, while organ-level traits failed to explain the observed differences in RUE.

There may also be opportunities to improve yield through genetic effects on reproduction. It has often been suggested in the literature that alleles of *PPD-H1* have pleiotropic effects on yield, but it has not been clear whether these effects are truly pleiotropic or simply a "domino effect" of modifying time to flowering. This question has been particularly difficult to address because, under long photoperiods, the insensitivity alleles delay flowering. By growing *PPD-H1* isogenic lines under extremely long days, we were able to isolate the effects of the alleles on floret development beyond those on flowering time.

We demonstrated that the insensitivity allele improves floret survival and increases spike fertility as a true pleiotropic effect.

SESSION THEMES

04 – Resource use efficiency

Title: Barley cultivars use light and energy in different ways.

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The objective of this work was to evaluate the response of photosynthetic activity in barley plants grown in different light environments. Two barley genotypes (INIA Arcadia and Norteña Carumbe) were used, which were developed to different light environments. The plants were cultivated in growth chambers under a constant temperature of 12 ºC and a relative humidity of 60%. Two photoperiods were defined: a short day (SD,12 hours of light and 12 hours of dark) and a long day (LD, 16 hours of light and 8 hours of dark). The spectral quality of the LED luminaires used was identical, but four levels of light intensity were employed (1600, 1200, 400, 300 µmol photons m^{-2} s^{-1}). The combination of photoperiod and light intensity permitted the creation of high-energy light (HE) and low-energy (LE) environments during plant development. Once the third leaf developed, the energy partitioning was measured by chlorophyll fluorescence using the PAM (pulse amplitude modulated) method. The energy partitioning in photosystem II was evaluated with two actinic light intensities (400, 1600 µmol photons m^{-2} s^{-1}). The energy partitioning was calculated based on three main parameters (Φ_{PSII}, Φ_{NPQ}, Φ_{NO}) which were extracted from the fluorescence measurement profile. A correlation was observed between PSII quantum yield (Φ_{PSII}) and basal dissipative processes (Φ_{NO}) in both genotypes. In SD, Arcadia exhibited a higher correlation when the actinic excitation light was high (1600 µmol photons m^{-2} s^{-1}), irrespective of the energy level of the developmental light environment. Conversely, in Carumbe, this correlation was high (> -0.80) at both excitation levels (400 and 1600 µmol photons m^{-2} s^{-1}) across all light environments. In Arcadia, the correlation between Φ_{NO} and non-basal dissipative processes (Φ_{NPQ}) is only significant (>0.5) in high actinic light, irrespective of the light environment. Conversely, in Carumbe, this negative correlation is observed at this actinic light in LE. However, in LD, a negative correlation between Φ_{NO} and Φ_{NPQ} is observed in Carumbe in both energy environments when PSII is excited at 1600. It is therefore confirmed that the light environment (photoperiod and light intensity) during leaf development in barley has an effect on energy partitioning in photosystem II, with this effect being genotype dependent. No genotype exhibited consistent behavior across different environments, indicating the presence of a genotype-by-light environment interaction in energy partitioning during the initial stages of photosynthesis.

Genetic Adaptation of Barley to Extreme Environments: Estimation of genotype by environment interaction in spring barley between Iceland and Sweden

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The production of cereals is the weakest link of Iceland's food security chain, with only 1% of the country's cereal consumption being produced domestically. The Icelandic barley population has been developed over several decades, with emphasis on earliness, straw strength, yield and quality, but yield and quality are still insufficient under the extreme sub-arctic growth conditions. Increased genetic gain is therefore of importance, leading to a revision of the breeding program. The Icelandic genotypes are of Northern European descent, and are genetically diverse due to crossing with other Nordic material. A long-term collaboration between Iceland and Sweden has resulted in many of the barley genotypes from each population being tested in both countries for decades. Due to the Icelandic and Swedish genotypes' relationship and the interlinked pedigree, there is an opportunity to study the genetic performance of barley variants in different environments; Iceland, North and South Sweden.

The aim of this study is to estimate genotype by environment interactions of barley in Iceland, North, and South Sweden using multi-trait linear mixed models. Covering decades of barley trials in both countries, the dataset includes tens of thousands of observations. The phenotypes will be modelled as separate traits for the three unlike regions which allows the estimation of genetic correlations, focusing on dry matter yield, weight by volume and thousand grain weight as an indicator of quality. Furthermore, each barley genotype gets an estimated breeding value (EBV) for each of the three regions, even if it has not been tested in that region. Prediction accuracy of the model will be validated using cross validation. Three types of linear mixed models will be compared, a model using pedigree information (PBLUP), genomic information (GBLUP), and a combination of genomic and pedigree information (single step GBLUP). The prediction accuracy based on PBLUP, GBLUP and ssGBLUP will indicate whether pedigree information is valuable to increase accuracy of genomic evaluations for barley.

Keywords: Genotype by environment interactions, Barley at extreme environments, Nordic barley

Accelerated barley breeding to meet the new climate realities

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Canada produced just under ten million metric tons of barley in 2023. The Ottawa barley breeding program has an established record of developing and releasing both two-row and six-row barley varieties within eastern Canada. The current breeding objective of the program is to incorporate grain yield, lodging resistance, and disease resistance traits to develop next-generation barley varieties more quickly and precisely for Canada. Even though genetic improvement has contributed significantly to barley productivity in Canada, barley breeding programs need to deliver an even higher rate of genetic gain to increase productivity and the crop's competitiveness. Conventional breeding strategies take 10-12 years to develop new barley varieties, which means slow variety improvement and long varietal turnover. The breeding program needs innovative breeding approaches to accelerate the genetic gains in barley varieties. The use of rapid generation advancement of segregating populations towards homozygosity will facilitate genetic gain for key traits and rapid development of the improved varieties. We are using speed breeding and genomic selection techniques to shorten the breeding cycle time from 10-12 years to 8-10 years and increase the capacity of multi-location testing systems for advanced breeding lines to capture the genotype by environment interaction.

Battling the Blight: Strategies for Ameliorating the Impact of FHB on Barley

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Fusarium head blight (FHB) is one of the most devastating diseases of barley because it can severely reduce the yield and quality of the grain produced and also contaminate it with mycotoxins. It is caused by a number of different *Fusarium* species which can produce various mycotoxins hazardous to humans and animals. Over the past few decades, the disease has become more widespread and severe in many cereal-producing regions of the world. Management of FHB and its associated mycotoxins is very difficult, requiring an integrated strategy of various management practices, fungicide application, and the use of resistant cultivars. Nearly 30,000 accessions of *Hordeum vulgare* have been evaluated for their reaction to FHB in the field, but unfortunately none exhibited a high level of resistance. Mapping studies were conducted with accessions possessing partial resistance to identify the number, chromosomal position and allelic effect of quantitative trait loci (QTL) contributing to FHB resistance and DON accumulation. A meta-analysis based on a consensus map revealed 96 QTL for FHB resistance and 57 for DON accumulation scattered across the barley genome. Many of the QTL explained a low percentage (<10%) of variation for the traits and were often found significant in only one or a few environments in multi-year/multi-location field trials. Moreover, many of the FHB/DON QTL mapped to chromosomal positions coinciding with various agro-morphological traits that may influence the level of disease (e.g. heading date, height, spike density, and spike angle), raising the important question of whether the former are true resistance factors or are simply the result of pleiotropy with the latter. Considering the magnitude of effect, consistency of detection across environments and independence from agro-

morphological traits, only a few QTLs for FHB/DON were considered priority targets for breeding. In spite of the challenge for having a limited number of useful QTL for breeding, genomic selection holds promise for increasing the efficiency of developing FHB-resistant barley cultivars. Indeed, several breeding lines with 20-43% lower disease severity levels than older standard cultivars were identified in the Minnesota barley breeding program. The development of hulless malting barleys is another strategy being advanced for reducing DON levels in harvested crops since a large percentage of mycotoxins are retained in the hull. An experimental multi-parent population composed of eight parents possessing different haplotypes for FHB/DON resistance is being tested to identify progeny with as many positive alleles for low disease and mycotoxins as possible. Finally, transgenic barley lines with various constructs implicated in reducing FHB levels have also been developed and tested in the field. Several of these lines show some promise in reducing FHB and DON. Through all of these efforts, it should be possible to ameliorate the threat of FHB and DON contamination for barley producers.

Improving climate resiliency of barley

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Climate change mediated increase in ambient temperature and reduced precipitation are two major abiotic factors that negatively impact agriculture globally. The combined occurrence of heat and drought stress especially during reproductive stages are detrimental to barley yields and malting quality. Most of the barley varieties in the US are sensitive to concurrent heat and drought stress. Multiple strategies for developing climate resiliency in barley are being pursued. A collection of spring barley accessions was screened for tolerance to short-term heat and drought stress at heading stage. A recombinant inbred line population derived from stress tolerant Otis and sensitive Golden Promise was used for QTL mapping to identify candidate genes associated with drought tolerance. Transcriptomics was undertaken to identify genes responsive to abiotic stress in Otis and GP. A preliminary case study on the utility of transgenics for improving abiotic stress tolerance in barley will be discussed.

Title: Application of RNA interference for improving barley fusarium head blight (FHB) resistance

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Fusarium head blight (FHB) is a devastating disease of barley and wheat in the world as it can cause severe yield loss and diminish grain quality due mostly to the contamination of deoxynivalenol (DON). Unlike the situation in wheat, no barley germplasm showed high and stable FHB resistance. Thus, use of host resistance for improving barley FHB resistance remains a big challenge. In this study, we attempted to apply RNA interference (RNAi) technology for FHB management. We first established a new meristem-based barley transformation system through shoot organogenesis and transferred the RNAi constructs containing Tri6 gene, a key transcriptional regulator for DON generation in *Fusarium graminearum*, into the elite barley cultivar GemCraft. A total of 25 independent T0 transgenic plants were generated including 15 events for which transgene-specific PCR amplicons were observed. To further determine the presence of transgenes, the T1 progenies of all 15 T0 plants were analyzed, and the expected PCR products were obtained in 10 T1 lines. Droplet digital (dd) PCR analysis revealed various copy numbers of transgenes in the transgenic plants. We determined the insertion site of transgenes using long-read sequencing data and observed the rearrangements of transgenes. We found phenotypic variations in both T1 and T2 generation plants. FHB disease was evaluated under growth chamber conditions. The transgenic lines without selection marker are being developed by crossing with the stable lines containing maize Ac transposase. The most popular barley transformation system using young embryos as the explants is genotype-dependent and usually takes 7-12 months, our new transformation method can generate transgenic barley in 10 weeks and may less genotype-dependent, it opens the door for applying this system for genetic improvement and gene function research in other commercial barley cultivars. The marker-free transgenic lines will be submitted to USDA seed bank for sharing with the barley community.

A novel resistant gene *rym7H-1* protects barley against barley yellow mosaic virus (BaYMV) in the disease prevalent regions of China

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Soil-borne yellow mosaic virus disease is a long-lasting threat to winter barley production in East Asia and Europe. Breeding for resistance barley varieties is the optimal way to prevent viral infection. To date, about 20 genetic loci conferring resistance against barley yellow mosaic virus (BaYMV) and/or barley mild mosaic virus (BaMMV) have been reported, among which the resistant gene *rym4*/*rym5* and *rym1*/*11* have been cloned. The allelic variants *rym4* and *rym5* have been widely adopted in breeding scheme in Europe and East Asia, respectively, while *rym1/11* was often found in Chinese barley landraces but rarely detected in modern varieties. A latest survey on the pathogen diversification and the performance of barley varieties carrying those known resistant genes in disease prevalent regions of China, showed that *rym4*, *rym5* and *rym1/11* had been overcome by local viral strains. Exploiting novel resistant gene is of significant importance to protect barley production. Through Genome-wide association study (GWAS) within two distinct populations which contain 900 worldwide collected landraces and 532 Chinese historical/modern varieties, respectively, a novel resistant gene *rym7H-1* on the short arm of chromosome 7H was detected within both populations. Marker assisted selection of this resistance locus was carried out among segregants of a bi-parental population, and heterozygous segregants were subjected to rounds of self-pollination in order to obtain near-isogenic lines (NILs). Through investigation in two years field trials, significant difference on BaYMV accumulation was detected between four pairs of F_8 NILs that carry the resistant or susceptible allele, respectively, and this gene was allocated at a 1.2 Mb interval. Haplotypes analysis showed that, pyramiding of *rym7H-1* and either *rym5* or *rym1/11* largely guaranteed the long-term and broad-spectrum resistance of those Chinese varieties throughout the past decades.

Keywords: BaMMV/BaYMV, *rym4*/*rym5*, *rym7H-1*, Genome-wide association study (GWAS), Near-isogenic lines (NILs)

Utilizing artificial intelligence techniques like deep neural networks for barley disease detection

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Thematic Session: Biotic and abiotic stresses

Abstract

Timely diagnosis of plant diseases is crucial in agriculture due to its adverse effects on product quality and yields. Barley (*Hordeum vulgare L.*), one of the main crops in Australia and around the world, can get infested with several diseases viz. net form net blotch (NFNB), spot form net blotch (SFNB) and scald (Sc). We investigated artificial intelligence deep learning (DL) techniques for barley disease detection and recognition from images captured at trial sites by consumer grade cameras. Our method involved image acquisition, pre-processing, feature learning and classification. Three hundred and twelve color images (6000 × 4000 pixels, 72 dpi) were collected from barley disease paddocks in natural daylight. The images were divided into patches of 448 × 448 pixels, which were manually annotated into four barley disease categories as NFNB, SFNB, Sc and no-disease to establish the ground truth. We investigated binary classifiers to distinguish between no-disease versus a disease class. Multiclass classification was also investigated to classify between all four classes. Several well-known pre-trained DL networks such as DenseNet, ResNet, InceptionV3, Xception, and MobileNet in a transfer learning pipeline were tested. The results are an average of 10-fold cross-validation. The training data was augmented using random flip and random rotation to increase the data pool and capture variations in data. The results show that the DL methods achieved high performance with MobileNet, Xception, and InceptionV3. For binary classification the average (for the three diseases) accuracy, F1-score and area under the receiver operating characteristic curve (AUC) were 98.63%, 98.60% and 99.55% by MobileNet, and 98.07%, 98.06% and 98.73% by Xception respectively. For multiclass classification accuracy, F1-score and AUC were 93.50%, 93.49% and 97.55% by MobileNet, and 92.96%, 92.97% and 97.92% by InceptionV3 respectively. These networks achieved top performance due to their lightweight architectures that are suitable for small datasets. These findings can

be deployed for disease identification and screening barley materials in a timely manner for resistant varietal development.

Image-based phenotyping to understand the genetic basis of waterlogging barley

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Barley is increasingly vulnerable to climate change-induced extreme rainfall, leading to heightened occurrences of waterlogging. To improve barley waterlogging tolerance, it is essential to capitalise existing genetic diversity and develop resilient varieties for a sustainable agricultural production. This project addresses the limitations of traditional phenotyping by employing advanced high-throughput phenotyping for non-destructive, continuous, and quantitative data collection to study waterlogging tolerance. In this initiative, we established a core collection known as the core European Heritage Barley collection (ExHIBiT), consisting of 230 lines of 2-row spring barley. This collection was genotyped with a 50K SNP array, agronomically characterised and validated for association mapping. The ExHIBiT core collection was phenotyped under both field and controlled conditions. In controlled conditions, we used a high-throughput imaging platform with RGB, Chlorophyll Fluorescence, and Hyperspectral (VNIR & SWIR) cameras. The core collection was subjected to 14 days of waterlogging stress followed by a 7 days recovery, during which various traits, including growth and spectral indices were examined. In field conditions, the core collection was planted for two consecutive years and exposed to four days of waterlogging stress. Unmanned Aerial Vehicle (UAV) were deployed throughout the growing season to monitor the effects of stress and recovery and several agronomic traits such as flowering time, grain yield, and plant height were assessed. Our aim is to establish the correlation between imaging data in the field and traditional agronomic traits, and to determine whether genotypes that show resilience under controlled conditions also exhibit resilience under field conditions. Finally, a genome-wide association study (GWAS) is ongoing on all three datasets, identifying several genes involved in waterlogging tolerance.

Barley (*Hordeum vulgare* L.) diversity set reveals complex genetic architecture of net blotch resistance on chromosome 6H

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Leaf blight diseases like net blotch (*Pyrenophora teres*) and spot blotch (*Bipolaris sorokiniana*) are important diseases in all barley growing regions of the world which can cause average yield losses of up to 40%. Multiple QTL mapping studies and GWAS have identified numerous QTL associated with net blotch resistance located on all seven barley chromosomes. Additionally, ten major genes for net blotch resistance have been reported. Chromosome 6H is suspected of harbouring several major QTL. However, the exact dissection of the QTL is difficult as they are in LD with *Rpt5* located in the centromeric region.

A worldwide barley diversity set consisting of 449 accessions originating from 50 different regions of the world, including landraces and cultivars, was genotyped with the 50k iSelect barley SNP chip. The set showed a weak population structure and clustering of genotypes that corresponds with other large population genetics studies. The LD decay of the set ranged between 2.07 Mbp and 2.7 Mbp for individual chromosomes and decayed genome-wide at 2.32 Mbp.

The barley set was phenotyped under controlled and field conditions (Germany, Russia, Australia) for resistance against three diseases, i.e., net form net blotch (NFNB), spot form net blotch (SFNB), and spot blotch. Four genotypes carrying resistance against both forms of net blotch were identified. In addition, two lines carry dual resistance against NFNB and spot blotch were found.

Subsequently, GWAS was conducted for each individual isolate and field location. For spot blotch, one QTL was identified on chromosome 1H and two on 7H, one of which corresponds to the resistance locus *Rcs5*. Additionally, all three were in close proximity to QTLs identified for NFNB.

For net blotch, 33 QTL were identified: 20 QTL were unique for NFNB, four were unique for SFNB and 9 QTL were identified for both forms. Seven distinct QTL were located on chromosome 6H. One corresponds to the susceptibility locus *Spn1* located on the short arm of chromosome 6H at around 30 to 90 Mbp. Five QTL were scattered over the large, predicted region for *Rpt5*, which spans from 110 to 466 Mbp. The first QTL was located at 125 to 161 Mbp (NFNB), the second at 249 Mbp (SFNB), the third at 361 to 367 Mbp (NFNB), the fourth at 386 to 402 Mbp (NFNB) and the fifth at 457 to 483 Mbp (NFNB). The LD decay in the analysed set suggests that these are independent loci and distinct from *Rpt5*.

These results can be used to resolve the complex genetic architecture surrounding the previous *Rpt5* locus that was in strong linkage disequilibrium with loci spanning chromosome 6H.

Barley endophytes as a bioprospecting platform for Fusarium head blight control

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The cereal pathogen, *Fusarium graminearum*, infects barley spikes and reduces the grain yield. The pathogen produces harmful mycotoxins in grains, thus the economic impact is significant. Endophytes are asymptomatic, plant tissue-dwelling microorganisms representing a diverse potential source of new products for use in agriculture, medicine and industry. Bioactive metabolites produced by endophytic microbes have demonstrated socio-economic importance and found applications in agriculture (as biofertilizers/bio-stimulants/biocontrol agents) and the environment (bioremediation), as biofuels/biocatalysts, in addition to their pharmacological attributes. Limited research is available and not studied enough to use endophytes as a platform for agricultural applications although these organisms were extensively studied as a production platform of novel pharmacological metabolites. We aim to survey and study the bacterial and fungal endophytes in different Canadian grown barley genotypes under clean and Fusarium head blight (FHB) infection. The Illumina sequencing platform was used to barcode the 16S and 18s rRNA of gene from the DNA extracted from surface sterilized barley samples. The microbial profiles were highly diverse in roots compared to stems and grains. Sequencing analysis revealed a greater number of endophyte species in FHB-infected barley compared to clean barley. Few Isolated endophyte organisms showed the inhibition of *F. graminearum* pathogen in vitro. The research findings provide meaningful insights on the endophyte microbiome of barley which may be implemented in plant growth improvement and disease management. Benefits of endophyte based platforms in agriculture could move towards sustainable crop production minimizing the use of chemical fertilizers and pesticides.

Grain-filling rate improves physical grain quality in barley under heat stress conditions during the grain-filling period

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Abstract

The incidence of heat stress during grain filling is rising. Responses to high temperatures at the reproductive stage in barley have received less attention than those of crops such as wheat and rice. This study tested physical grain quality and a range of physiological, developmental, and agro-morphological traits concurrently for their responses to natural heat events during grain-filling in a diverse set of barley genotypes.

Heat stress is a constraint to Australia's barley production. In addition to impacting grain yield, it adversely affects physical grain quality and market value. However, breeding for heat-tolerant genotypes has been challenging due to the narrow sensitivity window, the unpredictable nature of heat stress and its frequent co-occurrence with drought stress. Greater scientific knowledge regarding traits and mechanisms associated with heat tolerance would help develop more efficient selection methods. Our objective was to assess 157 contrasting barley genotypes under delayed sowing to examine the effects of heat stress on physical grain quality. Delayed sowing increased the likelihood of daytime temperatures above 30°C during grain-filling. Supplementary irrigation of field trials ensured a reduced impact of drought stress.

Heat tolerance appeared to be the primary factor determining grain plumpness. A wide variation was observed for heat tolerance, particularly among the Australian varieties. Genotypic variation was also observed for grain weight, grain growth components, stay-green and stem water-soluble carbohydrates (WSC) content and mobilisation under normal and delayed sown conditions. Compared to normal sowing, delayed sowing reduced the duration of developmental phases, plant height, leaf size, head length, head weight, grain number, plumpness, grain width and thickness, stem WSC content, green leaf area retention and harvest index (HI), but also increased screenings, grain length, grain-filling rate (GFR), WSC mobilisation efficiency (WSCME) and grain protein. Genotypes with heavier and plumper grains under high temperatures had higher GFR, longer grain-filling duration (GFD), longer green leaf area retention, higher WSCME, taller stature, smaller leaf size, greater HI, higher grain weight/plumpness potentials and earlier flowering. GFR played a significant role in determining barley grain weight and plumpness under heat-stress conditions. Enhancing GFR may provide a new avenue for improving heat tolerance in barley.

Additionally, the results suggest that WSC mobilisation and stay-green may contribute to better physical grain quality performance by stabilising GFR and GFD, respectively. However, the stable GFR correlated with WSC mobilisation might be more influential in determining physical grain quality performance under heat stress conditions than GFD correlated with stay-green.

Heat-tolerant genotypes useful for barley breeding programs and traits for complementary selection criteria for heat tolerance were identified. Heat tolerance, the physical grain quality potential, and heat escape (early flowering) all appeared to play a role in determining the barley genotypes' physical grain quality assessed under the delayed sown conditions. However, they differed in their relative contribution, with heat tolerance and physical grain quality potential more important than heat escape. The accelerated development with later sowing may have reduced the contribution of heat escape as a mechanism.

Unraveling genomic regions associated with preharvest sprouting resistance in barley using genome-wide association and transcriptomic analyses

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The quality and yield of barley is adversely affected by preharvest sprouting (PHS), where seeds germinate on spikes before harvest due to wet and rainy conditions. PHS is attributed to low level of seed dormancy, a polygenic trait that prevents germination under favourable environmental conditions. Thus, identification of genomic regions and genes regulating seed dormancy is critical for developing barley cultivars with enhanced resistance to PHS. To this end, this study performed genome-wide association study (GWAS) of seed dormancy/PHS in a mapping panel consisting of 255 diverse barley genotypes grown over four environments using 31,140 single nucleotide polymorphisms (SNPs). Statistical analysis of the phenotypic data revealed significant variation in the dormancy levels among the genotypes. GWAS identified 16 significant SNPs and found two quantitative trait loci (QTLs) on chromosomes 3H and 5H associated with dormancy/PHS, explaining 6.9% to 11.1% of the observed variation. The QTL.5H contains 14 SNPs, and 12 of these SNPs passed a stringent false discovery rate (FDR) threshold at $\alpha = 0.05$. Based on its position on chromosome 5H, this QTL likely corresponds to the SD2 locus. The QTL on 3H consists of one SNP that was found to be significant at FDR of 0.1 but not at FDR of 0.05. This study also revealed that the expression patterns of genes harboring these SNPs including *HvRCD1*, *HvPSRP1*, and *HvF3H* are closely associated with seed dormancy/PHS resistance. Haplotype analysis of the SNP markers within QTL.5H detected a haplotype associated with PHS resistance. The SNP markers and candidate genes revealed by this study have the potential to enhance the development of barley cultivars with improved resistance to PHS.

Note: Abstract submitted for consideration for poster presentation under Session: 05 – Biotic and Abiotic stresses.

Identification of QTL for preharvest sprouting associated with alpha-amylase activity in barley

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Preharvest sprouting (PHS), which refers to the germination of seeds on the spike prior to harvest, negatively affects barley production worldwide. Because of the low level of seed dormancy they exhibit at maturity, most of the modern barley cultivars are susceptible to PHS, which induces alpha-amylase activity and thereby breakdown of starch deposited in the seeds, leading to significant reductions in yield and quality. Therefore, developing barley cultivars resistant to PHS is crucial. The main goal of this study was to identify QTL associated with alpha-amylase activity using genome wide association analysis. A mapping panel of 160 barley accessions was genotyped using 50K SNP Illumina iSelect array. Seeds of the mapping panel grown in two environments were examined for variation in alpha-amylase activity via the Rapid Visco Analyser (RVA). Marker-trait associations were identified by integrating the alpha amylase activity and the genotypic data comprising 30,494 polymorphic single nucleotide polymorphisms (SNPs) using a mixed linear model with Kinship (MLM+K). Our analysis identified nine significant markers representing a quantitative trait locus (QTL) on chromosome 5H based on linkage disequilibrium (LD) decay of 2.81 cM and a false discovery rate (FDR) threshold of $\alpha = 0.05$. These markers explain 11.9% to 21.2% of the phenotypic variation, with four SNPs accounting for over 20% of the variation. The markers identified as linked to alpha-amylase activity could potentially be deployed for marker-assisted selection when developing PHS-resistant barley cultivars.

Note: Abstract submitted for consideration for poster presentation under Session: 05 – Biotic and Abiotic stresses.

Protein-protein network hubs in host-pathogen interactions: Targets for next-generation breeding

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Disease phenotypes are the result of dynamic changes in gene and protein interactions at multiple levels in multiple cellular compartments. To establish a regulatory network view of protein-protein interactions (PPI) critical to pathogen infection and disease resistance in cereals, we have constructed PPI networks of barley (*Hordeum vulgare* L.) in response to powdery mildew, caused by the ascomycete fungus, *Blumeria hordei* (*Bh*). The barley MLA nucleotide binding, leucine-rich repeat (NLR) receptor was used as a model regulator to interrogate host immune response, as it's alleles and orthologs confer recognition specificity to diverse fungal diseases, including powdery mildew, stem rust, stripe rust, spot blotch, and rice blast. On the pathogen side, 47 representative *Bh* effector proteins, including AVR_{A1}, AVR_{A7}, AVR_{A9}, and AVR_{A13}, were selected from time-course RNA-sequencing on CI 16151 progenitor and fast-neutron derived immune mutant hosts. Next, all MLA domains and *Bh* effectors were used as baits in yeast two-hybrid next-generation interaction screens (Y2H-NGIS), where batch matings with a 3-frame infection prey library were followed by quantitation and ranking of Illumina 20-million read samples via custom NGPINT and Y2H-SCORES software, and subsequent binary confirmation. Results were integrated with the HvInt barley interactome, enabling assembly of a high-confidence host-pathogen network of 1085 proteins and 1497 interactions to further probe cellular localization and immune activation for next-generation breeding to new and emerging pathogens.

Supported in part by Fulbright Colombia & Schlumberger Faculty for the Future Fellowship to VVZ, USDA-NIFA-ELI Postdoc Fellowship 2017-67012-26086 to JME, and USDA-NIFA-PBI 2020-67013-31184 & USDA-ARS 5030-21220-068-000-D to RPW.

Genetic mapping of *Sph1* conferring susceptibility to leaf rust in barley

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Caused by the biotrophic fungal pathogen *Puccinia hordei,* leaf rust is one of the important foliar diseases in barley. Although a few dominant resistance genes to leaf rust have been identified and cloned in barley, resistance conferred by major genes has been frequently defeated by the pathogen. A recessive resistance was identified in a spring barley accession by using the VA90-34 isolate which is virulent to most of major resistance genes. For fine mapping towards cloning of this recessive resistance gene (hereafter named *Sph1*, *Susceptibility to P. hordei 1*), we conducted genetic mapping with biparental populations in the present study. The *Sph1* gene was anchored close to the telomere of the short arm of 3H, delimited within a 260 kb region. Of the six predicted genes in the *Sph1* region, two genes encoding putative receptor-like kinase were selected for functional validation. Therefore, our study provides high-resolution genetic map and candidates of *Sph1*, paving foundation for cloning of this important gene.

The novel non-transgressive extreme hybrid susceptibility locus *Spt2* exploited by *Pyrenophora teres* f. *maculata* maps to a single heterozygous candidate gene

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Hybrid crops are coveted for their superior performance such as yield by exploiting heterosis or hybrid vigour. However, hybrids can also display unintended negative consequences such as extreme pathogen susceptibility. The necrotrophic pathogen *Pyrenophora teres* f. *maculata* (*Ptm*) causes spot form net blotch, which has caused significant losses to barley worldwide. A hybrid susceptibility locus in barley was initially identified because the three parental lines CI5791, Tifang and Golden Promise exhibited resistance to *Ptm* isolate 13IM.3, whereas F_2 progeny from CI5791 × Tifang and CI5791 × Golden Promise crosses exhibited extreme susceptibility. The susceptible phenotype segregated in a ratio of 1 resistant:1 susceptible representing a genetic segregation ratio of 1 parental (res):2 heterozygous (sus):1 parental (res) suggesting a single hybrid susceptibility locus. Genetic mapping using 715 CI5791 × Tifang F_2 individuals and 149 targeted SNPs delimited the hybrid susceptibility locus designated *Susceptibility to Pyrenophora teres 2* (*Spt2*) to an ~198 kb region on chromosome 5H of the Morex V3 reference assembly. This single locus was independently mapped with 83 CI5791 × Golden Promise F_2 individuals and 180 genome wide SNPs that colocalized to the same *Spt2* locus. Analysis of Golden Promise × Tifang F_2 individuals and CI5791 × Tifang recombinant inbreed lines suggest that this phenomenon only occurs in the heterozygous state when CI5791 is a parent. The CI5791 genome was assembled and comparative analysis against Golden Promise and the barley pangenome delimited the *Spt2* region to one high confidence candidate gene predicted to encode a pentatricopeptide repeat-containing protein.

Mining of wild barley alleles in the nested association mapping population HEB-25 to improve abiotic stress tolerance in elite barley.

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Crop yield is under permanent threat through abiotic stresses. In a worldwide field trial, we tested plant performance under the abiotic stresses nitrogen deficiency, drought and salinity using a subset of the nested association mapping (NAM) population HEB-25 (Wiegmann et al. 2019). We showed that the barley flowering time genes *Ppd-H1, Sdw1, Vrn-H1* and *Vrn-H3* exert pleiotropic effects on plant development and grain yield under a wide range of field conditions. For example, in Al-Karak, Jordan, the day length-sensitive wild barley allele of *Ppd-H1* was associated with an increase in grain yield by up to 30% compared to the insensitive elite barley allele. The observed yield increase was accompanied by pleiotropic effects of *Ppd-H1* resulting in a shorter life cycle, an extended grain filling period and an increased grain size. Our study indicates that the adequate timing of plant development is crucial to maximize yield formation under harsh environmental conditions. We provide evidence that wild barley alleles, introgressed into elite barley cultivars, can be utilized to support grain yield formation.

The action and interaction of flowering regulation genes to control yield and yield components was also studied in HEB-25 based on heterogenous inbred families (HIFs), segregating at the *early flowering locus 3 (ELF3)*. We could show that the wild barley *ELF3* allele caused a accelerated plant development, resulting in higher yields under drought stress in field trials (Zahn et al. 2023). In a separate study, we found that the joint action of wild barley alleles of *Ppd-H1* and *ELF3* were associated with a significantly accelerated plant development under speed breeding conditions by reducing time to heading and maturity (Rossi et al. 2024). In a further study we found that the observed G669W substitution in elite barley *ELF3* alleles contributed to a significant increase in phase duration and in phytomer initiation for both vegetative and reproductive structures, which may have supported the northward expansion of barley after domestication in the Fertile Crescent (Huang et al. 2024).

In addition, we found evidence that the composition of the bacterial microbiome in the rhizosphere is controlled by the presence of wild barley genes present in HEB-25, as indicated by the identified *QRMC-3HS* locus. In future, this knowledge may be used to specifically enrich the barely rhizosphere with microbiome species, which support abiotic stress tolerance. The presented knowledge may be transferred to related crop species like wheat and rice securing the rising global food demand for cereals (Escudero-Martinez et al. 2022).

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Efficacy of Nanoparticles and Bacteria in Mitigation of Cadmium Toxicity in Barley (*Hordium Vulgare* L.)

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ABSTRACT

This study analyzes the potential role of glycine betaine loaded chitosan nanoparticles and bacteria in mitigation of Cadmium (Cd) toxicity in barley plants by enhancing the overall growth of plant. Our findings are novel revealing the beneficial influence of association between betaine loaded chitosan nanoparticles and bacteria in plant tolerance against Cd toxicity. In our work we isolated Cd and nanoparticles tolerant bacterial isolate and analyzed the ability of this isolate and nanoparticles to alleviate the impact of Cd toxicity in barley plants. Out of six strains LSN-6 (*Acinitobacter johnsanii*) showed maximum tolerance to Cd and its MIC was determined as 6 mM which highlights its ability to detoxify Cd from soil/water. LSN-6 was also capable of solubilizing zinc, potassium and phosphorus. Barley plants were exposed to eight treatments in hydroponics: (1) Control (CK), (2) $CdCl_2$, (3) nanoparticles, (4) Bacterial strain, (5) $CdCl_2$ + nanoparticles, (6) $CdCl_2$ + bacteria strain, (7) nanoparticles + Bacterial strain, (8) $CdCl_2$ + nanoparticles + Bacterial strain. Each treatment was independently run-in triplicate. Levels of $CdCl_2$ and nanoparticles were screened before applying in hydroponics. Overall, a decrease in growth performance was observed in plants exposed to $CdCl_2$ stress, however bacterial inoculation and foliar application of nanoparticles significantly increased growth parameters of barley. Thus, betaine loaded chitosan nanoparticles and *Acinitobacter johnsanii* could be effective biosource for enhancing tolerance in barley plants in response to $CdCl_2$ toxicity. However, in depth studies are recommended to understand the tolerance mechanism by which betaine loaded chitosan nanoparticles and bacteria alleviate $CdCl_2$ stress in barley plants.

Diversity of spot blotch resistance in barley: an haplotyping approach

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Spot blotch (SB), caused by *Cochliobolus sativus* is an economically important barley foliar disease in warm and humid areas worldwide, including Uruguay. The narrow genetic base of current donors of SB resistance used in many breeding programs as well as recent changes in pathogen virulence has driven the search for novel sources of resistance for this disease. In this study, we examined seedling and adult plant stages resistance to SB in a diverse collection of 40 barley entries, and analyzed their genetic relationships, population structure and haplotype diversity using 27 SSR and 8 STS markers linked to nine SB resistance QTL. These QTL are located on chromosomes 1H, 2H, 3H, 4H and 7H (*Rcs-qtl*-1H-1-2, *Rcs-qtl-1H-5-7*, *Rcs-qtl-2H-7-8*, *Rcs-qtl-3H-1-3*, *Rcs-qtl-3H-4-6*, *Rcs-qtl-3H-9-12*, *Rcs-qtl-4H-10-11*, *Rcs-qtl-7H-2-4*, *Rcs-qtl-7H-7*), for which resistant donors were genotypes Calicuchima-sib, Bowman-BC, Morex and Harrington (reference genotypes). Fourteen of the 40 barley genotypes expressed high and moderate SB resistance at seedling and adult plant stages. Both distance- and model-based cluster analyses of molecular marker data revealed that these 14 lines clustered in four groups consistent with geographical origin and pedigree structure. Few lines with resistant behavior presented similar chromosome haplotypes as the reference genotypes, with the exception of Bowman-BC haplotype in the *Rcs-qtl*-3H-1-3 region. Yet, further studies should address this region, as its haplotype diversity was consistently low. This work suggests that 14 barley resistant lines, genetically diverse from NDB112 and Bowman, have the potential of carrying-novel genes for SB resistance. Further molecular genetic analysis of the SB resistance expressed by these lines may expand the understanding of the genetic architecture of this trait and provide genotyping-based approaches for their use in barley breeding.

Pathogenicity of *Fusarium graminearum* and *F. poae* causing Fusarium head blight in barley under controlled conditions

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Fusarium head blight (FHB) is one of the most devastating diseases of barley. FHB is caused by a species complex of *Fusaria*, of which *Fusarium graminearum* Schwabe is the species responsible for most FHB epidemics. Field surveys show that two or more *Fusarium* species often co-exist within the same field and *F. poae* is as another dominant species in barley. This study investigated the effect of the interactions between *F. graminearum* and *F. poae* on FHB and mycotoxin accumulation. Two susceptible barley genotypes were spray-inoculated with *Fusarium* conidiospore suspensions and the disease severity and fungal accumulation was evaluated based on symptom and genomic DNA. There was a significant difference in FHB severity between *F. graminearum* and *F. poae* infections, where *F. graminearum* produced severe FHB disease symptoms while *F. poae* did not cause FHB. When heads were co-inoculated with both *Fusarium* species, the resulting FHB severity was unchanged relative to heads inoculated with *F. graminearum* which was reflected in the DNA quantification of the species. The mycotoxin profile of the co-inoculated treatment appeared to be most influenced by *F. graminearum*-related metabolites with a minor influence by *F. poae*-related metabolites. Forty-six features were annotated with metabolite study and which shows *F. graminearum* appears to outcompete *F. poae* in its ability to establish infection in barley and as a result contributes the majority of mycotoxin contamination within this crop.

Modelling a phenotype for adult plant resistance to barley leaf scald (*Rhynchosporium commune*) independent of plant maturity

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The disease barley leaf scald is caused by the pathogen *Rhynchosporium commune* and can cause up to 30-40% yield loss in susceptible varieties. Phenotyping for resistance to *Rhynchosporium* in field nurseries often confounded by the effect of plant height or growth stage of the host genotype.

Later flowering time and taller plant height can physically slow the upward spread of splash-dispersed *Rhynchosporium* spores and contribute to disease escape. While disease escape traits are important, it is also important to identify sources of resistance that are independent of relative maturity or plant height, as this resistance is more likely to be useful in breeding programs selecting for a constrained agronomic plant type and window of flowering time for their target environments.

Under field conditions, *Rhynchosporium* resistance was negatively correlated with early relative maturity. A linear mixed model using a bivariate approach was used to analyse the data for *Rhynchosporium* resistance and relative maturity. This model structure permits the fitting of a linear relationship at the residual level between the two pairs of traits. The linear relationship between *Rhynchosporium* resistance and relative maturity was plotted as a trend line, and the difference (residual) between the BLUP from the mixed model for *Rhynchosporium* resistance of a genotype and the predicted value on the trend line was calculated. These values are referred to as the deviation from the regression of scald resistance on relative maturity (DRSRRM) and are a phenotype more independent of maturity.

In general terms, for phenotypes based on deviation from the regression of a correlated trait (like our DRSRRM measure of resistance), the most resistant lines are those with the largest negative values of residuals, also allowing breeders to select resistant lines with desirable relative maturity. This approach is especially useful where disease resistance is evaluated in experiments at a single date, rather than at critical development stages for each line, especially in genetic material with large variation for confounding traits such as maturity.

The value of quantitative PCR within annual crop disease surveys - learnings from incursion of Ramularia leaf spot in New South Wales (Australia) farming systems

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Crop disease surveys are an important element of integrated disease management. However, they are labour intensive and require access to specialist diagnostic plant pathologists. One solution to this problem is to implement technologies such as species-specific DNA probes via quantitative PCR (qPCR) to track pathogen species distribution and gain a measure of disease burden.

Here we present data from a spatial and temporal survey conducted using qPCR assays to understand cereal disease dynamics across New South Wales (NSW) Australia. Over five years, 1526 paddocks growing either wheat, barley, durum or triticale were randomly selected and sampled during grain fill. Structured samples of plant material was collected from each paddock and assessed both visually and by qPCR for disease infection and pathogen levels, respectively. Dried and ground plant material from each paddock was subject to 25 species specific TaqMan MGB assays conducted by the South Australian Research and Development Institute molecular diagnostic service. Only the results for Ramularia leaf spot (RLS, caused by *Ramularia collo-cygni*) are presented. Confirmation of the qPCR detection was carried out by pathogen isolation from a sub-sample of the tested plant samples.

The study reports widespread detection of the RLS in NSW in 2021. This detection was a significant range expansion by RLS into NSW. RLS was first detected in Australia on the island state of Tasmania in 2016 and subsequently in Western Australia during 2018. Analysis of stored qPCR DNA from annual crop surveys indicated that the NSW incursion could be traced back to as early as 2019. Since then, RLS has rapidly expanded its geographical range, becoming a significant concern for the NSW barley industry. Additionally, the study confirmed that RLS is non-preferentially hosting on alternative cereal crop types including wheat, oats and triticale.

The findings from this study highlight the dynamic nature of pathogen distribution and demonstrated the value of stored DNA from annual surveys in rapidly determining the distribution of an incursion to guide industry response. This study highlights the need to focus breeding efforts on priority pathogens, as initial experiments show a lack of resistance/and or tolerance to RLS in existing germplasm and current commercial Australian cultivars.

A Breeding Method for Selection of *Fusarium pseudograminarium* Tolerance and Partial Resistance Using qPCR Molecular Tools

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Fusarium crown rot (FCR) causes significant grain yield and quality loss to barley production around the world. Breeding for resistance or tolerance to FCR has been slow with relatively limited success due to the complex nature of inheritance and partial effectiveness of the trait phenotypes.

In this study, we use multi-species (barley and wheat) experiments to estimate the genotype yield potential in treatments with (inoculated) and without (non-inoculated) FCR infection in three target environments, across two years. FCR infection was measured at maturity using quantitative PCR (qPCR), TaqMan MGB assays conducted by the South Australian Research and Development Institute molecular diagnostic service. This allowed a quantitative measurement of *Fp* DNA loads to identify genotypes with partial resistance. Greater *Fp* DNA levels were strongly correlated with grain yield loss in the barley cultivars used in this study. Partitioning of tolerance and partial resistance of genotypes was achieved by using residual regression deviation modelling. This study is the first to measure *Fp* DNA at plant maturity in the field as a proxy for FCR infection severity and associate it with tolerance.

The results were consistent across three environments with different levels of disease expression. The improved measure of FCR infection along with genotype yield retention allows for identification and separation of both tolerance and partial resistance phenotypes, in a way that could be used to select for these two traits in barley breeding programs.

This new approach offers a more robust, unbiased way to select for both FCR traits within breeding programs compared with visual assessments. The increased throughput and accuracy of qPCR measurement of FCR means this method is likely to be more useful in assessment of lower infection measured as *Fp* DNA and tolerance in barley breeding programs, and as such is more likely to be useful as a selection tool.

Unravelling Barley Genetic Defenses Against Stripe Rust: Mapping Resistance to *Puccinia striiformis* f. sp. *hordei*

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Barley stripe or yellow rust (BYR) caused by *Puccinia striiformis* f. sp. *hordei* (*Psh*) is a major threat to barley production and quality. While fungicides offer control, host resistance remains the most cost-effective and environmentally sustainable approach. To broaden the genetic basis of resistance, breeders are continuously searching for new diversified sources of resistance, and subsequent characterization and mapping of these resistances for their effective utilization in breeding. This study focused on mapping resistance in five doubled-haploid (DH) and/or recombinant inbred line (RIL) populations (Baronesse/Stirling, Dash/VB9104, Klimek/Gus, Nagrad/Gus and CG15/Gus). These populations were phenotyped in Mexico and were genotyped using more than 10K DArT-Seq informative markers followed by development of linkage maps for each of the five populations. Genetic mapping detected 14 quantitative trait loci (QTL) distributed across barley chromosomes: 3 on 1H, 1 on 2H, 3 on 3H, 2 on 4H, 3 on 5H, and 1 each on 6H and 7H, and of these 14 QTL, 11 were detected in multiple populations. Among these identified loci, three loci (*Rpsh_QDsh* and *Rpsh_QStr* on 3H; and *Rpsh_QVB* on 5H) were the most significant in terms of their genotypic contribution (maximum number of linked markers), phenotypic contribution (R^2), and their detection in multiple mapping populations. *Rpsh_QDsh* spanning 0.01-38.13 Mbp on chromosome 3H was detected in four populations (Baronesse/Stirling, Dash/VB9104, Klimek/Gus and CG15/Gus) with closely associated marker *3985238* positioned at 18.77 Mbp. *Rpsh_QStr* was also detected in four populations (Nagrad/Gus, Klimek/Gus, CG15/Gus and Baronesse/Stirling) over an interval of 530.11-585.3 Mbp with closely linked marker *3666183* at 567.67 Mbp. *Rpsh_QVB* on chromosome 5H was mapped between the marker interval of 1.87-18.8 Mbp in two populations (Klimek/Gus and Dash/VB9104) with closely linked marker *3985711* located at 4.21 Mbp. The QTL detected in this study hold particular significance for countries like Australia, where *Psh* poses a serious exotic threat. Breeders can leverage these findings for pre-emptive breeding and pyramiding of resistance genes. Future work will focus on 'mendelising' the identified QTL, fine mapping, and developing of markers closely linked to QTL for their use in marker assisted selection.

Title: Loss of ice nucleation activity restricts barley pathogen transmission strategies

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Biological ice nucleation is critical for freezing water at temperatures near 0°C. InaZ, an ice nucleation protein, was first discovered in the pathogenic bacterium Pseudomonas syringae but is also present in other Gammaproteobacteria, like barley pathogen *Xanthomonas translucens*. Although the role in precipitation and frost damage are well known, the evolutionary links between bacterial ice nucleation and life history have not been explored extensively across Gammaproteobacteria. We therefore studied the association between InaZ evolution and transmission strategies, which control pathogen epidemiology. We determined that *inaZ* is an ancient, vertically inherited gene in *Pseudomonas*, *Pantoea* and *Xanthomonas*. The *inaZ* phylogeny was congruent with the whole genome tree of these Gammaproteobacteria, and notably, horizontal transfer of *inaZ* was not observed. *inaZ* was only found among ice nucleation active bacterial lineages, and as InaZ is required for ice nucleation activity across these genera, we discovered frequent functional loss of ice nucleation by *inaZ* deletions or disruption by transposable elements through synteny and functional prediction analysis. Organisms with no functional InaZ including some *X. translucens* isolates appear to have stronger phylogeographical structuring and transmission that depends primarily on the host (e.g. seed). We have evidence for selection for *X. translucens inaZ* loss and linkage between phylogeographic structure that is associated with zones of cultivation of crops. Given the role of biological ice nucleation in dissemination of bacteria via the atmosphere and rainfall, we hypothesize that the loss of *inaZ* limits bacterial dispersal and reinforces population structure via dissemination with plant tissues.

Preliminary study of net blotch resistance (*Pyrenophora teres* f. *teres*) in an elite barley breeding population in Uruguay

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Net blotch, caused by the necrotrophic fungus *Pyrenophora teres* f. *teres*, is a major disease of barley, resulting in significant yield losses and reduced grain quality. South American Barley production relies on European germplasm, which is highly susceptible to this disease. This study´s goal is to identify chromosome regions associated with resistance against the disease in Uruguayan conditions in germplasm representative of local breeding programs. To achieve this, a nested association mapping population of 150 double haploid lines derived from crosses between modern European cultivars and local well-adapted germplasm was used (family 1, CLE268/Kalena; Family 2, Kalena/CLE267; Family 3, Kalena/Conchita; and Family 4, Livia/CLE268). We carried out a field experiment during the 2023/24 growing season in Paysandú, Uruguay. We assessed net blotch severity by scoring the percentage of infected leaf area in the total plot. The population was genotyped by the Illumina barley 50K iSelect SNP array resulting in 6340 informative SNPs covering all chromosomes. The environmental conditions during the growing season were highly conducive to the development of net blotch, resulting in a large variability of net blotch resistance across and within families in the population. Our analysis identified two quantitative trait loci (QTL) for net blotch resistance located on chromosomes 2H and 6H, indicating the presence of alleles with contrasting effects on net blotch resistance, with local cultivars carrying the favorable alleles. These findings highlight the potential for using locally adapted germplasm to enhance disease resistance and reduce foliar fungicide applications. Moreover, these results provide valuable insights for barley breeding programs aiming to enhance disease resistance and improve overall crop performance under specific local conditions. Further field experiments are being conducted to validate these results and improve our understanding of the genetic mechanisms of this resistance.

Tattle-TALE: Live visualization of bacterial transcriptional reprogramming of plant cells for single cell analysis

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Barley leaves are cellular landscapes that dynamically express genes in response to biotic and abiotic stresses. Here we developed a tool for live visualization of bacterial pathogen transcriptional reprogramming of plant cells and for single cell RNA sequencing. During infection, many bacterial pathogens secrete protein effectors to promote virulence and overcome host immunity. Some effectors disrupt plant immunity via direct injection into host cells through a Type III secretion system. The spatiotemporal dynamics of Type III effector activity during early infection are not well characterized. Here, we developed a method of visualizing Type III-effector activity of *Xanthomonas translucens* during live infection of barley. Transgenic barley plants were developed with an inducible gene encoding green fluorescent protein (GFP). The promoter is activated by *X. translucens* carrying and secreting an artificial transcriptional activator-like effector, designated as dTALE-GFP. *X. translucens* (dTALE-GFP) are also carrying a reporter gene that can be simultaneously detected and differentiated from the plant GFP with confocal microscopy. We detected TALE-induced GFP expression in plant cells as early as 6-hour post-inoculation and cells preferentially targeted by distinct strain types during non-vasuclar and vascular leaf colonization. With this system, we demonstrate that stomatal guard cells and mesophyll cells are targeted by Type III effectors as disease progresses, and that epidermal cells are targeted by some bacteria from likely under the leaf surface. We have also developed time lapse imaging to document the single cell plant response with video. This system provides a valuable tool to the community for research into the dynamics of plant-microbe interactions during infection and can be used to identify effector-responsive genes and their spatiotemporal deployment during infection.

Comparative genomics and transcriptomics reveal fitness tradeoffs for barley pathogen host range

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Biotic stresses limit barley crop production, and often microbial pathogens have varying host ranges that limit plant colonization. The mechanisms of host adaptation remain largely unknown for most plant diseases and are critical for defining universal and unique biotic stress response. Plant pathogenic bacteria often deliver effector proteins into host cells via the Type III secretion system to modulate plant cellular pathways and promote host susceptibility. Conversely, recognition of effectors by resistance proteins encoded by plants triggers host resistance. Here, we used comparative bacterial genomics and barley and wheat transcriptomics to define the role effector gene loss plays in emergence of a broad host range subgroup in the re-emerging bacterial plant pathogen *Xanthomonas translucens*. Comparative analysis of whole genomes identified unique effector proteins encoded by the narrow host range, barley-infecting *X. translucens* pv. translucens (Xtt) but not by the related broad host range, wheat-infecting *X. translucens* pv. undulosa (Xtu). We found that individual targeted deletions of Xtt-unique effectors allowed for host expansion to wheat, but at the expense of decreased virulence in barley. Moreover, evolutionary genomics analyses revealed loss of the host range-limiting effector from the Xtu subgroup during its divergence from the common ancestor with Xtt. We also determined the barley responses that may explain the fitness cost for loss of these host range effectors, and presence of these effectors alters plant metabolic processes. This work overall demonstrates that effector loss modulates host range across plant genera at a fitness cost. Overall, our study provides a functional framework for predicting how pathogens might evolve based on gene loss.

Tracking a Decade of Change in Barley Leaf and Stem Rust Resistance in Uruguay

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Barley is an important crop for Uruguay, contributing significantly to the country's agricultural economy. In 2023, barley sowing reached 191.000 ha, making it the second most widely planted winter crop (~ 43% of the total winter crop area). Of this, 75% was dedicated to brewing barley, while 25% was used for forage barley. Last season, the average barley yield reached 4,789 kg/ha, with a total production of 914.712 tons, of which 684.000 tons were for malting purposes. Leaf rust, caused by *Puccinia hordei*, is considered the most important rust disease of barley worldwide. It is widely distributed in barley-growing regions, including Uruguay. Barley leaf rust epidemics since 2005 have significantly increased cultivation costs due to the necessity of fungicide applications. Damage estimates for leaf rust made before 2004 indicated yield losses of 17-25%. In 2006, during a severe leaf rust epidemic, grain yield was reduced by ~ 60%, and the yield of plump kernels dropped by ~ 85% in susceptible cultivars. This highlights the potential for substantial damage in years with severe epidemics, characterized by early onset and high infection levels. The *P. hordei* population in Uruguay is relatively stable and less diverse than wheat leaf rust, likely due to the lower prevalence of major resistance genes in commercial barley cultivars. To date, three races of *P. hordei* have been identified, each differing in their reaction to resistance and commercial cultivars. Race UPh1 was predominant until 1998, followed by the identification of race UPh2 in 1999, and race UPh3 in 2004, which has dominated the pathogen population since its emergence. Stem rust in barley is caused by *P. graminis* f.sp. *tritici*, the same pathogen that causes the disease in wheat. It does not appear every year and remains at low levels when it is observed, likely due to early planting and short maturity of barley cultivars. Since 2013, The National Institute of Agricultural Research (INIA) has been developing the characterization of the field resistance to various diseases affecting commercial cultivars based on information from the National Cultivar Evaluation Trials and specific disease nurseries managed by INIA. This process is critical for assessing the health status of plant varieties, providing farmers with essential information for making informed crop management decisions, leading to improved crop health, higher yields, and better economic returns. Drawing on a decade of data from the leaf and leaf rust characterization performed by INIA, along with information on the area sown to different cultivars, this study explores how resistance to leaf and stem rust in commercial barley varieties in Uruguay has evolved. over the past 10 years.

The Pacific Northwest Virulent Stem Rust Population on Barley: Current Status of Understanding the Host-Pathogen Genetic Interactions

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Wheat stem rust caused by the fungal pathogen *Puccinia graminis* f. sp. *tritici* (*Pgt*) is an important disease of barley worldwide. The Pacific Northwest (PNW) region is the center of stem rust diversity in North America due to the completion of its sexual cycle in primary hosts, barley, wheat, and wild grass species, and secondary sexual stage hosts mahonia species and common barberry. A PNW *Pgt* population was identified with virulence on lines containing *Rpg1, Rpg2, Rpg3, rpg4, Rpg5,* and *rpg8.* Alarmingly, 10% of the population including *Pgt* isolate Lsp21 was determined to be virulent on *Rpg1* and the *rpg4*/*Rpg5*-mediated resistance locus (RMRL) when stacked together; unprecedented *Pgt* virulence on barley that had not been previously reported. To identify and map effective resistances against this population a genome wide association study (GWAS) was conducted on 440 accessions from the World Barley Core Collection (WBCC), genotyped with the 9K Illumina barley iSelect chip and phenotyped at the seeding stage with the two PNW *Pgt* isolates, Lsp21 (virulent on both *Rpg1* and RMRL), and Lsp18 (avirulent on both *R*-genes). A total of 10 resistance loci were identified including four novel loci on chromosomes 1H, 2H, 5H, and 6H. The line Elliot (PI 592261) was identified as containing effective seedling resistance to *Pgt* isolate Lsp21. To genetically characterize this resistance, 129 recombinant inbred lines (RILs) from an Elliot (resistant) x Palmer (susceptible) cross were phenotyped with Lsp21 at the seedling stage and genotyped with the Illumina 50K bead express SNP chip. Analysis identified two significant resistance QTL (EP*Rpg*_4H-1 and EP*Rpg*_5H-1) contributed by Elliot on chromosomes 4H and 5H. EP*Rpg*_4H-1 is novel, while EP*Rpg*_5H-1 mapped to a region of the barley genome known to contain stem rust resistance. A second comprehensive screen was done on the Wild Barley Diversity Collection (WBDC) and a GWAS identified 12 novel loci on chromosomes 1H, 2H, 3H, 5H, 6H, and 7H associated with resistance to isolate Lsp21. Two lines (WBDC-94 and WBDC-238) were identified to have high levels of resistance against *Pgt* isolate Lsp21. Both lines contain the *R*-gene *Rpg7*. We genetically characterized, fine-mapped, and identified candidate *Rpg7* genes utilizing a Morex × WBDC-94 biparental population. We mapped *Rpg7* to a 51 kb region containing two candidate genes on chromosome 3H. We hypothesize that both the *Rpg7* candidate genes, a HvRIN4-like protein and the HvRPM1-like protein, are required for resistance. The resistances identified in these studies are currently being integrated into elite malting barley backgrounds to enhance resistance to the virulent PNW *Pgt* population. On

the pathogen side, GWAS was performed using 113 diverse *Pgt* isolates collected from the PNW and the Midwest region of the US to identify virulence and avirulence loci corresponding to the barley stem rust resistance gene *Rpg1*. The genotype data for 96 isolates were generated by whole-genome shotgun sequencing and for 17 isolates by RNA sequencing. The phenotype data for the *Pgt* isolates were collected by seedling disease assays on two barley lines, H228.2c (an *Rpg1* transgenic line) and Morex (a natural source of *Rpg1*). A total of five *AvrRpg1* effector candidate genes were identified at two loci. Further characterization of the host *R*-genes and pathogen avirulence genes will be done to enhance our understanding of host pathogen interactions in the barley-*Pgt* pathosystem.

An Island of Receptor-Like Genes at the *Rrs13* Locus on Barley Chromosome 6HS Co-locate with Three Novel Sources of Scald Resistance

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Three *Hordeum spontaneum*-derived resistances (referred to as 145L2, 41T1 and 40Y5) have demonstrated long-term effectiveness against barley scald, caused by *Rhynchosporium commune*, in western Canada. Genetic mapping of these resistances in three populations, and the use of five barley genome assemblies, revealed they co-located to a narrowly defined 0.58-1.2 Mbp region of chromosome 6HS containing the *Rrs13* scald resistance gene. Differential disease reactions among the three resistances and a *Rrs13* carrier to a panel of 24 scald isolates indicated that the four resistances were unique from one another. A marker created to target the 6HS scald locus was screened across a panel of barley germplasm that included *H. vulgare*, *H. spontaneum* and *H. bulbosum* lines. The marker showed specificity to *H. vulgare* lines known to carry the 6HS scald resistances and to two *H. spontaneum* lines that trace their origins to Jordan. Within the 0.58-1.2 Mbp region were 2-7 tandemly repeated Leucine-Rich Repeat Receptor-Like Proteins (LRR-RLP) and one Lectin Receptor-Like Kinase (Lec-RLK) genes with abundant sequence variation between them. The well-defined role that RLP and RLK genes play in plant defense responses make them logical candidate resistance genes, with one possible hypothesis being that each unique scald resistance may be encoded by a different RLP that interacts with a common RLK. It is suggested the three scald resistances be temporarily named $Rrs13^{145L2}$, $Rrs13^{41T1}$ and $Rrs13^{40Y5}$ to recognize their co-location to the *Rrs13* locus until it is determined if these resistances represent unique genes or alleles of the same gene.

Note: Abstract submitted for consideration for oral presentation. I think it would fit well within Session 1, 5 or 6.

Improved drought tolerance in barley (*Hordeum vulgare* L.) using plant growth-promoting rhizobacteria (PGPR)

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Abiotic stress significantly limits plant growth and production. Drought, in particular, is a severe constraint that affects growth and limits agricultural productivity on a global scale. Water stress induces in plants a set of morpho-anatomical (modification of root and leaf structure), physiological, and biochemical (relative water content, membrane stability, photosynthesis, hormonal balance, antioxidant systems, and osmolyte accumulation) changes mainly employed to cope with the drought stress. These strategies allow the plant to overcome the unfavorable period of limited water availability. Currently, a promising alternative is available to improve plant growth and tolerance under drought conditions. The use of osmotolerant plant growth-promoting rhizobacteria (PGPRS) as inoculants can alleviate water stress by increasing the water use efficiency of the plant. The PGPR improve the tolerance of plants to drought, through changes in the morphology and architecture of the root system, production of phytohormones, extracellular polysaccharides, and osmolyte accumulation. They may also enhance the antioxidant defense system and induce transcriptional regulation of stress response genes.

The trial was conducted in the expirimental station of the national institute of agronomic research of Tunisia during the season from November to June 2023 to test the effectiveness of using PGPRS as microbial inoculants in grains as well as inorganic fertilizers in the soil on growth, yield and some biochemical constitutions in Tunisian barley varieties and considering growth, yield and biochemical constitutions. The results showed that the application of PGPR and their combinations significantly influenced the growth, yield, quality and biochemical content of barley.

Australia: A barley success story

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Abstract

Over the past 25 years Australian barley production has doubled, driven by a combination of a 33% improvement in grain yields and a 50% growth in area sown to barley. Whilst larger growth rates have been observed in other countries, notably Argentina, no other large barley producing country has experienced such growth. Globally, barley production has stagnated during this period.

The barley production growth has been due to a conflux of four factors: proximity to Asian growth markets, a growing environment providing significant malting quality advantages over alternative grain origins, a relatively small range of crop options in Australia, and a successful competitive contest between genetic gains in barley versus wheat.

This paper seeks to describe the genetic drivers behind the success of barley in Australia.

The higher rate of genetic gain for grain yield of barley versus wheat over the 25-year period can be attributed to relatively simple genetic factors.

In a global context, Australia was relatively late in the successful deployment of semi-dwarfing genes in barley but, in the period of interest, area sown to such varieties has increased from less than 20% to more than 75%. In comparison, the earlier successful adoption of semidwarf wheat varieties has meant there has been less opportunity to further improve grain yields using this trait.

Increasing incidence of spring frost events in Australia, and barley's lower sensitivity to frost induced sterility compared to wheat, have resulted in farming practices favoring the sowing of barley before wheat. Yields of barley, relative to wheat, have increased consequently, with grain filling of barley now occurring into the cooler, late winter/early spring months.

The Australian barley breeding industry has been a leader in adoption of herbicide tolerance technologies. Initially the imidazolinone (IMI) tolerance trait, and more recently the CoAxium technology, have provided barley growers with significant tools in weed management.

Deployment of the IMI trait in market leading Australian barley varieties has occurred at a much faster rate than has been achieved in hexaploid wheat; yield progress in the development of (single gene) IMI tolerant barley has occurred at a faster rate than the development of (two gene) IMI tolerant wheat.

Significant investment by growers has underpinned genetic improvements, initially through GRDC research levies (now invested principally in upstream technology development) and End Point Royalties (paid to breeding companies). The EPR system has allowed a successful transition from Australian, public sector, breeding programs to highly competitive, private breeding companies. Successful companies have invested in adoption of technologies that enhance the rate of genetic gain, such as genomic selection (GS). Further, the EPR system has encouraged the importation, evaluation and commercialization of varieties developed in other countries, furthering grower genetic gain.

AAC Lariat: a new two-row general purpose barley

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Barley is grown across the Canadian Prairies (Alberta, Manitoba, and Saskatchewan) and can be used for malting, food, and general purposes (feed and forage). In 2023, in western Canada, more than 50% of the area seeded with barley was in Alberta, with general purpose barley exceeding the area seeded with malting barley in this province. The most grown general purpose barley variety in 2023 in western Canada was CDC Austenson.
At Agriculture and Agri-Food Canada's Brandon Research and Development Centre (AAFC-BRDC), Manitoba, Canada, a new two-row malting barley cultivar, AAC Lariat (TR19268), was developed and recently released. This is a promising barley variety developed from the cross AAC Synergy/TR09398 made in 2010, and it was evaluated in the Western Cooperative Two-row Barley Registration Test (2019-2020) before being registered in 2022 (No. #9759, Canadian Food Inspection Agency - CFIA). AAC Lariat is widely adapted to western Canada and significantly out-yielded the check cultivar CDC Austenson by 4% during the two years of testing. AAC Lariat demonstrated similar yields in the black soil zone and outperformed CDC Austenson by 5% and 8% in the brown and black & gray soil zones, respectively, across the Canadian prairies. It was similar to CDC Austenson in days to maturity and lodging resistance but significantly shorter than it. AAC Lariat had a lower test weight than CDC Austenson, while kernel weight and plumpness were slightly higher. During testing, AAC Lariat demonstrated susceptibility to scald, moderate susceptibility to fusarium head blight, intermediate resistance to spot blotch, and moderate resistance to spot-form net blotch. It has also displayed resistant reactions to stem rust, indicating the presence of the *Rpg1* gene as confirmed by molecular marker screening, as well as surface smut and net-form net blotch. With its high yield, good standability, and disease resistance, AAC Lariat will offer a good production choice for feed growers across the Canadian Prairies.

Note: Abstract submitted for consideration for poster presentation under Session: 06 – Barley breeding success stories.

How were the attributes associated with phenology modified in malting barley cultivars in Argentina over the last 40 years?

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Barley is the second most important cereal in Argentina after wheat, with the country being the largest exporter of malt in Latin America. During the past 40 years (1980-2020), the national average yield increased at a rate of 59 kg ha^{-1} year^{-1}, largely due to the introduction of varieties of European origin. These new varieties generally exhibited a longer phenological cycle, from emergence to maturity, compared to older materials, with the crop cycle duration increasing at a rate of 2 to 4 °Cd year^{-1} (0.25% year^{-1}) during the period 1982-2019. However, the attributes that determine these changes in the duration of the cycle, and specifically between emergence and anthesis, have not been analyzed. To understand the development attributes behind these changes, commercial cultivars released between 1982 and 2021 were sown for two consecutive years (2022-2023) across a wide range of sowing dates (April to August) under not restrictive conditions of water and nutrients and without biotic constraints. Photoperiod sensitivity, threshold photoperiod and intrinsic earliness were measured for different phenological stages. For the emergence (EM) to anthesis (ANT) phase, photoperiod sensitivity varied between -95 and -355 °Cd h^{-1} showing significant differences ($p<0.05$) among cultivars, while intrinsic earliness ranged from 875 to 1059 °Cd. Intrinsic earliness showed a negative trend with the year of released of the cultivars ($r^2=0.19$ $p<1.596$). Threshold photoperiod ranged from 12 to 14 h, with significance differences among cultivars and a positive trend with the year of released ($r^2=0.38$ $p<0.0341$). Breeding increased the duration of the EM-ANT phase at a rate of 2.9 °Cd year^{-1} in early sowing dates (11 h photoperiod), reducing this difference as the sowing date was delayed (i.e. 1.3 °Cd year $^{-1}$ at 12.5 h photoperiod). For the EM - first visible node (FVN) phase, photoperiod sensitivity ranged between -45.6 and -98.2 °Cd h^{-1}, with threshold photoperiod ranging from 14.7 to 18.8 h. Intrinsic earliness values for this phase varied between 372 and 427 °Cd. The duration of the EM-FVN phase showed a positive trend with the year of release for early sowing dates (11 h photoperiod) with a rate of 0.94 °Cd h^{-1}, which diminished as sowing dates approached threshold photoperiod values. No association was observed between intrinsic earliness or photoperiod sensitivity and the year of release for the EM-FVN phase. The FVN- ANT phase showed important variation in photoperiod sensitivity (-41 to -251 °Cd h^{-1}), although to a lesser extent than the EM-ANT phase. Threshold photoperiod for FVN-ANT ranged from 15.6 to 14.0 h, and the intrinsic earliness registered a range from 351 to 460 °Cd. As for the EM-ANT phase, a positive trend was observed between the duration of the FVN-ANT phase and the year of release at a rate of 3.6 °Cd year^{-1} for early sowing dates (11 h photoperiod) which decreased as the sowing date was delayed. Phyllochron values ranged from 60 to139 ºCd leaf^{-1} while the final leaf number on the main stem (FLN) ranged from 9 to 13 leaves. The larger duration of the EM-ANT phase observed in newer cultivars, respect to older ones, was mainly explained by greater phyllochron values rather than changes in the FLN. The findings highlight the impact of breeding on the phenological development of malting barley cultivars in Argentina, providing valuable perspectives for future cultivar selection.

From staple to supergrain: advances in food barley research

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Barley, one of the oldest crops cultivated for human consumption, lost its preeminent role as a food staple in human nutrition at the beginning of the 20th century, when grains like wheat and maize gained greater preference. However, over the last decade, its high adaptability to various climatic and growing conditions has led to worldwide production of barley averaging 148 million tonnes per year, making it the fourth most-produced cereal crop. Despite the fact that barley is still primarily used for animal feed and alcoholic beverages, interest in barley for food uses has revived in recent years. Tremendous advances have been made in breeding barley varieties destined specifically for food uses, in unraveling unique and valuable properties of barley constituents, and in developing processes to produce attractive barley ingredients and products that can also effectively deliver the health benefits of its constituents. The objectives of this presentation are (1) to discuss the distinctive chemical composition, molecular properties, and associated health benefits of specialty food barley varieties, (2) to demonstrate how dry grain fractionation techniques, milling conditions, and hydrothermal treatments can be tailored to optimize the concentration of bioactives and physical structure of barley products to improve their physiological efficacy, and (3) to emphasize the potential of conventional and novel products, such as barley bulgur or barley-based meat analogues, to be used as nutritious, attractive and healthy foods.

Title: Dry fractionation of barley flour and the use of its protein-rich fractions for plant-based meat analogue applications

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Abstract:

Pilot scale air classification of barley flours from 2 distinct barley varieties was performed with the objective of understanding the effect of repeat milling and air classification cycles (3 cycles in total) on the shift of proteins and other components including starch, β-glucan, fiber and ash. Notably, proteins gravitated towards the finer air-classified fractions, resulting in approximately 2.1-fold and 1.8-fold enrichments solely in the first fractionation cycle for hulled and hull-less barley varieties, respectively. Subsequent cycles, utilizing recycled coarse air-classified fractions, further accentuated protein separation. The fine fractions were also enriched with fat component. In contrast, the coarse fractions were enriched in total, soluble and insoluble dietary fibers as well as β-glucan. The protein-rich fine fractions showed promise for plant-based meat analog applications, potentially allowing for nutritional claims in the end product. Meanwhile, fiber-rich starchy coarse fractions demonstrated potential for producing high β-glucan-containing breakfast cereals and puffed snacks, possibly supporting health claims regarding cholesterol reduction. The fine fractions were blended with pea protein isolate at three ratios (0-30% barley) and extruded at three moisture contents (47.5-57.5%), with the objective of complementing the cereal and pulse amino acid profiles in the end product, namely a plant-based meat analogue. All barley protein substituted blends produced meat analogues with sufficient fibrous characteristics and texture comparable with recent studies on meat analogues,

with the highest anisotropy index (1.57) obtained at 52.5% moisture level for the 15% barley containing blend. Substitution of pea protein with barley led to increased hardness, and chewiness. Whereas increase of feed moisture content during extrusion resulted in lower values for hardness, chewiness, density, and color change. The flexibility obtained with varying feed formulations and moisture contents opens opportunities for mimicking different meats by producing a wide range of textures. This study showcased the effective integration of air-classified barley protein flour to produce high-moisture meat analogs, contributing to the diversification of plant protein alternatives and promoting the utilization of this nutritionally dense cereal in human food. Future investigations will delve into comprehensive sensory evaluations, flavor profiling, and assessments of consumer acceptance for meat analogs containing barley protein.

Spring barley breeding for organic farming: challenges and first results

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Due to development of organic farming in Latvia, first steps towards a breeding program for this farming system were initiated in 2003 with establishment of organic crop rotation with certified fields. Testing of existing varieties and some breeding lines from conventional breeding program was performed in the first stage. Gradual transfer of all breeding steps (except crossing, F_1 and partially F_2 generations) to organic fields was done after that. Currently breeding of barley cultivars appropriate for growing under organic farming is the main goal at AREI Priekuļi Research Centre.

The main challenges are the unstable growing environments since no synthetic substances are used for fertilization and plant protection as well as large amount of additional traits/breeding aims if compared to conventional breeding. The main end uses of our cultivars are hulled barley for feed and hulless barley for healthy food. In addition to grain yield and quality and disease resistance/tolerance attention needs to be paid to yield stability over contrasting environments and adaptability to poor organic conditions, competitive ability against weeds, and nutrient use efficiency under organic growing conditions. In terms of disease resistance seed born diseases, especially loose smut (*Ustilago nuda*) is more critical due to absence of effective organic seed treatments.

Diversity within a crop is another breeding direction on which we work since 2013. Heterogeneous materials (composite cross populations and similar) have ability to evolve and adapt to particular growing environment. They might have improved stability, disease tolerance and nutrient use efficiency in comparison to homogeneous varieties.

All selection work is performed on organically certified fields. Yield trails of advanced lines are in two organic locations and in one conventional location in order to get more information on yield stability and disease resistance. In addition, molecular marker developed from the candidate Un8 gene is used for selection for loose smut resistance along with artificial inoculation test.

First Un8 resistant candidate variety from organic breeding program, hulless barley 'Gunika', is under official testing for 3rd year. It is with comparatively high beta-glucan, protein and amino-acid content and large light-colored kernels, resistant to powdery mildew, with satisfactory competitive ability against weeds and nitrogen use efficiency. It is not superior for grain yield but has adaptability to unfavorable conditions. A problem can be its susceptibility to covered smut (*Ustilago hordei*).

An early maturing hulled barley breeding line PR-9275 with possible Un15 and/or Un3/6 loose smut resistances, stable grain yield, good nitrogen use efficiency and competitive ability against weeds is under registration for first year. More than 10 other advanced lines (F_7-F_{10}) with possible presence of different loose smut resistance genes are currently under pre-evaluation.

One of our CCP populations was registered by an organic farmer, and seed multiplication is going on. Another farmer is developing two other populations at his farm for feeding livestock. Around 10 new heterogeneous populations are under testing. A problem can be not complete resistance to smuts since only part of parents crossed usually have resistance. Selection of Un8 resistant plants from composite cross population by MAS was done in order to obtain improved population with resistance to loose smut.

Promoting Nutraceutical Cereal Hulless Barley: A Strategic Move for Public Health Enhancement

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Hulless barley, a mighty nutraceutical cereal, has emerged as a promising grain with significant health benefits. Upscaling its production and promotion can be a strategic move to boost public health. This cereal is rich in indispensable nutrients, including dietary fiber, protein, vitamins, and minerals. The high fiber content (7-8%) subsists a strong gut microbiome, which is central for overall well-being. Beta-glucan, a soluble fiber, found in barley grains, lowers total and LDL cholesterol levels thus reducing the risk of cardiovascular diseases. Barley has a low glycemic index (28) that makes it an exceptional dietary choice for individuals with diabetes, as it keeps optimum glucose level in blood. Its grains contain antioxidants including phenolic acids, flavonoids, and anthocyanins that combat oxidative stress, reducing the risk of chronic diseases such as cancer and cardiovascular diseases. As a nutrient-dense grain, hulless barley can play a significant role in addressing nutritional deficits, especially in areas where undernourishment is widespread. To maximize the benefits of hulless barley, persistent efforts are needed to encourage its production and consumption. Key strategies include: development and release of high yielding biotic and abiotic stresses resistant varieties and their cultivation technologies, seed production and distribution to the farmers. Educating the public about health benefits of hulless barley through media campaigns, nutritional workshops, and community programs can increase its demand. Health organizations and government agencies should consider including of hulless barley in dietary guidelines. Official endorsement can inspire its acceptance in schools, hospitals, and other institutions. Investing in research to improve the yield and resilience of hulless barley can make its cultivation more attractive to farmers. Developing new barley-based products can cater to consumer preferences and expand its market. Collaborating with the food industries to develop and market barley-based products, such as cereals, snacks, and health supplements, can enhance its visibility and accessibility. This grain, with its innumerable health benefits deserves a prominent place in our diets.

ICAR-Indian Institute of Wheat and Barley Research, Karnal, manoeuvres a strong hulless barley breeding programme in collaboration with ICARDA. ICARDA supplies advanced breeding lines of hulled and hulless barley each year for their evaluation and selection at its Amlaha centre and to IIWBR and five other centres of AICRP in form of nurseries namely, IBYT-FFM, IBYT-ASA, and IBON for evaluation and selection. Genotype DWRB 223, a selection from INBYT-HI 2012-13, out yielded best check over 28% in its final year of testing of All India Coordinated Research Project (Barley) at multi-location trials during 2023-24 and its proposal for release as a variety for commercial cultivation in North West Plain Zone to be submitted to Indian Council of Agricultural Research in the ensuing workshop. The 32 advanced breeding lines were evaluated in station yield trials and some of them yielded up to 7.6 tonnes per hectare grain yield. Thousands of segregating hulless barley lines from F_2 to F_7 generations are being handled. We have developed a massive awareness programme to educate people about the nutraceutical properties of hulless barley and its value added food products development.

Functional cereal products produced by supplementing wheat flour with high β-glucan hull-less barley flour

Gh.Salih, A.Jilal and H.Esbiba

Abstract:
Nutrient-dense cereal products play a crucial role in promoting health and well-being. Proportions of high β-glucan hull-less barley (Moroccan cultivar. Chifaa) in mixture with commercial bread wheat were used in functional pizza dough and cake production. Physical (dry matter, ash content, bulk density and minerals) and chemical (polyphenol, flavonoid and antioxidant activity) parameters of the mixture of flours, pizza dough and cake were evaluated. Sensorial analysis with semi-qualified panel was performed for pizza and cake products. High level of whole barley flour supplementation increased both physical and chemical criteria for pizza while 40% supplementation was significant for cake product. Sensorial analysis (general aspect, texture, color, aroma and taste) for cake and pizza revealed a good appreciation with increased proportion of barley flour. The results indicated that pizza and cake nutritional dense (β glucan, polyphenol, flavonoid and antioxidant) through incorporation of high β glucan barley (60% and 40% respectively) can be served as a good healthy meal.

Introgression, characterization, and use of *Hordeum bulbosum* genetic diversity for barley improvement

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Abstract

Barley has a unique and important place in the Moroccan agriculture. It is ranked second after bread wheat, it is grown in multiple agro-ecological zones especially in the semi-arid, arid and mountainous zones, it is considered by most farmers as a minimum-risk crop that will not require additional inputs. In Morocco this crop is used not only for animal feed but also for human consumption.

Because of the great interest of this cereal, ICARDA in collaboration with Morocco's National Institute for Agricultural Research has given a principal attention to improve barley's productivity through breeding. The development of new varieties more productive and nutritious, biotic stress resistant and climate smart requires an exploration and exploitation of new and broad genetic diversity. Wild species constitute an important reservoir of useful genes with high economic potential that control resistance/tolerance traits to various biotic and abiotic stresses. Ambitious research has been initiated, with the main objective is exploiting the genetic diversity of *Hordeum bulbosum* for barley improvement. Some *Hordeum bulbosum* introgression lines were provided by the ICARDA and NordGen gene bank. Crosses between these lines and modern barley varieties were performed at ICARDA-Rabat. Advanced F6 lines were obtained. An evaluation of these F6 lines for quality, diseases, yield performance and stability has been initiated in order to assess the contribution of these *Hordeum bulbosum* introgressions to the genetic improvement of barley. Another part of the study consists in developing and characterizing near-isogenic lines (NILs) by using speed breeding technique targeting each *Hordeum bulbosum* introgression to identify the traits that are affected by these introgressions. Furthermore, this research also aims to develop and validate a protocol to perform successful *Hordeum bulbosum* introgressions in common barley by overcoming the limitations related to interspecific incompatibility, F1 hybrid lethality, chromosomal elimination and F1 hybrid sterility.

The expected results of this project will allow to better exploit the genetic diversity of *Hordeum bulbosum* and to regenerate new *Hordeum bulbosum* introgression lines in order to register new barley varieties with increased grain and straw productivity, adapted to the constraints of climate change.

Enzyme quality as a breeding aim – the demand for key enzymes showing higher heat stability in the future brewing process

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In the past decades breeding efforts have provided a high potential of mashing relevant enzymes in raising their quantity in the malt. However, new challenges like climate change, its impact during growth and ripening of the barley on substrate quality, requirements for stricter cost savings and improved capacity utilization could be parried by an improved quality of enzymes. This term means a higher robustness against detrimental effects of temperature, pH or oxygen or a higher turn-over frequency.
The goals of mashing are high extract formation and overall high wort quality for easy further processing and good drinkability of the beer. Crucial for accomplishing these aims are the action of enzymes and their interaction with substrate. The set screws for steering the process are the mashing parameters, where - besides pH - temperature and time are the most important factors with high economic impact due to energy consumption during mashing. The extent of starch breakdown catalyzed by α- and β-amylase clearly has the strongest effect on extract formation. But the team play of both enzymes is very limited due to the different optimum temperatures: the step maker enzyme (α-amylase, 72 °C) has a significant higher optimum than the fermentable sugar forming β-amylase (62 °C). Furthermore, the gelatinization behavior of starch in recent years frequently requiring temperatures higher than the optimum of β-amylase provokes her rapid inactivation. Decoction procedures with a tricky setup of mashing rests could solve both problems but are considered too energy consuming.
An at least partial harmonization of the two enzymes could be achieved by crossing alleles for more heat-stable β-amylases found in wild or cultivated barley. In a joint project with a German breeder, a selected set of 392 barley accessions from the primary and secondary gene pool (*Hordeum vulgare*, *H. spontaneum*, *H. bulbosum* and introgression lines with *H. bulbosum* components) were screened for promising alleles for heat stability of β-amylase using a half-life-span method. Suitable candidates were tested for typical sequence differences in the relevant alleles. This enabled markers for higher heat stability to be developed, which can be used in the breeding of new barley varieties allowing abbreviated mashing procedures. Splitting populations and elite material were created with heat-stable β-amylase. The procedure could be repeated addressing other enzymes of technological value, e.g. limit-dextrinase, β-glucanases or AX-degrading enzymes.
Finally, as an addendum, in considering brewing enzymes also the role of intrinsic inhibitors of proteinic nature should be taken into focus, a field not yet handled well. Inhibitors of α-amylase (e.g. BASI), part of the stress metabolism of barley, may have a strong impact on activity of enzymes of starch breakdown. Selecting lines providing lower inhibitor amounts also among heat or pest stress could be a promising strategy for future breeding.

Transgenic barley expressing the algae starch branching enzyme Gomez Ibarra A. R.1, Souza Canada

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Barley is a crop that has been domesticated, selected, and improved over many years, but the continued introduction of new genetic variations is relevant for plant breeding programs. One of the points with more emphasis is improving the industry's nutritional quality and malting efficiency. A starch branching enzyme (SBE) from the algae Ostreoccocus tauri was recently identified, promising the improvement of these characteristics. It has been evaluated under rigorous scientific criteria in Arabidopsis thaliana, and has been shown to structurally change the shape of the starch grain, reducing its size and increasing the percentage of starch that is in constant rearrangement in addition to improving its digestibility. The objective of this work is to express the O. tauri SBE transgene in barley through transgenesis, by biolistic bombardment, under the hordein promoter for their expression in the endosperm, and with a bar selector gene. For this purpose, isolated immature embryos from two varieties (Andreia -A-, and Golden Promise -GP-) were used. The bombardment conditions were: a helium gas pressure of 900 psi and 6 cm bombardment distance to target explants, including pre- and post-bombardment osmotic treatment of 4 and 16 hours, respectively. They were then subjected to four different in vitro culture media (MI, MT, MR, and MR2). Their viability across the in vitro culture and the regeneration was evaluated under 1 or 2.5 mg/L-1 ammonium glufosinate to select transgenic plants. Eight T0 fertile transgenic plants (six from A, and two from GP) were obtained from 270 embryogenic calli bombarded in the conditions described above, and revealed by specific PCR assays. Plants are being conducted to the next generation T1 to evaluate starch morphology, degradation, and micromalting quality tests. The data were statistically analyzed and showed significant differences in the response of transformation according to the variety used, as well as for two of the media assayed (MT and MR), suggesting a genotypic dependence in the regeneration and transformation efficiency. As a conclusion, we set up a protocol to develop transgenic barley for expressing a nutritional quality transgene in commercial varieties.

Evidence-based breeding for malting quality

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In barley breeding, effective selection to maintain and improve malting quality is constrained by the cost and complexity of micromalting and malt analysis. Malting quality traits are typically assessed only in advanced stages and for limited numbers of lines, using grain samples from limited numbers of field environments. With little or no replication within environments and limited connectivity between environments, it is difficult to evaluate sources of variation, including genotype-by-environment interaction (GEI). Advanced experimental design, statistical analysis and genomic selection methods that are now routinely applied in breeding for grain yield have not been applied for malting quality traits. To generate an evidence base for designing genomic selection strategies for malting quality, we carried out a series of analyses and experiments using data and materials from a commercial barley breeding program.

To understand the extent and nature of GEI for malting quality traits, we fitted one-stage factor analytic mixed models (FALMMs), to a multi-environment trial (MET) data set (including genotypic data) involving almost 3000 breeding lines and 25 environments, and classified environments into iClasses. To evaluate the extent to which malting quality results can vary even among genetically identical samples, we conducted a uniformity trial. To account for and understand all sources of variation from the field to the laboratory, we designed and conducted a multi-phase experiment in which malting quality traits were evaluated for 622 barley lines. Collectively, these analyses and experiments provide an evidence base for designing effective strategies for accurate genomic selection for malting quality traits.

Exploring yield and malting quality stability through genome-wide association in an elite barley mapping population

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Yield and malting quality traits, as all quantitative traits, are controlled by multiple genes and highly influenced by environmental conditions. A better understanding of the genetic components conferring stability may help to improve cultivar development for contrasting environments. Barley production in the Southern cone of South America has a strong dependence on modern European germplasm combining high grain yield potential and excellent malting quality. Unfortunately, this germplasm has limited stability and adaptation, since it was developed to perform in optimal environments. Our objective was to identify quantitative trait loci (QTLs) controlling the stability of yield and malting quality traits in South American (specifically Uruguayan) environmental conditions in germplasm representative of the crosses used in local breeding programs. To this goal, we used a nested association mapping population of 150 double haploid lines obtained from crosses between modern European cultivars and local well-adapted germplasm. Phenotypic data for grain yield was obtained from 13 field experiments conducted over 4 years (2015-2018) in contrasting environments. To obtain malting quality traits data (beta-glucan content, malt extract, protein content, and soluble nitrogen), the population was phenotyped at three locations in Uruguay in 2016. We calculated the following stability indexes that describe stability in terms of genotypes and environment: Finlay Wilkinson index, Genotypic superiority index, Stability variance, Environmental variance, and Ecovalence. A genome-wide association study using a K+PC model with a Bonferroni-adjusted p-value < 0.05 was conducted with 6340 informative SNPs distributed through the genome. Our preliminary results identified a single QTL for yield stability located in chromosome 1H, and two significant QTLs for soluble nitrogen and beta-glucan stability, both located in chromosome 5H. No QTLs associated with malt extract and protein content stability were identified. The parental

line CLE267 carries the favorable allele for identified QTLs. The distribution of favorable alleles is a reminder of the importance of local breeding and cultivar development for non-optimum environments.

ASSOCIATION MAPPING OF HAGBERG FALLING NUMBER IN AN ADVANCED GENERATION BARLEY BREEDING GERMPLASM

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Pre-harvest sprouting (PHS) is an important factor limiting stable supply of high-quality barley (*Hordeum vulgare* L.) grains for the malting industry in regions that regularly experience high temperature and rainy conditions around harvest time. Barley varieties with lower level of dormancy are more susceptible to PHS; on the other hand, high level of dormancy can negatively affect the uniformity of germination during malting. An increased level of α-amylase activity that leads to starch degradation in the grain has been used as an indicator of PHS damage in cereals. On industrial scale, the Hagberg-Perten Falling Number (FN) assay is used on malting barley during harvest receival to detect possible PHS damage. Low values of FN may lead to malting-grade barley grain being downgraded to feed-grade, with a consequent financial loss for the farmers. Breeding of malting barley has made considerable progress to generate varieties with high yield and high malting quality. However, high malting quality is often associated with susceptibility to PHS and low FN. The expected frequency and intensity of heat waves and an erratic rain pattern associated with climate change may expose the barley growers to further risks. Therefore, it is necessary to release barley cultivars with high malting quality, but also with lower susceptibility to PHS, and therefore displaying stable levels of FN across different environmental conditions. Since FN analysis is time consuming and expensive, and PHS shows high genotype x environment interaction, the use of genotypic information may contribute to increased selection precision. To assist in breeding barley cultivars with low susceptibility to PHS, identifying genomic regions that affect FN is essential. This study was performed using a panel of 484 spring barley genotypes, including commercial cultivars and advanced generation breeding lines of the INIA Barley Breeding Program. The breeding panel was evaluated in replicated field trials in La Estanzuela and Young, Uruguay, during the 2023 growing season. The environmental conditions around harvest time at the Young field trial were predisposing to low FN due to rain events at physiological maturity. Heritability calculations for FN showed that the trait was highly heritable (0.70-0.78). Phenotypic BLUP values were used for further analysis. The barley panel was genotyped using skim-sequencing technology. As a reference population, 25 genotypes representing the genetic diversity of the breeding panel were sequenced with 10X coverage. The rest of the panel was sequenced with 0.08X coverage and imputed against the reference population. Single-nucleotide polymorphism (SNP) calling was performed with reference to the genome of RGT Planet. After filtering, 22 307 SNP markers were retained for further analysis. Genome-wide association study (GWAS) was performed to detect marker-trait associations for FN using GWASpoly package in R. Heading date as the days from emergence to awn tipping was used as a covariate in the analysis. Significant marker-trait associations that had a false discovery

rate adjusted *P* value < 0.05 were retained. The results of this study are useful for determining the presence of previously described QTLs for FN in the local breeding germplasm, and for marker-assisted selection that can be directly employed in the breeding program.

Title: Optimizing Malt Quality by Integrating Markers Assisted and Genomic Selection within the Washington State Barley Breeding Program.

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Barley (*Hordeum. vulgare* L.) is an economically important cereal crop in the United States (US) that is mainly used for malt production to support the brewing and distilling industries. Barley breeders strive to meet end use malting standards set by the American Malting Barley Association. However end-use malt quality traits are under complex, highly quantitative genetic control. Furthermore, phenotyping experimental lines for malt quality traits is time-consuming and expensive. To address these limitations and to assist the development of WSU malt barley lines, genome-wide association studies (GWAS) and genomic selection (GS) pipelines were implemented to reduce cycle time and increase genetic gains and selection accuracy. To do this the top yielding 250 experimental lines from the WSU barley breeding single replicated yield trials in 2021, 2022, and 2023 were selected and malted at the USDA-ARS Cereal Crops research unit in Madison, WI. Malt quality phenotypes tested were kernel weight, kernel plumpness, kernel protein, malt β-glucan, free amino nitrogen, soluble protein over total protein, extract, barley color, wort color, wort clarity, wort protein, α-amylase, and diastatic power. These lines were also genotyped using the Illumina iSelect 50k platform. The phenotyping and genotyping data were utilized in GWAS to identify marker-trait associations (MTAs) with 13 malt quality traits. Using the GAPIT package in R studio, the BLINK model was used to identify 57 MTAs with malt quality across all seven chromosomes anchored to the cv Morex v3 genome assembly. These MTAs will assist breeding programs in identifying lines enhanced for malt quality by utilizing marker-

assisted selection (MAS). To complement MAS, GS used phenotyping and genotyping from all three years to create training population to improve selection accuracy and increase genetic gain.

What QTLs are affecting Kernel Discoloration Resistance and Grain Protein Content in Spring Malting Barley(*Hordeum vulgare* L.)?

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Kernel discoloration in malting barley (*Hordeum vulgare* L.) is a visual assay for maltsters to avoid buying low-quality grain. Discoloration is influenced by factors such as genetics, fungal infection, and environmental conditions. The low-protein trait inherited from the barley cultivar 'Karl' has been reported to be associated with kernel discoloration. Previous research at NDSU indicated that these two traits are co-inherited, with the most significant quantitative trait loci (QTL) associated with the Karl low-protein trait mapped to the centromeric region of chromosome 6H. 252 breeding lines from the NDSU barley breeding program and commonly grown cultivars were tested at multiple locations in North Dakota from 2023 and 2024, to identify QTLs associated with kernel discoloration resistance and low grain protein content. A color spectrometer was used to measure the L-value, which indicates the kernels brightness or whiteness for all lines evaluated. Grain protein content was assessed using near-infrared reflectance. The 50K Illumina Infinium iSelect array for barley was used to genotype the lines. Best linear unbiased estimators (BLUEs) were calculated by location and best linear unbiased predictors (BLUPs) were calculated across locations for both kernel color and protein content. BLUEs and BLUPs were used in a genome-wide association study to detect the significant QTLs for both traits. The most significant QTLs for these traits were found in chromosomes 4H and 6H. In chromosome 4H, the QTL for protein was located in the short arm, while the QTL for kernel color was in the long arm. To our knowledge, this is the first detection of this low-protein QTL. Unsurprisingly, the QTL for both traits on chromosome 6H were co-located in the centromeric region, confirming previous findings. The fact that the QTLs for protein content and kernel color in chromosome 4H do not co-segregate suggests the potential to develop low-protein lines with acceptable kernel color.

Achieving Genetic Gain under Climate Instability in the Global Barley Breeding Program

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Extreme climatic events and large year by year variations hinder breeding efficiency, more so when a breeding program aims at developing new elite germplasm for more than 10Mha in the Developing World. To address the challenges, novel approaches to develop better adapted varieties faster are needed.

To mobilize new diversity faster, many breeding programs have adopted SpeedBreeding as their main advancement method. At the Global Barley Breeding Program of the CGIAR, Speed Breeding coupled with selection for diseases is the generation advancement strategy from 2021. This approach allows the program to advance 4 generations in merely one year and helped increase the proportion of resistant progenies to diseases such as net form of net blotch from 26% to 49% without increasing undesirable plant types.

To increase the potential for adaptation of the new elite genotypes developed to target environments, the program uses a wide range of field stations representing important agroecologies for barley cultivation. Since 2021, to increase the number of genotypes tested at these locations, the program implemented a Genomic-Assisted Sparse Multi-location approach that has allowed increasing the selection accuracy to these environments up to 8-fold. However, the year-by-year variation still creates a distortion for selection. To overcome this problem, Genotype by Location analysis performed with the GEBVs of past environments is being implemented. This approach allows increasing the selection accuracy at target locations and minimizing year by year variation, boosting breeding efficiency.

KeyWords: ICARDA, CGIAR, Genomic Predictions, Developing countries, Genotype by Environment, drought, heat, diseases, breeding

QUILMES BREEDING PROGRAM

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Quilmes research activities began in Argentina in 1974. At that time, the situation of barley crop was unsustainable due to low yield potential, low plumpness and high dispersion in grain size and incidence of diseases, mainly leaf rust. First varieties were released to the market in 1982, and from that moment on, crop improved substantially, since the yield relationship between barley and wheat was reversed, genetic resistance to the most common barley diseases was obtained, and the stability of grain size and industrial quality increased.

In the period 1970 – 2022 (FAOSTAT, 2024) the yield at comercial level increased at a rate of 59 Kg/Ha.year ($R^2 = 0.88$). However, dividing the germplasm change periods, the trends were 22.6 Kg/ha.year (1970 – 1982, $R^2 = 0.15$), 45 Kg/ha.year (1983 – 2000, $R^2 = 0.43$) and 65.5 Kg.ha.year (2001 – 2022, $R^2 = 0.49$). That is, the varieties developed by Quilmes allowed doubling the yield of the first cycle (from 22.6 to 45 Kg/ha.year) and provided 45% more yield (from 45 to 65.5 Kg/ha.year) since the introduction of European germplasm, although in this case the varieties developed in the country were not exclusive to Quilmes but also to other companies. Regarding grain size, in the first cycle (prior to the release of the Quilmes varieties), the average was 74.10 ± 7.09%, while the contribution of the new varieties represented a substantial improvement in this commercial quality parameter: 88.75 ± 3.12% (that is, not only was the average improved but the dispersion was reduced).

In 1997, Quilmes signed two fundamental agreements that contributed to the increase in yield and industrial quality of the varieties developed since 2000. The first one with Bayerische Landesanstalt für Landwirtschaft Institute of Germany, and the second one with Ackermann Saatzucht GmbH barley breeding company. Andreia, the most stable variety in yield, size and industrial quality, being the most planted cultivar in the entire history of the barley crop in Argentina, was the result of the joint work between Quilmes and Ackermann. In 2011, the Global Barley Research Program was created, which integrated all of Anheuser-Busch InBev's R&D programs. In addition, Quilmes is regionally integrated with the programs in Brazil and Uruguay, making this regional program part of the GBR.

As an example of the improvement in industrial quality of the Quilmes/Ackermann varieties, in the period 2008 – 2017 the level of extract weighted by malt production went from 80.58% to 81.98%.

Currently, relationships with other breeders, local (Instituto Nacional de Tecnología Agropecuaria) and European (Secobra, Breun) allow testing germplasm from other sources. Malkia (Quilmes/Secobra) and Adeline (Quilmes/Ackermann) are the latest varieties registered (2023) in stage of industrial approval.

The premises of the program have always been yield, disease resistance, grain size stability (through high photoperiodic response or high filling grain rate), and commercial and industrial quality. Regard to industrial quality, Quilmes breeding program has been successful in factors such as reduction of wort filtration time and increase of amino acids for the fermentation phase, in addition to increased extract, controlled attenuation, and diastatic power according to the needs of each beer design.