The 3rd International Barley Mutant Conference

October 8 – 10, 2023
Kurashiki, Japan

Contents

Organization

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Sponsorships

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Program

Sunday, October 8, 2023

15:00-18:00 Registration at IPSR https://www.rib.okayama-u.ac.jp/english/
(Katsuyuki Kakeda, Shun Sakuma, Ryo Matsushima)

Mixer; Refreshments and snacks.

16:00- IPSR tour (Hiroshi Hisano and Kazuhiro Sato)

18:00- Dinner on your own

Monday, October 9, 2023

09:00-17:00 Registration at Kurashiki Geibunkan Hall (https://arsk.jp/geibun/)

10:00-10:10 **Opening Session**
Kazuhiro Sato (IPSR, Okayama University, Japan)

10:10-12:00 **Session I (Morphology I)**
Chair: Robbie Waugh (JHI, Scotland)

10:10-10:40 Nils Stein (IPK, Germany)
The genetic control of *short rachilla hair 1*

10:40-11:00 Mats Hansson (Lund University, Sweden)
Identification of a dense spike gene deficient in Japanese landraces and in a Scandinavian barley collection

11:00-11:20 Takao Komatsuda (Shandong Academy of Agricultural Sciences, China)
Identification of *Super-open flowering 1* mutant gene in barley

11:20-11:40 Mattew Tucker (University of Adelaide, Australia)
Establishing a Genetic Blueprint for Floral Organ Development in Barley

11:40-12:00 Hiroyuki Tsuji (Nagoya University, Japan)
Surgical manipulation of barley inflorescence meristem

12:00-14:00 **Lunch and Poster Preparation**

Group photograph

14:00-15:50 **Session II (Morphology II)**
Chair: Mats Hansson (Lund University)

14:00-14:30 Thorsten Schnurbusch (IPK, Germany)
Genetic factors controlling grain number per spike

14:30-14:50	Agatha Walla (Heinrich Heine University Düsseldorf, Germany) Hunting the Hidden: Exploring a Developmental Gene in Barley
14:50-15:10	Muhammad Awais (IPK, Germany) Genetic control of awn roughness in barley
15:10-15:30	Shun Sakuma (Tottori University, Japan) Mutant collections derived from wild barley with chronic irradiation accelerate gene cloning
15:30-16:10	**Coffee break and Poster**
16:10-18:00	**Session III (Physiology and quality)** Chair: Nils Stein (IPK, Germany)
16:10-16:40	Jian Feng Ma (Okayama University, Japan) Exploring mineral transporter genes for better and safe barley production
16:40-17:00	Kelly Houston (JHI, Scotland) Functional mutations of *HvHKT1;5* influence yield related traits and composition of barley grain
17:00-17:20	Qiufang Shen (Zhejiang University, China) Ca2+ signal pathway negatively regulates barley salt tolerance
17:20-17:40	Zhou Zhou (McGill University, Canada) Interplay of starch debranching enzyme and its inhibitor is mediated by Redox-Activated SPL transcription factor
17:40-18:00	Morten Egevang Jørgensen (Carlsberg Research Laboratory, Denmark) Natural and induced variations in the grain dormancy gene *MKK3*
18:30-	**Dinner** (Chateau de Felicion; https://felicion.net/access/)

Tuesday, October 10, 2023

08:30-12:00	**Registration**
08:50-9:20	**Poster presentation I (odd numbers)**
09:20-9:50	**Poster presentation II (even numbers)**
10:00-12:10	**Session IV (Genomics)** Chair: Chengdao Li (Murdoch University, Australia)
10:00-10:30	Martin Mascher (IPK, Germany) How will cheap genome sequences help mutant research?

10:30-10:50	Soichiro Asuke (Kobe University, Japan) Cloning of *Rmo2*, a gene for resistance in barley to various host species-specific pathotypes of the blast fungus
10:50-11:10	Brian J. Steffenson (University of Minnesota, USA) Genome-wide Association Mapping of Leaf Rust Resistance in Wild Barley Based on Whole Genome Sequence Data
11:10-11:30	Guillermo Garcia Gimenez (La Trobe University, Australia） Manipulation of mixed-linkage (1,3;1,4)-β-glucan in barley using gene editing technology
11:30-11:50	Miriam Schreiber (JHI, Scotland) Using whole genome shotgun sequencing to explore mutants of the Bomwan NIL collection
11:50-12:10	Christoph Dockter (Carlsberg Research Laboratory, Denmark) JUST FIND-IT. Harnessing the powers of induced mutagenesis
12:10-14:00	**Lunch**
13:40-14:00	**Poster removal**
14:00-15:50	**Session V (Genetics and Breeding)** Chair: Martin Mascher (IPK, Germany)
14:00-14:30	Robbie Waugh (JHI, Scotland) Rapid evolution of domesticated barley
14:30-14:50	Paolo Pesaresi (Università degli Studi di Milano, Italy) Boosting photosynthesis to deliver next generation barley plants for the circular bioeconomy
14:50-15:10	Chengdao Li (Murdoch University, Australia) Utilization of mutation genes in Australian barley breeding
15:10-15:30	Ping Yang (Chinese Academy of Agricultural Sciences, China) HTX mutagenesis population for forward and reverse genetic studies in barley
15:30-15:50	Lingzhen Ye (Zhejiang University, China) *HvGA20ox2* synergistically regulates plant height and malt quality traits in barley
15:50-16:30	**Closing Session**

List of Posters

O-1

The genetic control of the rachilla hair phenotype in barley

Hélène Pidon[1], Murukarthick Jayakodi[1], Luke Ramsay[2], Twan Rutten[1], Robert Hoffie[1], Sudharsan Padmarasu[1], Ravi Koppulu[1], Corinna Trautewig[1], Magnus W. Rasmussen[3], Robbie Waugh[2], Christoph Dockter[3], Michael Melzer[1], Jochen Kumlehn[1], Martin Mascher[1], Nils Stein[1]

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The rachis is the primary (main) axis of the cereal inflorescence, whereas its side branches are called rachillae (sg. rachilla). While in related cereals like wheat and rye the rachilla carries multiple fertile florets, partly explaining the higher yield potential of these cereal crops compared to (2-rowed) barley, the rachilla in barley is a rudimentary structure found at the base of the sessile florets. Rachillae are covered by trichomes with unknown function, however, since hairs are very long and rigid in wild barley and the rachillae remain attached to the seeds at dehiscence, both may fulfil a functional role as part of the seed dispersal unit of wild barley. Long rachilla hairs are single-celled trichomes. Short hairs, however, are not just shorter but in fact are multicellular and the morphological / developmental distinction is due to endopolyploidy-dependent cell size and mitotic control. Although representing a rudimentary structure, the trait 'short rachilla hair', controlled by the single genetic locus *Srh1*, is famous as number 24 (rachilla hair type) of the 29 UPOV characteristics that are used for discrimination of varieties in the process of barley cultivar registration (https://www.upov.int/test_guidelines/de/list.jsp). The underlying gene, known for many decades, has not been isolated until recently and its function remained unknown so far. This gene was now identified by high resolution mapping and GWAS and functionally validated by chemically or Cas9-induced mutagenesis. While mutagenesis of the structural gene reproduced the short rachilla hair phenotype, natural variation of the trait can be attributed to structural variation outside the coding region.

O-2

Identification of a dense spike gene deficient in Japanese landraces and in a Scandinavian barley collection

Hansson, M[1]

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Erectoides mutants are among the most common mutants in barley affecting plant architecture; more than 1,200 mutant accessions are available at the Nordic Genetic Resource Center (www.nordgen.org). The mutants have various degrees of erect and compact spikes, often accompanied with short and stiff culms. The Erectoides mutants have been grouped in 31 allelic groups through diallelic crosses. The *ert-a* mutant group consists of 43 mutants. Though crosses to Dense spike 1 mutants we identified a handful of additional alleles. The genome of a selection of *ert-a* mutants induced in cultivars Bonus and Foma were sequenced. Through bioinformatic analyses a candidate gene was identified encoding an "ABC transporter ATP-binding protein". Sequence analyses of this gene in the available *ert-a* and *dsp1* mutants confirmed that the correct gene had been identified since almost all mutant accessions had severe mutations in the "ABC transporter ATP-binding protein". Since *dsp1* mutants have also been associated with many cultivars of Oriental origin, we also analysed seven Japanese landraces. Severe mutations in the *ert-a* gene were confirmed in six of them.

O-3

Identification of *Super-open flowering 1* mutant gene in barley

Yuji Hamada[1,2], Nadia Anwar[1], Gang Chen[1,2], Kohei Mishina[1], Shun Sakuma[3], Atsushi J. Nagano[4], Shuichi Fukuoka[1], Sara Milner[5], Hiroshi Hisano[6], Shunzong Ning[1,2], Mohammad Pourkheirandish[1], Akemi Tagiri[1], Shinji Kikuchi[2], Hidenori Sassa[2], Martin Mascher[5], Kazuhiro Sato[6], Youko Oono[1,2], Takato Koba[2], Takao Komatsuda[1,2,7]

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Plant domestication is a consequence of the gradual accumulation of natural mutations in wild plants. As wild barley (*Hordeum vulgare* subsp. *spontaneum*) is an immediate ancestor of cultivated barley (*H. vulgare* subsp. *vulgare*), gene function presumably is better retained in wild barley than in cultivated plants. Here we mutagenized a wild barley strain OUH602 originated from the Caspian Sea region. Grains were sown in the autumn and plants were grown in the "Gamma field "of the Institute of Radiation Breeding and irradiated chronically from germination to maturation by gamma-ray dose at 0.24 – 0.77 Gy/day and 5 days irradiation/week from a cobalt 60 (^{60}Co) source. As a generation of the irradiated plants was defined as M_1, 1,600 M_2 plants and their M_3 lines were cultivated in an ordinal experimental field and forward-screened by eyes for morphological mutation. One M_3 line, #44205, included a single plant showing a mutant with lemma and palea (the pair of bracts which encase the floret) gaped at a wider angle than that of the wild type. When the floret opens, the palea and the lemma are forced apart by the swelling of the lodicule. Lodicule is orthologous to the petal of the hermaphroditic angiosperm flower. Lodicule of this mutant was larger in length, depth, and width than that of the wild type. This gene was named "*Super-open flowering 1"* as a single gene that controls the phenotype, and the mutant phenotype was recessive, so the gene symbol was *sof1*. Identification of *sof1* is reported.

O-4

Establishing a Genetic Blueprint for Floral Organ Development in Barley

Selva C[1], Yang X[1], Shirley NJ[1], Houston K[2], Li G[3], Baumann U[1], Whitford R[1], Tucker MR[1]

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Floral development is the result of a sophisticated balance of molecular and environmental cues. Floral mutants provide insight into the genetic determinants that integrate these cues, as well as opportunities to assess functional variation across species.

We have been investigating flower development in barley (*Hordeum vulgare*) using induced mutants, gene edited lines, and natural diversity panels, with a view to understanding the role of key regulatory genes involved in organogenesis and tissue patterning.

Of particular interest are genes that influence the formation of female tissues, which contribute directly to grain development and yield. For example, in recent work we have characterised and proposed causative genes for the *multiovary* (*mov*) mutants *mov2.g*, *mov1*, and *mov5.o*; a C2H2 zinc-finger gene *HvSL1*, a B-class MADS-box gene *HvMADS16*, and the barley homologue of *LEAFY*, respectively. Molecular studies suggest these factors are acting upstream of other MADS-box genes to ensure correct patterning of the carpel and ovule.

In this presentation I will discuss how these mutants provide an opportunity to piece together the network of genes required for female fertility and yield. The findings lay the foundation for a better understanding of floral development in Triticeae crops, and new genetic targets for crop improvement.

O-5

Surgical manipulation of barley inflorescence meristem

Tanaka M[1], Akashi H[1], Tsuji H[1,2]

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The establishment of positional information is essential for the proper developmental progression of multicellular organisms. A primary mechanism for establishing the positional information is effective communication between regions of different degrees of differentiation. A simple method to verify the presence of such communication is to excise and transplant developing tissues. However, few such experiments have been performed in plants.

Here, we developed a unique experimental technique to excise stem cell regions and differentiate inflorescences in developing barley. The leaf sheaths of a 2-3 week old barley plant were carefully incised with a scalpel in a 500 μm square area, creating a "window" to expose the central inflorescence. Once a window was created in the barley, inflorescence development was temporarily halted. As the leaf primordium grew, the window eventually closed, and inflorescence development continued. This technique allowed us to design an experiment in which part or all of the barley inflorescence could be excised immediately after the window was created, and the subsequent developmental events could be observed after the window closed. Consequently, we found different developmental processes depending on the location of the incision. Through our experiments, we determined the significance of the meristem region in the development of the basal regions. Additionally, by observing the developmental stages of the vertically split inflorescence meristem, we discovered that inter-regional communication within the barley inflorescence meristem is intricate and multifaceted.

O-6

Genetic factors controlling grain number per spike

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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. In this presentation, I will focus on the genetic basis of inflorescence architecture in barley, highlighting recent insights that have helped to improve grain yield by, for example, extra spikelet formation or reducing the pre-programmed abortion/degeneration of floral organs. The accumulating information on inflorescence development can be harnessed to enhance grain yield potential of grain crops.

O-7

Hunting the Hidden: Exploring a Developmental Gene in Barley

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Recent developments in barley genomics and cost-effective NGS technologies have expanded the pool of candidate genes associated with traits that hold significant agronomic value. Most of these traits, including seed number, spike and tiller count, and plant height, collectively contribute to the overall shoot architecture. Discovering underlying genes enhances our comprehension of the genetic regulation governing shoot architecture development, opening new avenues to create high-yielding barley ideotypes. This study presents the process of identifying a gene in a forward genetics approach. Our focus was on a pleiotropic high-tillering mutant that exhibited short culm nodes, resulting in a dwarf plant architecture. Unlike other high-tillering mutants categorized as *many noded dwarf*, this specific mutant had only one additional node at the main stem compared to the wild type. However, we observed negative correlations between biomass and yield, similar to other *many noded dwarf* mutants previously reported. Initially, attempts to identify the causal mutations through mapping by RNAseq were unsuccessful. Only through a combination of WGS and maker-assisted mapping could we identify a large inversion on chromosome 7H as the candidate mutation. Nevertheless, this structural mutation did not reveal the causative gene. It was only by integrating data from WGS, RNAseq, and complementation tests that we could propose that the underlying mutation is located in the cis-regulatory region of a previously described tillering gene, strongly affecting its expression. Using CRISPR lines with changes in the cis-regulatory region, we confirmed that these variations and subsequent expression changes of the known tillering gene could modulate the phenotypic expression, resulting in milder consequences on shoot architecture traits. These insights shed light on the intricate regulatory mechanisms influencing plant architecture and tillering.

O-8

Genetic control of awn roughness in barley

Muhammad Awais[1], Muhammad Khan[1], Matthias Jost[2], Hélène Pidon[3], Twan Rutten[1], Michael Melzer[1], Robert Hoffie[1], Jochen Kumlehn[1], Götz Hensel[5] and Nils Stein[1,4]

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Awns in cereals are considered to play a significant role in seed protection and dispersal as well as assimilation. Most barley awns are covered with upward-oriented single-celled trichomes (barbs) with a silicified cell wall giving them a "rough" surface. On the contrary, some barley awns that are lacking such barbs, feel smooth and thus are called smooth awns. A major gene (*Raw1*) on chromosome 5H controlling barb formation of barley awns has been isolated and one additional locus was detected by genome-wide association study. To characterize further this second locus, F_2 mapping populations between the "semi-smooth" spring barley cultivar 'Morex' and a very smooth mutants 'MHOR597', both carrying the recessive semi-smooth *"raw1*" allele, were used for genetic mapping using genotyping-by-sequencing. A single locus (named *Raw7*) could be assigned to a 10 Mbp interval of the short arm of chromosome 7H. With the help of improved phenotyping and high-throughput genotyping of ~3000 F_2 plants, using KASP (kompetitive allele-specific PCR) markers, we identified a candidate gene (*Raw7*) which is validated using CRISPR-based site-directed mutagenesis. Furthermore, the interaction of both genes (*Raw1, Raw7*) was studied in the F_2 progenies of wild and mutant parents at both loci. The results demonstrate that both genes control different characteristics (size, frequency of occurrence) of barb formation. The current study identified a new genetic basis for the emergence of barbs on barley awns, providing insight into the mutual interaction and the functional roles of the two genes.

O-9

Mutant collections derived from wild barley with chronic irradiation accelerate gene cloning

Shun Sakuma[1,2], Martin Mascher[2], Mohammad Pourkheirandish[3], Thorsten Schnurbusch[2], Takao Komatsuda[4]

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Wild barley (*Hordeum vulgare* ssp. *spontaneum*) is the sole member of 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, respectively, suggesting that wild barley leaves many intact genes for lesions. Here we have developed a large-scale mutant population from wild barley accession OUH602 to accelerate forward genetics mainly focusing on inflorescence structure. From sowing to harvest, plants were exposed to weak irradiation by cobalt 60 in the NIAS gamma field from the time of sowing to harvesting. This system is called chronic irradiation as opposed to acute irradiation commonly in plant mutagenesis. M2 seeds were harvested manually and through the phenotypic screening of about 50,000 M3 plants, we 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 gene cloning with next-generation sequencing technology. This wild barley mutant population is instrumental in improving barley and the discovery of novel genes and their mode of action.

O-10

Exploring mineral transporter genes for better and safe barley production

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Barley is widely cultivated in different areas, however its productivity is often limited due to various mineral stresses including deficiency of essential mineral elements and excess of mineral elements. We found that transporter genes play important roles in improving mineral stress tolerance as well as grain quality. One example is that a citrate transporter gene *HvAACT1* is involved in detoxification of Al toxicity, which is a major limiting factor for production of barley on acid soil. We found that the differential Al tolerance is strongly associated with the expression of *HvAACT1*, which is controlled by insertions in the upstream genomic sequence of *HvAACT1*. Introgression of these insertions into Al-sensitive cultivars resulted in higher productivity on acid soil.

On the other hand, cadmium (Cd) is a highly toxic heavy metal, which threats our health through food chain. We found that the genotypic difference in barley Cd accumulation is caused by different expression of *HvHMA3*, which encodes a tonoplast-localized transporter for Cd. Furthermore, we revealed that an upstream insertion of a 3.3-kb Sukkula-like transposable element functions as a promoter and enhances the expression of *HvHMA3*. Introgression of this insertion to an elite barley cultivar resulted in decreased Cd accumulation in the grain grown in Cd-contaminated soil without yield penalty.

High P in barley grain is associated with low bioavailability of essential metals such as Zn and Fe, and eutrophication because most P in the grain is present in the form of phytic acid. We recently identified a node-based P transporter, HvSPDT (SULTR-like phosphorus distribution transporter). *HvSPDT* is expressed in both xylem and phloem region of enlarged and diffuse vascular bundles in the nodes. Knockout of *HvSPDT* significantly decreased P allocation to the grains, resulting in great reduction of grain yield especially under P-limited conditions, indicating that the node-based HvSPDT plays a crucial role in loading P into the barley grains.

O-11

Functional mutations of *HvHKT1;5* influence yield related traits and composition of barley grain

Kelly Houston[1], Pauline Smith[1], Lawrie Brown[1] Christoph Dockter[3], Pai Pedas[1], Tim George[1], and Robbie Waugh[1,2,*].

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Soil salinity, most prominently the product of NaCl accumulation induced by a combination of natural and anthropogenic events that include irrigation and climate change, is a major factor affecting agricultural production worldwide. In adapting to saline soils plants have evolved a range of mechanisms, including Na^+ transporters and antiporters, that co-operate to control Na^+ ion homeostasis. Previously, we identified a Class 1 HIGH-AFFINITY POTASSIUM TRANSPORTER (HKT1;5) known to be involved in regulating plant Na^+ homeostasis as being responsible for the variation in grain Na^+ in our GWAS panel of cultivated barley[1]. We show that an L189P substitution disrupts Na^+ transport in the high Na^+ lines, disturbs the plasma membrane localisation typical of HKT1;5 and induces a conformational change in the protein predicted to compromise function. Under NaCl stress, lines containing P189 accumulate high levels of Na^+, but show no significant difference in biomass to individuals with L189. P189 increases in frequency from wild-species to elite cultivars leading us to speculate that the compromised haplotype is undergoing directional selection possibly due to the value of Na^+ as a functional nutrient in non-saline environments. We used a mutant line, which contains a P189 in HKT1;5, from the FIND-IT population[2] generated in RGT Planet (which contains L189, and therefore excludes Na^+) to explore this potential role for Na^+.

1. Houston, K., Qiu, J., Wege, S. et al. Barley sodium content is regulated by natural variants of the Na+ transporter HvHKT1;5. Commun Biol 3, 258 (2020).

2. Knudsen, S., et al. "FIND-IT: Accelerated trait development for a green evolution." Science Advances 8.34 (2022): eabq2266.

O-12

Ca^{2+} signal pathway negatively regulates barley salt tolerance

Yishan Tu[1], Yunfeng Xu[1], Fengyue Wang[1], Liangbo Fu[1], Dezhi Wu[2], Guoping Zhang[1], Qiufang Shen[1]

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Calcium (Ca^{2+}) signals have been recognized as a core regulator in plant salt tolerance. However, the mechanism underlying salt tolerance mediated by Ca^{2+} sensor, i.e. calmodulin (CaM), in regulating sodium (Na^{+}) transport is not completely clarified. We report a barley CAMTA (calmodulin-binding transcription activator) transcription factor HvCAMTA4 can directly bind to a salt-responding calmodulin HvCaM1 by Pull-down, Co-IP, BiFC and yeast-two-hybrid assays. HvCAMTA4 had conserved sequence and showed a flexible increased expression under salt stress. Both knock-out or knock-down of *HvCAMTA4* by CRISPR/Cas9 and RNAi techniques enhanced barley salt tolerance, reflected by lower shoot Na^{+} concentration and Na^{+} transportation rate relative to the WT plants under salt stress. In contrast, the expression of *HKT1;1* and *HKT1;5* genes were significantly modulated in *cam1* and *camta4* mutants. Moreover, HvCAMTA4 had the potential activity of transcriptional regulation in the nuclei that can bind to the CGCG-core of *HKT1;1* and *HKT1;5* promoters. The Ca^{2+} signals were also decreased in the roots of *camta4* and *cam1* mutants in comparison with WT, indicating the complex of CaM1-CAMTA4 was participating in Ca^{2+} signal pathway. This study revealed a novel role of HvCAMTA4 in regulating salt tolerance by interacting with HvCaM1 to transit Ca^{2+} signal and modulate Na^{+} transport. This Ca^{2+} signal pathway negatively regulates salt tolerance, which highlights its potential use in developing crop cultivars with high salt tolerance.

O-13

Interplay of starch debranching enzyme and its inhibitor is mediated by redox-activated SPL transcription factor

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Pullulanase (PUL), a starch-debranching enzyme, plays an important role in mobilizing stored endosperm food reserves during seed germination. It is known to be inhibited by pullulanase inhibitor (PULI), regulation of which is not fully understood. Barley has one *HvPUL* and two *HvPULI* (*HvPULI-1* and *HvPULI-2*) genes. During the time-dependent processes of barley seed germination, only HvPULI-1 expression shows an antagonistic relationship with that of HvPUL. Our data have indicated that the expression of HvPULI-1 is modulated by SPL (Squamosa-promoter-binding Protein Like) transcription factors, known to be targeted by miR156. Of all the *SPL* genes expressed during germination, *HvSPL3* co-expressed with *HvPULI-1* at early stages of seed germination. We showed that binding of HvSPL3 protein to *HvPULI-1* promoter occurs under reducing but not under oxidizing conditions. Replacement of Cys residues with threonine in HvSPL3 abolishes the binding, indicating an essential role of the redox state in the expression of HvPULI. We further investigated the effect of interaction between HvSPL3 and HvPUL/HvPULI complex on the starch structure and composition in barley grains through CRISPR-Cas9 mediated gene editing. The binding motifs of HvPULI promoter and SBP domain of HvSPL3 were targeted to generate mutants for subsequent phenotypic analysis. Our findings may have important implications for the industrial use of barley starch.

O-14

Natural and induced variations in the grain dormancy gene *MKK3*

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MKK3 (*Mitogen-activated Kinase Kinase 3*) is a genetic determinant of increased post-harvest grain dormancy (PHD) in East Asian barley and is associated with decreased PHD in North American and Australian elite malting varieties. PHD modulation is crucial for agricultural production and industrial application to control the risk for pre-harvest sprouting (PHS). PHS-susceptible cultivars show a high risk of moisture-induced germination prior to harvest which jeopardizes the quality of grain and agronomic performance. PHS is a global problem in barley and is at risk of being further aggravated by climate change. To effectively breed new barley varieties with reduced risk of PHS, while maintaining malt quality, it is imperative to understand the molecular nature of the loci responsible for low PHD and high PHS susceptibility.

Here, we unravel the complexity of the barley *MKK3* locus through multi-year field trials of *MKK3* near-isogenic lines (in an EU elite spring malting barley background) exposed to PHS-inducing conditions, combined with *in vitro* kinase activity assays, FIND-IT variants and droplet digital PCR genotyping of contemporary and historical barley accessions. In-depth *MKK3* haplotype and expression analyses in the new, high-resolution barley pan-genome assemblies (BPGv2) and barley pan-transcriptome datasets (BPTv1) reveal extensive structural variation at the *MKK3* locus including copy-number variation and corresponding changes in transcript abundance. The presence of variable copy number and specific hyperactive *MKK3* alleles coincide with traditional and modern farming practices that are important for yield gain and grain quality. Our results suggest the *MKK3* gene is a major determinant of PHD selected across the globe to adapt to local climate and agricultural practices and that breeders must carefully balance *MKK3* copy-number and protein haplotype to mitigate the risk of PHS.

O-15

How will cheap genome sequences help mutant research?

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Genomic methods complement traditional mapping approaches and help identify the sequence variants that cause mutant phenotypes. Mapping-by-sequencing in barley has relied on the genome sequence of cv. Morex, short-read sequencing of mutant genomes, and sequence-based genotyping of segregating offspring. High-throughput sequencing technology has improved a lot in recent years. As sequence reads have become longer and more accurate, sequence contigs now extend over megabases without intervening gaps. This has made structural variation in the intergenic space accessible to genetic analysis. Thanks to cheaper long-read sequencing, it is now possible to assemble and annotate the genome sequences of many barleys, including those of mutants and their parents. A pangenome, a collection of sequences of diverse and representative barley genomes, has been constructed. In this talk, I will review the emerging role of pangenomics in barley mutant research. I will report on genome sequences of the transformation-amenable variety Golden Promise and the parents of several mutant populations. A duplication at the *Intermedium-c* locus in six-rowed barleys serves an illustrative example for the importance of structural variation in natural and induced mutants.

O-16

Cloning of *Rmo2*, a gene for resistance of barley to various host species-specific pathotypes of the blast fungus

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Blast disease of various gramineous plants is caused by *Pyricularia oryzae* (syn. *Magnaporthe oryzae*). This fungus is composed of pathotypes that show host specificity at the plant genus level, but there is no *Hordeum*-specific pathotype. Barley cultivars shows various patterns of reactions to *P. oryzae* isolates. Resistant reactions in those interactions are all regulated by a single locus on barley chromosome 7H, designated as *Rmo2*. *Rmo2d* is an allele at the *Rmo2* locus that recognizes *PBY2*, an avirulence gene carried by *Eleusine* and *Triticum* pathotypes of *P. oryzae*. We isolated *Rmo2* through fine-mapping and complementation assays. In addition to the barley *Rmo2*, we also isolated *Rwt7*, a common wheat (*Triticum aestivum*) resistance gene which recognizes the avirulence gene *PWT7* of the *Eleusine* pathotype. Interestingly, *Rwt7* was an ortholog of *Rmo2* in spite of recognizing the sequence-unrelated avirulence genes, *PBY2* and *PWT7*. This result indicates that *Rmo2* and *Rwt7* have diversified from an ancestral resistance gene of Triticeae, and potentially play a central role in the resistance to multiple pathotypes of *P. oryzae*.

O-17

Genome-wide Association Mapping of Leaf Rust Resistance in Wild Barley Based on Whole Genome Sequence Data

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Leaf rust is an important disease of barley in many production areas. Extensive phenotyping assays revealed that most of the described *Rph* (Reaction to *Puccinia hordei*) genes for leaf rust resistance in barley have been overcome by virulent isolates of the pathogen. To identify new *Rph* genes for use in breeding, an ecogeographically diverse panel of *Hordeum vulgare* ssp. *spontaneum* accessions (i.e., the Wild Barley Diversity Collection or WBDC; N=314) was evaluated to a suite of 11 *P. hordei* isolates with unique virulence spectra. None of the accessions was resistant to all isolates; however, 9 accessions were resistant to at least 8 of the 11 isolates tested. The highest frequency of resistance was found in accessions from the Levant where *P. hordei* co-evolved on wild barley and its alternate host of *Ornithogalum*. The WBDC was sequenced at ~10x depth. Genome-wide association studies based on 171,000 single nucleotide polymorphisms (SNPs) (filtered based on minor allele frequency and missing marker data) identified a total of 62 marker trait associations (MTAs): nine on chromosome 1H, five on chromosome 2H, eight on chromosome 3H, 17 on chromosome 4H, six on chromosome 5H, eight on chromosome 6H, and nine on chromosome 7H. Many of these MTAs mapped to regions not known carry any *Rph* genes and therefore represent new resistance loci. Annotations of genes positioned on the same contigs as the most significant SNPs identifying leaf rust resistance loci revealed, in many cases, nucleotide binding site-leucine rich repeat motifs--typical of many resistance genes. The many new *Rph* genes identified in the WBDC may be useful for gene pyramiding in cultivars to increase their longevity.

O-18

Manipulation of mixed-linkage (1,3;1,4)-β-glucan in barley using gene editing technology

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Mixed linkage (1,3;1,4)-β-glucan (MLG) is a major non-cellulosic polysaccharide of commelinid monocot cell walls and an important soluble dietary fibre component found at high levels in some cereal grains (primarily the endosperm), such as barley and oats. Despite its importance, relatively little is known about the regulation of MLG biosynthesis. The *Cellulose synthase-like F6* (*HvCSLF6*) is the main gene contributing to MLG biosynthesis in barley[1]. To date, studies on *CslF6* transcriptional regulation identified a GT-rich motif in the second intron of *CslF6*, bound by *Trihelix1* transcription factor (*THX1*) in *Brachypodium distachyon*[2] and candidate MYB transcription factors using a *HvCslF6* promoter deletion series[3]. We therefore decided to target these putative regulatory regions using a multiplex CRISPR/Cas9 gene editing strategy to finetune *HvCslF6* expression by targeting either the barley ortholog of *BdTHX1* or putative MYB transcription factor binding sites within the *HvCslF6* promoter and assessing the impact of any change in *HvCslF6* expression on the amount of MLG in the mature grain. For the *HvTHX1* construct, a dead Cas9 fused to a tripartite activation complex (dCas9-VPR) was used to activate transcription. For *HvCslF6* promoter editing, five guide RNAs were designed to target a regulatory region containing putative MYB motifs which could affect the expression of this gene. We obtained 7 primary transformants using the *HvTHX1* construct and 31 for the *HvCslF6* promoter construct. For the latter, 45% of primary transformants carry edits, mostly deletions from 2 to 31 bp, all in heterozygous state. Grain phenotypic studies quantifying MLG will be carried out in subsequent generations. We will present our latest results describing the characterisation of these gene-edited lines.

References:

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O-19

Using whole genome shotgun sequencing to explore mutants of the Bomwan NIL collection.

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The development of new resources in the form of pangenomes and the reduction in cost of sequencing technologies have resulted in new approaches to identifying mutations. Here, I'll be showing how we have used whole genome shotgun sequencing of multiple mutants to identify underlying mutations. For this we used mutants from the extensive Bowman near isogenic line (NIL) collection. Most mutants within the collection have been back-crossed multiple times into Bowman, resulting in clearly defined introgressions. I'll highlight the advantages of using whole genome shotgun sequencing, point out the challenges which can arise, and show where further approaches are needed to find the causal gene of interest.

O-20

JUST FIND-IT. Harnessing the powers of induced mutagenesis

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The powers of induced mutagenesis were realized a century ago and Nobel Prizes were awarded for its discovery and for its application in the crop improvements facilitating the *green revolution*. To meet the ever-growing global food demands and to minimize the effects of the man-made climate change, agricultural and industrial production organisms must be constantly improved. The Carlsberg Research Laboratory designed FIND-IT (*Fast Identification of Nucleotide variants by droplet DigITal PCR*) to efficiently harness the potential of extremely large mutant populations. This new pipeline for crop and microbe improvement provides isolation of predetermined, targeted genetic variants in short screening cycles. Using large-scale sample pooling in combination with sensitive droplet digital PCR we have greatly increased the size of low mutation-density and screen-able variant libraries and the probability of identifying the variant of interest. Examples highlighting the power and applicability of FIND-IT – in barley and beyond - will be given.

O-21

Rapid evolution of wild barley (R-Evolve)

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The genepools of our major domesticated cereals, including barley, have narrowed considerably due to the practices of modern plant breeding. This restricted diversity leaves barley vulnerable to emerging biological and environmental challenges because the genes and alleles that may have allowed the crop to respond positively will frequently have been left behind by the breeding process. Traditionally, introgression from diverse germplasm has been an effective strategy for incorporating major, single genes, particularly for dominant disease resistance traits. However, this approach has largely failed for complex multigenic traits such as abiotic stress tolerance. Consequently, we and many others argue that radically new approaches to exploiting the extensive genetic diversity available in the relatives of our cultivated crops are urgently required. We recently proposed to explore the development of such an approach by accelerating the domestication of wild barley germplasm, exploiting knowledge and understanding of key domestication genes that we and others in the barley community have generated in recent years. We suggested reversing the traditional introgression breeding process by transferring optimal 'domestication alleles' (*btr1*, *Vrn-H1*, *vrnH2*, *sdw-1* and *ppdH1*) from elite cultivated barley into wild species using a combination of crossing, marker assisted selection, accelerated generational advance and increasing recombination by environmental manipulation during the process of SSD. The outcome would be a collection of partially domesticated F6 inbred lines. We then proposed to use Recurrent Introgressive Population Enrichment (RIPE) (*rob1*, *msg6*, *sex1*) to generate sufficient F1 seed to allow assessment of SSD line performance as F1 hybrids and to identify germplasm with significant potential for plant breeding. Our initial hypothesis was that the outcome will be a radically new, partially domesticated barley genepool that we anticipate could be used immediately by the scientific and breeding communities. During my presentation I will provide an update on the progress we have made towards our objectives.

O-22

Boosting photosynthesis to deliver next generation barley plants for the circularbioeconomy

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There is a need for a ground-breaking technology to boost crop yield (both grains and biomass) and its processing into materials of economic interests. Novel crops with enhanced photosynthesis and assimilation of green-house gasses, such as carbon dioxide (CO_2) and ozone (O_3), and tailored straw suitable for industrial manufacturing will be the foundation of this radical change. We are an alliance of European plant breeding companies, straw processing companies and academic plant scientists aiming to use the major advances in photosynthetic knowledge to improve barley yield and to exploit the variability of barley straw quality and composition. We will capitalize on very promising strategies to improve the photosynthetic properties and ozone assimilation of barley: i) tuning leaf chlorophyll content and modifying canopy architecture; ii) increasing the kinetics of photosynthetic responses to changes in irradiance; iii), introducing photorespiration bypasses; iv) modulating stomatal opening, thus increasing the rate of CO_2 fixation and O_3 assimilation.

Beside the higher yield, the resulting barley straw will be tailored to: i) increase straw protein content to make it suitable as an alternative feed production source; ii) control cellulose/lignin contents and lignin properties to develop construction panels and straw reinforced polymer composites. To do so, we aim to exploit barley natural- and induced genetic variability as wellas gene editing and transgenic engineering. Based on precedent, we expect that improving our targeted traits will result in increases in above ground total biomass production by 15-20% without modification of the harvest index, and there will be added benefits in sustainability via better resource-use efficiency of water and nitrogen. A public dialogue will be established to ensure stakeholder engagement and explore the acceptability of a range of technologies as potential routes to crop improvement and climate change mitigation.

O-23

Utilization of mutation genes in Australia barley breeding

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Mutation and utilization of mutant genes have played significant role to transform Australia to one of the world largest barley exporters in the last 70 years. Mutant genes for semidwarf, short vegetative period (BVP) and disease resistance were successfully utilized to release commercial barley varieties and some of the genes are still widely utilized in Australian barley breeding programs. Genes and alleles were identified, and gene-specific-molecular markers were developed to support molecular marker-assisted selection. Short BVP gene is essential to breed barley for adaption to the Australian environment. Four alleles of the sdw gene were utilized in the breeding programs for different purposes. The dep gene become the most successful gene to breed high yield barley varieties for the low rainfall environment in the last decade. New mutant populations were created from the current commercial varieties Vlamingh, RGT Planet and Baudin. Mutants and mutant genes were identified to enhance height stress tolerance and yield potential.

O-24

HTX mutagenesis population for forward and reverse genetic studies in barley

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Barley 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. The recent development of barley genomics has created a need for a high-throughput platform to identify genetically uniform mutants for genes functional investigation. We produced an ethyl methanesulfonate (EMS)-mutagenized population consisting of 10,109 M_3 lines in the Chinese barley landrace 'Hatiexi' (HTX), which we complement with a high-quality *de novo* assembly of a reference genome for this genotype. The mutation rate within the population ranged from 1.51 to 4.09 mutations per Mb, depending on the treatment dosage of EMS and the mutation discrimination platform employed for genotype analysis. We implemented a three-dimensional DNA pooling strategy combined with multiplexed amplicon-sequencing to create a highly efficient and cost-effective TILLING (Targeting Induced Locus Lesion IN Genomes) platform in barley. We discovered abundant allelic mutants for over 100 genes from >20 research units in China, supporting the classification of their genes functions. In addition to test a proof of concept via rapidly determining the causal gene responsible for a chlorotic mutant M4009, a number of mutants were further isolated via the MutMap strategy and the classic mapping approach. Collectively, we demonstrate the value of this resource to support forward and reverse genetic studies in barley.

O-25

Withdrawn

P-1

Genome editing of *TaQsd1* in common wheat

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To analyze the gene function, knockout mutants give a definitive information. However, it takes a lot of years to produce the knockout mutant using conventional techniques in polyploid plants such as hexaploid wheat. Genome editing with site-directed nucleases is a promising approach for obtaining knockout mutations in all homoeologous genes. Here, we produced hexaploid wheat lines with loss-of-function of homoeoalleles of *Qsd1*, which controls grain dormancy in barley, by Agrobacterium-mediated CRISPR/Cas9. The *TaQsd1* genome-edited line was shown to have a significantly longer grain dormancy period than wild type when grown in a growth chamber. Furthermore, a transgene-free null-segregant was identified by PCR and whole-genome shotgun sequencing. Using genome editing technology, we were able to produce a transgene-free triple recessive mutant within only 14 months. This technique serves as a model for trait improvement in wheat, particularly for genetically recessive traits, based on locus information from diploid barley. The *TaQsd1* genome-edited wheat is currently undergoing a field cultivation trial.

This work was supported by MAFF Commissioned project study on “Development of new varieties and breeding materials in crops by genome editing” Grant Number JPJ008000, and JSPS KAKENHI Grant Number 19H00943 and 23H00333.

P-2

Identification of a new family of small prolamins in the search for better barley breads

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Barley bread has a high content of β-glucans and other fibers which makes it a low glycemic food product; and the consuming of barley bread is associated with lower blood cholesterol levels and reduced risk of colon cancer (Mendis and Simsek., 2014; El-Khoury et al., 2012). However, using barley flour in bread making is difficult because it decreases the sensory characteristics and qualities of bread, such as color, texture, and loaf volume.

We identified the existence of a new family of barley seed Proteins (WSPN) that could be related to the qualities of barley bread. We found three WSPNs in the prolamin fraction of barley flour. They are encoded by three linked genes. None of them have previously been annotated in the barley genome. All three genes were expressed specifically in seeds and have no detectable expression in the leaves. When comparing barley varieties, we found that higher gene expression levels of the WSPN genes were correlated with better baking qualities.

P-3

Cancelled

P-4

Strengthening spring barley breeding in the Nordic region: A pre-breeding partnership

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Enhancing crop resilience to biotic and abiotic stress through pre-breeding is resource-intensive and time-consuming. Currently, global plant breeding is dominated by large multinational corporations, leaving the unique Nordic market overlooked. In response, the Nordic Council of Ministers (NMR) initiated a public-private collaboration framework in 2011, funded equally by the NMR and breeding entities, to support smaller Nordic breeding companies through sustained pre-commercial cooperation.

One such collaboration, initiated in 2012, concentrated on the pre-breeding of spring barley for disease resistance and Nordic adaptability. Spanning three phases, the initial phase involved exploring the genetic reservoir of the Nordic breeding gene pool, yielding novel resistance markers against various diseases, including cereal cyst nematodes and powdery mildew, along with allele combinations conducive to regional adaptation.

The subsequent phase aimed to diversify disease resistance and early maturation genes in adapted materials. Approximately 200 diverse genotypes, encompassing traditional varieties, breeding lines, and cultivars, underwent evaluation to pinpoint genetic resources relevant to disease resistance and other vital agronomic traits. Informed by the outcomes of the initial two phases, specific genotypes were selected and interbred to create nine multi-parent advanced generation inter-cross (MAGIC) populations combining disease resistance or early maturation traits.

The final phase focused on genotyping and evaluating MAGIC offspring across multiple Nordic field trial locations for disease resistance and various agronomic traits. Additionally, the third phase encompassed high-throughput, non-invasive phenotyping of a spring barley panel for early seedling growth under controlled settings in collaboration with IPK, Germany, aided by supplementary funding from an EPPN2020 grant.

A prerequisite for successful collaboration was building mutual trust and understanding among partners. The benefits of this public-private partnership far exceeded the challenges. The spring barley PPP project, which ended in 2020, provided Nordic researchers and breeders with valuable insights into genetics and phenotypes, along with several MAGIC populations, invaluable resources for developing locally adapted, disease-resistant spring barley cultivars. Using the barley platform, a new Nordic PPP project on spring wheat, CResWheat, was initiated in 2021 with a similar design and aim of developing climate-resilient spring wheat adapted for Nordic conditions.

P-5

Transcriptional and metabolic analyses of a tocotrienol-deficient *hvhggt* mutant revealed a relationship between vitamin E and starch synthesis in barley endosperm

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Endosperms constitute the main volume of cereal grains and supply a substantial portion of calories and food worldwide. Homogentisate geranylgeranyl transferase (HGGT), found mainly in monocot endosperm, is essential for the synthesis of tocotrienols, which are forms of vitamin E. In our previous study, we found that the tocotrienol-deficient *hvhggt* mutant exhibited shrunken grains with low starch content. However, the relationship between the vitamin E levels and endosperm development in cereals remains unclear. Here, we report that *hvhggt* mutant altered the endosperm structure with a hollow starch endosperm, defective endosperm transfer cells, and few aleurone layers but had little effect on plant growth and embryo viability. *HvHGGT* mutation significantly decreased grain carbohydrate and total starch content in endosperm by approximately 20% and 23%, respectively. KEGG pathway analysis of significantly downregulated differently expressed genes from RNA-Seq showed that the top pathways included starch and sucrose metabolism. Many genes encoding the isoforms of AGPase, granule-bound starch synthase, starch branching enzymes, soluble starch synthase, isoamylase, and other starch phosphorylases were consistently significantly downregulated. Metabolome analysis revealed a general increase in the accumulation of soluble sugars in the mutant endosperm, suggesting that soluble sugar level was not responsible for the decreased starch synthesis. Further analysis revealed that *HvHGGT* mutation increased the reduction states of NAD(H) and NADP(H) and decreased levels of ADPGlc, suggesting that redox-balance-regulated ADPGlc levels may be involved in the decrease in starch biosynthesis in tocotrienol-deficient endosperm development. Interestingly, exogenous spraying of α-tocotrienol on developing *hggt* spikes partially restored the phenotype and starch content of *hvhggt* mutant grains, verifying the relationship between vitamin E and starch synthesis. In conclusion, our study provides novel insights into the biological significance of *HvHGGT* in starch biosynthesis and endosperm development, which will pave the way for regulating vitamin E levels and grain size in cereal crops.

Keywords: Barley, *hvhggt* mutant, metabolome, endosperm starch, transcriptome

P-6

Correlation Between Seed Dormancy and Pre-harvest Sprouting Resistance in Barley

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Pre-harvest sprouting (PHS), a significant hazard in crop cereals, often leads to yield reduction and quality deterioration. Previous studies have reported that seed dormancy plays a crucial role in PHS resistance. To investigate the correlation between these two factors, we conducted tests on 274 barley cultivars to assess seed dormancy and PHS resistance. The experiments were conducted in germination chambers at a temperature of 15°C, and the results were evaluated after 7 days of germination. PHS resistance was assessed by calculating the spike germination score. The score was determined based on the average number of germinated seeds from 3-5 spikes, ranging from 0 to 10, with 10 indicating 10 or more germinated seeds. Seed dormancy was evaluated by measuring the average germination percentage of seeds from three threshed spikes. The results revealed a significant correlation between PHS resistance and seed dormancy, with a Pearson correlation coefficient of 0.38 ($P<5.27e-9$). Furthermore, the proportion of varieties with low spike germination values was higher than the proportion of varieties with low seed germination percentages, while the proportion of varieties with high spike germination values was lower than the proportion of varieties with high seed germination percentages. Additionally, the average spike germination score and seed germination percentage were lower in 2-row barley than in 6-row barley, demonstrating a significant difference in seed dormancy trait between these two types (t-test, $P<0.014$). These findings emphasize the relationship between seed dormancy and spike germination resistance in barley, albeit with a weak correlation. The presence of mechanisms within the spikes that inhibit seed germination indicates the importance of spike germination experiments in assessing PHS resistance in barley varieties.

P-7

The homology of *RBP-A* between rice and barley

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Rice *RNA binding protein A* (*RBP-A*; *Os11g0637700*) has two RNA recognition motifs (RRMs) and is annotated as a *heterogeneous nuclear ribonucleoprotein A1* (*hnRNPA1*). *hnRNPA1* is highly conserved in living organisms, and its function is related to cell differentiation and proliferation. Our previous study had shown that *RBP-A* is a negative regulator of rice grain size. There are two copies of *hnRNPA1* in rice, on chr 11 and 7; however, only one copy could be found in the barley genome (Chiou et al. 2019). In this study, the BLAST was applied using the latest released barley genome assembly (MorexV3_pseudomolecules_assembly), and two matched results were on chr 4H and 2H. It is consistent with the synteny between rice chr 11 and barley chr 4H, and rice chr 7 and barley chr 2H (Thiel et al. 2009). The locus on chr 4H is more conserved at full length, but the one on chr 2H might barely match the conserved motifs. It needs further investigation to know the functions of both genes.

P-8

Pyrenophora teres f. *maculata* induces extreme susceptibility at the heterozygous barley *Spt2* locus

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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 (1430 recombinant gametes) and 149 targeted SNPs delimited the hybrid susceptibility locus designated *Susceptibility to Pyrenophora teres 2* (*Spt2*) to an ~249 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 (166 recombinant gametes) and 180 genome wide SNPs that colocalized to the same *Spt2* locus. The CI5791 genome was sequenced using PacBio Continuous Long Read technology and comparative analysis between CI5791 and the publicly available Golden Promise genome assembly determined that the *Spt2* delimited region contained two high confidence candidate genes including a zinc finger and a pentatricopeptide repeat-containing protein.

P-9

Transcriptomic and physiological changes of spring barley in response to water deficit stress

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Drought is a common environmental factor that reduces crop productivity around the world. Drought responses in barley are influenced by multiple genes, yet specific mechanisms that contribute to this tolerance are still mostly unknown. We combined morpho-physiological and comparative RNA-sequencing analysis to identify core genes and regulatory networks associated with drought tolerance in the barley cultivar 'Giza134'. The drought-induced declines occurred in crop growth rate, relative water content, leaf area duration, flag leaf area, contents of chlorophyll (Chl) a, b and (Chl a + b), Net photosynthesis, and yield components. In contrast, the Chl a/b ratio, stoma resistance, and proline content increased significantly. After the water stress treatment, 2,462 differentially expressed genes (DEGs) were recognized, of which 1,555 genes were upregulated and 907 were downregulated in well-watered (WW) compared to water-deficit (WD) conditions, respectively. Functional enrichment analysis showed that DEGs were mainly enriched in molecular functions and biological processes related to lipid transport, lignin catabolic, regulation of abscisic acid, asparaginase activity, and beta-aspartyl-peptidase activity. Comparative transcriptomics here shows, however, that carbohydrate metabolism, iron ion binding, and oxidoreductase activity were affected in both up and- down-regulation. Regulation of photosynthesis and gibberellin biosynthetic gene expression was severely impacted, in response to water deficit. The combined analysis identified several potential key regulatory factors/ genes that may control drought tolerance in barley. Taken, those genes can be utilized for the identification of gene-associated markers in breeding programs, thus providing new insights into the extra understanding of the mechanisms involved in water stress responses in barley.

P-10

Life course monitoring of plant hormones of barleys grown in the field conditions

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As sessile organisms, plants need to sense and adapt to changes in their surrounding environment to survive and optimize their growth for reproductive success. To achieve this, plants have developed specific strategies involving unique developmental processes. Both shoot and root meristems play a pivotal role in producing various tissues and organs, while an intricate environmental stress response system ensures their survival. Key signaling molecules, known as plant hormones, are essential for orchestrating these processes. These plant hormones interact to cooperatively fine-tune and balance their physiological effects. It is widely accepted that these plant hormones are synthesized, transported, and accumulated in cells or tissues where they are required. They are also degraded or inactivated when no longer needed. Consequently, the endogenous levels of plant hormones serve as promising biological markers for assessing the developmental and physiological status of plant cells or tissues. In this study, we analyzed the endogenous levels of seven plant hormones in barley plants from four different accessions. These plants were cultivated in two distinct test fields over the course of two seasons. The analysis of the gathered data revealed that the levels of most plant hormones fluctuated, likely in response to environmental cues. For example, abscisic acid and jasmonic acid were observed to accumulate at low temperatures, while the accumulation of another hormone correlated with increasing temperatures. Comparing these hormone levels with transcriptome data, we found novel linkages between hormone levels and cellular functions. Additionally, our study demonstrated that the growth status of the plants could be predicted from their plant hormone profiles. This supports the concept of deep phenotyping as a powerful tool for comprehending crop behavior in the field.

P-11

Regulation of pre-harvest sprouting by site-directed mutagenesis of grain dormancy genes in barley

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Site-directed mutagenesis is a promising new breeding technique to efficiently generate desired mutants. Barley requires longer grain dormancy to increase tolerance to pre-harvest sprouting, on the other hand, too long grain dormancy is unsuitable for malt production. In this study, our group employed CRISPR/Cas9-based targeted genome modification to generate mutants of the *Qsd1* (*QTL seed dormancy 1*) and *Qsd2* genes for fine-tuning grain dormancy in barley. Two guide-RNAs (gRNAs) were designed for each exon of *Qsd1* and *Qsd2*, and barley transformation was performed by the *Agrobacterium*-mediated method. As a result, 19 and 24 mutation events were obtained for each gene, respectively. Molecular analysis of the T_1 generation confirmed that the mutations were inherited. These progenies included individuals in which the mutation was fixed as homozygous and the transgene was removed by segregation. After six weeks of after-ripening treatment of grains at 25°C, germination tests showed that all control lines, including the non-transgenic lines, had a germination rate of over 90%. However, the *Qsd1* and *Qsd2* mutants showed 0% for seven days after imbibition. As well, grains that did not germinate for seven days finally germinated when germination was hastened with 5% hydrogen peroxide. This suggested that the mutant grains were not lethal and had an extremely long dormancy period. These results indicate that induced mutations in *Qsd1* and *Qsd2* inhibit rapid grain germination and may contribute to the breeding of tolerance for pre-harvest sprouting in barley.

P-12

HvSPDT-L is probably involved in redistribution of phosphorus in barley

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Phosphorus (P) is an important nutrient for plant growth, but its deficiency is often a limiting factor of crop production in many areas. Improvement of P use efficiency within the plants has been proposed an important way to overcome P-deficiency, however, the mechanisms underlying internal transport of P is largely unknown, Here, we functionally characterized a novel type of P transporter gene, *HvSPDT-L* in barley. HvSPDT-L belongs to SPDT (SULTR-like phosphorus distribution transporter) subgroup in sulfate transport family. Its members in rice (OsSPDT) and barley (HvSPDT), which are highly expressed in the nodes, have been reported to be involved in preferential distribution of P to the panicles. HvSPDT-L shares 60% and 57% identity, respectively, with OsSPDT and HvSPDT. Similar to *OsSPDT* and *HvSPDT*, *HvSPDT-L* also encodes a plasma membrane-localized Pi influx transporter. However, in contrast to *HvSPDT*, *HvSPDT-L* showed much higher expression level in the leaves at both the vegetative and reproductive stages. Tissue-specificity analysis of genes expression showed that *HvSPDT-L* was mainly expressed in the phloem region of leaf sheath and leaf blade. Knockout of *HvSPDT-L* resulted in higher P accumulation in old leaves, but less P in the new leaves under P-limited condition. A short-term ^{32}P labeling experiment showed that knockout of *HvSPDT-L* hardly affected the P distribution to different organs. Taken together, our results suggest that different from HvSPDT, which is mainly involved in P distribution, HvSPDT-L probably mediates P redistribution from old leaves to new leaves for internal P translocation under Pi-limited condition.

P-13

A novel resistance resource found in Sv196, a Turkish accession of barley (*Hordeum vulgare)*, conferring resistance against *Triticum* isolates of *Pyricularia oryzae*

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Wheat blast disease is caused by a hemi-biotrophic fungus *Pyricularia oryzae Triticum* pathotype (MoT) which is posing a great threat to global wheat production. After its inception in Brazil in 1985, wheat blast disease was confined in Latin America for 3 decades. Then, this fearsome disease expanded to South Asia (Bangladesh) in 2016 and Africa (Zambia) in 2018. In the present study, we identified a unique Turkish barley landrace, Sv196, as a novel resistance resource to MoT. Screening of 274 barley landraces collected from worldwide revealed that Sv196 was resistant to Br48 (a Brazilian MoT isolate) at 22 °C at the seedling stage. When F_2 populations derived from the cross between Sv196 and cv. Nigrate (susceptible) were inoculated with Br48, resistant and susceptible seedlings segregated in a 15:1 ratio, suggesting that the resistance of Sv196 to Br48 is controlled by two major genes. Heading stage inoculation against Brazilian (Br48, Br5) and Bangladeshi isolate (T-109) at 22°C confirmed that Sv196 is resistant against both Brazilian and Bangladesh isolates. Based on the resistance reaction, these novel resistance sources will be successfully exploited for wheat blast resistance breeding.

P-14

Dissection of developmental state transition in the shoot apical meristem of barley by single meristem RNA-seq

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The timing of flowering and inflorescence development greatly affect the propagation and yield of cereal crops like barley and wheat. In the case of barley with indeterminate inflorescence, the end of the flowering period is tightly regulated to ensure reproductive success. These developmental processes continuously occur at the shoot apex and can be influenced by genetic and environmental factors. The shoot apical meristem (SAM) plays a crucial role in determining the timing and duration of the flowering period and inflorescence architecture. The SAM maintains a stem cell pool that serves as the origin of all tissues in the aerial parts of plants, allowing for prolonged developmental plasticity. However, limited molecular and temporal resolution has hindered our understanding of how developmental transitions in SAMs progress, coordinate and adapt to the environment through interaction with other organs. To address this, we developed a unique method to generate hundreds of transcriptome profiles of individual SAMs from four barley accessions with different heading dates grown in the field. Barley SAMs were computationally ordered by pseudo-time analysis, revealing the continuum of state transition, and temporally expressed genes changing over pseudo-time. We also obtained transcriptome data from leaves originating from the same individual used for SAM sampling and performed a comparative analysis of state transitions between the leaf and SAM. In this presentation, the results of these analyses will be discussed.

P-15

Mutation breeding, an affordable crop improvement strategy in challenging times: Barley mutation breeding projects in Syria and the urge for acceleration

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Countries in prolonged conflicts suffer from economic instability and inflations, factors that challenge research and development in private and public research institutes. The problem is magnified when the latter factors go together with losses of elite local varieties against climate change. Syria, centre of origin and domestication for barley (*Hordeum vulgare* L.), currently a conflict-affected region, has only 9 accredited barley varieties and two local varieties covering five agricultural zones. Critical actions for broadening of elite resilient barley germplasm and the introduction of new barley varieties with vigorous early growth, early maturity, lodging resistance, drought tolerance and increased yield are needed. Since 1960s, the currently known as classical mutation breeding by gamma rays and X-rays irradiation, has proved as reliable, safe and affordable methodology for middle- and lower-income countries. Two barley gamma irradiation mutation breeding programmes supported by the IAEA have been running in the last 12 years in Syria. Advanced mutant lines (M8) developed from Furat 9 using 100 and 200 Gy, were screened in multiple fields by minimum number of staff, using affordable and basic screening methods that fit with shortages and constraints stemmed from prolonged conflict. Four potential drought tolerant mutants (HvM7, HvM23, HvM32 and HvM69) have outperformed parent Furat 9 under drought conditions. Twenty-four M4 mutant populations developed from Furat 6, irradiated with 150, 200 and 300 Gy, were screened and five were selected to advance as lines in testing fields. Accelerating breeding programmes by half time is now achievable through precise and speed breeding, still a costly approach in low-income countries that are in most need to secure food and feed. Governments and funding agencies are urged to support components of precise and speed breeding in countries prone to food insecurity.

P-16

A chasmogamous mutant induced from a cleistogamous barley cultivar 'Haruna Nijo'

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A single recessive gene at the *Cleistogamy 1* (*cly1*) locus confers cleistogamous flowering in barley, in which the palea and lemma remain tightly closed throughout the pollination period. The *Cly1* gene encodes an AP2 transcription factor, HvAP2. The cleistogamous allele (*cly1.b*) contains a synonymous nucleotide polymorphism (SNP) at the microRNA (miR172) target site, which would inhibit the binding of miR172 to *cly1* mRNA, resulting in the expression of HvAP2 protein. The HvAP2 protein is thought to be a negative regulator of lodicule development, leading to floret closing during anthesis by inhibiting lodicule swelling. At the last Barley Mutant Workshop, we reported an open-flowering (chasmogamous) mutant (MGC) induced from a cleistogamous cv. 'Misato Golden' carrying the *cly1.b* allele and identified a novel chasmogamous *cly1* allele (*cly1.b3*) containing a missense mutation within the AP2 domain (Kakeda et al. 2018, Wang et al. 2021). In this paper, we report another chasmogamous mutant (BM) that was newly induced from cv. 'Haruna Nijo' carrying the *cly1.b* allele. The BM mutant was examined for flowering characteristics and floral organ development. Molecular and genetic analysis revealed that the BM mutant had a unique nonsense mutation in the *cly1* allele, which had not previously been found in the *Cly1*/*HvAP2* gene.

P-17

Agronomic and malting characteristics of a barley mutant line with low β-glucan content in grains

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β-Glucan is one of the major cell wall components in barley grains and is highly viscous, which causes problems such as slow beer filtration and slow lautering in brewing process. Therefore, it is considered desirable to have lower β-glucan content in grains. Several mutants with extremely low β-glucan content in grains have been screened and the causal mutant genes have been identified. However, this trait has not yet been commercially utilized because of the lower yield of the progeny lines as compared with their parent lines.

In this study, we investigated the agronomic and malting characteristics of 'Sainohoshi-975 (S-975)', a mutant line with quite low β-glucan content (less than 1 %) obtained from mutant population of 'Sainohoshi'. There was no significant difference between 'S-975' and its parental line Sainohoshi in the agronomic characteristics. Malt analysis showed lower β-glucan content in 'S-975'. We identified that the gene responsible for the low β-glucan trait in 'S-975' is mutated at a different sequence position than in the previous reports. Furthermore, we compared the agronomic characteristics between 'S-975' and back-crossed lines, which had the mutant gene from 'S-975' with the genetic background of 'Sainohoshi' to clarify the effect of this mutant gene on the agricultural characteristics. The number of spikelet and plumpness (% >2.5 mm of grain) of 'S-975' were significantly lower compared to 'Sainohoshi'. These results indicate that the extremely low β-glucan trait of 'S-975', as well as other low β-glucan mutants, negatively affect agronomic characteristics and grain morphology.

P-18

Using barley gene information to modify seed dormancy of synthetic hexaploids with genome editing technique.

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The synthetic hexaploids generated from *Triticum turgidum* (2*n* = 4*x* = 28; AABB) and diploid species in *Triticeae* tribe have been used as the novel genetic resource to improve wheat traits. For the purpose to directly alter the traits in synthetic hexaploids, we started to perform genome editing experiment. We tested 13 lines of synthetic hexaploid lines for suitability of immature embryo cultivation, propagation of callus, and regeneration of shoots. We chose two synthetic hexaploid lines, which were derived from *Aegilops tauschii* (2*n* = 2*x* = 14; DD) or *Ae. umbellulata* (2*n* = 2*x* = 14; UU). Grain dormancy is important trait for cereal production to prevent pre-harvest sprouting, which cause severe decrease of the product quality. The *quantitative trait locus on seed dormancy 1* (*qsd1*) gene was the major grain dormancy gene in barley. The repression of *Qsd1* gene expression prolongs the grain dormancy. In wheat (*Triticum aestivum* L. 2*n* = 6*x* = 42; AABBDD), a triple mutation of *TaQsd1* generated using CRISPR/Cas9 system, also resulted in longer grain dormancy. To alter the grain dormancy of the synthetic hexaploids, we determined the *Qsd1* gene sequences and induced mutation using CRISPR/Cas9 system via biolistic bombardment. We used more than five hundred embryos for transformation and finally obtained 8 regenerated plants with mutation on *Qsd1* gene. We will evaluate the grain dormancy of *Qsd1* mutant on these synthetic hexaploids.

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P-19

FLOURY ENDOSPERM 6 mutations enhance the sugary phenotype caused by the loss of ISOAMYLASE1 in barley

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Starch is a plant-derived glucose polymer that finds extensive usage in both food and industrial applications. Within the storage organs, such as seeds and tubers, starch is synthesized as semicrystalline starch granules (SGs). Morphologically, SGs vary among plant species and can be classified as compound or simple SGs. Compound SGs form from assemblies of smaller starch particles, whereas simple SGs consist of single starch particles. In rice endosperm, compound SGs typically range from 10 to 20 μm in diameter and comprise individual sharp-edged polyhedral smaller starch particles, each with 3 to 8 μm diameter. Barley and wheat endosperms have two types of simple SGs, namely the smaller B-type and the larger A-type, coexisting within a single cell, a storage type termed bimodal simple SGs. Because SG morphology affects starch properties, mutants with altered SG morphology may be useful in breeding crops with desirable starch properties, including potentially novel properties. In this study, we employed a simple screen for mutants with altered SG morphology in barley. We isolated mutants that formed compound SGs together with the normal simple SGs in the endosperm and found that they were allelic mutants of the starch biosynthesis genes *ISOAMYLASE1* (*HvISA1*) and *FLOURY ENDOSPERM 6* (*HvFLO6*), encoding starch debranching enzyme and CARBOHYDRATE-BINDING MODULE 48-containing protein, respectively. We generated the *hvflo6 hvisa1* double mutant and showed that it had significantly reduced starch biosynthesis and developed shrunken grains. In contrast to starch, soluble α-glucan, phytoglycogen, and sugars accumulated to higher levels in the double mutant than in the single mutants. In addition, the double mutants showed defects in SG morphology in the endosperm and in the pollen. This novel genetic interaction suggests that *hvflo6* acts as an enhancer of the sugary phenotype caused by *hvisa1* mutation.

P-20

Exploring the Structure of a Diverse Set of Naked Barley

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Hull adherence in barley is controlled by a single gene at the *Nud* locus on the long arm of chromosome 7H. Naked barley arose by a spontaneous mutation around 6500 BCE, likely in a domesticated covered type. In covered barley, the *Nud* allele results in production of a lipid based 'cement' secreted from the pericarp which causes the lemma and palea to adhere to the caryopsis. The *nud* allele prevents this from occurring, allowing the grain to thresh freely from the hull during harvest. Currently, there is fragmentation in barley production and end-use potential due to the presence/absence of hulls and variation in β-glucan levels. Naked barley shows potential as a crop that can be used for food, feed, and malt. A diversity panel of 384 naked entries from breeding programs and gene banks around world was assembled to characterize and analyze genetic resistance to biotic and abiotic stresses, and assess agronomic and end-use quality traits in order to establish a breeding program for multi-use naked barley in organic systems. The panel includes winter, facultative, and spring growth habits, different seed coat colors, waxy and non-waxy starch, and lines with mostly unknown end-use properties. The panel contains 130 lines that require vernalization and 254 lines that do not. This panel was genotyped on a 50K Illumina SNP chip and has been phenotyped for resistance to stripe rust, leaf rust, and stem rust, resistance to spot blotch and FHB, resistance to covered smut, lodging, grain protein, test weight, early plant vigor, resistance to embryo damage, and threshability. A structure analysis of the population resulted in genotypes clustering into six subpopulations based on origin, row type, and vernalization requirement.

P-21

The mutation frequency of the wild barley mutant collections derived from chronic gamma irradiation

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The genomic characterization of the mutant collections accelerates the forward genetics of cereal crops. Recent advances in genome sequencing technology have dissolved the bottlenecks to genome-wide genotyping even in large genome-size species such as barley (*Hordeum vulgare*). Cultivated barley (*H. vulgare* ssp. *vulgare*) was domesticated from its conspecific wild progenitor *H. vulgare* ssp. *spontaneum*, which is native to Western Asia. Three characters are most conspicuous in wild barley: brittle rachis, long seed dormancy, and two-rowed spike. These traits are undesirable in the domesticate and have been selected against early on. But they still complicate genetic analysis in wild-derived experimental populations. Despite these challenges, a large mutant population derived from wild barley accession OUH602 has been successfully developed via weak irradiation with cobalt 60 and phenotypically characterized mainly focusing on inflorescence shapes. Through the phenotypic screening of about 50,000 M3 plants, we collected several morphological mutants: six-rowed spikes, zero-rowed spikes, super-open spikes, and malformed spikes with absent tillers. Whole genome shotgun sequences (4–40x coverages) were obtained from these mutants. In this poster, we will present the mutation frequency of the mutant collections and discuss future perspectives of the mutant population for gene cloning and breeding.

P-22

Ectopic expression of *HvMADS58*, an ortholog of *AGAMOUS*, caused homeotic transformations of floral organs in Barley

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In core eudicots, the pattern of floral organs is governed by the ABC model, which is based on three classes of genes that specify floral organ identity: sepals (A-class), petals (A and B-class), stamens (B and C-class), and carpels (C-class). In the grass species, florets consist of lemma and palea (equivalent to sepal), lodicule (homologous to petal), stamen, and carpel. While rice follows the core eudicot's ABC model, it employs adapted genetic mechanisms diverging from eudicots due to gene duplication events. Notably, two rice orthologs of the C-class gene *AGAMOUS* (*AG*), *OsMADS3* and *OsMADS58*, have partially subfunctionalized roles. *OsMADS3* primarily determines lodicule development and stamen identity. *OsMADS58* mainly contributes to floral meristem determinacy and carpel morphogenesis. Barley also contains two C-class genes *HvMADS3* and *HvMADS58*, orthologous to rice *OsMADS3* and *OsMADS58*, respectively. However, the functions of *HvMADS3* and *HvMADS58* remain unclear.

In this study, we focus on the role of *HvMADS58* through its overexpression in barley. We constructed *HvMADS58* overexpressed lines that exhibit HvMADS58 cDNA expression under the control of a ubiquitin promoter in cv.‘Golden Promise’. The observations of the florets revealed several homeotic changes in its transgenic barley. In the transformants, lodicules exhibited stamen-like characteristics, and palea formation was incomplete, sometimes resulting in carpel-like alterations. These changes align with the eudicot’s ABC model and are consistent with ectopic *AG* expression in Arabidopsis.

Taken together, similar to *AG* in eudicots and rice, *HvMADS58* plays a crucial role in determining carpel identity, highlighting its significance in floral organ development in barley.

P-23

Analysis of six-rowed mutants from a two-rowed barley cultivar "Haruka Nijo"

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"Haruka Nijo" is two-rowed hulled Japanese elite barley cultivar released in 2012 by Kyushu-Okinawa agricultural research center, NARO. To produce mutant lines with useful phenotypes for barley breeding program in Japan, we mutagenized "Haruka Nijo" with sodium azide and then obtained about 2000 mutant M2 seeds. During cultivation of the M2 mutants in the field, several tens of mutants exhibited morphological alterations such as dwarf, waxless, and albino. Among them, we identified three six-rowed spike mutants and designated them as *six-rowed spike 1* (*srs1*), *srs2*, and *srs3*, respectively. *srs1* showed more proportional six-rowed spike morphology compared to *srs2* and *srs3*. Sequence analysis of *six-rowed spike 1*(*VRS1*) gene revealed that *srs1* has a nonsense mutation which leads Trp-209 to stop codon in VRS1 protein. However, *srs2* and *srs3* did not have any mutations in the coding sequence of *VRS1*. Allelism tests using *srs1* and "Haruka Nijo" (two-row, harboring *VRS1* allele) or six-rowed cultivar harboring loss-of-function *vrs1* allele confirmed that causal mutation in *srs1* is recessive and the mutation in *VRS1* is responsible for six-rowed spike phenotype of *srs1*. Further analyses revealed that causal mutation in *srs2* and *srs3* are also recessive. All the F1 plants obtained by crossing *srs1* and *srs2*, *srs1* and *srs3*, and *srs2* and *srs3* showed two-rowed spike phenotype indicating that *srs2* and *srs3* possess different causal genes other than *VRS1* for spike morphogenesis.

P-24

Analysis of deletions in wheat zygotically induced by a gametocidal chromosome

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Gametocidal (Gc) chromosomes in wheat induces chromosome breakage both gametophytically and zygotically. Zygotic induction of chromosome breakage was first notified by Tsujimoto and Tsunewaki (1985) as seed shriveling associated with chromosome mutations in the offspring. They found that *Gc1* gene from *Aegilops speltoides* causes mutations only when paternal gamete with *Gc1* was hybridized with maternal gamete without it. In this study, we studied zygotic induction of chromosome breakage by *Gc2* from *Ae. sharonensis*. First, parent-of-origin effect on zygotic induction was confirmed by cytological observation of the isolated zygotes after a given period of time after artificial fertilization. Chromosome breakage occurred in the first zygotic mitosis only in the cross between female gamete without and male gamete with *Gc2*, confirming the parent-of-origin effect on induction of chromosome breakage. Next, by utilizing genome-wide heterozygous conditions in an F_1 between wheat cultivars and a genome-wide marker system GRAS-Di, we observed loss-of-heterozygosity in the offspring of the cross to induce zygotic chromosome breakage. We recognized 189 deletion events in total and there were no preferences on the parental genomes. Deletion frequency was 0.98 per plant and most of them were terminal deletions or deficiencies. Lastly, we tried to delete genes conferring undesirable traits in a synthetic wheat. A hexaploid synthetic wheat (2n = 6x = 42, genome constitution AABBA^mA^m) between *Triticum durum* cv. Langdon (2n = 4x = 28, AABB) and an accession of *T. boeoticum* (2n = 2x = 14, A^mA^m) was crossed with the *Gc2* carrier to induce chromosomes breakage zygotically. As examples of undesired genes, we targeted to delete three genes *Btr1*, *Rht1*, and *wPCL1*, for which we could design specific PCR markers to distinguish the A^m homoeologue from others. In 288 plants of the direct offspring of zygotic induction, we found 8, 4, and 56 individuals lucking the *Btr1-*A^m, *Rht1-*A^m, and *wPCL1-*A^m.

P-25

QTL analysis of flowering time in barley using a rapid genotyping method, MIG-seq

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Multiplexed inter-simple sequence repeat genotyping by sequencing (MIG-seq) is known to be an effective genotyping method for wheat with large genome size (Nishimura et al. 2022). Since the MIG-seq workflow is a simple two-times PCR to construct NGS libraries, this method is expected to be applied to genetic analysis of various crops. In this study, we evaluated the effectiveness of MIG-seq in barley, a species closely related to wheat with a relatively large genome size, through QTL analysis on flowering time. First, a MIG-seq library was constructed using DNA from F_7 RILs derived from a cross between two Japanese barley cultivars 'Kashimamugi' and 'Ishukushirazu'. We succeeded in constructing a linkage map consisting of 12,681 markers detected via MIG-seq. Next, QTL analysis was performed by r/qtl using days from sowing to heading for F_7 and F_8 generations. QTL analysis using this linkage map revealed QTLs on chromosomes 2H, 4H and 5H. The 2H and 5H QTLs were considered to correspond to the *HvCEN* and *VRN-H1*/*HvPhyC* regions, respectively. For the 4H QTL, there were no corresponding known genes. A three-way ANOVA using genotypes of the 2H, 4H and 5H QTLs showed a significant interaction between the 2H and 4H QTLs for both F_7 and F_8 generations. These results indicate that MIG-seq is effective in barley genotyping.

P-26

The exploration of novel alleles affect heading time by exome-sequencing of barley mutants

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Barley is a major grain crop used for brewing, food, and livestock feeding. For its stable production, heading at the optimum time is very important. For efficient improvement of heading time, it is essential to elucidate the underlying mechanisms through the analysis of the single gene effects and intergenic interactions. Several heading-time genes have been identified so far. However, allelic variations are limited for many of the genes (e.g. only functional alleles are known in the *HvPhyC*). This has been a limiting factor for the analysis of intergenic interactions. To exploit novel allelic variations that do not exist in natural variations, we screened a mutant population of the early-heading variety 'Haruna Nijo' that was provided by NBRP Barley. 40 heading-time mutants (early: 15, late: 25) were selected and subjected to exome-sequencing. The result showed that a single mutant possessed mutations (missense and nonsense) in 282 genes on average. Several mutant genes were found to be the Arabidopsis flowering-time orthologs such as *FLD*, *FCA*, *PhyB*, and *PhyC*. To identify causal genes for heading time, we developed eleven F2 populations derived from the cross between the mutants and 'Haruna Nijo'. The heading time of three populations clearly segregated early ('Haruna Nijo')-type and late (mutant)-type plants with segregation ratios of 3:1, indicating the existence of one recessive gene for late heading in three parental mutants. We are conducting the linkage analysis and Mutmap to identify the causal genes in these mutants.

P-27

Empirical genetic research for sustainable barley production under warming environment

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Crop production plays a crucial role in achieving Planetary health by ensuring the well-being of billions of people and the planet. The impact of global warming on crop growth extends throughout their life cycle, with numerous predictive studies indicating that crops are vulnerable to its impacts, leading to significant yield reductions. However, previous studies often overlook factors such as the plasticity in response and genetic variability, while empirical studies in field environments are still limited. Global warming profoundly affects crop growth across the life cycle with broadly developmental and physiological aspects, resulting in severe productivity consequences. To preemptively address climate change scenarios through breeding, it is essential to understand the genetic structure underlying the effects of warming and their genetic variations. Particularly in the case of barley, genetic insights into high-temperature responses are scarce. To advance preemptive breeding strategies, empirical genetic research is crucial. This study focuses on elucidating the genetic structures necessary for stable barley production under a warming field environment, simulating a sub-tropical climate. Diverse responses among barley germplasm were revealed under the cultivation environment, with notable effects on yield components such as grain number and weight per plant. The study involves crossbreeding involving a malting variety and a Japanese landrace, aiming to evaluate their influence on agricultural traits. The results highlighted the genetic regions influencing grain numbers per plant, indicating the potential to mitigate the adverse effects observed in the malting variety. Through these findings, the study emphasizes the importance of understanding genetic factors to enhance barley's resilience to climate change and ensure future food security.

P-28

Evaluation of brewing characteristics of 9-/13-HPL-less mutant barley

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Several enzymes involved in lipid metabolism are present in barley. Lipoxigenase-1(LOX-1) and LOX-2 catalyse the formation of 9-hydroperoxy-octadecadienoic acid(9-HPOD) and 13-HPOD respectively, and subsequent reactions are catalysed by 9-/13-hydroperoxide lyase(9-/13-HPL) and 13-HPL. 9-HPOD is metabolized into substances such as *trans*-2-nonenal (T2N) and trihydroxyoctadecenoic acid (THOD), which are known to negatively affect beer freshness and foam stability. It has been reported that beers using barley varieties lacking LOX-1 have reduced T2N and THOD.

In this study, a large mutant population was generated by ion beam treatment of the LOX-1 lacking barley variety "Satuiku 2 go" for further reducing T2N and THOD. Results of screening for mutants lacking enzymes involved in lipid metabolism such as LOX-2, 9-/13-HPL and 13-HPL, mutant barley lacking 9-/13-HPL was obtained. Evaluation of wort from this mutant barley showed lower T2N content compared to the control variety "Satuiku 2 go". In addition, brewing trials using this mutant barley showed a slight decrease in T2N content, although no significant differences were observed in sensory tests.

P-29

Barley *albino lemma 1* mutants

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Barley (*Hordeum vulgare* L.) *albino lemma 1* (*alm1*) mutants exhibit conspicuous albinism in spikes, whereas leaf blades are normal green. Our positional cloning identified that the *alm1* mutation represents a gene locus encoding one of two GOLDEN2-LIKE transcription factors in barley (HvGLK2). GLK2 is a plant specific transcription factor and regulates chloroplast development. Photosynthesis was measured using the second leaf of the three leaf stage seedlings of wild type (cv. 'Misato Golden') and its *alm1.g* mutant. Leaf photosynthesis ability was similar between them. The *alm1.g* mutation caused a 34% reduction in spike photosynthesis at the heading stage, which likely accounts for the 15.8% reduction in the grain weight. Expression analyses of *HvGLK2* and its homologue *HvGLK1* suggested distinct, non-redundant roles for *HvGLK2*. *HvGLK2* could be an important target for yield improvement in barley breeding.

P-30

SNP markers associated with grain quality traits in a barley collection harvested in Kazakhstan

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Barley (Hordeum vulgare L.) is an important cereal crop with high genome plasticity that is cultivated in all climatic zones. Traditionally, barley grain is used for animal feed, malting, brewing, and food production. Depending on the end-use product, there are individual requirements for the quality traits of barley grain, particularly for raw starch and protein contents. This study evaluates a collection of 406 two-rowed spring barley accessions, comprising cultivars and lines from the USA, Kazakhstan, Europe, and Africa, based on five grain quality traits (the contents of raw starch, protein, cellulose, and lipids, and grain test weight) over two years. The results of population structure analysis demonstrate the significant impact of geographical origin on the formation of subclusters in the studied population. It was also found that the environment significantly affects grain quality traits. Heat and drought stresses, particularly during grain filling, led to higher protein and lower starch contents. A genome-wide association study using a multiple-locus mixed linear model allowed for the identification of 26 significant quantitative trait loci (QTLs) for the five studied grain quality traits. Among them, 17 QTLs were found to be positioned close to known genes and previously reported QTLs for grain quality in the scientific literature. Most of the identified candidate genes were dehydration stress and flowering genes, confirming that exposure to heat and drought stresses during grain filling may lead to dramatic changes in grain quality traits, including lower starch and higher protein contents. Nine QTLs were presumably novel and could be used for gene mining and breeding activities, including marker-assisted selection to improve grain quality parameters.

P-31

Pre-breeding for pre-harvest sprouting and pathogen resistance in Nordic spring wheat

Ronja Wonneberger[1] and the CResWheat Consortium[1-9]

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The cultivation of spring wheat (*Triticum aestivum* L.) holds significant importance under the climatic conditions of northern Europe, particularly in regions where winter wheat cultivation faces challenges. Cool and wet winters can delay sowing, while wet autumns can increase the risk of pre-harvest sprouting and loss of grain yield and quality. The impacts of climate change, including heightened occurrences of extreme weather events such as heat and drought stress and changed precipitation patterns, are anticipated to further exacerbate these challenges. Additionally, pests and diseases are expected to become an increasing problem in this region.

The CResWheat project is a collaborative pre-breeding effort involving researchers and spring wheat breeders from the Nordic region. Its primary objective is to improve climate resilience in Nordic spring wheat by improving yield, yield stability and self-sufficiency in this region. We have screened Nordic and exotic spring wheat in multi-environment trials for agronomic traits, pre-harvest sprouting resistance and resistance to various pathogens and are now focusing on identifying quantitative trait loci (QTL) and genetic markers linked to these traits to be utilized for marker-assisted selection.

Based on phenotypic performance and genetic analyses, we have selected promising genotypes as donors of desired traits and have initiated crosses with adapted elite material. In the next phase of the project, these resulting populations will be genotyped and screened in multi-environment trials across the region to identify genetic markers. Suitable, adapted lines with combinations of desired traits will be identified for use in Nordic breeding programs.

To further future-proof Nordic spring wheat, we are going to screen a collection of landraces, cultivars and exotic accessions from the Nordic Genetic Resource Center (NordGen) to identify resistance sources against gout fly (*Chlorops pumilionis*), a newly emerged pest in Nordic spring wheat, to be introgressed into adapted elite germplasm.

P-32

Comparing Enzymatic Activities in Barley and Sorghum During Grains Germination

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The germination of grains initiates internal transformations, activating intrinsic enzymes and augmenting attributes such as nutritional composition, flavour profiles, and textural qualities. To enrich the variety of raw materials available for craft beer production in Taiwan, this study primarily focused on the germination-malting processes of six-rowed barley sourced from Taichung and "San-Lin-Sui" sorghum from Changhua. A comparative assessment of the α-amylase and β-amylase activities was conducted across different germination durations. The results revealed a significant increase in the α-amylase and β-amylase activities of six-rowed barley throughout the germination process. On the third day of germination, the α-amylase and β-amylase levels reached their maximum values at 103.70 CU/g and 26.03 BU/g, respectively. Similarly, the α-amylase content of "San-Lin-Sui" sorghum also exhibited an upward trend with progressing germination time, rising from 0.10 CU/g to 7.29 CU/g. These results demonstrate that germinated six-row barley exhibits α-amylase activity comparable to commercial varieties, while its β-amylase activity surpasses that of commercial varieties. Regrettably, both α- and β-amylase activities in sorghum are notably lower than those in barley. These outcomes imply that the starch composition of sorghum may not be conducive to successful malting for subsequent mashing, or that our malting process may be optimized for barley but not for sorghum. In forthcoming research, we aim to enhance grain processing versatility by refining malt processing methods and enzyme extraction conditions.

P-33

Elucidation of barley response to *Barley yellow mosaic virus* and *Japanese soil-borne wheat mosaic* virus in the mixed infestation field

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Soil-borne viruses, such as *Barley yellow mosaic virus* (BaYMV) and *Japanese soil-borne wheat mosaic virus* (JSBWMV), cause mosaic diseases that severely reduce yield and quality in winter barley. BaYMV belongs to the genus *Bymovirus* within the family Potyviridae, while JSBWMV belongs to the genus *Furovirus* in the family Virgaviridae. BaYMV and JSBWMV are transmitted by the ubiquitous soil-borne plasmodiphorid protist *Polymyxa graminis* to the roots. However, the dynamics of mixed infection involving these two viruses within a host plant remain unexplored. The purpose of this study is to understand the mixed infections of BaYMV and JSBWMV within the same individual barley plant. In the mixed infested field, we analyzed the titer changes of BaYMV and JSBWMV in the roots and leaves of the susceptible cv. Kashimamugi from December to April by RT-qPCR assay. JSBWMV infection in barley roots manifested approximately four weeks after sowing, which was four weeks earlier than BaYMV. Both viruses were transferred to and multiplied in the leaves in close proximity, occurring in early January. Our investigation also encompassed the localization of the virus coat proteins and viral RNA in barley root and leaf, which were detected in the cortex (roots), phloem, and mesophyll (leaves). Furthermore, we examined the responses of various barley pan-genome accessions against BaYMV and JSBWMV. cv. Morex was resistant to BaYMV but susceptible to JSBWMV, while cv. Igri was susceptible to BaYMV but resistant to JSBWMV in leaf. This study provides new insights into mixed viral infections in barley, not only for studying virus-virus interactions within the same host plant but also for understanding their effects on the development of soil-borne virus epidemics and the evolution of virulence.

Keywords: *Barley yellow mosaic virus*, *Japanese soil-borne wheat mosaic virus*, mixed infection, barley

P-34

Collection, preservation and distribution of *Oryza* genetic resources by National Bioresource Project RICE (NBRP-RICE)

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Biological resources are the basic infrastructure of bioscience research. Rice (*Oryza sativa* L.) is a good experimental model for research in cereal crops and monocots and includes important genetic materials used in breeding. The availability of genetic materials, including mutants, is important for rice research. In addition, *Oryza* species are attractive to researchers for both finding useful genes for breeding and for understanding the mechanism of genome evolution that enables wild plants to adapt to their own habitats. NBRP-RICE contributes to rice research by promoting the usage of genetic materials, especially wild *Oryza* accessions and mutant lines. Our activity includes collection, preservation and distribution of those materials and the provision of basic information on them, such as morphological and physiological traits and genomic information. NBRP-RICE is managed by National Institute of Genetics and Kyushu University. National Institute of Genetics mainly provides about 1700 accessions of wild *Oryza* species covering 21 species in genus *Oryza*, and their genetic/genomic information through our database, Oryzabase (https://shigen.nig.ac.jp/rice/oryzabase/). Kyusyu University mainly provides experimental lines, such as mutation lines of cultivars, chromosome segment substitution lines (CSSLs) of wild *Oryza* species and cultivars. Genomic information of the mutation lines is provided through MiRiQ database (https://miriq.agr.kyushu-u.ac.jp/index.php). In our poster, we will introduce the activities of NBRP-RICE and our database, Oryzabase and MiRiQ database, which facilitate the access to NBRP-RICE resources and their genomic sequences.

P-35

The National BioResource Project (NBRP) KOMUGI, Japan

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The National BioResource Project (NBRP) KOMUGI (= wheat), Japan, is a gene bank to collect, store and provide *Triticum* and *Aegilops* species. It was initially started in 1996 by a voluntary group of Japanese scientists who were worried about genetic resources of wheat and its related species conserved in several institutions in Japan. Our genetic stocks include *Triticum* and *Aegilops* species (15,652 lines) collected during scientific expeditions by Dr. Kihara, Dr. Tanaka and their colleagues, experimental lines (1,576 lines) including barley chromosome dissection lines, and nested association mapping population lines of Asian common wheat (4913 lines). NBRP KOMUGI is unique in a large resource of landraces, wild-related species and experimental strains compared with other gene banks. We developed KOMUGI, an integrated database site for wheat research. The KOMUGI database is the contact point for seed distribution requests to the National BioResource Project (NBRP) Wheat. In addition, the KOMUGI database provides polymorphisms among 48 accessions including three *Hordeum* species for 827 SSR markers, spike pictures of 2287 accessions, 169 chromosome images, and wheat current and classical classification tables. We are updating the website highly user-friendly by linking seeds with biological information such as genomes and phenotypic traits and collection site information based on field notebooks of the expeditions. We are also developing novel recombinant inbred lines integrated with genome information and will plan to distribute these seeds to researchers in future.

P-36

National Bioresource Project –Barley–
Genetic resources for research of barley genome diversity

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Barley is an important crop plant that serves many purposes, including malting, food, and animal feed. Barley is used as a model plant of cultivated wheat for the purpose of genetics and genomics, since barley is diploid and has a simpler and smaller genome in Triticeae species. National Bioresource Project of Japan –Barley– (NBRP-Barley) preserves the germplasms of ca. 20,000 barley accessions including landraces, breeding cultivars/lines, experimental lines, and wild relatives collected from the habitat area of the world. In addition, the barley genetic resources, particularly mapping population, core collection, and mutants have been collected in this project. On the other hand, genomic resources including full-length cDNA libraries, genome DNA samples of seed collections as well as the fingerprint data of germplasms have been also developed and provided. Furthermore, genome assemblies of the several accessions have been released. This activity contributes to the research community, and several outstanding papers have been published related to genome diversity and important agronomic traits; grain dormancy, non-brittle rachis, aluminum tolerance, salt tolerance, and cadmium accumulation in barley. Recently a part of barley germplasms had been deposited in the Global Seed Vault at Svalbard, Kingdom of Norway as a safety duplication of genetic resources.