eMonogram 2021

1st–4th June 2021

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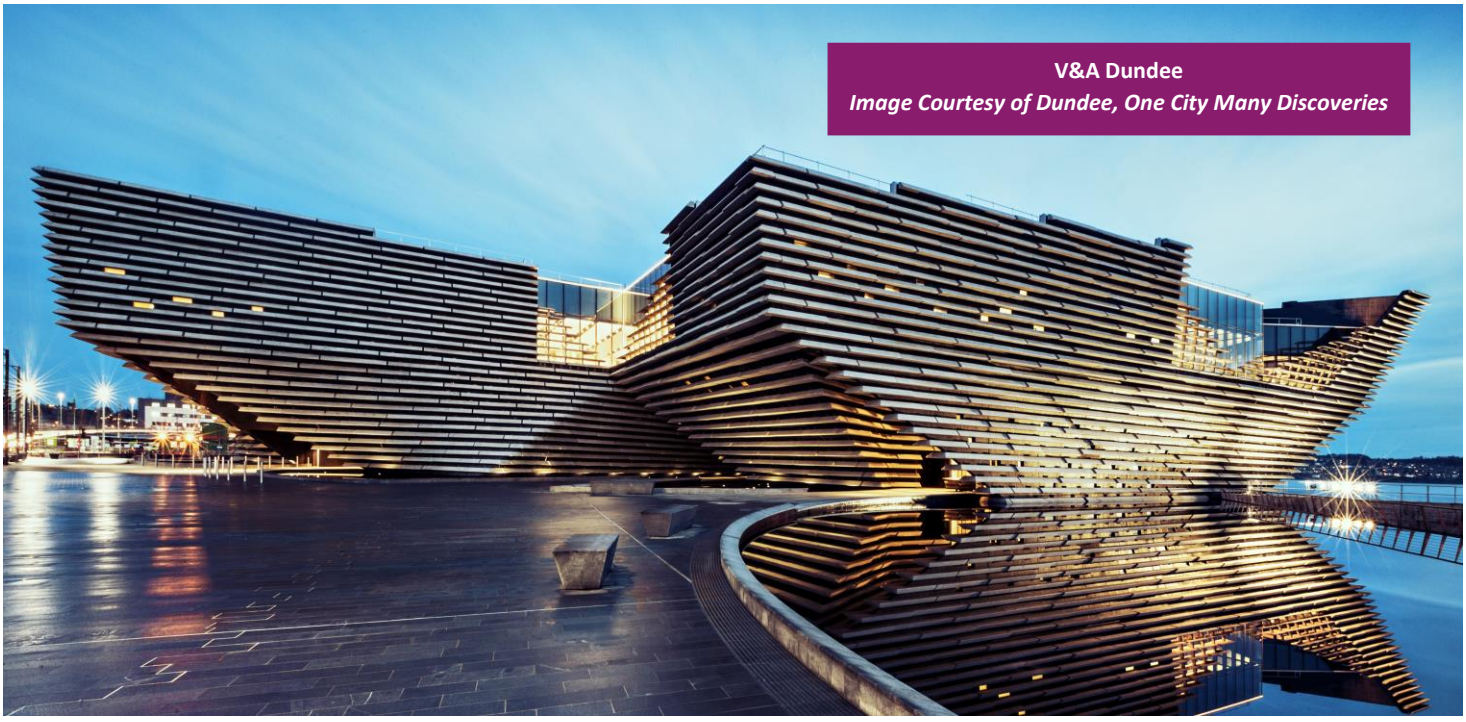
The James Hutton Institute

Conference Programme

#monogram21

We are delighted by the generous support and collaboration we have received from our Partners. Our thanks and gratitude go to everyone involved.



V&A Dundee
Image Courtesy of Dundee, One City Many Discoveries

Welcome

It gives us great pleasure to finally welcome you to Dundee for Monogram 2021. As we all know, Monogram 2020 was postponed due to COVid19 and unfortunately due to serious technical issues we had to postpone Monogram21 therefore we feel like it's third time lucky in terms of organising this conference! We'd like to thank everyone for their patience and fantastic support over the last few weeks and your excellent abstract contributions and we can't wait to finally meet up albeit virtually and enjoy a great week of excellent science.

Please interact with other delegates using the virtual conference platform and remember to use the hashtag #monogram21 if you mention the conference on twitter. Throughout the week, please take the opportunity to visit posters booths and speak to the presenters about their work and please chat to fellow delegates in the networking section of our conference platform.

Due to the sensitive and confidential nature of the content being shared at the conference, we will not be recording this event and we kindly ask that you respect the privacy of the content within the presentations and posters. If you require technical assistance during the conference, please contact us using monogram21@hutton.ac.uk as this inbox will be monitored during the conference.

On Thursday evening we are delighted to have the opportunity to listen to Sir Geoff Palmer OBE speak about his "Life Scientific" during our evening lecture. We are sure this will be an inspiring talk which will encourage discussion during the Q&A session.

We are grateful to our sponsors for providing support to allow the conference to take place this year. In particular we would like to thank Dundee & Angus Convention Bureau who have provided practical support to help us make this conference happen. We would also like to thank the James Hutton Institute communications team for their help in organising and delivering this conference, in particular Nicola Strachan & Zoe Bonar, for running the virtual meeting platform.

Please enjoy the virtual experience and thanks for your continued support for the Monogram community.

Warm regards Kelly Houston & Malcolm Macaulay

Join the Conversation #monogram21

We will be active on social media throughout the conference. We encourage you to share stories, photographs and comments.

International Barley Mutant Workshop 2018 - Tour of the barley mutants which was set up on the James Hutton Institute site in Invergowrie, Dundee

Acknowledgments

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Conference Booklet designed by Malcolm Macaulay, Paul Shaw, Tracy Duncan & Debbie Ree

Our Invited Speakers

We are delighted to introduce you to our invited speakers and look forward to hearing more about their research and expertise at our first virtual Monogram 2021 Network Meeting.

Sir Geoff Palmer OBE

Sir Geoff Palmer OBE
Professor Emeritus, School of Life Sciences, Heriot-Watt University, UK

Sir Geoff Palmer OBE is Professor Emeritus in the School of Life Sciences at Heriot-Watt University in Edinburgh, Scotland, and a human rights activist.
He discovered the barley abrasion process whilst a researcher at the Brewing Research Foundation from 1968 to 1977. In 1998, and became the fourth person, and the first European, to be honoured with the American Society of Brewing Chemists Award of Distinction, considered the "Nobel prize of brewing". In 1989, he became the first black professor in Scotland, becoming a Professor Emeritus after he retired in 2005. He was knighted in the 2014 New Year Honours. We will hear Sir Geoff's inspiring 'Life Scientific' story including arriving in the UK from Jamaica in 1955, revolutionising the malting industry to his human rights work.

Dr Martin Mascher

Dr Martin Mascher
Independent Research Group Leader 'Domestication Genomics', Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany

Martin Mascher is head of the research group "Domestication Genomics" at IPK Gatersleben. A mathematician by training, his research focusses on topics in plant bioinformatics and computational genetics. He has developed algorithms and pipelines for assembling genome sequences and analysing resequencing data of crop diversity panels. Recently, his group has analysed sequence data from plant genetic resources, crop wild relatives and archaeological samples to understand domestication and crop evolution in cereals.

Prof Anne-Katrin Mahlein

Prof Dr Anne-Katrin Mahlein
Head of the Institute of Sugar Beet Research, Göttingen, Germany

I am a trained Phytopathologist and studied agriculture at the Rheinischen Friedrich-Wilhelm-Universität Bonn, Germany.

Since 2017, I am Head of the Institute of Sugar Beet Research (IfZ) Göttingen, Germany. Study plant-pathogen interactions and ways to visualize complex reactions going on inside plants, with optical sensors and digital technologies. At the IfZ, my team and I are responsible for developing innovative sugar beet cultivation practices. This considers all aspects of sugar beet and table sugar production, like yield and quality forming, and plant diseases and protection. For plant phenotyping, we develop and use modern machines, sensors, robots, analyzing tools, field, and laboratory studies. I am currently involved in the Cluster of Excellence 'Phenorob' and we lead the digital experimental field 'Farmerspace'.

Dr Manny Delhaize

Dr Manny Delhaize
Honorary Visiting Scientist, ANU (Australian National University), Canberra, Australia.

Manny Delhaize is an Honorary Visiting Scientist at the ANU (Australian National University) in Canberra, Australia. He received his PhD from Murdoch University, Western Australia in 1987 and then undertook a Postdoctoral Fellowship at the Los Alamos Laboratories in New Mexico USA. After another Postdoctoral Fellowship with CSIRO Plant Industry (as it was known then) he became a research scientist. He was with CSIRO for about 30 years to become a Principal Research Scientist with Agriculture and Food in Canberra before joining the ANU.

His early work focussed on acid soils and in particular on unravelling mechanisms of aluminium tolerance in cereals and cloning the underlying genes. Subsequently his research included work on phosphorus nutrition with a focus on identifying genes that are important for improved efficiency of phosphorus uptake from soil. More recently he studies wheat mutants with altered root morphologies for developing wheat germplasm with improved traits and to understand underlying mechanisms and genes.

Prof Julie Gray

Prof Julie Gray
Professor of Plant Cell Signalling, University of Sheffield, UK

Julie Gray is the Professor of Plant Cell Signalling at the University of Sheffield. Her group study stomatal development and function. Recently, they have created barley, wheat and rice varieties with fewer stomata that have improved drought tolerance and reduced water requirement. Her research group's work on rice has been highly commended by UNESCO and recently featured in a BBC World documentary, and their work on Mexican beans won a Newton Prize. They also study the molecular pathways that allow stomata to open and close and have shown that these pathways evolved soon after the appearance of stomata in the fossil record over 400 million years ago.

Julie studied Biochemistry at Liverpool University, and was a PhD student at Nottingham University where she researched genes that control fruit ripening and was part of the team who created slow ripening tomatoes. She won Royal Society funding to carry out postdoctoral work at the University of Melbourne where she investigated how flowers prevent self-pollination.

Dr Tomislav Cernava

Dr Tomislav Cernava
Assistant Professor, Graz University of Technology, Austria

Dr Tomislav Cernava is an Assistant Professor at Graz University of Technology (Austria) and a guest professor at Guizhou University (China). Currently, he is holding several national as well international research projects at the Institute of Environmental Biotechnology (Graz University of Technology), where he obtained his PhD in 2015.
Dr. Cernava's research is focused on the plant microbiome, targeted modulation of microbial communities, host-microbe interactions, and the application of "meta-omics" techniques as an explorative tool in environmental microbiology. So far, he has authored more than 50 peer-reviewed publications, which mostly focus on potentially useful traits of the plant microbiome and specific microbes therein. He is serving as associate editor for the Nature Springer journal Microbiome, which aims to deliver substantial advances in the field of microbiome research by providing detailed insights into host/community interactions.

Dr Kay Trafford

Dr Kay Trafford
Head of the Grain Quality Research Group, NIAB, Cambridge

Kay Trafford is head of the Grain Quality research group at NIAB, Cambridge.
She was trained in plant biochemistry, and her research focus has been primarily on starch metabolism.

Current research projects include control of starch granule size and number in wheat, barley and oats, control of amylose content in wheat, the influence of embryo-size on grain quality and the genetic determination of transformability in barley.

Dr Mónica Pradillo

Dr Mónica Pradillo
Associate Professor/Lecturer in the Department of Genetics, Physiology and Microbiology (Faculty of Biology), Universidad Complutense de Madrid, Spain

I graduated in Biology with a major in Genetics at the Universidad Complutense de Madrid (Spain).

Since 2019, I am an Associate Professor/Lecturer in the Department of Genetics, Physiology and Microbiology (Faculty of Biology) in the same University.

My research has been mainly focused in plant meiosis. I am particularly interested in elucidating the mechanisms that control pairing, synapsis and recombination, as well as the relationships between them, using a combination of molecular cytogenetics and molecular cell biology approaches. I apply these techniques mostly in the plant model Arabidopsis thaliana. I consider that my research could contribute to gain control over meiotic recombination. This knowledge will be essential for breeders to effectively engineer the allelic composition of plant chromosomes.

Dr Philippa Borrill

Dr Philippa Borrill
Lecturer in Plant Biology at the University of Birmingham, UK

Dr Philippa Borrill is a Lecturer in Plant Biology at the University of Birmingham, UK. Her research uses genetic and genomic approaches to understand how polyploidy influences gene expression and ultimately phenotype in wheat. Her lab is particularly interested understanding the genes regulating senescence and nutrient remobilisation to improve grain nutrient content. Her work on wheat genomics and transcriptomics has been recognised by awards such as the International Wheat Genome Sequencing Consortium Leadership Award 2017 and the New Phytologist 2019 Tansley Medal for Excellence in Plant Science.

Philippa studied Natural Sciences at the University of Cambridge and obtained her PhD from the John Innes Centre studying the genetic control of wheat senescence. She won a BBSRC Anniversary Future Leader Fellowship, held at the John Innes Centre, in which she analysed the gene networks that control senescence and nutrient remobilisation in wheat.

Prof Anthony Hall

Prof Anthony Hall
Head of Plant Genomics, Earlham Institute, UK

Professor Anthony Hall is the head of Plant Genomics at the Earlham institute in the UK, previously he was the Holbrook Gaskell Chair of Botany at the University of Liverpool. He has published over 70 papers including Nature (3), Science (1) and PNAS (1), together with discipline specific journals, Plant Cell, Genome Biology and Molecular Systems Biology. His plant genomics group started in 2009 and has focused on using and developing next generation genetic approaches to address key questions in plant science. In 2010 he was awarded a senior BBSRC research fellowship entitled "Developing Next Generation Genetic Tools for Wheat". To date he has played a leading role in generating the first draft wheat genome(s) (Brenchley *et al*, Nature 2012, Walkowiak et al. Nature 2020) and epigenome (Gardiner *et al.* Genome Biology 2015). He led an international consortium to develop a wheat exome capture and re-sequencing platform in collaboration with NimbleGen (Winfield *et al.* 2012, Allen *et al.* 2011, Jordan *et al.* 2015). His group have developed novel strategies for the identification of EMS induced point mutations in *Arabidopsis* using high throughput sequencing (Ashelford *et al.* Genome biology 2011) and extended these to wheat (Gardiner *et al.* Plant Journal 2014, Gardiner *et al.* Plant Journal 2016).

He has worked as an Arabidopsis molecular geneticist for 25 years, focusing on the field of plant circadian biology. His groups work has provided key insights into the molecular basis for temperature compensation of the Arabidopsis circadian oscillator (Gould et al. Plant Cell 2006), this has been expanded to include a first temperature compensated mathematical model of the clock, identifying an interaction between light and temperature in the control of period (Gould *et al.* 2012). His more recent work in collaboration within a collaboration with James Locke at the Sainsbury Laboratory Cambridge has used experiment and mathematical modelling approach to investigate the clock at the single cell level in a multi-cellular context.

Conference Programme

Tuesday 1st June 2021

Session 1	Development
	This session is kindly sponsored by KWS
	Chaired by Chiara Campoli & Thorsten Schnurbusch
09:20	**Robbie Waugh** - Welcome
09:30	**Julie Gray,** *Engineering Stomatal Development*
09:50	**Bethany Love**, *Raising wheat yield potential through optimised plant hormone spike signalling*
10:05	**Annis Richardson**, *Insights into the evolution of grass leaves*
10:20	**Nikolai Adamski**, *A dosage-dependent response to the Triticum polonicum VRT-A2 allele causes elongated glumes and grains in hexaploid wheat*
10:35	**Q & A**

10:50	**BREAK**

Session 2	Recombination & Pollen Development
	This session is kindly sponsored by Biosearch
	Chaired by Malcolm Macaulay & Isabelle Colas
11:10	**Mónica Pradillo,** *Meiotic nuclear envelope is required for proper chromosome dynamics and homologous recombination*
11:30	**Abdul Kader Alabdullah**, *A novel pollen profiling approach and its application in studying wheat meiosis*
11:45	**Andrea Matros,** *GrainCorder: A hand-held sensor for monitoring fructan profiles in cereal grain*
12:00	**Mikel Arrieta**, A *suppressor screening on MLH3 mutants show the role of a RECQL4 mutation on restoring fertility and increasing overall recombination in barley*
12:15	**Q & A**

12:30	**SESSION ENDS**

Tuesday 1st June 2021

Bioinformatics session

	New and updated Triticeae resources **Chaired by Philippa Borrill & Jemima Brinton**
13:30	**Ricardo Ramirez-Gonzalez**, *Haplotype viewer*
13:45	**Guy Naamati**, *Pangenome on Ensembl Plants*
13:55	**Rachel Rusholme-Pilcher/Ben White**, *Pan-transcriptome*
14:10	**Q & A**
14:25	**BREAK**
	Databases and pipelines for integration and visualisation **Chaired by Philippa Borrill & Jemima Brinton**
14:40	**Wenbin Guo**, *3D RNA-seq*
14:50	**Mark Winfield**, *Cereals DB inc. minimal marker sets for variety identification*
15:00	**Paul Shaw**, *Bits to Bytes: Software development for genetic resources, genetics and plant breeding*
15:15	**Keywan Hassani-Pak**, *Update on KnetMiner*
15:20	**Q & A**
15:35	**BREAK**
15:50	**Panel Q & A "Current and future challenges in Triticeae bioinformatics"** **Chaired by Philippa Borrill**
	Robbie Waugh/John Baison/Sarah McKim/Jemima Brinton/Rowena Haardt
16:30	**SESSION ENDS**

Wednesday 2nd June 2021

Session 1	Genomics & Technologies I
	This session is kindly sponsored by DSV
	Chaired by Donal O'Sullivan & Davide Bulgarelli
09:30	**Tomislav Cernava,** *Implications of the seed microbiome for plant health*
09:50	**Benedict Combes**, *genomic analysis of 20 hexaploid wheat/AM. muticum introgression lines reveals new introgressed segments, homoeologous translocation of segments, extensive structural disruption, and patterns of gene expression*
10:05	**Carmen Escudero-Martinez**, *Mapping barley genes shaping the rhizosphere microbiota*
10:20	**Helen Brabham**, ***MECEA 2020 PhD Prize Winner***, Recognition of Mildew, Rust, and Blast by the Immune Receptor *Mla.*
10:35	**Q & A**

10:50	**BREAK**

Session 2	Genomics & Technologies II
	This session is kindly sponsored by SSCR
	Chaired by Laura Dixon & Matt Moscou
11:10	**Martin Mascher,** Long-read sequence assembly in barley: the updated MorexV3 reference genome and prospects for pan-genomics
11:30	**Jemima Brinton**, *A haplotype-led approach to increase the precision of wheat breeding*
11:45	**Goetz Hensel**, *Precise gene editing using ribonucleoprotein-complexes*
12:00	**Q & A**

12:15	**SESSION ENDS**

Thursday 3rd June 2021

Session 1	**Abiotic & Biotic Stress**
	This session is kindly sponsored by Novogene
	Chaired by Tim George & Ruth Bryant
09:30	**Manny Delhaize,** *Root mutants of wheat: a source of diversity for improved phosphorus nutrition*
09:50	**Helen Pidon**, *Positional cloning of two resistance genes to barley viral diseases from a wild relative*
10:05	**Surbhi Grewal**, *Molecular characterisation and trait analysis of wheat-triticum timopheevii introgression lines reveals new source of FHB resistance*
10:20	**Kelly Houston**, *A Grain of Salt: naturally occurring variants of HVHKT1;5 determine barley grain sodium content*
10:35	**Q & A**
10:50	**BREAK**
Session 2	**Quality & Nutrition**
	This session is kindly sponsored by James Hutton Institute
	Chaired by Alison Lovegrove & Rowan Mitchell
11:10	**Kay Trafford,** *Understanding starch granule morphology in cereal grains.*
11:30	**Sarah Raffan**, *Genome-editing for low acrylamide wheat*
11:45	**Rowan Mitchell**, *Mutations in a candidate gene underlying a dietary fibre QTL in wheat cause high dietary fibre*
12:00	**Sophie Harrington**, *High fe-lines: improving iron levels and bioavailability in wheat grains*
12:15	**Q & A**
12:30	**SESSION ENDS**
19:20	**Evening Lecture with Sir Geoff Palmer OBE, 'A Life Scientific'**
	This session is kindly sponsored by RAGT
	Chaired by Malcolm Macaulay & Kelly Houston
20:15	**Q&A**
20:30	**SESSION ENDS**

Friday 4th June 2021

Session 1	Phenotyping
	This session is kindly sponsored by University of Dundee
	Chaired by Sonia Negrao & Tim Langdon
09:30	**Anne-Katrin Mahlein,** *Digital Phenotyping for the Assessment of Plant Traits* **(TBC)**
09:50	**Dominika Lewandowska**, *Barley anther proteome dynamics in early meiosis*
10:05	**Oluwaseyi Shorinola**, *Fifteen Years of UK Wheat Breeding: Using Historical Dataset for Trend Analyses and Association Mapping in UK Wheat*
10:20	**Sebastian Raubach**, *Gridscore: Plant phenotyping using mobile applications*
10:35	**Q & A**

10:50	**BREAK**

Session 2	Rank prize
	This session is kindly sponsored by Rank Prize Fund
	Chaired by Malcolm Bennett & Keith Gardner
11:10	**Philippa Borrill, Rank Prize New Lecturer,** *The alternative dwarfing gene Rht13 encodes an autoactive NB-LRR gene*
11:30	**Anthony Hall, Rank Prize Lecture,** *Beyond the single wheat reference genome*
11:50	**Yeorgia Argirou**, **MECEA 2021 PhD Prize Winner** *Large-eared lines as a resource for increasing yield in hybrid wheat*
12:05	**Cara Wheeldon**, **MECEA 2021 PhD Prize Winner** *Understanding crop responses to soil volume and neighbour density in barley*
12:20	**Q&A**
12:40	**Kelly Houston** Closing remarks

12:45	**SESSION ENDS**

Oral Presentation Abstracts: Development

ENGINEERING STOMATAL DEVELOPMENT

Julie Gray

University of Sheffield

Stomata allow carbon dioxide to enter leaves for photosynthesis. They close in the dark or under drought conditions to restrict water loss and, during pathogen attack to prevent infection. The identification of signalling components regulating stomatal development has allowed us to manipulate model and crop species, and to begin to investigate what might be the optimal stomatal number for current and future climate scenarios. We have produced plants with abnormally low stomatal densities by overexpressing or gene-editing epidermal patterning peptide signals that regulate stomatal development. These plants with greatly reduced stomatal density have substantially lower levels of transpiration and enhanced drought and pathogen tolerance. They require less water to grow yet maintain seed yields. For example, rice seedlings with approximately half the usual number of stomata, use only 60% of the normal amount of water, are better able to survive drought and still yield well.

RAISING WHEAT YIELD POTENTIAL THROUGH OPTIMISED PLANT HORMONE SPIKE SIGNALLING

Bethany Love[1], Gemma Molero[2], Matthew Reynolds[2], Carolina Rivera-Amado[2], Sergi Munné-Bosch[3], Maren Müller[3], Ali Babar[4], Anthony Hall[5] and John Foulkes[1]

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[2] CIMMYT, El Batan, Mexico City, Mexico

[3] University of Barcelona, Barcelona, Spain

[4] University of Florida, Gainesvillle, Florida, United States

[5] Earlham Institute, Norwich, NR4 7UZ, UK

Plant growth regulators (PGRs) are organic substances that influence specific physiological processes, such as floret fertility, and move throughout the plant. Previous studies suggest that genetic variation in grain number in cereals is associated with PGRs such as cytokinin, which is crucial in controlling cell division and lateral meristem activity. This has been demonstrated in cereals, when QTLs coding for reduced expression of cytokinin oxidase/dehydrogenase (*OsCKX2*) increased grain number in rice and wheat. However, how PGRs interact with grain number traits such as fruiting efficiency (FE, ratio of grain number to spike dry weight at anthesis) and grain dry matter partitioning at harvest in wheat is not fully understood. Our objective is to test for associations between grain number traits and grain yield with PGR levels in the spikes and using a bespoke target sequence capture strategy generate SNPs and establish molecular markers for the PGR traits with a focus on cytokinin metabolism. A high biomass association panel (HiBAP II) of 150 CIMMYT spring wheat genotypes was phenotyped for grain number and partitioning traits in the field and spike PGR levels were sampled at anthesis in NW Mexico. A subset of 10 lines representative of field variation for FE was grown in the glasshouse at the University of Nottingham, UK in three years for detailed PGR analysis at GS49 (booting) and 65 (anthesis). Results will be presented on the PGR data in both the glasshouse and field experiments, and associations with grain number traits as well as potentially promising SNPs with spike PGR levels for deployment in breeding programmes will be discussed.

INSIGHTS INTO THE EVOLUTION OF GRASS LEAVES

A. E. Richardson[1,2,3†*], Jie Cheng[1,4,5], R. Johnston[6,7], R. Kennaway[1], A.B. Rebocho[1], H. Kong[4,5], M. J. Scanlon[6*], S. Hake[2], E. Coen[1*]

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There are over 10,000 species of grass (Poaceae), which show diversity in floral structures but share a common leaf shape. In contrast to eudicots, which have a narrow proximal petiole and broad distal lamina, the grass leaf has an ensheathing base, a middle hinge region and an upper lamina which bends away from the main axis of the plant. The specialised shape of the grass leaf enables vertical growth during the vegetative phase without stem extension, protecting the shoot apical meristem by keeping it at, or below, ground level until the transition to flowering. This evolutionary innovation is thought to have contributed to the world-wide ecological success of the grasses, yet its origin and relationship with the eudicot leaf has been a subject of longstanding debate. By combining developmental genetics and computational modelling we have found that the grass leaf likely arose through *WOX*-gene-dependent extension of a primordial zone around the shoot apex. Gene expression patterns within this zone underlie growth patterns and can account for wild-type and mutant leaf development. By contracting the primordial zone, but maintaining gene expression patterns, we found that we can regenerate a eudicot like leaf shape. Differences in grass and eudicot leaf shape most likely arose through modification of the downstream effects of these conserved patterning genes on growth rate patterns. By comparing our grass leaf model and eudicot model we find that that the grass leaf sheath likely derives from petiole, whereas the blade derives from the rest of the eudicot leaf, consistent with homologies proposed in the 19th century. Given this conservation of domains, our future work will test the hypothesis that the same genes function in the development of leaf-like organs such as the lemma.

Sea Eagle sculpture by Ellen Brown, to celebrate the return of sea eagles to Scotland. Stannergate Cycle Path, Dundee
Image Courtesy of Dundee, One City Many Discoveries

A DOSAGE-DEPENDENT RESPONSE TO THE *TRITICUM POLONICUM VRT-A2* ALLELE CAUSES ELONGATED GLUMES AND GRAINS IN HEXAPLOID WHEAT

Nikolai M. Adamski[1], James Simmonds[1], Jemima F. Brinton[1,3], Anna E. Backhaus[1], Yi Chen[1], Mark Smedley[1], Sadiye Hayta[1], Tobin Florio[1], Pamela Crane[1], Peter Scott[1,4], Alice Pieri[1], Olyvia Hall[1,5], J. Elaine Barclay[1], Myles Clayton[2], John H. Doonan[2], Candida Nibau[2], Cristobal Uauy[1]

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Abstract:
The tetraploid wheat subspecies *Triticum turgidum* ssp. *polonicum* (here: *T. polonicum*) is characterized by highly elongated glumes (2-4 times longer than wildtype glumes) as well as long grains, which are controlled by the *P1* locus on chromosome 7A. We introgressed the 7A interval containing *P1* into hexaploid wheat cultivar 'Paragon' and measured its phenotypic effects in a set of near isogenic lines (NILs). Across six field trials, we observed consistent significant effects for thousand grain weight (+5.5%), test weight (2.3%), grain length (+5%), crop height (+7.1%), as well as spike length (+13.8%) in NILs with the *P1* introgression, whereas yield was not significantly different between the NILs. The effect on grain length however was not uniform across the florets of a spikelet, with basal grains (floret 1 and 2) showing significant differences in length, while later grains showed only small (floret 3) or no (floret 4) differences in grain length. We genetically mapped *P1* to a single candidate gene, the MADS-box transcription factor *VRT-A2*. Compared to the 'Chinese Spring' wildtype sequence, the *VRT-A2* allele from *T. polonicum* contains only a single polymorphism in its first intron; here, a 563-bp sequence from the wildtype allele (*VRT-A2a*) was substituted by a 160-bp in the *T. polonicum VRT-A2* allele (*VRT-A2b*). This polymorphism is unique to accessions of *T. polonicum* (and species thought to have naturally hybridized with *T. polonicum*) and absent from wild emmer, durum, or bread wheat. We hypothesize that the underlying mutation occurred only once and defines the sub-species *T. polonicum*.
We measured transcription levels of the two *VRT-A2* alleles within our NILs via qRT-PCR and observed ectopic expression of the *VRT-A2b* allele in glumes and grains. For the latter, we observed ectopic expression in all grains, regardless of floret position. We transformed the *VRT-A2b* allele with its native promoter into hexaploid cultivar 'Fielder'. The phenotypic effects varied with the copy number of the transgenic construct, with low copy number lines showing similar phenotypes as the *VRT-A2b* NILs, while high copy number lines showed vastly longer organs. For the latter, even grains from floret 4, which showed no difference in length in the NILs, were significantly elongated. This suggests a dosage-dependent mechanism that controls the length of glumes and grains in a basipetal gradient.
Future work will focus on understanding the regulatory network of *VRT-A2* in developing spikes and floral organs to further elucidate its mode of action.

Oral Presentation Abstracts: Recombination & Pollen Development

MEIOTIC NUCLEAR ENVELOPE IS REQUIRED FOR PROPER CHROMOSOME DYNAMICS AND HOMOLOGOUS RECOMBINATION

Nadia Fernández-Jiménez, Francesco Blasio, **Mónica Pradillo**
Departamento de Genética, Fisiología y Microbiología; Facultad de Ciencias Biológicas; Universidad Complutense de Madrid; Madrid; Spain.

Interactions between proteins of the inner and outer nuclear membrane link chromatin to cytoskeletal dynamics and have a role in meiotic chromosome movements. These movements not only facilitate the identification of homologous sequences on the homologous chromosome, but also help to remove unwanted contacts between non-homologous chromosomes. In *Arabidopsis thaliana*, AtSUN1 and AtSUN2 localize to the nuclear envelope (NE) in pollen mother cells (PMCs) in prophase I, in a pattern resembling telomere location. In an *Atsun1 Atsun2* double mutant, the normally polarized location of telomeres to the NE in leptonema does not take place, prophase I is delayed, with incomplete synapsis and unresolved interlocks, there are univalents at metaphase I and missegregations at anaphase I that lead to the formation of aneuploid gametes. Recently, we have also demonstrated a meiotic function for the plant specific nucleoporin AtNUP136, located in the nuclear basket subcomplex of the nuclear pore complexes (NPCs). The absence of this protein produces interlocks among non-homologous chromosomes, univalents, unbalanced nuclei at second meiotic division, and reduced fertility. In addition, other Arabidopsis nucleoporins seem to be important for normal meiotic chromosome behavior. Since the role of NE proteins in the regulation of meiotic chromosomal movements seems to be conserved across different species, the knowledge about these proteins in the model *Arabidopsis thaliana* can potentially be extrapolated and applied to crop species that secure the majority of our food and feed.

A NOVEL POLLEN PROFILING APROACH AND ITS APPLICATION IN STUDYING WHEAT MEIOSIS

Abdul Kader Alabdullah, Azahara C. Martín, Tracie Draeger and Graham Moore
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Meiosis, a specialized type of cell division, is an essential process for sexual reproduction in eukaryotes. In plants, pollen mother cells (meiocytes) divide by meiosis to form four haploid microspores, which undergo a post-meiotic process that involves two successive mitosis divisions to produce four mature pollen grains. The normal and harmonious course of male meiosis ensures normal pollen development. Thus, meiotic abnormalities caused by genetic or environmental disruptions are likely to result in unviable and/or variable sized pollen grains and reduced fertility. In polyploids, meiosis must adapt to ensure proper chromosome segregation to produce balanced gametes, thus maintaining fertility. Polyploid wheat, a key staple crop for global food security, behaves like diploid during meiosis thanks to the major meiotic gene *ZIP4* on chromosome 5B (*TaZIP4-B2*). This gene promotes homologous pairing, synapsis and crossover, and suppresses homoeologous crossover, thus maintaining wheat genome stability and fertility. In this study we developed and validated a novel pollen profiling method for the measurement of wheat pollen size and number and applied it to meiotic studies. Pollen analysis showed that the average wheat pollen size was 49.0±0.4 µm and 44.6±0.2 µm in hexaploid and tetraploid wheat, respectively, while the average pollen number per anther was 2709±614 in keeping with those reported in previous studies. Our method showed similar and consistent pollen profiles in the different *Tazip4-B2* mutants, showing near to 50% small pollen grains. This agreed with the meiotic scores at metaphase I (56% of the meiocytes having meiotic abnormalities) and at tetrad stage (50% of the tetrads having micronuclei), thus proving to be a reliable method for wheat meiotic studies.
We also applied this method in a forward genetics screening for temperature-related meiotic genes. About 40 hexaploid wheat cv. paragon lines with gamma radiation induced-deletions were used in this study. Pollen analysis of the plants exposed to heat stress (30 ^{o}C day and 25 ^{o}C night) at the meiotic stage showed that at least in four lines,

pollen profiles were dramatically altered compared to those of the same lines under non-stress conditions and the paragon wild type under heat stress, which indicates their sensitivity to elevated temperature during meiosis. The selected lines represent good candidates to identify temperature-related meiotic genes. Thus, our pollen profiling approach can facilitate large-scale meiotic studies to enhance the discovery of new meiotic genes in plants.

BARLEY ANTHER PROTEOME DYNAMICS IN EARLY MEIOSIS

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Anther development is a multi-step process, resulting in the release of mature pollen. It can be divided into three main phases: organ patterning and cell differentiation, meiosis, and post-meiotic pollen biogenesis. Understanding anther and pollen development have key implications for crop yield and biodiversity.
Most studies to address fundamental questions in anther development are focused on investigating transcriptome, although there is only defined evidence of the germline protein-encoding transcripts being translated to functional proteins. We have applied our previously published 'micro-proteomics' sampling approach to study proteome changes of barley anther during early meiosis development. To better understand the molecular basis of barley meiotic-stage anther progression, six consecutive anther sizes, spanning the prophase I, have been dissected and proteins in each of them were identified and quantified, using label-free high-resolution mass spectrometry. We built a comprehensive Barley Anther Proteome (BaAP) containing more than 6000 non-redundant proteins and investigated its dynamics during meiotic prophase I.

A SUPPRESOR SCREENING ON MLH3 MUTANTS SHOW THE ROLE OF A RECQL4 MUTATION ON RESTORING FERITLITY AND INCREASEING OVERALL RECOMBINATION IN BARLEY

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Plant breeding relies on meiotic recombination generated by crossover (CO) formation to generate new allelic combinations, but this process is constrained in crops such as barley by number and position of COs. We have carried out a suppressor screen using a characterised barley mutant of the meiotic gene Hvmlh3, which is involved in the resolution of crossover events. Lines with restored fertility resulting from this screen allowed the identification of a mutation in the anti-crossover gene *RECQL4.* The suppressor mutant was crossed onto a wildtype barley cultivar to explore the effect of this mutation on the recombination landscape. Genotyping F_3 populations with the barley 50K genotyping platform, detected nearly double the recombination levels in *Hvrecql4* compared to wildtype. The effect of the *Hvrecql4* in the *Hvmlh3* background was stronger more than quadrupling the recombination indicating the non-independence of the two genes in CO promotion and resolution. These results confirm the effect of RECQL4 in a large genome cereal such as barley albeit within the normal recombination distribution and opens the possibility for increasing recombination in a breeding context in this crop.

Oral Presentation Abstracts: Genomics & Technologies I

IMPLICATIONS OF THE SEED MICROBIOME FOR PLANT HEALTH

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With the development of high-throughput DNA sequencing techniques, our understanding of plant-associated microbial communities has greatly improved. While the association between plants and microbes was discovered more than 100 years ago, only recently have various technological developments enabled us to resolve the complexity of such systems in more detail. Interestingly, only recently the seed microbiome of plants came into the foreground. This is in contrast to the plant rhizosphere and various endosphere microenvironments that have been intensively studied. Our studies in recent years have shown that microbial communities (bacteria, fungi and archaea) in the seed endosphere are shaped by the local environment, the plant genotype as well as certain agricultural practices such as seed coating. We also found that seeds can serve as natural carriers for beneficial microorganisms across plant generations. In our most recent study, we observed that one specific rice cultivar (cv. Zhongzao 39) is not affected by rice seedling blight in certain growing regions although the causative pathogen *Burkholderia plantarii* was present. Such phenomena have previously been attributed to disease triangles in which the environment plays an elusive role in shaping disease resistance in plants. We followed an investigative approach and found that seeds of the resistant phonotype harbor a specific microbiota with predominance of *Sphingomonas melonis*. The isolate ZJ26 was characterized in detail and it was found to produce anthranilic acid which interferes with the pathogen's virulence signaling cascades and confers holistic disease resistance to host plants. We also found that the isolate's protective features are not restricted to the rice cultivar where it was initially detected. The overall findings expand our knowledge related to disease resistance mechanisms in plants and will likely serve as a basis for future biotechnological developments.

GENOMIC ANALYSIS OF 20 HEXAPLOID WHEAT/AM. *MUTICUM* INTROGRESSION LINES REVEALS NEW INTROGRESSED SEGMENTS, HOMOEOLOGOUS TRANSLOCATION OF SEGMENTS, EXTENSIVE STRUCTURAL DISRUPTION, AND PATTERNS OF GENE EXPRESSION

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Wheat is a globally vital crop whose continual improvement is being limited by a lack of genetic variation in the gene pool accessible by breeders. A key component of overcoming this lack of genetic variation in the years to come will be wild relative introgression breeding, where genetic variation from wheat's wild relatives will be bred into elite wheat lines. While technical advances are making the wider adoption of such breeding strategies feasible, we lack a fundamental understanding on various aspects of the genomic impacts of these wide crosses. The wheat wild relative *Ambylopyrum muticum* has a diploid TT genome and possesses genetic variation for a range of agronomically important phenotypes including disease resistance such as powdery mildew, and salt tolerance. The King group at The University of Nottingham have previously developed a set of homozygous hexaploid wheat/*Am. muticum* introgression lines with most of the *muticum* genome represented in chromosomal and sub-chromosomal segments in an elite wheat background. Here, we utilise whole genome sequencing data from introgression and parent lines to explore the structural landscape of 20 *Am. muticum* introgression lines. Using SNPs specific to *Am. muticum*, we identify previously missed introgressed segments and using a mapping coverage-based approach, performed in silico karyotyping and

found large deletions and duplications across the set of lines. Comparing SNP data and coverage data between pairs of DH lines that derive from the same BC3 plant, we identified cases of homoeologous pairing, including a translocation of part of a *muticum* segment between homoeologous chromosomes. Utilising a draft nanopore genome assembly and annotation of *Am. muticum*, we produced a list of introgressed genes for each line and using RNAseq data for 5 introgression lines, the 3 wheat parents and *Am. muticum* we analysed how *Am. muticum* genes are expressed in a wheat background and how existing wheat genes are affected by the introduction of a wild relative segment, including how this changes based on the proximity of the wheat gene to the introgressed segment and how triad expression balance changes when one homoeologue is replaced by a *muticum* gene.

MAPPING BARLEY GENES SHAPING THE RHIZOSPHERE MICROBIOTA

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Harnessing the beneficial functions of the microbial communities thriving at the root-soil interface, the so-called rhizosphere microbiota, is a promising strategy to enhance sustainable crop production. We previously demonstrated that wild and domesticated barley genotypes host a contrasting microbiota and we proved that these differences are modulated, at least in part, by the plant genome. To gain novel insights into the genetic mechanisms underpinning microbiota recruitment in barley we used metagenomics information as a 'quantitative phenotype' in QTL mapping using a segregating population between the elite cultivar Barke and a wild parent. The genetic mapping, conducted at a depth of thousands of SNPs in the barley genome, resulted in the identification of a single locus on chromosome 3H associated with the recruitment of taxonomically distinct bacteria, possibly representing a major regulator of plant-microbiota interactions. By mining the recently released pangenomic information we discovered that, in Barke, the locus 3H encompasses ~60 genes encoding for functions putatively implicated in plant-microbiota interactions, such as cell wall remodelling and immune recognition. Next, we selected introgression lines with contrasting alleles, i.e., either elite or wild, at the 3H locus. Strikingly, metagenomic profiling revealed that introgression at locus 3H is sufficient to shape, at least in part, microbial composition validating the role of locus 3H as a regulator of the rhizosphere microbiota. We are currently exploring RNA-seq information obtained from the roots of our introgression lines to identify genes differentially expressed in the region of interest.

Broughty Castle, Dundee
Image Courtesy of Dundee, One City Many Discoveries

MECEA PhD Prize Winner 2020:

RECOGNITION OF MILDEW, RUST, AND BLAST BY THE IMMUNE RECEPTOR MLA

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A primary threat to stable food production is epidemics caused by plant pathogens. However, it remains unclear how plant breeding approaches may increase the vulnerability of agricultural systems. The majority of NLR-encoding resistance genes recognise single pathogen species; few NLRs have the capacity to recognise multiple pathogens. The *Mla* locus has over 30 described alleles conferring isolate-specific resistance to *Blumeria graminis* f. sp. *hordei* (powdery mildew), contains three NLR encoding gene families (*RGH1*, *RGH2*, and *RGH3*), and is associated with resistance to multiple pathogens including *Puccinia striiformis* f. sp. *tritici* (wheat stripe rust; *Rps7*) and *Magnaporthe oryzae* (rice blast; *Rmo1*). In addition, sensitivity to the *Cochliobolus victoriae* toxin victorin (*Lov1*) is in coupling with *Mla3*—in *Arabidopsis*, sensitivity to victorin is mediated by an NLR-dependent plant immune response. We found that only three of eleven *Mla* haplotypes had functional resistance to *P. striiformis* f. sp. *tritici*, including *Mla7*, *Mla8*, and *Mla15*. Using *Agrobacterium*-mediated transformation, we have established that *Mla8* confers resistance to *P. striiformis* f. sp. *tritici*, but not *P. striiformis* f. sp. *hordei*. In the *Mla3* haplotype, we performed a high-resolution recombination screen and confirmed the genetic coupling of *Mla3*, *Rmo1*, and *Lov1*. Using sequence capture and RNAseq, we discovered copy number variation and high expression levels for *Mla3* (*RGH1;* three copies), with one expressed copy (*Mla3Δ6*) containing a 6 bp deletion in the LRR region. Evaluation of stable transgenic barley found that *Mla3* alone conditions *Blumeria graminis* f. sp. *hordei* and *M. oryzae* resistance. This work, coupled with the recent discovery of direct interaction of MLA and AVR_a effectors (Saur *et al*., 2019), suggests that MLA has the capacity to recognize molecular structures conserved among plant pathogen effectors. The role of *Mla* in conferring multiple pathogen recognition implies that selection for resistance to a host pathogen can directly influence resistance to non-adapted pathogens. This observation impacts our understanding of the evolution of plant immunity and how breeding resistance to one pathogen may have unintended consequences.

Glamis Castle, Angus

Oral Presentation Abstracts: Genomics & Technologies II

LONG-READ SEQUENCE ASSEMBLY IN BARLEY: THE UPDATED MOREXV3 REFERENCE GENOME AND PROSPECTS FOR PAN-GENOMICS

Martin Mascher[1,2], Thomas Wicker[3], Jerry Jenkins[4], Christopher Plott[4], Thomas Lux[5], Chu Shin Koh[6], Jennifer Ens[7], Heidrun Gundlach[5], Lori B. Boston[4], Zuzana Tulpová[8], Samuel Holden[9], Inmaculada Hernández-Pinzón[9], Uwe Scholz[1], Klaus F.X. Mayer[5], Manuel Spannagl[5], Curtis J. Pozniak[7], Andrew G. Sharpe[6], Hana Šimková[8], Matthew J. Moscou[9], Jane Grimwood[4], Jeremy Schmutz[4], Nils Stein[1,10]

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Sequence assembly of large and repeat-rich plant genomes has been challenging, requiring substantial computational resources and often several complementary sequence assembly and genome mapping approaches. The recent development of fast and accurate long-read sequencing by circular consensus sequencing (CCS or “HiFi”) on the PacBio platform may greatly increase the scope of plant pan-genome projects. Here, we compare current long-read sequencing platforms regarding their ability to rapidly generate contiguous sequence assemblies in pan-genome studies of barley (*Hordeum vulgare*). Most long-read assemblies are clearly superior to the current barley reference sequence based on short-reads. Assemblies derived from accurate long reads excel in most metrics. The CCS approach was the most cost-effective strategy for assembling tens of barley genomes. We present MorexV3, an updated reference genome assembly of barley cv. Morex with near-complete representation of the repeat-rich intergenic space. Long-read assembly will underpin the construction of accurate and complete genome sequences to expand pan-genome infrastructures in barley and its wild relatives.

A HAPLOTYPE-LED APPROACH TO INCREASE THE PRECISION OF WHEAT BREEDING

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Crop breeding involves assembling desired combinations of traits to generate improved cultivars. Most of these traits are governed by genomic regions defined by underlying genetic variation. These genomic regions are often co-

inherited as blocks of variation, or haplotypes, which are the effective units of selection by breeders. Exploiting natural variation for the genetic improvement of crops plays a central role in increasing productivity, which must increase at unprecedented rates to meet the needs of the growing worldwide population. Although current genomic technologies can be used for high-throughput identification of genetic variation, methods for efficiently exploiting this genetic potential in a targeted, systematic manner are lacking. Here, we developed a haplotype-based approach to identify genetic diversity for crop improvement using genome assemblies of 15 hexaploid wheat cultivars representing modern-day diversity across wheat breeding programmes. We used stringent criteria to identify identical-by-state haplotypes and distinguish these from near-identical sequences (~99.95% identity). We showed that each cultivar shares ~59 % of its genome with other sequenced cultivars and we detected the presence of extended haplotype blocks containing hundreds to thousands of genes across all wheat chromosomes. However, we found that genic sequence alone was insufficient to fully differentiate between haplotypes, as were commonly used array-based genotyping chips due to their gene centric design. We developed an interactive visualisation resource to allow exploration of the haplotype blocks we identified (www.crop-haplotypes.com). We exemplify the use of this approach for focused discovery of novel beneficial haplotypes using a highly conserved region on chromosome 6A, which is associated with many traits of agronomic interest. We defined haplotype blocks in this region and developed a suite of haplotype-informed markers to accurately identify these haplotypes in large germplasm panels. We show that a single haplotype covering ~60% of the physical chromosome sequence dominates in European germplasm. We identified novel haplotypes across this region in the Watkins landrace collection that were not present in modern germplasm. Using publicly available biparental QTL data for Watkins lines we identified a subset of novel 6A haplotypes that were associated with increased grain weight. These beneficial haplotypes represent targets for introduction to elite cultivars for yield improvement. Overall, this study provides a framework for defining and exploiting haplotypes to increase the efficiency and precision of wheat breeding towards optimising the agronomic performance of this crucial crop.

PRECISE GENE EDITING USING RIBONUCLEOPROTEIN-COMPLEXES

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Targeted mutagenesis employing sequence-specific endonucleases (SSE) such as CRISPR/Cas technology has been demonstrated in a variety of plant species. This technique's frontiers still lack predictability of the outcome, since SSE-introduced double-strand breaks are resulting in insertions and deletions (InDels), which are itself not predictable. Using the homology-directed repair (HDR) mechanism in the cell, a predicted allele-exchange can be introduced into the loci by providing a synthetic repair template including the desired gene modification. One way to achieve this precise allele exchange is the use of ribonucleoprotein complexes (RNP). A synthetic sequence-specific gRNA and a Cas protein are assembled *in vitro* and transferred together with the allele exchange-specific repair template, into the cell. To facilitate easy detection of homology-directed genomic modifications, we follow two approaches. Employing *Gfp*-transgenic barley plants, we use *Gfp*-specific guides for Cas9/Cas12a-mediated double-strand break induction and application of a custom *Yfp* repair-template. After successful HDR, Yfp fluorescence can be used as readout. In a second attempt, non-fluorescing *gfp* mutants can be converted to Gfp fluorescence following a similar approach just introducing *Gfp*-specific repair template.
Presented are data comparing SpCas9 and AsCas12a endonuclease in barley epidermal leaf cells considering their different features. To interfere with the ratio between preferred non-homologous end joining (NHEJ) and HDR, RNAi was used to repress key genes involved in NHEJ. RNAi constructs targeting *Ku70*, *Ku80* and *Ligase IV* genes were generated and used for transient and stable barley integration

MECEA Post Doc Prize Winner 2020:

WHOLE GENOME SEQUENCE FROM 3,000-YEAR-OLD EGYPTIAN EMMER WHEAT REVEALS DISPERSAL AND DOMESTICATION HISTORY

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Tetraploid emmer wheat was one of the first cereals domesticated in the old world. In Egypt, it was a dominant crop from the earliest settlements (5500-4500 BCE) until at least 332BCE. We report whole genome sequence from a museum specimen of emmer wheat chaff that was harvested during the New Kingdom (^{14}C dated 1,130 – 1,000 BCE) and excavated 90 years ago. Its genome shares haplotypes with modern domesticated emmer at shattering, seed size, and germination loci, and within other putative domestication loci, suggesting these traits share a common origin prior to emmer's introduction to Egypt. Its genome is otherwise unusual, indicating genotypes potentially lost among modern emmer. Genetic similarity with modern Arabian and Indian emmer landraces connects ancient Egyptian emmer with early South-Eastern dispersals, while inferred gene flow with wild emmer from the Southern Levant signals a later connection. Our results show the importance of museum collections as sources of genetic data to uncover the history and diversity of ancient cereals.

Barley grain filling in a typical sunny Scottish summer

Oral Presentation Abstracts: Abiotic & Biotic Stress:

ROOT MUTANTS OF WHEAT: A SOURCE OF DIVERSITY FOR IMPROVED PHOSPHORUS NUTRITION

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Phosphorus (P) is one of the major nutrients regularly applied to cereal crops to ensure adequate yields. Phosphorus applied as phosphate is poorly mobile in most agricultural soils with much of the P found near the surface. Germplasm able to explore the surface soil is a strategy aimed at improving the efficiency of crops in taking up P. Key traits for improving the P use efficiency of cereal crops include shallow roots and long root hairs that enable effective exploration of the topsoil. To identify major genes governing root morphologies, we developed high throughput screens of wheat seedlings. An elite Australian cultivar was mutagenized with sodium azide to generate pools that could be screened. The first mutants identified had severe phenotypes that would not be considered of direct value to agriculture but are scientifically valuable. One of the mutants isolated was named Stumpy as it has long root hairs associated with short roots. While the long hairs are a beneficial trait for P efficiency, the short roots would be a disadvantage. Nevertheless, by understanding the physiology of the mutant and cloning the underlying gene we argued that this would provide us with a way of understanding the mechanisms controlling root hair length in wheat. We also identified mutants with shallow roots due to an altered ability to sense or respond to gravity. After screening about 20,000 seedlings we have a collection of root gravity mutants with phenotypes ranging from severe to subtle. To date all the characterized mutants are due to single genes and are dominant or semi-dominant mutations. While some of the mutants with severe phenotypes such as antigravity nodal roots are not of agronomic value, mutants with more subtle phenotypes could have benefits for P nutrition. The large and complex genome of bread wheat has long meant that it was difficult to identify the genes underling particular traits. However, the advent of a sequenced genome along with high throughput sequencing technologies has helped enable the cloning of genes based solely on mutant phenotypes. We used fine mapping along with chromosome purification and high throughput sequencing to identify a strong candidate gene for Stumpy and a similar strategy could be applied to the various gravity mutants. Future work aims to establish if the candidate gene encodes Stumpy and to assess the various mutants in field trials to determine if any have improved P-uptake efficiency.

POSITIONAL CLONING OF TWO RESISTANCE GENES TO BARLEY VIRAL DISEASES FROM A WILD RELATIVE

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Virus diseases are causing high yield losses in crops worldwide. In Europe, *Barley yellow dwarf virus* (BYDV), transmitted by aphids, and the soil-borne *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) complex, transmitted by *Polymyxa graminis*, are of prime importance in this respect in barley. To date, only two genes providing resistance to BaYMV/BaMMV have been cloned. To be able to achieve durable protection of the crop, additional resistance genes are urgently needed. We are aiming at cloning *Ryd4*^{Hb} and *Rym14*^{Hb}, two dominant

resistance genes from *Hordeum bulbosum*, a wild relative of barley, providing resistance to BYDV and to the BaMMV/BaYMV complex, respectively. In a previous study, *Ryd4^{Hb}* was allocated to chromosome 3H. Recombination in crosses between *H. vulgare* and *H. bulbosum* are scarce: by screening 16,000 F_2 plants, we identified less than 120 recombinant plants in a 13.3 Mbp interval. However, by fine-mapping, we were able to reduce the interval to 67 kb on the barley reference genome. Genome assembly of the introgression line carrying *Ryd4^{Hb}* revealed a 700kb-long interval in which four NB-LRR genes are annotated. Functional validation is ongoing using CRISPR-Cas9 knock-outs in the introgression line. Using GBS genotyping of 427 F_5 plants, we reduced *Rym14^{Hb}* interval on chromosome 6H to 4 Mb. To further reduce it, 7,500 F_2 plants were screened for recombination. We identified 28 recombinants and a resulting 2Mbp telomeric interval.

MOLECULAR CHARACTERISATION AND TRAIT ANALYSIS OF WHEAT-*TRITICUM TIMOPHEEVII* INTROGRESSION LINES REVEALS NEW SOURCE OF FHB RESISTANCE

Surbhi Grewal[1], Paul Nicholson[2], Andrew Steed[2], Anthony Hall[3], Benjamin Coombes[3], Caiyun Yang[1], Stephen Ashling[1], Duncan Scholefield[1], Lolita Wilson[1], Stella Hubbart-Edwards[1], Manel Othmeni[1], Jack Walker[1], Peter Isaac[4], Ian King[1] and Julie King[1]

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For future food security it is important that wheat, one of the most widely consumed crops in the world, is able to survive the threat of abiotic and biotic stresses. New genetic variation is currently being introduced into wheat through introgressions from its wild relatives. *Triticum timopheevii*, a tetraploid wild relative of wheat (2n = 4x = 28, A^tA^tGG), is an important source of resistance to various diseases such as wheat rusts, powdery mildew and Fusarium head blight (FHB), as well as fertility restorer genes and tolerance for various abiotic stresses. Its practical utilisation in wheat improvement is being facilitated through the generation of genome-wide introgressions leading to a variety of different wheat–*T. timopheevii* introgression lines. For trait discovery, the recombinant lines require stable homozygous introgressions. Breeding programs rely on efficient genotyping platforms for marker-assisted selection (MAS). Through whole genome sequencing of *T. timopheevii* and filtering for single-copy regions of the wheat genome, a large number of chromosome-specific single nucleotide polymorphisms (SNPs) between Chinese Spring wheat and *T. timopheevii* have been generated and converted into chromosome-specific Kompetitive allele-specific PCR (KASP™) assays. These markers 1) allow rapid detection of *T. timopheevii* segments in a hexaploid background 2) distinguish between segments originating from the A^t and G genomes of *T. timopheevii* 3) provide information on the segment's homozygosity in bread wheat and 4) indicate the potential site of introgression of the segment in the wheat genome. Here, we report the generation of 29 wheat–*T. timopheevii* homozygous introgression lines which were characterized using 355 chromosome-specific KASP markers and multi-colour genomic in situ hybridization (mc-GISH).

Spray inoculation with *Fusarium graminearum* revealed very rare Type 1 FHB resistance in two introgression lines. Molecular analysis of these lines indicates the source of resistance to be on the distal end of chromosome 3GS of *T. timopheevii.* Phenotyping analysis of an F6 mapping population between two *T. timopheevii* accessions, showing resistance and susceptibility to FHB (genotyped using the Axiom® Wheat Breeders Array), also indicated a potential QTL for FHB resistance corresponding to wheat homoeologous group 3. Further characterisation to find the novel source of this resistance and its incorporation in commercial breeding could save the worldwide wheat breeding industry billions of dollars in yield and quality losses due to this economically devastating disease.

A GRAIN OF SALT: NATURALLY OCCURRING VARIANTS OF *HVHKT1;5* DETERMINE BARLEY GRAIN SODIUM CONTENT

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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. Under normal and salt stressed conditions Na^+ taken up from the soil is transported via the xylem to the shoot, where excessive Na^+ negatively impacts a range of physiological processes that include photosynthesis, transpiration, stomatal conductance and K^+ concentration, and leads to depressed yields. 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. Class 1 HIGH-AFFINITY POTASSIUM TRANSPORTER's (HKT1's) actively and specifically transport Na^+ from the root xylem into the xylem parenchyma cells, where it is extruded through the root cortex to the epidermis and then back into the soil. Genetic studies have identified clear associations between specific *HKT1* alleles and salinity tolerance, highlighting one potential genetic route towards reducing the impact of soil salinity on crop production. We quantified grain sodium (Na^+) content across a barley GWAS panel grown under optimal conditions. We identified a strong association with a region containing two low and one high Na^+ accumulating haplotypes of a Class 1 HIGH-AFFINITY POTASSIUM TRANSPORTER (HKT1;5) known to be involved in regulating plant Na^+ homeostasis. The haplotypes exhibited an average 1.8-fold difference in grain Na^+ content. 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. 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.

Oor Wullie Statue in Bronze
Image Courtesy of Dundee, One City Many Discoveries

Oral Presentation Abstracts: Quality & Nutrition

UNDERSTANDING STARCH GRANULE MORPHOLOGY IN CEREAL GRAINS.

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The way in which starch polymers are synthesized in plant storage organs is largely understood: the enzymes involved have been identified and characterized. However, the way in which starch polymers are built into insoluble granules of differing size and shape is not yet understood. There is a wide variation in starch granule morphology, even amongst closely related species such as cereals and their wild-grass relatives. Most grass species accumulate many small starch granules within each plastid in the endosperm cells. However, some accumulate a few, large starch granules. The shape of the granules is also genetically determined and is sometimes very complex. Unusually, *Triticeae* species (eg. wheat, barley and rye and *Aegilops*) have both small and large starch granules within the same plastids. The identification and charaterization of genes involved in the determination of starch granule initiation and morphology is in progress. In my lab, we have identified a gene that influences both the number of the small, B-type starch granules in Triticeae grains and the morphology of the large, A-type granules and we have found distinctly different starch phenotypes depending upon gene dosage.

GENOME-EDITING FOR LOW ACRYLAMIDE WHEAT

Sarah Raffan

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Acrylamide (C_3H_5NO) is a processing contaminant and a Group 2a human carcinogen which was found in many foodstuffs in 2002. Acrylamide forms from the reaction of free (non-protein) asparagine with reducing sugars during the Maillard reaction. All major cereal products are affected, and wheat represents one of the major sources of dietary acrylamide intake in Europe, with products such as biscuits, breakfast cereals and toast all showing high acrylamide concentrations. Asparagine concentration is the determining factor for acrylamide formation in cereal products. Asparagine biosynthesis is catalysed by a family of enzymes called asparagine synthetases (ASNs). Five *ASN* genes (*TaASN1, 2, 3.1, 3.2 and 4)* have been identified in wheat (*Triticum aestivum*), with *TaASN2* showing grain-specific expression. CRISPR/Cas9 was used to knock out the *TaASN2* gene of wheat cv. Cadenza. The subsequent edits were characterised in the T1 and T2 generations using Next Generation Sequencing nucleotide sequence analysis. Triple (A, B, and D genome) nulls were identified, alongside an AD and an A genome null. Amino acid concentrations were measured in the T2 and T3 seed, with one triple null line showing a substantial reduction in the free asparagine concentration in the grain (90 % in the T2 seed and 50 % in the T3 seed compared with wildtype). The free asparagine also reduced as a proportion of the total free amino acid pool. A poor germination phenotype was seen in some of the edited lines, but this could be overcome by application of exogenous asparagine. Some of these genome-edited lines are being prepared for a 2021-2022 field trial. Acknowledgement: Sarah Raffan was supported by the Biotechnology and Biological Sciences Research Council through a South West Biosciences Doctoral Training Partnership CASE studentship supervised by Professor Nigel Halford (Rothamsted Research) and Professor Keith Edwards and Dr Gary Barker (University of Bristol), with the following partners: AHDB, KWS UK Ltd., Saaten Union UK Ltd, RAGT Seeds Ltd; Syngenta UK Ltd, and Limagrain UK Ltd.

MUTATIONS IN A CANDIDATE GENE UNDERLYING A DIETARY FIBRE QTL IN WHEAT CAUSE HIGH DIETARY FIBRE

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Genetic enhancement of the dietary fibre content in the endosperm of bread wheat is a target to deliver healthier foods made from white flour. Of particular interest is soluble dietary fibre (SDF) which confers many health benefits. Wheat grain SDF is consists mainly of water-extractable arabinoxylan (AX), the main polymer present in endosperm cell walls. Two wheat varieties with naturally high SDF content are the Chinese variety Yumai34 and the French variety Valoris. From biparental populations, QTLs have been identified on 1B and 6B with high SDF being conferred by the Yumai34 and Valoris alleles, respectively. From RNAseq on developing endosperm in lines from a Yumai34 x Valoris DH population contrasting for the two QTLs, we identified candidate genes underlying the QTL regions where there was a change in expression and/or change in coding sequence. We focussed on one leading candidate for each QTL, developing homozygous lines carrying detrimental mutations in these genes from the sequenced mutagenised populations of Cadenza and Kronos. We analysed viscosity of grain extracts from these lines as a measure of soluble dietary fibre. We found no difference between lines carrying mutations and controls for 1B candidate, but a more than three-fold increase in viscosity for the 6B candidate. The 6B candidate gene is from a family that has never been shown to be involved in AX synthesis and confirmation of this result will not only be of practical importance in facilitating breeding for high SDF wheat, but also an important advance in our understanding of grass cell walls. We lacked homozygous controls derived from the same cross in our first experiments on the 6B candidate but have now obtained these as BC2F2 lines. We will present recent analyses of extract viscosity and AX characteristics in grain from the control and mutant BC2F2 lines at the meeting.

HIGH FE-LINES: IMPROVING IRON LEVELS AND BIOAVAILABILITY IN WHEAT GRAINS

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Micronutrient deficiencies are a global health issue, in part because the main staple cereal crops do not provide enough bioavailable iron and zinc. This is due to high levels of phytate, an anti-nutrient which prevents iron and zinc absorption in the gut, and standard processing practices. These essential minerals are unevenly distributed in wheat grains, located predominantly in the phytate-rich aleurone layer and embryo (reviewed in Balk *et al*. 2019). In contrast, the phytate-poor starchy endosperm is deficient in these minerals. Milling produces white flour by stripping the outer bran layers of the grain, removing most of both the phytate and iron and zinc. Chemical fortification is therefore required to reach the legally mandated minimum iron content for wheat flour in the UK. To improve white flour iron content, we previously developed transgenic wheat lines with ectopic expression of wheat *Vacuolar Iron Transporter 2 (TaVIT2*) in the endosperm, which increased iron levels in the white flour up to four-fold, reaching the levels required by UK law (Connorton *et al*. 2017). However, this strategy only modestly increased iron bioavailability and did not alter zinc content. We then developed a gene stacking approach combining *TaVIT2* with the rice gene *Nicotianamine Synthase 2* (*OsNAS2*) with the aim of improving both iron and zinc levels and bioavailability. *OsNAS2* produces the metal chelator nicotianamine (NA) which is involved in the long-distance transport of iron within the plant and which enhances iron and zinc bioavailability (Beasley *et al*. 2019). Characterisation of the *TaVIT2/OsNAS2* lines showed that they maintain the high level of iron in the white flour seen in the *TaVIT2*-only lines, while also having increased levels of zinc in the wholegrain flour. However, we saw no increase in total grain iron content. Grain nicotianamine levels are increased up to 50-fold in the*TaVIT2/OsNAS2* lines, and preliminary results suggest this is associated with improved iron release in simulated intestinal digests. Work is ongoing to determine whether the *TaVIT2/OsNAS2* lines have improved iron bioavailability in intestinal cell culture. In addition to this transgenic approach, we are exploring TILLING lines in selected iron homeostasis genes for biofortification. These projects demonstrate the utility of both transgenic and traditional approaches to improve the nutritional quality of wheat grains and bring us closer to delivering biofortified wheat.

Oral Presentation Abstracts: Phenotyping

DIGITAL PHENOTYPING FOR THE ASSESSMENT OF PLANT TRAITS

Prof Dr Anne-Katrin Mahlein

For precision plant protection, the detection and identification of plant diseases is crucial. Robust and reliable optical sensors, able to perform this task in the field are demanded but are recently far away from commercial maturity. Also, in the field of plant phenotyping, optical sensors combined with robotic technologies and artificial intelligence provide the potential to speed up and automatize the assessment of plant traits or stress reactions. Especially hyperspectral cameras have a high potential to characterise plants and to detect and identify leaf diseases caused by fungal pathogens in the field. Hyperspectral imaging captures the spatial information of disease symptoms and additionally a continuous reflectance spectrum for each pixel. The value of the assessed information was demonstrated in various publications within the context of plant diseases. However, most of the studies were applied in the laboratory or greenhouse under controlled environmental conditions or use satellite data with insufficient spatial resolution. The potential to transfer these models over different scales and based on results derived under these artificial conditions is, in general, unknown. We present results of different studies for the detection of cereal diseases, from the lab to the field scale. Within the focus are relevant foliar diseases, and compatible and incompatible interactions. Multi- and hyperspectral imaging technology were applied at multiple scales (microscope, laboratory, field and aerial imagery) to close the gap between accuracy of information and required throughput. Furthermore, this multi-scale imaging allows integrating annotations and training data supporting a more disease specific data analysis. Using machine learning based methods for data analysis unbounds the results from prior experienced and expectations allowing finding new relations between the hyperspectral signal and the disease symptoms. The achieved results may support the definition of tailored optical sensors and analysis approaches and provide a perspective for improved plant protection and plant phenotyping.

GRAINCORDER: A HAND-HELD SENSOR FOR MONITORING FRUCTAN PROFILES IN CEREAL GRAIN

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Fructan determination typically relies on hydrolysis reactions coupled to quantification of total fructose in relation to glucose, which is technically challenging, time-consuming, and expensive, therefore hindering the application in large-scale phenotyping approaches, breeding programs and agricultural practice. To improve analytical speed and costs some spectroscopic approaches have been introduced for the analysis of water-soluble carbohydrates. We have utilized hyperspectral signatures which can be mapped against biochemical compositions by machine learning algorithms, also non-destructively in mature grain. From the data set, spectral signatures indicative for fructan patterns have been evaluated and employed for the development of a hand-held fructan sensor. We will present our approach and discuss potential pitfalls.

FIFTEEN YEARS OF UK WHEAT BREEDING: USING HISTORICAL DATASET FOR TREND ANALYSES AND ASSOCIATION MAPPING IN UK WHEAT.

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Advances in sequencing technologies have led to an increased availability of large-scale genomic and genotype data, but the generation of high-quality phenotype data at enough scale and dimension to capture the gene x environment interactions influencing crop traits remains a challenge. Due to their large scale and multi-environment (years and locations) design, historical dataset from national variety performance trials provides ample statistical power to examine phenotypic trends and study gene x environment interactions. This dataset can also be used to dissect the genetic architecture of economically important traits in national breeding programmes. Taking advantage of this resource, we re-analysed historical dataset obtained from the Agriculture and Horticulture Development Board (AHDB) Recommended List trials conducted across 15 years (2002 – 2017). The dataset consists of over 100,000 measurements on 213 winter wheat varieties that were phenotyped in more than 150 locations across the UK. The dataset covers measurements across a range of economically important traits including yield, height, Hagberg Falling Number (HFN), protein content, days-to-ripening, specific weight, lodging and disease scores. Trend analysis on the phenotypic distribution across the examined period highlights a non-stationary trend for most of the traits examined except for plant height and lodging. There was a continuous increase in yield and days-to-ripening values while protein content and HFN values showed progressive decrease across the same period. There was also a decreasing trend in diseases infection scores for yellow rust, mildew and septoria, possibly pointing to improved disease resistance gene pool used in the UK breeding programme or better disease management practices. When combined with high-density genotypic information (Axiom 35K SNP), we show the value of this historical dataset for conducting association mapping to examine the genetic architecture of high-value traits in a national breeding programme. Our association mapping uncovers previously identified economically important loci, further highlighting the high-quality nature and potential of this historical dataset for trait mapping. We identified a significant peak for days-to-ripening peak on chromosome 6A, which co-localises with *NAM-A1* - a gene previously associated with variation to senescence in European germplasm. We also identified a strong (-log(p) > 30) peak on chromosome 2B, which co-localises with *Sm1*- the major gene underlying variation to orange blossom midge resistance. Our work highlights the value of readily available and publicly accessible historical dataset from national varietal performance trials for assessing breeding progress and studying the genetic landscape of crops trait in a national breeding programme.

GRIDSCORE: PLANT PHENOTYPING USING MOBILE APPLICATIONS

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Efficient and accurate phenotypic data collection underpins large areas of plant breeding and crop research. Historically, the recording of plant phenotyping data ranges from hand-written records on paper through rudimentary data collection applications or spreadsheets run on portable devices, to advanced applications running on mobile phones or tablets. Each approach exhibits both unique advantages and potential sources of errors. Hand-written notes are quick and familiar but require an additional digitization step and are prone to errors such as typos, number swaps and field layout column or row mix-ups. Spread sheets are the natural next step from hand-written notes and are a widely used tool for data recording. They offer basic mechanisms for data verification with most users familiar with their operation. When used on mobile devices outdoors, spreadsheets have major limitations relating to the accurate input of data due to the application complexity, sunlight, and often fiddly input methods.

Modern mobile applications (apps) represent a current approach towards efficient plant phenotyping. They aim to work on low-cost mobile devices and address many of the issues that previous approaches exhibit. While some apps try to reduce user input to a minimum by completely adopting barcodes for the identification of plants as well as traits and phenotypes, others use a more guided approach taking the user through the field plot by plot. What these apps lack is the intuitiveness of hand-written notes and printed field plans where data was recorded in each cell of the plan. This approach allowed immediate identification of plots requiring scoring and how trait data compared between plots. The next logical step is an application that would harmonize all three approaches – barcoding, guided data recording and field overview – into a single tool that is fast, easy to set up and intuitive to use. To this end, we present a new phenotyping app which we call 'GridScore'. GridScore represents the field layout in the form of a table. During the trial setup characteristics like row and column counts, trait definitions and germplasm lists are defined. Data is collected by means of scanning barcodes or selection in the interface – either way, GridScore uses text-to-speech for audio

feedback. The collection progress is visually displayed on the table and the current location is highlighted using geographic position referencing. Data and configurations can easily be shared between devices using a QR-code based system and exported to databases like Germinate.

Oral Presentation Abstracts: Rank Prize

Rank Prize New Lecturer Award 2020

THE ALTERNATIVE DWARFING GENE *RHT13* ENCODES AN AUTOACTIVE *NB-LRR* GENE

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The introduction of semidwarfing genes in wheat led to major improvements in cereal yields, through reduced height, improved lodging resistance and increased assimilate partitioning to the grain. However, the widely used gibberellin insensitive genes *Rht-B1b* and *Rht-D1b* reduce coleoptile length, and hence seedling emergence, which makes them less suitable for use in water-limited environments. Therefore, there is a need to identify alternative dwarfing genes that overcome the limitations of the conventional semidwarfing genes. *Rht-B13b* is a gibberellin sensitive semidwarfing allele that has normal seedling emergence but has not yet been widely deployed in breeding programmes. Through a combination of conventional genetic mapping, RNA sequencing and flow sorted chromosome sequencing, we were able to identify the gene underlying the *Rht13* locus as an autoactive *NB-LRR* gene. The candidate gene was validated using the Cadenza TILLING population and transgenic overexpression lines. Moreover, we found that *Rht-B13b* had a comparable height reducing effect to the conventional *Rht-B1b* and *Rht-D1b* genes in a range of genetic backgrounds. Unlike other recently identified alternative dwarfing genes, this gene does not encode a component of the gibberellin metabolism pathway. Instead, our results show that *Rht-B13b* influences height through genes associated with the growth-defense trade-off and cell wall remodelling. This discovery will enable the use of *Rht-B13b* in breeding programmes and opens up new avenues to manipulate wheat height beyond the gibberellin pathway.

Rank Prize Lecture Award 2020

BEYOND THE SINGLE WHEAT REFERENCE GENOME

Benjamen White[1], Thomas Lux[2], **Rachel Rusholme-Pilcher**[1], Hannah Rees[1], Susan Duncan[3], Benedict Combes[1], Ben Ward[1], Anil Thanki[1], Daniel Lang[2], Philippa Borrill[4], Cristobal Uauy[3], Wilfried Haerty[1], Manuel Spannagl[2], **Anthony Hall**[1]

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More than 215 million hectares of wheat are planted annually, and it is the most widely cultivated cereal in the world. In order to keep up with the demands of a growing global population, wheat production needs to increase by around 60% within the next 40 years. The challenges for wheat breeders and growers are, therefore, tremendous. Continued development of genomic resources to support wheat research and breeding are at the forefront to supporting this global challenge. To address this, the international community has recently sequenced and assembled multiple elite

wheat varieties in order to quantify diversity and identify genomic regions under selection (human and natural). Having a genome or a genotype is only one part of the story, what is key is how the genomes functions to generate phenotypes. An important step is do understand how the transcriptome varies between cultivars. A pan-transcriptome for wheat is being generated, including all international elite varieties from the previous study, with multiple tissues sampled at key developmental time points. Analysis of this pan-transcriptome, as part of a global partnership, will provide a unique understanding of how gene-expression changes between these lines and will characterise core and dispensable genes; those expressed all varieties or in only one. Further to this, we are performing co-expression analysis to identify specific and/or conserved modules between cultivars, that when combined with existing haplotype and phenotype data may act as a predictive measure of expression. As such, the resources developed from this will be directly applicable to the community and breeders towards the identification of genes that can be used to improve key traits, such as increased yield and disease resistance.

MECEA PhD Prize Winner 2021 (Joint)

UNDERSTANDING RESPONSES TO SOIL VOLUME AND NEIGHBOUR DENSITY IN BARLEY

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Plants experience many external stimuli throughout their life which they must precisely respond to in order to optimise their growth and development. Plants use both nutritional and non-nutritional information from the rhizosphere to proactively modulate their shoot growth to avoid future resource limitation. We have previously highlighted the importance of soil volume and neighbour density as non-nutritional stimuli to which wheat plants proactively modulate their shoot growth in response to soil volume, in a two-step root exudate-based system. However, plants do not necessarily all respond to these stimuli in the same way; this may vary between species, varieties or even different developmental stages of the same plant. To understand the role of genetic background in plant responses to soil volume, we have tested responsivity in a panel of diverse barley accessions, in collaboration with KWS. We have identified lines which have very high and very low volume-response plasticity, and now plan to use these extreme lines to identify genetic loci controlling soil volume responses. Complementing this approach, we are also investigating the role of root-shoot hormonal signalling in controlling growth responses to cytokinin. We hope our work will identify mechanisms and germplasm that can reduce non-nutritional inhibition of crop growth by rhizosphere stimuli.

MECEA PhD Prize Winner 2021 (Joint)

LARGE-EARED LINES AS A RESOURCE FOR INCREASING YIELD IN HYBRID WHEAT

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Significant increases in wheat (*Triticum aestivum*) yield need to be achieved to ensure food security for the future population. Hybrid breeding, where two inbred lines are crossed to create superior F1 plants, has the potential to increase yield and yield stability in wheat. Currently, one of the major issues with hybrid wheat is its economic unreliability, which can be improved both by reducing production costs and increasing yield. Yield and yield traits were observed in a replicated, multi-year field trial to compare 30 'large-eared' lines bred from synthetic hexaploid (SHW) and tetraploid-hexaploid (THW) donors, and eight commercial elite varieties, when sown at conventional (100%) and hybrid (70%) seeding densities. My hypothesis was that the large-ear trait is a positive yield component at low planting densities, and thus desirable for hybrid wheat. Of the 11 ear and seed traits measured, all were on average higher at the lower (70%) planting density; 5 were significantly higher ($P<0.005$) in one year, and 5 more significantly higher ($P<0.005$) in both years. For most traits, SHW lines outperformed both commercial and THW lines, and for many THW outperformed the commercial varieties. Overall yield was the same for both densities in the first year, despite a 30% lower seeding rate, showing the positive effect of the large ear trait.

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6 row barley maturing in the field - 2019

READING BETWEEN THE LINES: THE ALTERNATIVE MESSAGE OF THE RICE TRANSCRIPTOME IN RESPONSE TO HEAT STRESS

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Rice (*Oryza sativa* L.), one of the most consumed foods in the world, is particularly susceptible to heat stress. Research has shown the importance of alternative splicing (AS) in regulating several stress responses, including heat stress. However, transcriptome studies that analyse heat stress in rice have completely overlooked at AS. In this context, this study aims at understanding the complexity of rice leaf response to heat through robust data meta-analysis of publicly available RNA-seq data. Particularly, we will explore the unanswered question of the extension of AS regulation of the rice heat response. To achieve our goals, we obtained sequencing data from RNA-seq datasets available on the Sequencing Read Archive database. So far, we have analysed 360 datasets generated from 6 different studies. Each dataset has gone through a quality-control pipeline using the following applications: fastq-dump v.2.8.2 and Trimmomatic v.0.35. Next, high-quality reads were used for transcript quantification using Salmon v.1.3.0 and the rice transcriptome Os-NipponbareReference-IRGSP v.7. The analysis of differential alternative splicing for identification of genes/transcripts with AS were carried out with the tool 3DRNA-seq under the following parameters: adjusted p-value = 0.01, log-2 fold-change = 1 and DeltaPS = 0.3. We identified 1209 genes under AS regulation, suggesting a strong link of this mechanism in modulating the response to heat stress. Among the differentially alternatively spliced genes, we identified transcription factors, splicing factors and chromatin-modifying enzymes. Moreover, hundreds of genes have shown differential transcript usage where every transcript code for a different protein, confirming the importance of AS in shaping the proteome diversity. Preliminary results corroborate the hypothesis of the influence of AS on the modulation of the rice heat response. Our next step is to conduct an integrative data analysis of the genes/transcripts in order to select the best candidates for functional studies. The knowledge resulting from this study will provide information and strategies for rapidly advancing rice breeding in response to climate change.

µCT 3D IMAGING REVEALS EFFECTS OF OZONE ON WHEAT GRAINS

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Elevated levels of ground-level ozone, the dominant air pollutant affecting crop growth, are estimated to reduce global wheat yields, on average, by 7 – 10 %, with grain quality also affected. Determining how and where the yield loss occurs, both on the spike and in changes in grain shape, provide insights into the abiotic stress response. X-ray microcomputed tomography (µCT) imaging enables 3D visualisation of spikes with their grains *in situ*, along with the extraction of grain morphology data with positional information. A line of spring Primary Synthetic Hexaploid (SHW) wheat, along with *Triticum aestivum* c.v. 'Paragon', and nine lines of synthetic hybrids derived from these parent lines were grown for 3 months in solardomes (Bangor, North Wales) under low (30 ppb), medium (55 ppb), high (80 ppb) and very high (110 ppb) levels of ozone. Chlorophyll measurements of the flag, 2nd and lower leaves were made before, at, and after flowering. At harvest, spike biomass was used as an indicator of tolerance/sensitivity and four lines were selected for µCT imaging. The three heaviest spikes from each replicate were scanned and 3D grain traits (volume, surface area, circularity, length, width and depth), as well as grain number and position along the spike, were extracted from the 3D images. A manual count of grain numbers per spikelet was also made. Total grain weight and 1000 grain

weight were obtained following threshing, along with protein and starch levels using Near Infrared Spectrometry. 'Paragon' and BC1 maintained their total and 1000 grain weights under medium and high ozone levels by preserving higher grain numbers per spikelet, particularly across the middle of the spike. They also maintained grain size and protein/starch levels. Both BC5 and BC7 had reductions in total grain weight and 1000 grain weight between the medium, high and very high ozone levels, linked to reduced grain volume and grain number per spikelet. Grain volume reductions were driven more by reduced width and depth than by reduced length. Before flowering, there was no effect of ozone on chlorophyll levels in the flag leaves of all four lines, but there were reductions in both 2nd leaves and lower leaves in the high and very high ozone levels at flowering and post-flowering, with the least effect shown in Paragon and the greatest in BC7. The causes of ozone-related grain reductions, and potential commonalities with other abiotic stresses, are discussed.

SILENCING GENES REQUIRED FOR BARLEY POWDERY MILDEW INFECTION TO INVESTIGATE CANDIDATES OF VIRULENCE OR SUSCEPTIBILITY DURING BIOTROPHY.

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Blumeria graminis is a major obligate biotrophic fungal pathogen causing powdery mildew disease in cereals. For successful infection, *Blumeria* produces haustoria, localised exclusively in the epidermal cell layer of infected leaves. Haustoria are involved in nutrient uptake, delivery of virulence factors or effectors to compromise the host immunity. Therefore, haustoria are at the centre of the biotrophic interaction. Haustoria are composed of a fungal core surrounded by an extra-haustorial complex, some of which is of plant origin. The extra-haustorial membrane is believed to be a determinant of host susceptibility, but its protein make-up is largely understudied. A proteomics investigation was undertaken to identify barley proteins associated with the extra-haustorial membrane or infected epidermis. To validate protein candidates as modulators of susceptibility, a transient gene silencing assay was performed using gene specific Antisense Short Oligodeoxynucleotides (ASOs) delivered into excised barley leaves. Two proteins associated to the extra-haustorial membrane, an aquaporin, and an early nodulin-like protein, as well as the secreted PR5 thaumatin-like protein TLP5 were shown to promote barley susceptibility to powdery mildew. Interestingly, TLP5 was previously shown to interact with the *Blumeria* effector BEC1054 (CSEP0064). This novel gene silencing approach was also adapted for Host Induced Gene Silencing (HIGS), allowing to overcome the difficulties associated with the functional genomic analysis of obligate biotrophs, such as rusts or powdery mildews. The silencing of known virulence factors and new predicted candidate secreted effector proteins (CSEPs) led to decreased powdery mildew infection as monitored by the reduced formation of *Blumeria* secondary hyphae. This HIGS tool was also suitable to investigate putative *Blumeria* transcription factors required for upregulating the expression of effectors in haustoria. We believe that the identification of haustoria-associated barley proteins, the identification of *Blumeria* effectors and their host binding partners, together with the silencing approach, is invaluable to gain first insights of key players of fungal virulence as well as host resistance and susceptibility. As such, this could potentially provide a useful screening tool to assist traditional breeding programs, or for the generation of CRISPR-Cas gene edited plants with decreased susceptibility to pathogens.

BACK TO THE FUTURE: IDENTIFYING MICRONUTRIENT EFFICIENCIES IN HERITAGE BARLEY LINES FOR IMPROVED AGRICULTURAL SUSTAINABILITY

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Limited availability of micronutrients (Fe, Zn, Mn, Cu, B and Mo) is a common problem in agriculture. It has been exacerbated by the success of the green revolution, which has led to increased demand for micronutrients by fast-growing, nutrient-demanding and high-yielding varieties of cereals. A recent study suggests that Bere barley is superior in Mn-efficiency. However, it is not known whether this ability transfers to other micronutrients. Here we measure the leaf ionome of a population of landrace and heritage lines of barley in comparison to modern elite cultivars to ascertain whether landraces have enhanced micronutrient efficiencies in the field. An ionomic study of leaf element concentrations was performed on material from a population of landrace and heritage genotypes of barley and compared to elite modern-day cultivars. A population of 120 Heritage barley genotypes were grown in the field in Orkney. The population consisted of Bere, other heritage lines and modern lines (including both 6-row and 2-row barley lines). Diagnostic leaf samples from the trial were digested and analysed using inductively coupled plasma-optical emission spectroscopy (ICP-OES) to determine nutrient concentrations including the micronutrients Mn, Cu and Fe. By correlating the ionomes of the heritage barley, strong correlations were found between the micronutrients Cu, Zn and Mn. There were significant ($p<0.01$) correlations between Mn and both Zn and Cu concentration and it was also apparent that the modern genotypes were less able to accumulate all three micronutrients than the heritage genotypes with many of the Bere barley genotypes outperforming all other lines. Our data suggest that Bere barley genotypes have a superior ability to accumulate Mn, Zn and Cu compared to elite and other heritage genotypes and that the mechanisms of accumulation are related to one another. Many Bere barley genotypes have been selected over generations on marginal soils of the Highlands and Islands of Scotland. These include calcareous and sandy soils with limited plant available micronutrients which may have applied a selection pressure for traits involved in micronutrient efficiency. Identifying and utilising the genes and phenotypes from ancient germplasm has the potential to provide breeders with markers to develop crops for future agricultural sustainability.

ROUTES TO IMPROVED THERMOTOLERANCE IN BARLEY

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Sustainable agriculture in the face of the climate crisis depends on identifying genetic routes to increase crop resiliency. While barley is well known to endure a wider range of environmental stresses (Newton et al., 2011), many elite cultivars are sensitive or intolerant to heat stress during post-reproductive growth, leading to decreases in photosynthetic efficiency, pollen viability, grain number and weight, and grain malting quality (Mahalingam, and Bregitzer, 2017). Our research aims to understand how the *APETALA2* gene in barley (*HvAP2*) contributes to resiliency in barley. We recently discovered that specific *HvAP2* alleles provide a significant protective effect against acute heat stress. These roles may reflect our findings that *HvAP2* may regulate flowering time, plant height and quality by influencing pathways normally involved in plant defence (Patil et al., 2019; Shoesmith et al, 2021). Here, we will describe our controlled environment experiments exploiting an *HvAP2* allelic panel to understand how *HvAP2* variation contributes to thermotolerance following post-reproductive heat stress, including *HvAP2*-depending stay-green phenotypes and changes in gene expression. We will also describe our plans to test whether *HvAP2* improves resiliency in response co-occurring stresses.

***BLUMERIA GRAMINIS* TRANSCRIPTION FACTORS THAT PROMOTE GENE EXPRESSION IN HAUSTORIA AND BARLEY POWDERY MILDEW VIRULENCE.**

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Blumeria graminis f.sp. *hordei* (*Bgh*), is an economically important obligate biotrophic fungus that causes powdery mildew in barley. Powdery mildews produce haustoria which are crucial for biotrophy, since haustoria are required

for the uptake of nutrients, as well as for the expression and delivery of effectors and virulence factors into host cells. Transcription factors (TFs) and other mechanisms that orchestrate upregulation of gene expression of effectors in haustoria are mostly unknown and therefore need to be investigated. *Blumeria* transcription factors that were either upregulated in haustoria, *bZip1* (Pap1), a *Zn-knuckle* (*ZnF*) and *TF4072* or *BghPacC*, an homologue to *PacC*, which is associated with the alkaline pH response and virulence in other fungi, were investigated for their role in virulence and gene expression in haustoria. A suitable Host Induced Gene Silencing (HIGS) approach using short antisense oligodeoxynucleotides (ASOs) with phosphorothioate modifications (PTOs) was optimised for *in planta* silencing of genes from this obligate pathogen. The barley "*Mildew locus O*" (*MLO1*), a well-known barley gene conferring susceptibility to powdery mildew and the seed protein *Z* were used as positive and negative control silencing targets respectively. The silencing of the three TFs upregulated in haustoria, namely *bZip1* (Pap1), *Zn-knuckle* (*ZnF*) and *TF4072* showed decreased infection, with more than 50% reduction in hyphal formation when silencing *bZip1*(*Pap1*) and *ZnF* transcription factors. These initial data suggest that the four TFs *BghPacC, bZip1* (Pap1), *Zn-knuckle* (*ZnF*) and *TF4072* have a virulence role for *Bgh* infection of barley, possibly by controlling gene expression in haustoria.

Wakeboarding, Dundee
Image Courtesy of Dundee, One City Many Discoveries

Development

WHY ARE WHEAT BASAL SPIKELETS RUDIMENTARY?

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Grain-bearing spikelets are attached along the inflorescence of wheat (*Triticum aestivum*) in a distichous manner. But not all spikelets are equal: Central spikelets are more productive with more and larger grains than spikelets at the apical and basal end (Bonnett, 1966). Various explanations exist for why the apical spikelets gradually become smaller, for example the reduced availability resources (Whingweri, 1981; Ghiglione, 2008) and the fact that apical spikelets are formed last during development (Kirby, 1974). But thus far it remains a mystery why the most basal spikelets are rudimentary (i.e. of small size and failing to produce grain). We imaged developing wheat spikes and showed that this bi-directional gradient in spikelet productivity is established very early on, just after the double ridge stage. At this point in development, the previously bilateral-symmetric apex starts to elongate horizontally, and the spikelet primordia are established. To investigate the genetic causes of developmental deviations in basal spikelets we hand-dissected spikes into apical, central and basal sections at different developmental stages. We performed RNA-sequencing of single sections from single plants using novel low-input RNA-sequencing techniques (Giolai, 2019). Unsupervised clustering of the sequencing libraries showed that, transcriptionally, larger differences exist across a spike than between the same sections from different developmental timepoints. Therefore, spatial dissection of the wheat spike could bring an important new dimension to the analysis of its development, which mainly has been investigated in a temporal dimension. Among the differentially expressed genes we identified the MADS-box transcription factor *VEGETATIVE TO REPRODUCTIVE TRANSITION 2* (*VRT2*) to be significantly upregulated in the basal sections compared to the apical sections at all timepoints. We confirmed this through an independent qRT-PCR study. *VRT2* is one of three *Short Vegetative Phase* genes in wheat and known to be downregulated at floral transition. Recently, it was shown that *T. polonicum* carries a *VRT-A2* allele that is ectopically expressed throughout spike development (Adamski *et al*., 2020). We found that *T. polonicum VRT-A2* near-isogenic lines (in 39ereal39ion cv. Paragon) have significantly more rudimentary (none-grain producing) basal spikelets in both field and glasshouse. Light microscopy of the *T. polonicum* developing meristem at double ridge stage revealed that the basal spikelet anlagen in *T. polonicum* have larger bract ridges and smaller spikelet ridges. We therefore hypothesise that ectopic expression of *VRT-A2* in the base of *T. polonicum* spikes delays bract suppression, which in turn negatively affects spikelet establishment, leading to their rudimentary form.

MANIPULATION OF AXILLARY MERISTEM INITIATION IN HEXAPLOIDY WHEAT

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Tillering in grasses is an important agronomic trait for grain production, which has been subject to artificial selection during the domestication of wild ancestors. Tillers are formed by the initiation and outgrowth of axillary meristems that determine the number of grain-bearing branches formed. Differences in axillary meristems formation and development produces the diversity of plant architecture seen amongst the grasses. The molecular mechanisms underpinning meristem formation in dicots have been well characterised through the study of mutants with altered patterns of shoot development. Most mutants exhibiting increased meristem formation have been found to be involved in the regulation of apical dominance and branching by phytohormones. However, the identification of key components controlling plant architecture and seed yield has been more challenging in polyploid grasses such as wheat. We have found a transcription factor that controls the formation of axillary meristems in 39ereal39ion wheat.

The ectopic expression of this transcription factor results in a significant increase in tiller number and seed yield both in wheat and rice. Transcriptional network analysis has revealed some of the components implicated in meristem initiation and maintenance in wheat. Identifying genes implicated in tiller formation could be a useful strategy to enhance seed productivity in hexaploidy wheat and other grasses.

WALKING A WAXY PATH: MOLECULAR CHARACTERISATION OF BARLEY *ECERIFERUM-YY*

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Current climate variations and future projections are posing an unprecedented challenge to XXI century sustainable agriculture. Plant waxes, found primarily in the cuticle, provide a protective barrier to a range of abiotic and biotic stresses including water loss, ultraviolet radiation, pathogens and insects. Therefore, identification of genes regulating waxes biosynthesis, is a key step towards the development of innovative, climate-smart crop varieties. Barley is the fourth most cultivated cereal worldwide and an excellent model to study the genetic basis of crop adaptation to the environment. To date, 1,580 barley *eceriferum* (*cer*) mutants have been isolated and described based on the glaucous appearance of the leaves, leaf-sheaths and spikes, and represent an attractive tool to dissect the molecular mechanisms underlying wax synthesis and deposition in cereals. Here, I will describe the characterisation of *Cer-yy*, a dominant, organ-specific suppressor of wax accumulation present in numerous wild and cultivated barley accessions. The composition and crystal structure of cuticular waxes showed that the non-glaucous appearance of the mutant spikes is due to the absence of β-diketones. An RNA-seq analysis corroborated this finding revealing that *Cer-yy* mutants have strongly down-regulated expression of the *Cer-cqu* gene cluster, a key element for β-diketones synthesis. We mapped *Cer-yy* to a sub-centimorgan region on barley chromosome 1H, allowing the identification of candidate genes, and we are currently exploring the recently published barley pangenome to gain insights into this locus. I will present the major findings of this research and outline future developments aimed at identifying the *Cer-yy* gene.

BEYOND ANTHER EXTRUSION: TRAIT DISCOVERY FOR EFFICIENT HYBRID WHEAT PRODUCTION

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Hybrid production of crops has been an ongoing major issue for decades for many crops such as corn, rice and rye. Hybrid performance is generally associated with higher grain yields, better adaptation and improved yield stability. Higher grain productivity is urgently needed in view of a growing world population and increased environmental calamities such as climate change. For wheat (*Triticum aestivum* L.), even though one of the world's top-three crops, no broad commercial success has yet been achieved due to its low efficiency for hybrid wheat production. To help overcome this low efficiency, we decided to step back and have a closer look at wheat's flower biology within the project HYFLOR. Anther extrusion is a trait well-known for its importance in hybrid wheat production. But what about other factors such as the amount of pollen and its viability or the receptivity of the female reproductive structures? All appear decisive for successful hybrid grain production but have been fairly underrepresented until very recently.
In order to identify relevant and cost-efficient traits for hybrid wheat production, we divided the whole topic into 'males' and 'females'. Both components will be analyzed individually in separated field trials. Depending on the grain set of the corresponding females, we will select good and bad males/females. These genotypes will be further examined in greenhouse experiments regarding their flower structure. For the male approach we will investigate pollen and anther traits, and for the female approach we will investigate receptivity traits. The grain set data from the field trials will be used for QTL analyses to reveal genetic loci contributing towards improved pollination capability and

receptivity. By colocalizing the QTLs with gene expression data gathered from contrasting parental lines, we will also try to identify candidate genes and genetic markers for the selection of good parents for hybrid breeding. In summary, this project aims to investigate the flowering biology of wheat in order to ensure an efficient and cost-effective production of hybrid wheat grains.

BARLEY DEVELOPMENTAL RESPONSES OF DIFFERENT *VRN-H1*, *VRN-H2* AND *PPD-H1* ALLELES TO HIGH TEMPERATURES AND VERNALIZATION TREATMENTS

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Variation on flowering time allows to optimise cereal adaptation to a wide range of agronomics conditions. Flowering time can be divided into two sub-phases, in which yield and components are being determined: (i) vegetative and (ii) reproductive. Additionally, flowering time can be understood as (i) final leaf number and (ii) phyllochron (thermal time between the appearance of two successive leaves). Ambient temperatures are increasing as consequence of climate change. Therefore, exploring physiological responses of barley to rising ambient temperatures are required. In this study, we examined how different allelic combinations of *VRN-H1*, *VRN-H2* and *PPD-H1* genes control barley developmental traits under two ambient temperatures (18˚C and 25˚C) with contrasting vernalizing treatments (V0 and V60 days) using eight near isogenic lines (NILs) differing in allelic constitution at these genes. Unvernalized plants carrying winter *vrn-H1* and *VRN-H2* alleles did not progress in the development. Recessive *ppd-H1* alleles caused photoperiod insensitivity and delayed flowering time and duration of sub-phases compared to sensitive *PPD-H1* alleles. High temperatures delayed flowering time in unvernalized plants. Otherwise, when lines were vernalized, higher temperatures delayed flowering time exclusively in spring barleys with insensitive *ppd-H1* alleles whilst accelerated flowering time in spring barleys with sensitive *PPD-H1* alleles. This finding evidenced an interaction between temperature and vernalization. Overall, differences on flowering time caused by the combination of three genes, temperature and vernalization treatments were mostly related to changes on the length of the late reproductive phase and variations in phyllochron. Regarding yield and components at 18˚C, insensitive *ppd-H1* alleles yielded more and generated more grains than plants with sensitive *PPD-H1* alleles. At high temperatures, *PPD-H1* lines presented more grain yield and higher grain number than *ppd-H1* lines. Furthermore, wild type *PPD- H1* lines yielded best in spring backgrounds (*VRN-H1-7*), whereas, recessive *ppd-H1* lines were best in winter lines (*vrn-H1*). These observations provide a potential source of information for breeding high-yielding barley cultivars with specific combination of *PPD-H1* and *VRN-H1* alleles. In conclusion, we report new findings on the effect of three key barley genes in interaction with environmental conditions that are relevant to barley breeding for upcoming climate conditions.

GENETIC CONTROL OF FLOWERING TIME IN A WIDE BARLEY CROSS

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Barley is the most diverse and drought tolerant cereal crop, and even adapted to arctic, subarctic and tropical environments outside the cultivation area of the other cereals. Appreciation of the complex genetic control of the key developmental stage of flowering has become a substantial scientific goal to minimize abiotic and biotic stresses under changing climate conditions. This project aimed to identify phenotypic segregation and diagnostic markers in the bi-parental subpopulations of crosses between the French elite cultivar RGT Planet and three landraces from Syria (WB-340), Ethiopia (WB-468) and a former cultivar from Mexico (WB-168) for maximum variation.

MODELLING ILLUSTRATES THAT GENOMIC SELECTION PROVIDES NEW OPPORTUNITIES FOR INTERCROP BREEDING

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Intercropping is an agricultural practice in which two or more component crops, commonly in combination between a cereal and a legume, are grown and managed in the field simultaneously. The practice can positively influence the agricultural land, provide a farmer with more resilience and can also improve dietary diversity. Especially in subsistence agriculture, intercropping has great potential to optimise farming and increase profitability. In spite of these potential benefits, intercrop breeding has received very little attention to date, with varieties specifically bred for intercrop production systems unavailable primarily due to preferences for simpler monoculture production systems and lower operational costs associated with these. As a result, sub-optimal monoculture varieties are being grown in intercrop production systems. Genomic selection may offer opportunities to overcome some of the traditional obstacles that may have hindered the development of intercrop breeding programs. However, it is unknown whether the benefits gained outweigh the extra costs that come with genomic selection. In our study, we hypothesized that genomic selection can significantly simplify and accelerate the process of breeding crops for intercropping. We used stochastic simulation to design and compare four different intercrop breeding programs implementing genomic selection and an intercrop breeding program entirely based on phenotypic selection. We assumed three different levels of genetic correlation between monocrop grain yield and intercrop grain yield to represent different degrees of genotype-by-cropping system interaction. All five breeding programs were constrained to have the same annual operational costs and were further tested using three different annual operational costs to determine the benefit of genomic selection under different budgetary scenarios. We found that all four simulated breeding programs using genomic selection produced significantly more intercrop genetic gain than the phenotypic selection program regardless of the genetic correlation with monocrop yield and annual operational costs available. To our knowledge, the use of simulations to explore genomic selection's value for intercrop breeding is unique. Moreover, the topic of our research has important implications for future farming system development.

DECIPHERING THE GENETIC MECHANISM UNDERLYING THE *CALCAROIDES-C* BARLEY MUTANT

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Barley is an important cereal crop and is farmed for grain production for food, malting and animal feed. To improve grain yield and quality, it is essential to understand the processes underlying grain development. The developing barley grain is protected by two leaf-like structures, the palea and the lemma. The lemma has a close relationship with the grain, but we know little about the genetic mechanism underlying lemma development or how lemma development relates to grain development. One way to shed light on this is to investigate how lemma shape can be altered and how this altered shape can affect grain traits. *Calcaroides-C* is a dominant mutant which develops a reduced awn and a novel sac-like structure on the lemma but has normal leaf development. To understand how the *Cal-C* mutant phenotype arises we are carrying out a developmental timecourse to track how lemma shape changes over time using confocal microscopy. Using this timecourse we will target a key developmental stage for RNAseq profiling of the developing lemma to identify potential candidates for the *CAL-C* gene and identify gene expression changes that may alter growth and polarity during *Cal-C* development to generate the novel lemma shape. Changes in spatial expression patterns will also be tested via RNA *in situ* hybridisation. We also plan to measure grain traits such as grain shape and carbohydrate and protein content to test whether the novel *Cal-C* shape influences grain development. Taken together we aim to determine what leads to the *Cal-C* phenotype and how this may impact on grain development.

DISSECTING THE EFFECT OF VARYING TEMPERATURE ON THE VERNALIZATION RESPONSE IN WHEAT

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Winter wheat is an important crop in both the UK and further afield. In winter varieties, exposure to prolonged cold temperatures in the early stages of wheat development facilitates the transition from vegetative to reproductive growth in a process known as vernalization. This enables flowering to be coordinated with the seasons and therefore increases the plants reproductive success and our final yield potential.
Vernalization can be observed in the field: winter wheat varieties are sown in the autumn and "overwinter" through the inhospitable winter conditions, before flowering in the following spring. The potential yield impact of increasing and variable global temperature on vernalization highlights the importance of understanding its effect in the field. Specifically, we are interested in whether warmer temperatures can fulfil the vernalization response. By recording the date of ear emergence and tracking the rate of apex development in several winter wheat varieties, we are able to determine when vernalization is completed. In addition, we have traced the expression of the key genes from the vernalization pathway (*VRN1*, *VRN2*, *FT1*, *VRT2*, and *ODDSOC2*, respectively). Investigating whether warmer temperatures during the early stages of growth affects the regulation of these genes is an essential part of understanding the molecular mechanism underlying the process of vernalization. Building on this, we have started to profile the gene expression responses, via RNA-Seq, across the vegetative to reproductive growth phase in the field with the aim of identifying new genes involved in the process. Protein activity will be investigated using two-hybrid screening and surface plasmon resonance to distinguish interaction between genes in the vernalization pathway and build a network where the temperature sensitive interactions can be regulated.

UNDERSTANDING THE MOLECULAR GENETICS OF GRAIN TO HULL ADHESION IN BARLEY

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Barley is one of the most important and oldest cultivated crops in the world. In most barleys, a protective and firmly adherent hull covers the grain fruit or caryopsis at maturity. Hulls adhere due to secretion of a sticky cementing layer onto the caryopsis cuticle. The NUDUM (NUD) transcription factor is required for cementing layer secretion, yet the regulatory cascade downstream of NUD is unclear. Poor hull adhesion can cause partial hull shed or "skinning" from the grain, an undesirable trait for the malting industry, which is controlled by both genotype and environment. We exploit barley mutants that show a stable skinning phenotype to investigate the underlying structural, chemical and genetic mechanisms of hull to grain adhesion. Here, I will present the characterisation of two mutants' skinning phenotypes, the identification and characterisation of the causal genes, and outline future work to understand downstream signalling pathways involved in grain to hull adhesion.

FINE-TUNING OF VERNALIZATION RESPONSE IN HYBRID BARLEYS

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Plant breeders must continuously deliver high-yielding and stable varieties adapted to increasingly unfavourable and variable environmental conditions. Fine-tuning crop phenology will be critical to mitigate the impacts of climate change on yield, to match the availability of resources with the most sensitive growth stages. Nowadays, hybrid

cultivars are increasingly used to raise productivity per unit of land and to ensure yield stability under fluctuating environmental conditions. Therefore, the optimization of phenology in hybrid cultivars is a strategy to improve yields under current and future climate conditions. However, there is lack of knowledge about flowering time control in a hybrid context. This study aims at assessing differences in development and gene expression in key genes controlling the duration of plant cycle, in a set of hybrid barleys and their parents under three different vernalization conditions (2,4 and 8 weeks of cold). A Central European female parent was crossed with three Mediterranean adapted inbred lines with different *VRN-H1* alleles. After vernalization, four plants of each genotype were transferred to a growth chamber with a 16 h photoperiod regime. The plants were regularly checked for the appearance of the first node (Z31), and awn tipping (Z49). Gene expression with qRT-PCR was monitored 17 and 35 days after the end of the vernalization treatment. In general, hybrids showed intermediate development compared to both parents. Genotypes presented different vernalization responses, whose variation was mostly explained by changes in the length until Z31, linked to the *VRN-H1* allele present and proportional to their genetic dosage. These differences were maintained until awn tipping but, in some cases, were modulated by the effect of other genes in the late reproductive phase, likely *VRN-H3* and *PPD-H2*. The inheritance of *VRN-H1* winter alleles was additive in hybrids throughout the vernalization treatments. Variation in the length of the late reproductive phase was mostly related to the *VRN-H3* allele present. Hybrids bearing one functional allele at *PPD-H2* accelerated flowering under insufficient vernalization, confirming the vernalization dependent effect of *PPD-H2* and its adaptive role in warm environments. We can conclude that hybrid combinations extend the available catalogue of genetic responses to vernalization. This opens new possibilities for fine-tuning barley phenology to the specific areas using hybrids.

UNDERSTANDING ENVIRONMENTAL REGULATION OF FLORET DEVELOPMENT IN WHEAT

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The timing of flowering is a critical factor that determines a plant's reproductive success in a changing environment. Decades of research have identified numerous environmental signals that determine when flowering begins in many species. However, very little is known regarding how these same environmental cues can control the termination of flowering. In wheat, inflorescence development takes place before heading. By the time the spike emerges, the spikelets and maximum number of florets have already been reached. Some of these florets will however subsequently degenerate. Various works have highlighted how changes in environmental signals such as photoperiod can impact floret development and modulate the final number of florets that survive this phenomenon. However, the relevant developmental and molecular events underlying this environmental integration at the floret level remain unknown.
In the angiosperm model *Arabidopsis thaliana*, the floral integrator *FLOWERING LOCUS T* (*FT*) controls the onset of flowering. Moreover, our work in Arabidopsis has identified that *FT* has a key role in regulating the shutdown of the inflorescence meristem and the end of the reproductive process. *FT* genes are also involved in controlling flowering time in wheat, raising the question of whether they may also control events surrounding the end of flowering, such as floret abortion. To investigate this possibility, we will characterise *FT1* in controlling the timing and duration of early apex development and floret abortion in wheat in response to environmental signals. This work will involve a detailed characterization of wheat TILLING lines and a study of wheat *FT* homologues using Arabidopsis as a heterologous system. The natural variation in the timing of these developmental processes will be assessed by screening a variety of landraces, and the specific role of environmental signals in their control will be evaluated under controlled conditions.

DIMERIZATION POTENTIAL OF 300 bZIP PROTEINS IN BREAD WHEAT

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Allohexaploid bread wheat (*Triticum aestivum*) is one of world's principal staple crop and a major source of calories with high nutritional value. Transcription factors including bZIPs are the primary conduit of environmental signals that ultimately results in gene expression. bZIP family contains one of the most abundant transcription factors in plants that regulate diverse biological processes such as pathogen defence, light and stress signalling, flower development and seed maturation. bZIPs regulate gene expression when they bind as homo- or heterodimer to short and specific promoter sequences. Theoretically, 300 bZIPs can form ~87,025 homo- and heterodimers, suggesting a remarkable transcriptional control. Dimerization specificity of bZIPs is well-studied in Drosophila, Mouse, Human and Arabidopsis but such studies are completely lacking in bread wheat. An unresolved functional aspect of bZIP proteins is how they favour to dimerize and how it affects their function? In order to address this problem, structural rules that define the dimerization potential of bZIP TFs, have been refined over the years and now are reliable enough to predict bZIP's dimerization with high confidence. Based on conserved structural motifs, we have found that wheat is predicted to have ~300 bZIP TFs. bZIPs consist of structural α-helical heptad repeats composed of seven amino acids with leucine or other hydrophobic amino acids occurring at 'd' position in each heptad. We have analysed the placement of amino acids in each heptad and at specific positions to infer their dimerization specificity based on (i) length of leucine zipper, (ii) presence of asparagine or charged amino acids at 'a' position in heptads and (iii) presence of interhelical electrostatic interactions. Presently, we predict the dimerization specificity of 60 seed-specific homo- and heterodimerizing bZIPs. We are using biophysical techniques such as CD spectroscopy, MALDI-TOF MS mass spectrometry for studying protein-protein interaction. In wheat most of bZIPs homodimerize, Interestingly, we have found four bZIPs, which due to the presence of repulsive amino acids in 1^{st},2^{nd} and 4^{th} heptad cannot form homodimer and shall preferentially heterodimerize. Unlike human bZIPs, heterodimerization in wheat is dictated by repulsive interaction in 5^{th} and 7^{th} heptads.

STRATEGIES OF GRAIN NUMBER DETERMINATION DIFFERENTIATE BARLEY ROW-TYPES

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Gaining knowledge on intrinsic interactions of various yield components is crucial to improve the yield potential in small grain cereals. It is well known in barley that increasing the grain number (GN) preponderantly improves their yield potential; however, to achieve this aim, it is pivotal to recognize the maximum yield potential (MYP) of the crop. In barley (*Hordeum vulgare* L.), the MYP is defined as the maximum spikelet primordia number of a spike. Previous barley studies often assumed the awn primordium (AP) stage as the MYP stage regardless of genotypes and growth conditions. From our spikelet-tracking experiments using the two-rowed cultivar Bowman, we found that the MYP stage can be different from the AP stage. Importantly, we find that the occurrence of inflorescence meristem (IM) deformation and its loss of activity coincided with the MYP stage, indicating the end of further spikelet initiation. Thus, we recommend validating the barley MYP stage with the IM shape and propose this approach (named Spikelet Stop) for MYP staging. Following this approach, we assessed different yield components like potential spikelet number (PSN), spikelet survival (SSL), spikelet number (SN), grain set (GS), and grain survival (GSL), as well as their interactions with GN by using a selected panel of two- and six-rowed barley types. Also, to analyze the stability of these interactions, we performed the study in two growth conditions, greenhouse and field. From this study, we found that in two-rowed, GN determination is strongly influenced by PSN rather than SSL and/or GS in both growth conditions. Conversely, in six-rowed, GN is associated with SSL instead of PSN and/or GS. Thus, our study exemplified that increasing GN might be possible by augmenting PSN in two-rowed genotypes, while for six-rowed genotypes, the ability of SSL needs to be improved. We speculate that this disparity of GN determination in barley row-types might be due to the fertility of lateral spikelets. Collectively, this study revealed that the GN of two-rowed largely depends on the developmental trait, PSN, while in six-rowed, it mainly follows the ability of SSL.

UNDERSTANDING THE REGULATION OF FLOWERING TIME THROUGH PHOTOPERIOD 1 (*Ppd-1*) and FLOWERING LOCUS T (*FT*) GENES IN BREAD WHEAT

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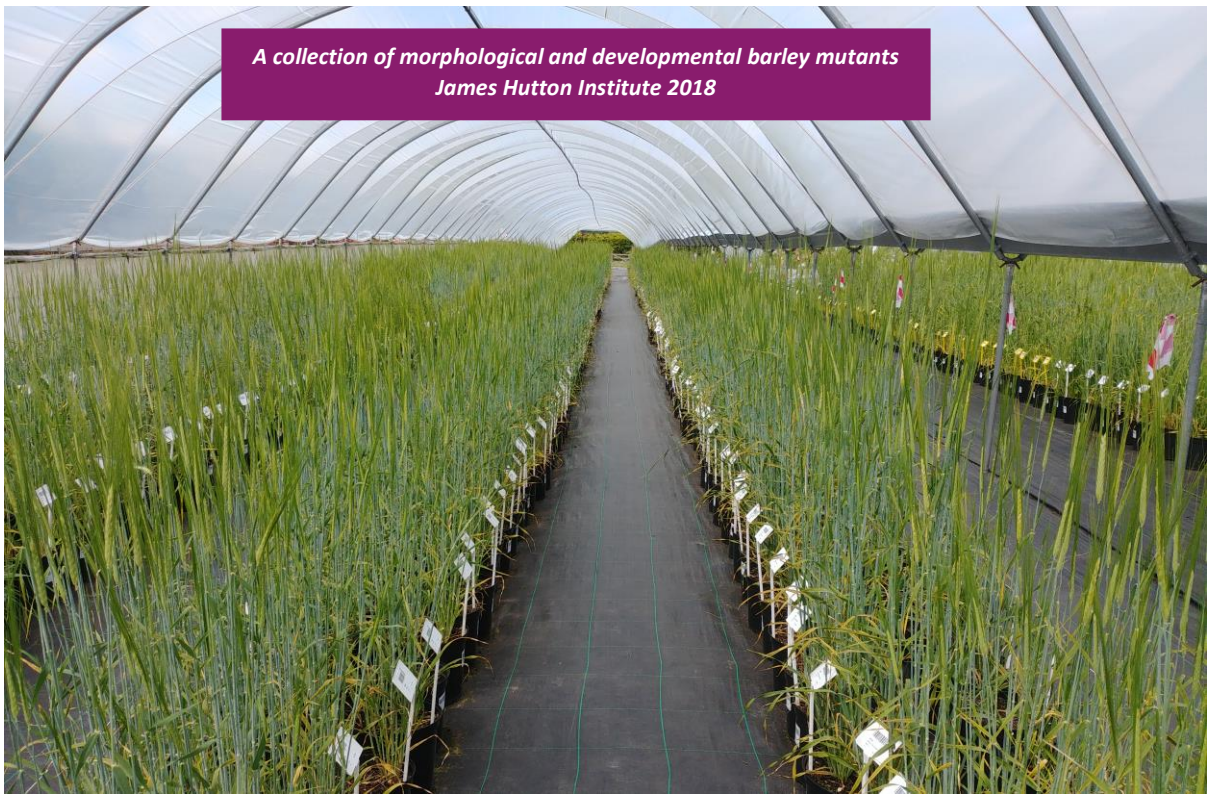
Wheat plants have evolved regulated ways to initiate flowering to maximise reproductive success. These aim to avoid the extremes of high and low temperatures associated with deep summer and winter to successfully produce viable offspring in the form of fertile seed. One of these systems of control is known as the photoperiod response and involves the sensing of daylength which when extended over 12 hours, means it is suitable for *Triticum aestivum* (bread wheat) to initiate flowering. The main gene responsible for the detection of the daylength extension is known as *PHOTOPERIOD 1* (*Ppd-1*). *Ppd-1* initiates flowering through the activation of FLOWERING LOCUS T 1 (*FT1*), which is believed to move from the leaf to the apex and begins the apical transition from being vegetative to reproductive. Previous work has identified and characterised *FT2* function, relating it to the commencement of the transition to terminal spikelet from lemma primordia. However, despite other *FT*s being identified (*FT3-12*), their functions remain unknown in 46ereal46ion wheat. My research aims to study the relationship between *Ppd-1* and the *FT* genes that have not yet been characterised and monitor their expression as the wheat apex develops. Four different genotypes of *Ppd-1* will be used: triple insensitive, single insensitive, triple knockout, Paragon wild-type. This is because allelic variations of *Ppd-1* have been shown to directly affect *FT1* and *FT2* expression so we postulate that this could be the same for *FT3-12*. Studying *FT* expression will involve growing the plants under controlled conditions and dissecting them at different apical stages such as vegetative and terminal spikelet. These dissections include the primary stems, primary tillers, and secondary tillers to compare gene expression between the three. The expression of *FT*s will then be studied using qPCR and using this data, the functions of the uncharacterised *FT*s could then be identified.



Genomics & Technologies

EXPANDING DURUM WHEAT DIVERSITY THROUGH *AE. CAUDATA* AND *T. TIMOPHEEVII* INTROGRESSIONS

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With the different challenges faced by durum wheat breeding programs, the use of wild relatives as sources of new genetic diversity could allow yield enhancement, mitigate disease/stress resistance and boost grain quality. This work outlines the incorporation of chromosome segments from *Aegilops caudata* (2n=2x=14; CC) and *Triticum timopheevii* (2n=4x=28; A^tA^tGG) into durum wheat (2n=4x=28; AABB) and their genotypic and phenotypic characterisation. A total of 146 backcrossed durum lines were generated, 59 derived from 47ereal47ion lines containing introgressions from *Ae. Caudata* and 87 derived from 47ereal47ion lines containing introgressions from *T. timopheevii*. The durum lines were screened using Kompetitive Allele Specific PCR (KASP) markers which allowed the identification and characterisation of the transferred introgressions. In total, 18 lines containing an introgression from *Ae. Caudata* linkage group (LG) 5 and 62 lines containing introgressions from *T. timopheevii* LGs 2, 3, 4, and 7 were identified. The lines were screened phenotypically at CIMMYT (Mexico), ICARDA (Morocco) and the Indian Institute of Wheat and Barley Research (India). Lines containing an introgression from LG5 from *Ae. Caudata* were found to have a higher test weight and grain protein content with reduced awns while higher grain protein and yellow colour were found in some of the lines containing introgressions from *T. timopheevii.* This strategy, involving the transfer of chromosome segments from wild relatives into durum wheat, together with their detection and characterisation, will allow breeders to maximize the use of the introgressions in their breeding programmes for the introduction of genetic diversity for agronomically important and novel traits.

CHARACTERISING AND VISUALISING 10 PLUS WHEAT GENE FAMILIES WITHIN GALAXY USING GENESEQTOFAMILY

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The phylogenetic information inferred from the study of homologous genes helps us to understand the evolution of gene families and plays a vital role in identifying ancestral gene duplication events as well as characterizing the nature, and quantifying the impact of the selective pressures acting driving the evolution of these genes. We developed GeneSeqToFamily (Thanki et al. GigaScience 2018), a computational workflow based on the Ensembl GeneTrees pipeline, to identify gene families using the Galaxy web-based bioinformatics platform. By using Galaxy we can perform a fully transparent and reproducible analysis and are able to easily tailor every workflow step if needed. It provides sequence alignments, associated phylogenetic trees, as well as details about exon conservation. One of the major computational challenges in identifying gene families is associated with the number of genes used as input either as a consequence of genome complexity or number of genomes analysed. The common bread wheat (Triticum aestivum) is a perfect exemplar of organisms owing to its size and complexity. The release of an increasing number of annotations across T. aestivum accessions to enable gene family identification across data including over 1.4 million genes. The characterization of the gene families and homologous genes across wheat varieties will provide key information about wheat evolution. The GeneSeqToFamily workflow and all tools composing it are open source and available to install from the Galaxy ToolShed as well as usable for free on the UseGalaxy.eu public instance. Source code is available on https://github.com/TGAC/earlham-galaxytools/.

LONG NON-CODING RNA ANALYSIS IN WHEAT PAN TRANSCRIPTOME

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All eukaryotes expressed tens of thousands of long non-coding RNAs (lncRNAs) whose function is often associated with expression regulation either in *cis* or *trans*. While the function and biological relevance of a few of those loci has now been well described, for the vast majority much remain to be learned. The release of extensive RNA-Seq across wheat cultivar and Triticeae species offer the unique opportunity to comprehensively annotate lncRNAs in *Triticum aestivum* and assess their variation in expression across tissues and accession. This large scale analysis enables us to identify loci that are likely inconsequential and to the characterise and prioritize of a set of lncRNAs for experimental validation based on their expression, regulation and conservation. Our annotation also give us the opportunity to investigate how lncRNAs have evolved in Triticeae and the impact of haplodiploidization on their diversity across species. Here, we present a bioinformatics approach to annotate and characterise more than 180,000 novel lncRNAs features across 148 RNA libraries, and 9 reference genome. And quantify lncRNAs expression across the tissues, accessions, and species. The pipeline has been developed to be species agnostic and is available from GitHub: https://github.com/TGAC/lncRNA-analysis

RAPID MASKING OF PLANT GENOMES BY COMBINING KMER COUNTING AND CURATED REPEATS

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The annotation of repetitive sequences within plant genomes can help in the interpretation of observed phenotypes. Moreover, repeat masking is required for tasks such as whole genome alignment, promoter analysis or pan-genome exploration. While annotation pipelines are computationally expensive, k-mer strategies for masking are orders of magnitude faster. Here we benchmark a two-step approach, where repeats are first called by k-mer counting and then annotated by comparison to curated libraries. This hybrid protocol was tested on 20 genomes from Ensembl Plants, using the kmer-based Repeat Detector (Red) and two repeat libraries (Redat and nrTEplants, curated for this work). We obtain repeated genome fractions that match those reported in the literature, but with shorter repeated elements than those produced with conventional annotators. Inspection of masked regions overlapping genes revealed no preference for specific protein domains. Half of Red masked sequences can be successfully classified with nrTEplants, with the complete protocol taking less than 2h on a desktop Linux box.

INVESTIGATING GRAVITROPISM AND THE *LAZY* GENE FAMILY IN WHEAT

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Gravitropism and the root gravitropic response help to shape plant root system architecture. The *LAZY* gene family has important roles in the gravitropic pathway and are known to influence root and shoot architecture in Arabidopsis and many other species. Several *LAZY* genes are predominantly root associated and can affect root growth angle, which is an important component trait of root system architecture. Root growth angle can affect nutrient uptake

where soil nutrient composition varies between soil layers. For example, water and nitrogen are highly mobile in soil and are likely found in deeper soil layers, so a steeper root growth angle phenotype can be advantageous in water and nitrate-limited conditions. From an EMS mutagenesis screen, we previously identified an Arabidopsis *lazy* mutant with more vertical lateral root growth angles. This root system architecture modification discovery has great potential to translate into other species, so we are investigating the *LAZY* gene family in wheat. We are characterising wheat *LAZY*s and exploring the gravitropic mechanism of non-vertical lateral root angles in the wheat root system. We screened Cadenza TILLING lines with *lazy* mutations to investigate their root growth angle phenotypes. Ongoing studies are investigating utilising the *LAZY* genes to modify crop root system architecture.

GWAS META-ANALYSIS OF A EUROPEAN FIELD TRIAL NETWORK OF SPRING BARLEY

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A panel of 151 spring two-rowed barleys were selected from larger panels assayed in field trials performed across Europe and North Africa, in the framework of EU projects ExBarDiv and ClimBar. After curation of phenotypic data, 16 trials were selected from Finland, Germany, Italy, Morocco, Spain and United Kingdom, carried out over 2009, 2010, 2016, and 2017. BLUEs were calculated for plant height, heading date, and grain yield. The panel was genotyped with the 50K Illumina Infinium SNP array. After imputation of missing values, markers with less than 5% MAF were excluded from the analyses. Single trial GWAS was performed with the mixed linear model (MLM) from the GAPIT suit, in R. Adjustment for population structure included the kinship matrix, as no significant principal components were detected. After this procedure, meta-analyses were run for the entire set of trials, and for the partitioning of trials that best captured genotype by environment interaction after AMMI analyses per trait. Meta-analyses increased markedly *p*-values of marker-trait associations. Commonly used thresholds for associations using this procedure (–log10*p*>8) were deemed too liberal in our case. A new threshold was selected as the minimum *p*-value found after 1000 meta-analyses of independent permutations of the actual *p*-values from each trial. These thresholds were stricter, but still allowed the identification of multiple marker-trait associations. A preliminary assessment of the width of the regions based on LD, and of the genes present in these regions will be presented.

NIAB DIVERSE MAGIC: DISSECTING TRAIT GENETIC ARCHITECTURE ACROSS 70 YEARS OF WHEAT BREEDING

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The NIAB Diverse MAGIC multi-parent population comprises over 500 recombinant inbred lines, descended from sixteen historical UK bread wheat varieties released between 1935-2004. The founders' genes and promoters were sequenced by capture and the MAGIC population by low-coverage whole-genome sequencing. We imputed 1.1M high quality SNPs that were over 99% concordant with array genotypes. Imputation accuracy only marginally improved when including the founders' genomes as a haplotype reference panel. Despite capturing 73% of global wheat genetic polymorphism, 83% of genes clustered into no more than three haplotypes. We phenotyped 47 agronomic traits over two years and mapped 136 genome-wide significant associations, concentrated at 42 genetic loci with large and often pleiotropic effects. Around half of these overlap known QTLs. Most traits exhibit extensive polygenicity, as revealed by multi-locus shrinkage modelling. Our results are consistent with a gene pool of low haplotypic diversity, containing few novel loci of large effect. Most past – and projected future – phenotypic changes arising from existing variation involve fine-scale shuffling of a few haplotypes to recombine dozens of polygenic alleles of small effect. Moreover, extensive pleiotropy means selection on one trait will have unintended consequences, exemplified by the negative trade-off between yield and protein content, unless selection and recombination can break unfavourable trait-trait associations.

GENERATING DOMINANT CIS-REGULATORY VARIATION IN WHEAT

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Wheat occupies a central place in human nutrition providing 20% of the global protein and calorie intake. Due to rising population and the concurring annual increase in wheat consumption, finding strategies to increase wheat yield is imperative. However, in polyploid species such as wheat, the study of quantitative traits is confounded by functional redundancy between the closely related homoeologous gene copies. Despite this, many domestication traits and natural variants that lead to dominant visible phenotypes in polyploids are due to *cis*-regulatory variants. Often these domestication traits are controlled by transcription factors (TFs). Thus, generating dominant *cis*-regulatory variation in TFs is a potentially valuable means to improve breeding targets. To pursue this, we selected two well-characterised TFs, *APETALA2* (*AP2/Q)* and *TEOSINTE BRANCHED 1* (*TB1)*, as a proof of concept on the consequences of generating variation in *cis*-regulatory elements in polyploids. We identified conserved non-coding sequences based on conservation with grass orthologs. We then designed CRISPR/Cas9 polycistronic constructs (two independent constructs, each based on six sgRNAs) to target these regions. Transformed plants are currently growing and our aim is to identify different versions of promoter-deletions and correlate this with phenotypic patterns. Ideally this method will help us to elucidate the mechanism underpinning the regulation of desirable genes. Secondly, we aim to identify open-chromatin configuration during spike and carpel development in wheat, as TF binding to *cis*-regulatory sequences has been associated with accessible chromatin. To do this, we are using ATAC-seq (Assay for Transposase Accessible Chromatin with high-throughput sequencing). Future work will involve the integration of accessible chromatin data obtained together with expression data, evolutionary conserved regions analyses and association studies, to then prioritise *cis*-regulatory sequences, TFs and candidate genes. These candidates will be edited, for example, via CRISPR/Cas9 with multiple guides as previously indicated, and evaluated for their potential in breeding programmes.

WHEAT HAPLOTYPING FOR BREEDING

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Whole Genome Re-sequencing (WGS) is now affordable as a variant calling method for many crops. Hexaploid wheat, however, has a polyploid, highly repetitive and 16 Gbp genome, making WGS relatively expensive. SNP arrays and capture probes are alternative options used for wheat genotyping, although pivotal information outside of gene-coding regions is usually not assayed. Using these technologies, a selection of individual markers, or a set of closely linked markers (haplotypes) can be assessed for genotype-phenotype association. In this project we are investigating the haplotype diversity of recent commercial lines, older cultivars (pre-1990's), and landraces wheat genotypes, aiming to generate an haplo-database for genotype-phenotypes association analysis. Recently, it has been shown that wheat genomes have conserved haplo-blocks among important world cultivars (Brinton *et al.,* 2020). Our results show that these haplo-blocks are being maintained for several generations across pedigree populations. For this study, we employed WGS at 10-fold coverage, and we are leveraging the recent pan-genome assemblies (Walkowiak *et al.,* 2020) to build an exhaustive haplotype database for wheat. Our strategy employs *k*-mers to call variants which are then used for the haplo-database building by pedigree-guided information. In the next phase of the project, we will employ skim sequences (~0.2x) of UK elite lines to validate a method for anchoring these shallow sequences and assign haplo-blocks from the aforementioned haplo-database. All these genotypes are being field-phenotyped for agronomic traits and we will use this data to test our haplotype-phenotype association model. Furthermore, we will use this haplo-database to detect haplotypes from landraces which are currently not present in UK cultivars. We will test the phenotypic value of those haplotypes using advanced ($F_{4:5}$) populations of elite cultivar Paragon crossed with landraces developed within the WISP and DFW institute strategic programmes. This approach will allow us to target the introgression of specific haplotypes into commercial cultivars.

MAKING SHORTS TALL AGAIN

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The inverse effects of wheat dwarf genes on yield in environments with extreme heat and constant drought like Kazakhstan are well documented. However, tall varieties have also such disadvantages as lodging and decreased harvest index. Thus, optimising plant height (HT) to drive wheat adaptation in Kazakhstan using modern genomic tools is the main priority. In our study, we found two HT increasing QTL on 5A and 6A wheat chromosomes which originated from a Kazakh wheat variety. Both were detected in RILs and have the additive effects of ~10cm. We developed NILs in the background of UK wheat variety Paragon for each QTL and conducted large scale glasshouse and field trials in various wheat growing environments of Kazakhstan. Results showed tall alleles increased not only the height, also the yield by ~20% in 5A and 6A NILs relative to the local check variety in the South-West Kazakhstan. In the North, however, we did not see such yield increase in NILs comparing to parents, but tall NILs performed well over shorts in many important traits and increased grain yield. Recently, double NILs have also been generated to examine the double effect of genes. Glasshouse experiment on double NILs revealed promising outcomes. Medium resolution mapping was carried out to bring the initial QTL regions down using map-based cloning and exome capture analyses.

TEMPORAL AND GENOMIC ANALYSIS OF ADDITIVE AND DOMINANCE GENETIC VARIANCE IN HYBRID BREEDING

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This study demonstrates a framework for temporal and genomic analysis of additive and dominance genetic variance in hybrid breeding programmes. Many crops rely on hybrids to yield high production. Hybrids are a product of selection and matching lines from different heterotic pools. In this context it is important to assess the amount of additive and dominance genetic variance that drive response to selection and heterosis as well as inbreeding depression. However, estimating these components of genetic variance over time and genome regions is challenging. Here, we describe a framework for such analysis using genome-wide marker data and demonstrate it using a simulated maize breeding programme involving a complex trait with additive and dominance gene action. The framework involves three steps: (i) fit a marker-based model to the dataset to estimate additive and dominance marker effects along with directional dominance parameter, (ii) sample realisations of the effects from the fitted model and for each sample calculate realisation of total, additive and dominance genetic values, and (iii) calculate mean and variance of the sampled genetic values over time and genome partitions. With this framework we can track trends in genetic means and genetic variances over time for different groups of genotyped individuals and different genome regions. This analysis gives insight into response to selection, heterosis, inbreeding depression and underlying genetic variances in hybrid breeding programmes. Furthermore, by dissecting genetic variance in different stages of a breeding programme, over time and different genome regions, breeders have a better insight into the potential of their programme to deliver future genetic gains.

CRISPR-CAS9 TARGETED NANOPORE SEQUECING IN BARLEY

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Targeted sequencing approaches reduce complexity in large genome species such as barley which contains high levels of repetitive sequences with genic regions accounting for less than 2% of the genome. However, current targeted sequencing methods can be restrictive due to short read lengths, high input requirements, loss of native modifications and PCR bias. We used a CRISPR-Cas9 targeted sequencing approach with Oxford Nanopore Technologies long read sequencing to define a 157bp deletion in HvMLH3 (*des10* mutation) contained in Bowman line BW230. This involved designing guide RNAs to excise an 8kb region of HvMLH3. Prior to library prep, Cas9 was combined with the guide RNAs and a tracer RNA creating a ribonucleoprotein (RNP) complex. High molecular weight genomic DNA was dephosphorylated to limit off-target sequencing, before adding RNPs to excise the region of interest. This released phosphorylated ends and the attachment of sequencing adapters to our target. After MinION Nanopore sequencing, we observed over 100-fold sequencing enrichment of our target region allowing for identification of the 157bp deletion causal of the *des10* mutant of HvMLH3. This method, through PCR-free enrichment and sequencing, enables enrichment of repetitive or GC-rich regions that cannot be amplified by traditional methods. It is also possible to characterise large regions of interest (tens of kb) containing SNPs, structural variants, repeat expansions and modified bases from a single native DNA sequencing run.

BaRT 2.0: A PACBIO ISO-SEQ BASED REFERENCE TRANSCRIPT DATASET FOR TRANSCRIPT RESOLUTION RNA-SEQ ANALYSIS IN BARLEY

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How plants respond to internal and external cues at the transcriptional level are important questions that could be answered using RNA-Seq analysis. Phenotypic responses reflect both changes in gene expression and alternative splicing (AS). Salmon/Kallisto generate rapid and accurate transcript quantification from RNA-Seq data but require an

accurate and comprehensive reference transcript dataset (RTD). RTDs have already been developed for Arabidopsis and barley but are largely based on short read sequencing. Here, we present BaRTv2.0, a new transcriptome based on the reference genome of barley *cv.* Barke, combining Pacbio Iso-Seq and Illumina RNA-Seq datasets made from 20 different tissues and treatments. Iso-Seq data has been analysed with Iso-seq3 and TAMA. Long read sequences have high error rates and a major source of transcript sequence errors are misalignments due to errors close to splice junctions. Transcript ends can also be false due to degradation of mRNA. We have developed methods to 1) identify and remove false splice junctions and 2) rationalise transcript start and end sites in Iso-seq reads. Combined with the highly accurate splice junction data from short reads, we defined high confidence splice junctions, transcription start sites and transcription end sites to generate Iso-seq transcripts. A parallel pipeline (RTDmaker) assembled quality-controlled transcripts from short reads using multiple assemblers with stringent filtering and generated transcripts for genes not covered by Iso-Seq reads and novel transcript isoforms. Merging the two datasets generated BaRT2.0 with 56k genes and 173,635 transcripts. Compared to BaRTv1.0 (a short-read based transcript dataset from Morex), BaRT2.0 gives improvements in several benchmarks, including transcript and AS quantification accuracy. The resulting RTD is currently being used to analyse a large RNA-Seq dataset comprising six tissues from over 200 European spring barley varieties to identify associations between transcript abundance and yield traits and to construct gene co-expression networks associated with yield characteristics. The analysis pipeline will also feed into ongoing work on the barley pan-transcriptome, generating accurate transcript assemblies for 20 genomes to compliment the recently published barley pan-genome.

IDENTIFICATION OF YIELD-RELATED GENES IN BARLEY USING A MULTI-LAYERED GENOMICS AND TRANSCRIPTOMICS APPROACH

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Increasing crop yield through breeding requires a thorough understanding of the genetic architecture of several morphological and physiological traits, as well as of the interaction and regulation of genes affecting these traits. In this project, we aim to identify and characterize genes regulating yield-related traits in a panel of 211 two-rowed spring barley cultivars representing the European breeding germplasm of the last century. The panel was phenotyped for more than 20 traits, mainly earliness, grain and spike traits, in field trials in Gatersleben (Germany), Dundee (Scotland) and St. Paul, Minnesota (USA) in 2019 and 2020. In addition to the phenotypic data, we also created a transcriptome atlas using RNA-sequencing data from six tissues across all 211 barley spring lines. RNA-seq quantification will allow us to elucidate how gene expression influences key traits in barley and how these genes are regulated throughout plant development. For further genetic analysis, RNA-seq derived SNP alleles will be complemented by variants identified through 4x coverage whole genome shotgun sequencing. The search for genetic variation commonly focuses on genome-wide association studies (GWAS) to find sequence variation associated with a trait, while gene expression variation as a driver of phenotype is often neglected. Transcriptome-wide association studies (TWAS) can complement GWAS by enabling the identification of associations between transcript abundance variation and the trait of interest. They also allow us to identify candidate genes, especially in genomic regions with high linkage disequilibrium. While the integration of GWAS and TWAS will facilitate candidate gene priorization, eQTL analyses will enable us to link sequence variation to transcript abundance variation to gain insight into the regulation of candidate genes. Gene co-expression networks will shed light on how key regulatory genes interact to determine yield.

GENETIC CONTROL OF BARB/TRICHOME FORMATION OF BARLEY AWNS

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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 trichomes (barbs) giving them a "rough" surface. In contrary, barley awns that are lacking such barbs feel smooth and thus are called smooth awns. The barbs are single-celled trichomes with silicified cell wall. A major gene (raw1) on chromosome 5H has been isolated, however, at least one additional locus has been detected by GWAS that contributes to barb formation of barely awns (Milner et al. 2019). In an attempt of characterizing further this putative second locus, F2 mapping populations between 'semi-smooth' spring barley cultivar 'Morex' (carrying the recessive semi-smooth allele at locus HvRaw1 on chromosome 5H) and two very smooth composite mutant stocks 'MHOR597' and 'MHOR598' from IPK genebank, were used for genetic mapping using reduced representation genotyping-by-sequencing (GBS). A single locus (named raw7HS) could be assigned to a 10Mbp interval of short arm chromosome 7H. With the help of improved phenotyping and SNP (single nucleotide polymorphism) genotyping using KASP (competitive allele-specific PCR) markers, the identified interval was further delimited to 1.4 Mbp comprising thirteen high confidence genes. Further screening of 2000 F2 plants is currently being performed to dissect the candidate gene, which will be verified by using CRISPR (clustered regularly interspaced short palindromic repeats) based site-directed mutagenesis. This study will help isolating a second gene controlling awn roughness in barley and will be used to determine the molecular mechanism underlying barb formation and to further dissect the correlation of smooth awns and stigma hairiness for the benefit of breeders, farmers and the consumers.

INTRODUCING A NEW EUROPEAN HERITAGE BARLEY COLLECTION (EXHIBIT)

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Modern barley cultivars have been bred under optimal conditions that led to an increased susceptibility to stress. Therefore, exploiting understudied genetic resources that potentially harbours tolerance genes and good malting qualities is vital to a flourishing Irish malting industry. A panel of 365 two-row Spring-barley accessions originating from all across Europe including Ireland is established and introduced as the European Heritage Barely collection (ExHIBiT). The collection comprises *landraces (~10%), old cultivars (~80%) and elite lines (~10%)* representative of European germplasm. The germplasm includes accessions from the Irish Heritage Barley Collection by DAFM (Department of Agriculture, Food and the Marine), the Nordic Genetic Resource Center (NordGen), and held by the James Hutton Institute (JHI). The ExHIBiT panel was genotyped using their 50K iSelect SNP array chip, and data summarizing preliminary findings will be presented. A preliminary PCA analysis on 224 lines with 29927 SNPs reveals clustering based on geographical origin. To efficiently preserve and evaluate the ExHIBiT collection, the construction of a core collection of approximately 230 accessions will be designed so that it captures most of the SNPs and will be representative of all of the phenotypes and geographic regions from the original panel. This core- ExHIBiT collection will be used GWAS purposes from both Field and controlled phenotypic data. In order to better understand the genetics and genomics of heritage barley germplasm, providing the opportunity to improve barley's yield and stress resistance and to further address environmental pressures from climate change to food security. Public availability of this new, and fully characterized, European heritage collection will be useful for breeders, geneticists, physiologists and pathologists around the world and will be extremely valuable for the innovation and premiumization of the malting industry.

SEEKING CEREAL CROPS WITH BALANCED PARTS FOR THE IDEAL WHOLE

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Agriculture is essentially the cultivation of genetically homogenous crops as a community, where cooperative behaviour is favoured over competition. Undoubtedly, crop domestication and breeding considerably increased productivity over centuries, but unconsciously lowered 'selfish-plant behaviour' or individual plant-fitness. Paradoxically, enhancing individual plant-fitness is mistakenly equated with crop improvement. Despite the recent technological advances, customization of crop genotypes to match target environment(s) is still a goal. One primary bottleneck hindering further progress may be the presence of trade-offs among major yield component traits. For instance, grain number and thousand grain weight are negatively related in wheat cultivars, as are increased panicle branching and reduced tillering in rice. These scenarios clearly indicate that efforts to solely improve a single trait often risk compromising other traits and negating the desired selection effect. Genetic analysis of wild-elite, landraces-elite recombinants might be a potential strategy to unravel key regulators of inter- and intra-plant trade-offs. In this context, we are currently analysing a large RIL population derived from CIRNO (elite durum) and TRI984 (landrace-miracle wheat) that are contrasting for major yield component traits. The initial phenotyping revealed significant diversity for plant height, tillering, heading time, flag leaf attributes, spike architecture etc., that indicates the potential to understand trait-trade-offs. In rice, previously we reported haplotype diversity of 120 key genes regulating major grain yield and quality traits across the 3K rice genomes (landraces and elite cultivars). Moreover, superior haplotypes were identified for tiller number (*MOC1*-H9 & *IPA1*-H14), *DEP3*-H2, *DEP1*-H2 & *SP1*-H3 for long panicles, *SD1*-H8 for semi-dwarfism, *LAX1*-H5, *OSH1*-H4 & *LP*-H13 resulting in increased panicle-branching, *PHD1*-H14, *AGO7*-H15 & *ROC5*-H2 for high yield and *OsNAS3*-H2 for increased Fe and Zn concentration in grains. Currently, these haplotypes are being validated and checked for possible epistatic interactions in three elite backgrounds. Taken together, our preliminary results indicate that exploring trait-trade-offs might be a potential option for increasing communal yield.

A FRAMEWORK FOR SHARING ENCRYPTED GENETIC AND PHENOTYPE DATA TO ACCELERATE CROP IMPROVEMENT

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Crop breeders need to mount an agile response to the challenge of a rapidly changing climate, and to do so the community must share data. Breeding improved crop varieties can be accelerated by first identifying the phenotypic effects of alleles segregating in the global germplasm and then using marker assisted selection to bring together beneficial alleles into a single genome. To do this efficiently, it is necessary to use large samples that ideally combine as many sources of public and proprietary genetic and phenotypic data as possible into a single analysis. This clearly presents a conflict between the need to share data in order to uncover these allelic effects, and the need to keep the genomes and phenomes of individual samples private. We describe a protocol for the sharing of encrypted genotypes and phenotypes between study participants that permits standard quantitative genetics analyses such as genome wide association, mixed models, ridge regression and heritability estimation on the pooled data, but that still preserves genetic privacy. This framework would allow commercial breeders to share encrypted versions of their proprietary genotypes and phenotypes – ciphertext – with each other and with public research organisations, in order that everyone can estimate allelic effects more accurately, which can then be fed back into proprietary breeding programmes, but without revealing any proprietary information. The only results made public are the more accurate estimates of phenotypic effects attributable to alleles, which we view as a shared public good, hence the slogan: Private Genomes and Public Alleles. Our methodology, described in PMID:32327562 uses random orthogonal transformations to mix plaintext genotype and phenotype data into ciphertext closely resembling random numbers, but in such a way that the standard quantitative genetic analyses are still possible and yield the same results as with

plaintext data. By presenting this framework at Monogram-21, we hope to engage stakeholders in both commercial and public domains and thereby establish a consortium of interested parties.

ENVIRONMENTAL EFFECTS ON GRAIN PROTEINS

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Gluten proteins are the major contributors to breadmaking quality. Among them, the high molecular weight glutenin subunits (HMW-GS) are accountable for 45 to 70% of the variation in baking performance of European cultivars (Payne et al., 1987). However, wheat quality is strongly affected by the environment (e.g. temperature, precipitation and nitrogen fertilisation). So consequently, millers have to make costly and time-consuming adjustments. Thereby, there is a strong demand from the milling industry to provide wheat varieties with consistent quality over year and location along with new quality attributes. Near-Isogenic lines (NIL) were developed from double haploid lines where 8 QTL related to breadmaking quality traits had been detected. 16 sets of NIL were studied on a small-scale dough analysis system (Reomixer) to assess the mixing behaviour and discriminate the quality. Wheat yields are strongly affected by the application of nitrogen fertilisers with an average fertilisation in the UK of 180kgN/ha/year for non-milling winter wheats. By contrast, wheat grown for breadmaking require a supplement of nitrogen of around 30kg/ha/year to achieve the 13% protein content threshold for breadmaking (Wray et al., 2016; Nabim, 2020). It is considered that an excess of fertilizer may damage the environment through leaching into the ground water or run-off into lake or river. The baking industry is also challenged by inconsistency of wheat quality over year and location. For particular year, when the UK wheat quality is poor, UK millers have to buy grain on the European market or from the USA which threaten the UK self-sufficiency. The grain protein content (GPD) is an important indicator of quality and for bread wheat a minimum of 12% is required. Several strategies to increase the protein content have been attempted but lead to no results due to a negative relationship with grain yield (GY). Grain Protein Deviation (GPD) is a promising trait that considers the relationship between GY and GPC. To investigate the genetic basis of this trait, 111 double haploid lines from the cross Malacca and Hereward were developed and will be observed in 3 environments. A QTL analysis on multi-environment will reveal potential genetic locus. The first environment data has been collected. The GPD values were highly variable across the lines and the negative relationship between GY and GPC observed. Future work will involve a QTL detection for GPD in the first environment as well as test baking and phenotyping of the NIL.

CLONING OF BARLEY NEC3 DISEASE LESION MIMIC MUTANT

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Disease lesion mimic or necrotic mutants are mutants that consistently display necrotic lesions in the absence of a pathogen infection. They have been described in several species, including barley and they can be useful in unravelling plant defence pathways. In this study, we cloned the historical barley gene *nec3,* controlling the production of orange necrotic leaf spots or lesions appearing on leaves from the jointing stage onwards. We screened the barley TILLMore population (cv. Morex background, chemically mutagenized using NaN3, currently including 56ereal56. 3,600 M_6 lines) and identified three distinct lines displaying orange lesions. Complementation tests involving the three TILLMore lines and the historical x-ray induced nec3 allele (Line BW630, with phenotype introgressed from cv. Villa) confirmed that the same locus was involved. A combination of WGS and BSA enabled us to map the causative locus on chromosome 6H. Allelic sequence variation at the candidate gene co-mapping at the target locus was revealed by WGS sequencing and confirmed by Sanger sequencing. The only mutated gene in common among the four orange-spotted nec3-like lines was our candidate. *Nec3* encodes a cytochrome P450 and through a phylogenetic analysis we demonstrated that

it belongs to the CYP71P1 family, as was previously described in its rice ortholog *SL* (Sekiguchi Lesion). Moreover, we found that CYP71P1 family emerged early in green plant evolution and was lost in some of the dicot lineages.

GRAINS OF FUTURE PAST: LEARNING FROM WHEAT LANDRACE DIVERSITY

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Traditional, pre-green revolution landraces represent a very important and largely untapped source of wheat genetic diversity. Given the geographical extremes to which they are adapted, and the traditional cultivation systems for which they have been bred, landraces are likely to contain highly useful genetic variation for breeding elite wheat to thrive in future climates, and lower-input agricultural systems. The 'Wheat Rose' consortium has assembled and analysed a panel of 340 landraces from across the globe. Each line has been transcriptome sequenced to identify both variation in exome sequence and gene expression levels across the genome. We have performed extensive phenotyping of the panel across each developmental stage, and in response to a range of stresses. This allows us to use genome association analyses to identify SNPs or gene expression markers (GEMs) that are associated with different phenotypes, thereby identifying new genetic targets for investigation and breeding. Furthermore, we can also use this phenotypic information to make phenotypic associations across the panel and understand how different aspects of growth and development are related to, or constrain each other. In this presentation, we present an overview of our approach and highlight some of the important results from our work so far, with a particular focus on understanding flowering time and reproductive shoot architecture.

Phenotyping

TOWARDS THE IMPLEMENTATION OF A MACHINE LEARNING (ML) FRAMEWORK TO IDENTIFY STRESS SIGNATURES IN BARLEY IN RESPONSE TO STRESS

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Although plant imaging and high-throughput phenotyping (HTP) have unlocked a significant crop research outcome, phenotypic data acquisition is still a bottleneck restricting crop breeding and functional genomics studies. New technological advances in data science allow data scientists to analyse data. The data generated causes a "big data problem"; thus, it is key to explore the challenges associated with the biological interpretation of plant performance. The research described in this project is based in Ireland and focuses on barley, which is the most important cereal crop in the country. Globally, farmers are facing severe yield penalties, mainly due to abiotic stresses caused by climate change. Waterlogging is a major abiotic stress, and improving waterlogging tolerance is imperative. To date, waterlogging has been studied through classical methodologies, and little is known about barley physiological responses to waterlogging throughout time. This project aims to elucidate the genetic basis of barley waterlogging tolerance by assembling and utilising a European HerItage Barley 58ereal58ion (ExHIBiT). This collection will be fully characterised, enabling the establishment of an association mapping population, which will be then phenotyped under waterlogging conditions with HTP. This approach combines machine learning (ML) and new association methods to identify genetic markers underpinning barley's temporal responses to waterlogging. A framework will be developed utilising ML to enable data assimilation and specific feature identification for stress phenotyping. The use of HTP will result in a deluge of high-resolution images of plants, which will require the use of ML tools to extract patterns and features from this large corpus of data. Four stages of the decision cycle in plant stress phenotyping and plant breeding activities where different ML approaches can be deployed are (i) identification, (ii) classification, (iii) quantification, and (iv) prediction of waterlogging induced stress. The data will be images extracted via three imaging sensors (fluorescence, RGB, hyperspectral) and be labelled (trait – such as barley accession & stress level). Different techniques for identification via supervised learning will be applied: K-nearest neighbours algorithm (K-NN), support vector machine (SVM), artificial neural networks (ANN), ultimately leading to the classification results. The data acquisition will also include the retrieval of phenotypic traits using UAV-based sensors at UCD Lyons Research Farm, which will be used to monitor physiological stress and quantify waterlogging effects on these traits. The project aims to elucidate barley stress adaptation in climate change using disruptive technologies, which can benefit plant scientists, malting industry and barley farmers.

PHENOTYPING OF FLOWER MORPHOLOGY RELATED TRAITS IN WILD RELATIVES OF WHEAT

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A sustainable solution to raise wheat yield is to improve its genetic potential as well as the exploitation of heterosis known as hybrid vigor. Successful hybrid production requires a female line that is male sterile, and a pollen donner male line. The male line needs to be taller than the female line, characterized by a high level of anther extrusion, having a long anther that carries a large amount of highly viable pollen. The female line needs to be shorter than the male line and have a gaping floret with a long-extruded stigma ideally characterized by a long period of receptivity. Wheat is an in-breeding species, with limited variation for flower morphology within its gene pool. The wild relatives

of wheat, however, represent a vast source of genetic variation for potentially all traits of agronomic importance, including flower morphology. In this study, nine wild relative species (two to four accessions per species with a total of 23 accessions) were phenotyped for flower morphology related traits such as anther extrusion (AE), anther length (AL), pollen area (PA) and viability, filament length (FL) and floret gaping. Results allowed the classification of the wild relatives into two sub-groups. The in-breeding sub-group included *Triticum timopheevii* and *T. 59ereal.* The out-breeding sub-group included *Secale 59ereal*, *Amblypyrum muticum*, *Aegilops caudata*, *Ae. Speltoides*, *Thinopyrum elongatum*, *Th. Bessarbicum* and *Th. Ponticum.* A variation between accessions within the same species for some traits was detected. Principle component analysis, explaining 91.82% of the variation, showed a positive correlation between AE, FL, and AL. Results highlighted the strengths of each species that could be used for a hybrid wheat system. For example, *T. timopheevii* and *T. 59ereal* were found to carry the smallest pollen grains, while *S. 59ereal*, *Th. Ponticum* and, *Th. Elongatum* had long anthers and a high level of AE. Hence, for each trait of interest, genes within these species could be of use for hybrid wheat production if transferred into wheat in the form of chromosome introgressions.

INVESTIGATING IRISH HERITAGE BARLEY RESPONSES TO WATERLOGGING

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Barley is the most important crop in Ireland and the key ingredient for the malting industry. Waterlogging is a major constraint to barley production and is expected to become a more significant problem as the frequency of flooding events is set to increase with climate change. Improvements to waterlogging tolerance are hindered by the variability of physiological responses to waterlogging stress and low heritability. There are extensive genetic resources for barley held in genebanks around the world, and its characterisation will assist crop improvement in response to the challenges posed by climate change and sustainable food production. The Department of Agriculture Food and the Marine (DAFM) holds a small collection of heritage barley lines, comprising of 'old cultivars' bred in Ireland for malting purposes from circa 1900. During seed bulking, researchers at DAFM have noted that many of these heritage lines possess larger root systems than modern counterparts. As waterlogging is a stress primarily affecting the roots, we aim to investigate this understudied Irish germplasm and compare waterlogging tolerance to that of modern commercial barley lines. Taking a three-pronged approach, we will assess heritage and modern cultivars genotypically and phenotypically in both the field and glasshouse conditions. Non- destructive measurements using a range of sensors (RGB, chlorophyll fluorescence and hyperspectral imaging) were obtained over the course of the waterlogging treatment. Preliminary glasshouse data show higher percentages of root biomass relative to control conditions in one heritage line compared to modern barley. Data from our pilot waterlogging field trial show that reduced tillering is the key yield reducing factor of waterlogging stress in barley. This effect will be assessed in greater detail using image-based phenotyping in glasshouse conditions. Non-destructive image-based phenotyping will provide a detailed characterisation of the waterlogging stress response over time, while field trials will provide an assessment of the agronomic potential of these heritage.

AGRONOMIC CHARACTERIZATION OF THE EUROPEAN BARLEY HERITAGE COLLECTION (ExHIBiT)

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Barley is the fourth most important cereal worldwide and is particularly important for food security. However, barley production is being seriously hindered due increased environmental pressures such as drought, waterlogging and salinity caused by climate change. Barley has the second largest germplasm collection in the world, yet this invaluable collection of genetic resources still remains largely uncharacterized. The phenotypic characterization of such resources provides a fundamental support to increase stress tolerance and accelerate breeding programs. In this project we assembled -the European Heritage Barley collection (ExHIBiT), which includes 365 lines of 2-row spring barley collected from northern Europe. The ExHIBiT collection comprises a mixture of landraces (~10%), old cultivars (~80%) and elite lines (~10%) and has been genotyped with 50K SNP array and cultivated at UCD Lyons Research Farm during Spring 2020. Here we present the agronomical characterization of the ExHIBiT collection, namely the preliminary results of the first year of field data. The three-hundred and sixty-five lines of the ExHIBiT collection were evaluated together with three check lines using an augmented design with 50 blocks for spatial analysis. The three check-lines were Propino and RGT Planet (nowadays premium malting lines) and Golden Promise (dominant malting line in the 70's). To characterize ExHIBiT, several traits were collected, including identification of main growth stages (tillering, flowering, ripening), lodging, height, shoot biomass, yield, harvest index and protein content. The ExHIBiT collection is agronomically diverse with some lines performing as well as the elite check-lines Propino and RGT Planet. Combination of this agronomical date with genotypic data will allow the selection of the ExHIBiT core population of approximately 230 lines, which will be both agronomically and genetically diverse. The fully characterized ExHIBiT core-collection will be then made available for plant researchers around the world for further studies ranging from agronomy to stress responses or inclusion in breeding programs.

2 Row Barley ready for harvest, Dundee 2020

Quality & Nutrition

IDENTIFICATION OF EIGHT QTL CONTROLLING MULTIPLE YIELD COMPONENTS IN A GERMAN MULTI-PARENTAL WHEAT POPULATION, INCLUDING *RHT24, WAPO-A1, WAPO-B1* AND GENETIC LOCI ON CHROMOSOMES 5A AND 6A

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Grain yield in wheat (*Triticum aestivum* L.) is a polygenic trait representing the culmination of many developmental processes and their interactions with the environment. Toward maintaining genetic gains in yield potential, 'reductionist approaches' are commonly undertaken by which the genetic control of yield components, that collectively determine yield, are determined. Here we use an eight-founder German multi-parental wheat population to investigate the genetic control and phenotypic trade-offs between 15 yield components. Increased grains per ear was significantly positively correlated with the number of fertile spikelets per ear and negatively correlated with the number of infertile spikelets. However, as increased grain number and fertile spikelet number per ear were significantly negatively correlated with thousand grain weight, sink strength limitations were evident. Genetic mapping identified 34 replicated quantitative trait loci (QTL) at two or more test environments, of which 24 resolved into eight loci each controlling two or more traits—termed here 'multi-trait QTL' (MTQTL). These included MT-QTL associated with previously cloned genes controlling semi-dwarf plant stature, and with the genetic locus *Reduced height 24* (*Rht24*) that further modulates plant height. Additionally, MT-QTL controlling spikelet number traits were located to chromosome 7A encompassing the gene *WHEAT ORTHOLOG OF APO1* (*WAPO-A1*), and to its homoeologous location on chromosome 7B containing WAPO-B1. The genetic loci identified in this study, particularly those that potentially control multiple yield components, provide future opportunities for the targeted investigation of their underlying genes, gene networks and phenotypic trade-offs, in order to underpin further genetic gains in yield.

NAC TRANSCRIPTION FACTORS IN THE REGULATION OF SENESCENCE AND GRAIN PROTEIN CONTENT

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Wheat provides 20% of the protein in our diets globally (FAOSTAT). Increasing wheat grain protein content would be beneficial for bread quality and human nutrition. The trade-off between protein content and yield makes this a challenge. During grain filling, wheat leaves senesce: cells break down proteins and remobilize nitrogen from the leaves to the developing grain. Transcription factors regulating senescence could be new targets for breeding to improve grain protein content. We aim to characterise two candidate senescence regulators, *NAM-2* and *NAC-5.* These two NAC-family transcription factors are upregulated throughout flag leaf senescence, and coexpression network analysis predicts a key role in the regulatory network (Borrill et al. 2019, *Plant Physiology*). *NAC-5* is the ortholog of rice *OsNAC5*. *OsNAC5* is upregulated in some high protein content genotypes (Sharma et al. 2019, *Journal of Genetics*), promotes drought tolerance (Takasaki et al. 2010, *Molecular Genetics and Genomics*) and can improve yield (Jeong,

2013, *Plant Biotechnology Journal*). *NAM-2* is the paralog of wheat *NAM-1. NAM-1* promotes earlier senescence and higher protein content with either reduced or unchanged yield (Uauy et al. 2006, *Science*). Loss-of-function of *NAM-2* delayed senescence in tetraploid wheat (Borrill et al. 2019, *Plant Physiology*). To test whether *NAM-2* regulates senescence, protein content, and yield in hexaploid wheat, we will cross together EMS-induced loss-of-function mutations in its homoeologs *NAM-A2, NAM-B2* and *NAM-D2*. Single, double and triple loss-of-function lines will be phenotyped in greenhouse and field. To test whether *NAC-5* is sufficient to alter senescence and protein content, *NAC-5A* was constitutively expressed in hexaploid wheat from a rice Actin promoter. We will compare the senescence timing, protein content and yield between *NAC-5A* overexpression lines and empty-vector controls in the greenhouse. These experiments will indicate whether *NAM-2* and *NAC-5* are members of the regulatory network that triggers senescence in wheat, and how they affect grain protein content and yield. If *NAM-2* and *NAC-5* regulate senescence, then variation in these genes could be used to breed wheat varieties with earlier or later senescence, optimising the balance between protein content and yield.

STARCH SYNTHASE 4 IS REQUIRED FOR NORMAL STARCH GRANULE INITIATION IN AMYLOPLASTS OF THE WHEAT ENDOSPERM

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Starch granule initiation is poorly understood at the molecular level. The glucosyltransferase, STARCH SYNTHASE 4 (SS4), plays a central role in granule initiation in Arabidopsis leaves, but its function in cereal endosperms is unknown. To study the role of SS4 in wheat endosperm, which has a distinct spatiotemporal pattern of granule initiation during grain development, we generated TILLING mutants in tetraploid wheat (*Triticum turgidum*) that are defective in both SS4 homoeologs. The morphology of endosperm starch was examined in developing and mature grains. SS4 deficiency led to severe alterations in endosperm starch granule morphology. During early grain development, while the wild type initiated single 'A-type' granules per amyloplast, most amyloplasts in the mutant formed compound granules due to multiple initiations. This phenotype was similar to mutants deficient in B-GRANULE CONTENT 1 (BGC1). SS4 deficiency also reduced starch content in leaves and pollen grains. We propose that SS4 and BGC1 are required for the proper control of granule initiation during early grain development that leads to a single A-type granule per amyloplast. The absence of either protein results in a variable number of initiations per amyloplast and compound granule formation.

REDUCED FREE ASPARAGINE IN WHEAT GRAIN RESULTING FROM A NATURAL DELETION OF *TAASN-B2*

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Free asparagine levels in wheat grain need to be reduced in order to mitigate the health risk posed by acrylamide, a processing contaminant formed from asparagine during high temperature processing such as roasting and baking. The asparagine synthetase gene family is responsible for synthesising asparagine in wheat and asparagine synthetase 2 (*TaASN2*) is the most highly expressed asparagine synthetase gene in the grain. Consequently, *TaASN2* is an important genetic target for developing wheat varieties with lower levels of asparagine in the grain. Previous work has established that *TaASN2* expression is uneven between homeologs and that *TaASN-B2* is variably present and absent across wheat varieties. Here, we determined the properties of the *TaASN-B2* deletion, its frequency across a collection

of commercial UK wheat varieties, and the differential expression of the *TaASN2* homeologs. Furthermore, we found that the*TaASN-B2* deletion reduced free asparagine levels in the grain and that differing levels of sulphur application in the field affected this reduction. These findings indicate that selection of varieties in which *TaASN-B2* is deleted can help to mitigate the risk associated with free asparagine.

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HOMOLOGUES OF IRON-REGULATED TRANSPORTER (IRT1) GENES ARE FUNCTIONAL IN IRON HOMEOSTASIS IN WHEAT

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Two strategies are understood to describe iron uptake in plants, Strategy I uses Iron Regulated Transporter 1 (IRT1) in the roots to take up Fe^{2+} from the soil. In contrast, Strategy II secretes chelators into the soil and takes up Fe^{3+}-chelate (Marschner, Römheld, 1994). Strategy I has been thought to be present in only non-graminaceous plants, while graminaceous plants utilize Strategy II. More recently, IRT1 has been found functionally active as an iron transporter in some graminaceous plants like rice (Ishimaru *et al*., 2006) and maize. However, the closest homologue in barley is likely to act as a manganese transporter (Long *et al.*, 2018). The *IRT1* homologues in bread wheat, *Triticum aestivum L*., have not yet been studied. Here we have identified six *IRT1* homologues in bread wheat and characterised their roles in iron uptake. Using RT-PCR and RNA-Seq, we found the expression of two of the *IRT* homologs, *IRT-A3* and *IRT-D2*, were consistently increased in the roots of seedlings grown in iron-deficient conditions. We hypothesised that these two *IRT* genes had a role in iron uptake. To test this, we identified mutants using the wheat Targeted Induced Local Lesions in Genomes (TILLING) resource and investigated the effect of this mutation on iron content within the plant. Using a quantitative colorimetric assay, we found significantly lower iron levels in the flag leaves of *IRT-A3* and *IRT-D2* single mutants ($p < 0.05$). These genes were also able to rescue growth and chlorophyll and iron concentration of an Arabidopsis *irt-1* mutant, suggesting the genes can function in iron uptake. Our results demonstrate that the *IRT1* genes in bread wheat are functional iron transporters and suggests that aspects of the Strategy I system for iron uptake function in wheat. Combined with previous work in rice and other graminaceous species, this suggests that the separation between Strategy I and Strategy II iron uptake is not as clear-cut as previously understood. In future work, we are interested in characterising the importance of *IRT1* on the iron content in the wheat grains. The ability to manipulate the expression of *IRT1* to possibly increase iron content in the grain could then lead to possible efforts to produce biofortified grain.

Ishimaru *et al.* (2006) *Plant J* 45: 335-346 Marschner, Römheld (1994) *Plant Soil* 165: 261-274

IDENTIFICATION AND CHARACTERIZATION OF *ZIP* GENES FOR IRON AND ZINC TRANSPORTATION IN WHEAT

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Wheat (*Triticum aestivum*) is one of the most essential staple crops and provides almost 20% of total protein in the human diet. However, wheat is a poor source of iron and zinc. Deficiency of these minerals affects almost 2 billion people in the world. These minerals are essential for normal growth and development. Moreover, iron and zinc deficiency causes severe chlorosis in food crops, including bread wheat and overall reduce the yield and quality. It is difficult to change the iron and zinc in wheat due to its genetic complexity and homeostatic mechanisms. In this study, we will understand the molecular basis of iron and zinc uptake and transportation. We will investigate the role of the *ZIP* (ZRT-, IRT-like proteins) gene family in the distribution of iron and zinc in the different wheat tissues. We chose three *ZIP* genes (*ZIP11*, *13* and *14*) considering that they are highly expressed in both rice and wheat in different tissues and orthologs of each other. We selected twelve TILLING (Targeting Induced Local Lesions IN

Genomes) mutants for these three *ZIP* genes based on consequence type. The consequence type defines the type of mutation predicted based on gene model. We selected most desirable consequence type i.e., "stop-gained", "missense variant" and "splice acceptor variant" for our experiment. We will generate F_2 population segregation for the mutations, which have homozygous double and single mutants and wild type plants. We will investigate the micronutrients especially iron, zinc, and agronomic traits (number of tillers, plant height, thousand-kernel weight) of the F_2 generation under soil and hydroponic conditions. We will do complementation of yeast mutants by *ZIP* genes to test their ability to transport of iron and zinc. The total iron and zinc contents will be determined by using Perls' staining and ICP-MS (inductively coupled mass spectrometry). This study will provide understanding of iron and zinc transport mechanisms in wheat and will help breeders to improve iron and zinc content in the grain.

POSTPRANDIAL BIOAVAILABILITY OF PHENOLIC METABOLITES FROM BARLEY CANNOT BE DETERMINED BY GRAIN ANALYSIS.

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Barley is currently underutilised as a human food source in the UK, with only 2% being used directly for food. Extensive literature indicates that consumption of wholegrains reduces the risk of non-communicable health disorders such as cancer, heart disease and type 2 diabetes. Strong evidence indicates that the health benefits are attributable to fibre. This includes a registered health claim for β-glucan, a soluble fibre found in barley known to lower cholesterol. A barley cultivar known to be high in β-glucan was analysed and shown to also be a rich source of micronutrients and anti-inflammatory phytochemicals, compared to commercially available barley products. However, levels of compounds present in the barley are not necessarily indicative of postprandial bioavailability, as shown in an acute dietary intervention. The consequential benefits to gut health are being explored in a longer-term human study.

GET A GRIP – MECHANISMS UNDERLYING VARIATION IN BARLEY GRAIN HULL ADHESION

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As the fourth most abundant cereal crop, barley is a key global cereal. The global malt extracts market alone is worth over £14bn (€16.6bn), primarily from the brewing and distilling industries. As such, understanding the genetic basis for agronomic traits that improve production quality and efficiency is of vital economic importance. Barley's normally adherent hull is an agronomic trait, distinctive among cultivated cereals and critical for malting. Grain skinning or partial shed of the hull decreases malting efficiency and output and causes devastating losses for barley growers, yet the underlying genetic basis is undefined. The NUDUM (NUD) transcription factor promotes adhesion between the grain and hull, but genes downstream of NUD are also unknown. My research addresses these knowledge gaps by studying the genetic, structural and chemical changes in stable skinning lines. Here, I will describe how I used high density genotyping and comparative gene expression to identify a gene which controls grain skinning and show that this gene is downstream of NUD function. I will also present preliminary characterisation of this gene and my investigations into the mechanisms of grain-hull adhesion, which we hope will provide potential routes to control this crucial grain quality trait in cultivated barley.

Recombination & Pollen Development

INVESTIGATION OF STIGMA DEVELOPMENT AND SURVIVAL IN BREAD WHEAT

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The establishment of hybrid breeding in wheat has been limited relative to other cereals (e.g. maize, rye, sorghum) due to the autogamous nature of its pollination. This restriction has been alleviated by the implementation of stable male sterility systems. However, a major factor limiting F_1 seed production has been the low outcrossing rate of the maternal parent, which may be due to the short functional life span of the stigma. Thus, research into understanding the processes controlling stigma survival in wheat could be key to achieving higher F_1 seed yields in a cost-effective manner. The aim of the study is to investigate the genetic regulations of wheat stigma biology using male sterile plants. To first address this, we have developed a high-throughput phenotyping approach using light microscopy, image analysis tools and machine learning techniques to identify natural variation in the dynamics of stigma shape and size during development. Preliminary clustering analysis on field data reveals the ability of this method to detect distinct growth patterns among different male sterile cultivars. Secondly, we have conducted an RNA-sequencing experiment to obtain a detailed gene expression time-course profile of unpollinated stigmatic tissues over a 3-week period (pre-anthesis to senescence). For this experiment, we sampled dissected stigmata of male sterile plants grown under field conditions at seven different developmental stages. The co-expression of down and up-regulated genes in a stage-dependent manner sheds new light on the metabolic processes, signalling pathways, and specific TFs that may regulate key stages of stigma development. This knowledge will equip breeders with genetic resources and tools to prolong the longevity of the wheat stigma to enhance the effectiveness of hybrid breeding programmes. In addition, this work will advance our understanding of the mechanisms controlling female fertility in wheat.

Barley field trials in Balruddery Research Farm, 2019

Abstracts:

IDENTIFYING GENES AND TRAITS FOR TOLERANCE TO MARGINAL SOILS IN ANCIENT BARLEY LANDRACES

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Micronutrient deficiency in cereals severely reduces yield and quality in marginal soils on a global scale. Ancient landraces represent a unique reservoir of genes and traits, selected over millennia, for adaptation to marginal soils. We screened extant Scottish barley (*Hordeum vulgare*) landraces (Bere) for their ability to cope with manganese (Mn) deficiency in hydroponics, mesocosms and field trials. Genetically the Scottish landraces were found to cluster according to their island of origin, and those adapted to soils with reduced fertility, had particularly larger manganese (Mn), but also zinc (Zn) and copper (Cu) concentrations in the shoot. Strikingly, when grown in marginal soils in Orkney, these adapted landraces demonstrated an exceptional ability to translocate Mn to leaves, maintain photosynthesis and generate robust yield, while modern elite varieties failed to complete their lifecycle. We conclude that ancient Scottish landraces contain untapped genetic diversity which could underpin future agricultural sustainability.

ROOTY: OPTIMIZING ROOT SYSTEM ARCHITECTURE TO SUPPORT IMPROVED WHEAT YIELDS

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'Rooty' is an International Wheat Yield Partnership (IWYP) and BBSRC-funded project that focuses on below-ground traits to support improved wheat yields. Below-ground traits have not been selected for in wheat breeding programs; therefore, optimizing root system architecture (RSA) is likely to be beneficial for the breeding of higher yielding crops. Moreover, improving wheat yield is likely to increase the need for water and nutrients, which root systems must capture. The Rooty project aims to develop a root ideotype toolbox through the development of novel germplasm and the delivery of a suite of genetic markers for key root traits for a diverse range of environments. One of the strategies adopted to develop these root ideotypes is through the identification and characterization of *de novo* or artificial alleles controlling RSA traits that could support improved wheat yields. Root hairs are crucial for root function and the acquisition of nutrients. However, little is known of the genetic control in wheat, while in rice and maize a few genes have been already characterised. Screening the hexaploid TILLING population cv. Cadenza enabled us to identify a *roothairless* mutant. Cryo-scanning electron microscopy of the mutant showed that in fact root hair primordia emerged from the epidermal cells but failed to elongate into fully grown hairs. To identify the genetic variant(s) associated with the *roothairless* phenotype, bulk-segregant analysis using exome capture and next generation sequencing was performed using a segregation population. A forward genetic strategy in the Rooty project is the physiological characterisation of RSA and *de novo* identification of root traits in elite germplasm with high yield potential. We selected a set of 37 lines that comprises current UK Recommended List varieties, NIAB's re-synthesised hexaploid lines and novel durum x bread wheat crosses, and a few lines with low yield potential lines for contrast. The lines were first evaluated at seedling and vegetative stages under controlled-environment conditions. A subset of 30 lines presenting the most contrasting RSA were selected for evaluations in the field, which took place in 2019-2020 in the UK and Germany and are being repeated this season. Combining the information collected in controlled environments and in the field should give us an indication of what root traits have been selected through selection for grain yield. Moreover, it should give us some indications on the degree of variation in RSA existing within as set of high yielding material.

A HIGHLY EFFICIENT *AGROBACTERIUM*-MEDIATED TRANSFORMATION SYSTEM DEPLOYING MORPHOLGICAL GENES IN HEXAPLOID AND TETRAPLOID WHEAT

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Wheat has lagged behind other major cereals in the advancement of gene transformation technology for its improvement. New breeding technologies such as genome editing allow precise DNA manipulation, but its potential is limited by low regeneration efficiencies in tissue culture and lack of transformable genotypes. We developed, in the hexaploid spring wheat cultivar 'Fielder', an efficient and reproducible *Agrobacterium*-mediated transformation system. This high-throughput robust transformation system has been used effectively to introduce genes of interest, for over-expression, RNAi and for CRISPR-Cas based genome editing. Transformation efficiencies of up to 33% in 'Fielder' and 10% in the tetraploid durum wheat 'Kronos' were achieved. A recent development for wheat transformation by the Dubcovsky Lab at UC Davis, USA, is a protein fusion consisting of two developmental regulator genes. The wheat Growth-Regulating Factor 4 (GRF4) and its cofactor GRF-Interacting Factor 1 (GIF1) when overexpressed as a protein fusion improve the regeneration of *in vitro* growing plant cells. When included and tested with our wheat transformation protocol, the transformation efficiencies increased to 77.5% in 'Fielder' and 70% in 'Kronos'. The GRF4-GIF1 technology results in fertile transgenic plants, giving a "normal" phenotype in low transgene copy plants without the need of specialized promoters or transgene excision, overcoming some of the limitations of transformation technologies with other morphogenic developmental regulating genes. It is envisaged that these developmental regulators will alleviate some of the cultivar dependence of wheat transformation and, so doing, enable the more efficient and rapid transformation and genome editing of elite wheat cultivars.

NITROGEN RESPONSIVENESS IS AN IMPORTANT TRAIT UNDERLYING SUSTAINABLE CEREAL CROP PRODUCTION

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Nitrogen (N) is a macronutrient necessary to plant growth and its availability must be carefully managed to achieve maximum yield and quality in cereal crops, while reducing potentially negative environmental effects. Crop varieties must be able to respond to available N, i.e. to induce morphological and physiological changes. Selecting crop varieties with high N responsiveness would prevent the cultivation of varieties having limited yield improvement for N fertiliser addition and drive a lowering of N requirements, thereby avoiding negative economic and environmental consequences. While the concept of N use efficiency is often used to address cereal N requirement, this only considers the ratio of yield produced at a given unit of N supplied. Considering N responsiveness requires measurements under both N deficient and N sufficient conditions in order to quantify the response variable. We investigated the genetic and molecular basis for N responsiveness in foxtail millet (*Setaria italica* L., a C4 lowland crop highly relevant to food security in arid and semi-arid regions of Asia, sub-Saharan Africa and China). We found significant variation in N responsiveness amongst accessions tested and used a genome wide association study to uncover potentially underlying genetic loci. We also investigated N responsiveness across a range of wheat (*Triticum aestivum* L.) varieties. These results indicate that using N responsiveness as a selection trait may provide new avenues to improve the sustainability of cereal crop production while maintaining yield.

SEASONALITY CONTROL OF BARLEY FLOWERING: FROM THE LAB TO THE FIELD

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One of the greatest challenges facing humanity is how to sustainably feed a growing population with our planet's limited resources. Complicating matters further, climate change and extreme weather events threaten agricultural productivity and food security. We must find novel ways to increase crop yields if we are to surmount these challenges.

The plant circadian clock is an internal timekeeping mechanism that integrates environmental information into a network of interlocking transcriptional feedback loops. The rhythmic outputs of the clock control a wide-ranging array of processes, including photosynthesis, resistance to biotic and abiotic stress, and floral induction. Consequently, having a circadian clock correctly matched to the photoperiod of a plant's environment confers a significant fitness advantage.

Flowering is a critical agronomic trait, and its timing has long been a target for cereal breeding. My iCASE industrial partner, Syngenta, has introgressed clock mutations into elite winter barley cultivars, and I will assess and characterise these lines under laboratory and field conditions. As clock mutations have historically been used to migrate barley cultivars to new climatic conditions, we hope my findings will feed into the development of cultivars with flowering times optimised to their agroclimatic zone. These cultivars would have the potential to be higher yielding or more resilient to stress than conventional barley.

Image Courtesy of Dundee, One City Many Discoveries