**DNA methylation variation in Barley is driven by both climate of origin and breeding efforts.**

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DNA methylation patterns strongly influenced by underlying genetics and are also sensitive to environment, suggesting a role in environmental adaptation or in the mediation of genome-environment interactions. Supporting this, we previously found that DNA methylation varies with the local environment in wild *Arabidopsis thaliana* populations where it is correlated with changes in gene expression and appears to be under selection (Dubin et al., 2015).

Here we extend this investigation to a worldwide panel of landrace and elite Barley cultivars using whole genome bisulfite sequencing. While the correlation between CG DNA methylation over gene-bodies and local climatic temperature extremes previously observed in *A. thaliana* is conserved in Barley (Spearman's Rho = 0.44), the majority of DNA methylation variation in Barley occurs on transposable elements (TEs). TEs in spring lines had significantly higher levels of DNA methylation compared to winter lines (*p* = 0.0023), while elite varieties had increased DNA methylation levels on TEs compared to landraces (*p* = 0.00083). Moreover, higher DNA methylation on TEs was associated with lower TE copy number and thus presumably lower genome size. Finally we found that both TE copy number and DNA methylation where correlated with agronomic traits such as 1000 kernel weight (Spearman's Rho = 0.30). Given that TEs are often not linked with neighbouring genetic markers such as SNPs (Stuart et al., 2016), they potentially represent an overlooked source of variation which could be beneficially exploited for the breeding of improved varieties.

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**A new collection of barley lesion mimic mutants**

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Several lesion mimic (LM) mutants showing necrotic spots on leaves and/or inflorescences have been described in barley and other species (Arabidopsis, maize, rice and others). Such necrotic spots or areas develop without any external stimulus (parasitic infection, physical damage, climatic event, etc) and are due to genetically-controlled dysfunctional processes. Often, LM mutants show phenotypes resembling plant defense-related processes similar to the hypersensitive response (HR). For these reasons, LM mutants can be potentially informative in the dissection of plant responses to pathogens. While LM mutants often showed enhanced disease resistance, knowledge of their molecular basis is limited (*mlo*, *nec1*) in barley.

By screening the TILLMore mutant collection (> 3,000 lines, in ‘Morex’ genetic background, mutagenized by NaN3. Talamè et al. 2008, PBJ 6:477-485), we collected 40 LM mutants showing obvious and heritable lesion mimic (ie. leaf necrotic) phenotypes. Mutants phenotypes ranged from lines with little black necrotics spots, to lines with larger (10 mm or more) spots of varying pigmentations. Three representative LM mutants are being studied in more details. LM#537 peculiarly extends necrotic areas in the mid vein blade region. LM#599 shows large ‘orange’ spots. LM#4118 produces small dense necrotic spots on leaf blades, with spots density related with leaf age. These three mutants were crossed out (with Barke cv.) and their Mendelian inheritance verified in F2 populations. Mapping-by-sequencing approaches are being considered in order to map and clone the underlying genes.

**Development of an improved assay for the detection of *Ramularia collo-cygni* DNA in barley tissues**

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Ramularia Leaf Spot (RLS) disease was first observed in South Africa in 2015. This disease, which is caused by the fungus *Ramularia collo-cygni* (Rcc), has the potential to reach epidemic proportions. *Ramularia collo-cygni* is both seed and air-borne, with the former playing a role in the long-range dispersal of the pathogen. Its ability to move from infected seed to emerging leaf tissue, without the development of visual symptoms, renders it difficult to detect until late in the growth season. This problem is exacerbated by the fact that other fungi infecting barley induce symptoms similar to RLS. A need thus exists to accurately detect Rcc in barley seeds and leaves. Currently used methods either rely on end-point PCR or use primers which have not been tested for specificity against South African isolates. The aim of the current research is to develop an improved method for the specific detection of Rcc DNA. Loop Mediated Isothermal Amplification (LAMP) has been identified as a method which has the potential to meet the aforementioned need. The LAMP assay is faster than currently used methods and obviates the need for post-PCR analysis steps. In addition to this, LAMP makes use of six primers per reaction and has the potential to be highly specific. We have designed seven sets of primers, confirmed that four sets failed to amplify *Pyrenophora teres* DNA, and will further test the primer specificity using DNA of ten Ramularia isolates from the Westerdijk Fungal Biodiversity Institute, and of other closely related fungal species.

**Understanding husk damage in barley**

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Barley grains have protective outer husks. Normally, the husk is firmly adhered to the caryopsis at maturity. However, mechanical processing can cause the husk to fragment or peel away, leaving the caryopsis exposed. This is known as skinning, which the malting industry views as a serious flaw in grain quality.

Skinning risk is affected by genotype and the environment. This PhD project aims to isolate the genetic risk factors underlying skinning. During the early stages of the project, a phenotypic screen identified major risk factors for skinning

In skinning-prone barley varieties, the husk spontaneously cracks during grain development. This may indicate an underlying problem with husk mechanical properties.

Preliminary results suggest that skinning-prone barley varieties do have weaker or more brittle husks. This project is now investigating why this might be the case.

Husk mechanical properties may be influenced by the husk cell wall. Therefore, barley mutants are being screened to see how changing lignin, cellulose or hemi-cellulose composition affects skinning.

Gene expression is also being used to evaluate any differences in cell wall synthesis and remodelling between skinning-prone and skinning resistant varieties.

Skinning tends to be more extreme and variable in modern malting barley varieties. Modern malting varieties have been selected to have: large caryopses, thin husks, low levels of (1-3),(1-4)-Beta-D-glucan, low viscosity and high friability. It is possible that this selection pressure has led to varieties with thin or brittle husks which are more susceptible to damage.

50 years a mutant – the Golden Promise story

WTB Thomas and others

The spring barley Golden Promise was first recommended to UK growers in 1968 making 2018 its 50th anniversary. The variety was the first semi-dwarf cereal to be widely cultivated in the UK and the direct product of a mutation breeding programme. It was so successful that around half a million tonnes of certified seed of the variety were produced with the variety dominating spring barley for 20 years. Golden Promise differed from its parent principally due to the mutation that resulted in its semi-dwarf stature, which made it attractive for farmers as they could realise the yield gains of increasing nitrogenous fertilisers with reduced lodging risk. It also proved attractive to maltsters as it recovered from dormancy quickly after harvest but did not sprout in the ear whilst producing a uniform grain sample. The variety therefore became the major distilling variety until it was superceded by higher yielding varieties in terms of grain and distillery yield. Despite this success, Golden Promise does not feature in the pedigrees of many varieties but a sister line, which gave rise to the cultivar Midas, proved a far more successful parent, giving rise to cultivars such as Tyne. This pedigree branch has disappeared from current UK recommended barleys but has found favour in Australia through varieties such as La Trobe and Hindmarsh. Golden Promise malt is now being sought by craft brewers all over the world and this may be due to some unique beer flavour attributes that have been associated with the variety.

**Genetic dissection of the barley Net blotch interaction using effectors.**

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The net blotch diseases of barley (net form and spot form) are caused by the Pleosporales pathogens Pyrenophora teres teres and Pyrenophora teres maculata respectively. They cause major yield losses in Australia and many other places. The control of these diseases is difficult as both genetic and chemical control methods are problematic.

Like other Pleosporales such as the wheat pathogens tan spot and septoria nodorum blotch, virulence in net blotch is at least partially controlled by the secretion of proteinaceous necrotrophic effectors. We are in the process of purifying and expressing such NEs. Genetic dissection of the host responses to NEs has proved to be a rapid way to map sensitivity genes to SNB and TS and we hope to replicate this work with the net blotch effectors.

**Understanding the basis of host and non-host defences during barley-aphid**

**interactions**

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Aphids are phloem-feeding insects that cause important yield losses on crops, including

cereals such as barley. Most aphid species are limited to one or few host species, but some

are able to reproduce on many plants belonging to different families. Interestingly, aphid

probing-behaviour can be observed on both host and non-host plants indicating a

requirement for molecular events to take place which may dictate the aphid host range. Here

we use barley as model monocot crop to understand the basis of host and non-host plant

interactions with aphids. We found that the barley specialist Rhopalosiphum padi and the

broad host range Myzus persicae showed strong differences in colonization, phenotype and

probing-behaviour on barley. Based on Electrical Penetration Graph (EPG) technique, we

found that barley resistance to M. persicae lies in the phloem. Analyses of barley

transcriptional responses revealed gene sets differentially regulated upon the different

barley-aphid interactions, where M. persicae induced the strongest response. Interestingly,

we identified several genes highly up-regulated upon M. persicae interaction, such as late

thionins or embryogenesis abundant gene (lea14). We have generated barley knock-out

lines, using CRISPR-Cas9 technology, for lea14 to investigate its role in plant-aphid

interactions. Moreover, aphid effectors have been implicated in the host range for model

plants; moving towards crops we used different barley transgenic lines overexpressing aphid

effectors to investigate their impact in aphid host range. Our work thereby provides novel

insights into host and non-host barley defences against aphids and uses barley as monocot

crop model to study aphid effectors.

**The characterization of *HvABI5* regulatory role during barley seed germination in the presence of ABA**

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Seed germination is regulated antagonistically by abscisic acid (ABA) and gibberellic acid (GA). The manipulation of hormone content (ABA or GA biosynthesis inhibitors) during early seed development in the mother plant can affect the germination of mature grains. ABA INSENSITIVE 5 (ABI5) is a basic leucine zipper transcription factor, which regulates ABA-responsive genes during seed germination. We identified a barley TILLING mutant in *HvABI5*, *hvabi5.d*, which showed insensitivity to 300 μM ABA at germination stage compared to wild type (WT). To reveal genes involved in ABI5-dependent regulation of germination in ABA presence, we conducted the gene expression analysis in ABA-treated seeds of *hvabi5.d* and WT. We analyzed the response of three gene groups: (1) ABA pathway genes, (2) *HvABI5* targets and barley orthologues of genes regulated by *AtABI5* in Arabidopsis seeds and (3) GA metabolism genes. We found that ABA induced higher expression of ABA biosynthesis and signalling genes in *hvabi5.d* than in WT. However, the activity of *HvABI5* and its targets was reduced in mutant when compared to WT under ABA. The similar results were observed for barley orthologues of *AtABI5* downstream genes. However, in the ABA presence the expression of GA biosynthesis gene was higher in *hvabi5.d* than in WT. Therefore, the disturbed *hvabi5.d* response to ABA is probably caused by decreased activity of *HvABI5* and its target genes, and the increased expression of GA biosynthesis gene. The higher activity of ABA-related genes in *hvabi5.d* may indicate a *HvABI5* role in a feedback loop in the frame of ABA pathway.

**Targeted mutagenesis in barley (*Hordeum vulgare*): Towards controlled meiotic recombination for efficient breeding**

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Barley (*Hordeum vulgare*) is a diploid crop with a large genome organized into seven pairs of chromosomes. The state-of-the-art tools and resources that are available for barley research make it an excellent model for other cereals. Functional validation by gene manipulation is a key step in quantitative trait locus (QTL) analysis. Here we report on our efficient TILLING and CRISPR/Cas9 based targeted mutagenesis and their application to the study of meiotic recombination in barley.

Meiotic recombination results in generation of genetic variation that underpins crop improvement. During meiosis, homologous chromosomes recombine and segregate under the control of a complex, dynamic and highly conserved molecular machinery. Although most structural and recombination components have been determined, the overall mechanisms that regulate the frequency and distribution of meiotic crossovers (CO) are still poorly understood. In grasses, COs are preferentially formed at sub-telomeric regions resulting in stable linkage blocks in centromeric regions containing ~30% of genes. Our aim is to elucidate the mechanism of this skewed pattern of recombination in barley and tap into this important gene pool.

In order to unravel key meiotic regulators, we are studying the dynamics of meiotic transcriptome and proteome in barley. In addition to the characterisation of a number of desynaptic mutants, we are mutagenizing several other key meiotic genes in barley cv. Golden Promise. The selected mutants are currently being crossed with barley cv. Bowman for genetic analysis of recombination - our key findings and methodology will be presented.

**Genetic analysis of canopy architecture traits linked to barley biomass and yield**

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Barley is mainly used for animal feed and malting industries and breeding traditionally focused on increase of grain yield by partitioning biomass from straw to grains. The increasing demand for renewable materials makes straw, and specially barley straw characterized by the largest content of carbohydrates among the cereals, a valuable product for its potential conversion into biofuels and other products. Tillers are a defining characteristic of shoot architecture and contribute directly to grain and biomass yield, making tillering an important trait for breeding. Despite this, regulatory genes and pathways and their interplay with other sources of variation that impact tillering throughout development are not well characterized in barley. As part of the BarPLUS project (<https://barplus.wordpress.com/)>, the main objective of my PhD project is the identification and characterization of genes controlling tillering ability in barley through (1) genetic analysis of high-tillering mutants and (2) study of natural genetic diversity in a germplasm collection. For the first approach, I am characterizing mutants previously identified from forward screening of the HorTILLUS population, which was produced by University of Silesia (Poland) by chemical mutagenesis of cultivar ‘Sebastian’. As a first step towards identification of the genes underlying these mutants, crosses were carried out with different wild-type cultivars to obtain F2 progenies for mapping by sequencing. In parallels, the HorTILLUS population was used for TILLING of the LBO (Lateral branching oxidoreductase) candidate gene, allowing the identification of lines carrying different nonsense and missense mutations: these will be phenotyped under controlled condition to evaluate their potential effects on plant architecture. In the second approach, allele mining for tillering CGs relies on the ‘Whealbi’ collection (www.whealbi.eu), which includes 403 diverse accessions whose exome sequences have already been captured. A subset of these will be phenotype under controlled conditions to analyze the morphological and physiological parameters and to estimate the allele effect on the tillering traits. These experiment will result into identification of useful genes, alleles and lines to breed a new barley ideotype with high tillering number.

**Barley short-awn *breviaristatum* mutants highlight amino acid residues critical for the activity of brassinosteroid-6 oxidase, Δ5-sterol-Δ24-reductase and the brassinosteroid receptor**

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Abstract

The development of cereal crops with short and sturdy culms is of importance for improving resistance of plants to lodging. Lodging leads to severe problems such as reduced yield and poor seed quality. In general short-culm cultivars are more resistant to lodging due to their compact and sturdy culms. Several hormonal pathways are responsible for the control of plant architecture. Brassinosteroid (BR) is one of the plant hormones known to regulate cell division and stem elongation. Both BR biosynthetic and BR signaling mutants obtain a short culm. In the present study we perform a phenotypic screen of 228 barley (*Hordeum vulgare* L.) *breviaristatum* mutants for BR deficient characters. 30 lines were tested in leaf-unrolling and leaf inclination bioassays, which suggested 28 BR biosynthetic and 2 BR signaling mutants. Crosses to four previously identified BR mutants revealed that eleven are deficient in *HvBRD* (*BRASSINOSTEROID-6-OXIDASE*), one in *HvDIM* (*DIMINUTO*) and two in *HvBRI1* (*BRASSINOSTEROID INSENSITIVE1*). *HvBRD* and *HvDIM* encode the biosynthetic enzymes BR-6-oxidase and Δ5-sterol-Δ24-reductase, respectively, whereas *HvBRI1* encodes the BR receptor. The mutations were analyzed at DNA level and their effects on the respective proteins were evaluated by homology modelling. The four mutations *ari-u.382*, -*u.264*, *-u.309* and –*u.465* affect amino-acid residues Thr287Ile, Cys421Tyr, Gly417Asp, Pro456Ser correspondingly for HvBRD. Mutation *uzu1.312* causes modification of a highly conserved amino acid in the activation loop of kinase domain of HvBRI1. Mutation *ari-o.415* results in a Gly165Asp substitutions affecting the FAD-binding domain.

**Closely related *Cellulose synthase-like* genes influence barley root growth and differentiation**

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The barley *Cellulose Synthase-like F* (*CslF*) gene family includes 9 members, which are related to the *CslD* genes that influence root hair development in Arabidopsis. Characterization of the *ClsF* genes in barley has focussed mainly on the grain, and to date, only CslF6 has been shown to play a significant *in planta* role in the synthesis of the primary cell wall polysaccharide, (1,3;1,4)-β-glucan. Gene expression analysis reveals that two other *CslF* genes, *CslF3* and *CslF9*, accumulate in the tip of barley root, suggesting a potential role in root development. We have investigated the role of these genes. *CslF3* and *CslF9* show peak expression in the elongation and young differentiation regions of root tip, and levels are notably higher than *CslF6*, which is known to be most highly expressed *CslF* gene in all previously studied tissues. In RNAi lines of *CslF3* and *CslF9*, all seedlings show slower growth rate during until 7 dpg. Shorter seminal roots, lateral roots and root hairs are also observed in transgenic lines in soil and agar environments. Overall, *CslF3* and *CslF9* RNAi lines have smaller root surface area, which may limit the water and nutrient uptake from surrounding environment. Consistent with this, we observed negative effects on shoot height and biomass in transgenic barley lines. Currently, we are examining CRISPR/Cas9 knockout lines for these genes in addition to determining the effects of these genes on a cellular level.

**GENE TRANSCRIPTIONAL REGULATION IN *LYS3a* MUTANT BARLEY SEEDS**

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Risø1508 is a Lysine-rich mutant whose mutation in the locus *Lys3a* segregates as a single Mendelian gene that produces pleiotropic effects, such as a drastic expression reduction of genes encoding the the B-, C- and γ-hodeins, but not of those encoding the D-hordeins. This later gene lacks in its promotor the GLM motif (5’-(G/A)TGA(G/C)TCA(T/C)-3’, that is recognized by bZIP transcription factors.

In this work, the barley mutant Risø1508 has been used as a tool for better understanding the gene regulation during the maturation and the germination phases of seed development. To this aim, transcriptomic analyses were performed comparing wild and mutant genotypes during seed maturation. The expression profile of the genes encoding the main transcription factors (TFs) involved in the gene regulation of seed maturation was analysed by RT-qPCR in both genotypes. The genes encoding DOF TFs were mis-regulated throughout the process in Risø1508, although significant differences were also found among some of those encoding bZIPs. Since these TFs have been shown to play a role, not only in seed protein expression during seed maturation, but also in the regulation of hydrolase gene expression in the germinating aleurone, its expression in the germination was also analysed. Besides, the germination kinetics in presence/absence of ABA demonstrated that Risø1508 behaves as an ABA-insensitive mutant.

These preliminary data have allowed us to propose a pioneering study linking together cereal grain development and abiotic stress response during seedling establishment using as a tool the barley ABA-insensitive mutant Risø 1508.

**Functional dissection of starch turnover during barley inflorescence formation**

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In plants, starch represents the major form of storage carbohydrate. Starch accumulation is well described in storage organs, but it can be transiently formed in photosynthetic tissues; in this case starch is synthetized in the chloroplasts during the day and it is later degraded during the night. Interestingly, transient starch accumulation is also observed in heterotrophic tissues where its role is less understood. Recently, three waves of transient starch accumulation have been reported during flower and seed differentiation in Arabidopsis. In this species, some of the candidate genes involved in the process have been identified.

Our laboratory in interested in dissecting the molecular mechanisms that control barley inflorescence and flower formation. Among the signaling molecules that play important roles in modulating plant growth, we are focusing the attention on sugars with the aim to understand how carbon availability is perceived by the plant to finally coordinate reproductive organs differentiation. As a first step, we are performing iodine staining to unveil the existence of transient starch accumulation during barley spike, flower and kernel differentiation. Next, gene co-expression analysis is supporting the identification of candidate genes involved in transient starch biosynthesis and degradation, followed by sugar transport. Finally, mutant development and characterization is performed to elucidate the biological function of the entire mechanisms.

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**A barley *Lys3* mutant has increased transformation efficiency.**

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Very few barley cultivars other than Golden Promise are susceptible to transformation.  The reasons for this cultivar specificity are not understood. Recently, we have been studying the control of organ size in *lys3* barley mutants that have abnormally-large embryos with irregular scutella epidermal cells. Our initial experiments suggested that the large-embryo phenotype was linked to increased transformation efficiency. Here, we describe our work to understand the genetic and molecular mechanisms underlying this phenotype.

Four independent *Lys3* mutants have been described to date and they all have shrunken endosperm as well as the larger-than-normal-embryo phenotype. Amongst the *lys3* mutants, M1460 (a mutant of Minerva) has the largest embryos. Initial experiments were performed using M1460 and we observed transformation efficiencies that were similar to those for Golden Promise and increased relative to Minerva. We subsequently tested the other three *lys3* lines (RISØ 1508, RISØ 18, RISØ 19) and found lower transformation efficiencies than for M1460. Our studies suggest that the immature embryos of M1460 are particularly efficient at producing embryogenic callus. To study this further, we crossed M1460 to the untransformable elite cultivar Optic. After two backcross generations, we found that the large-embryo phenotype (*lys3*) segregated away from transformability. This suggests that a locus other than *Lys3* is responsible for influencing transformation potential.

We are currently attempting to determine the genetic basis of transformability and ultimately, to generate new transformable barley lines.

**DNA-Methylation and meiotic recombination: Producing and characterizing barley *met1* mutants using TILLING by sequencing**

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One of the main challenges of the next decades resides in improving food production and agricultural yields to support an ever-growing world population. This can be achieved by creating new, high performing crop varieties through plant breeding, which relies on meiotic recombination or crossing over (CO) between parental varieties to recombine beneficial alleles into a single line or variety. However, in many cereal crops, and especially large genome species like barley, meiotic crossovers predominantly occur towards the ends of chromosomes and 20 to 30 % of genes are in regions that rarely recombine. Moreover many genes are trapped in poorly recombining linkage blocks, which leads to them being inherited together and thus unusable in breeding programs. In *Arabidopsis* *thaliana*, mutants in *Cytosine DNA-Methyltransferase 1* (*met1*) are characterized by a hypomethylated genome, which results in changes in the pattern of recombination and an increased number of COs in the centromere proximal region.

In barley, TILLING-induced mutations in *met1* are being screened to identify hypomethylated mutants showing an increased range of CO events in centromere-proximal regions. To this extent a new TILLING by sequencing method has been optimized to exhaustively identify mutations along the gene of interest. This allows to successfully isolate mutants which will be characterized for changes in the recombination patterns.

To further characterize the mutations’ effects, CRISPR-Cas9 mutants are also generated and cytologically phenotyped. Plants exposed to Zebularine, a hypomethylating agent, phenocopy *met1*, and are used to precisely assess the redistribution of CO, gene expression levels and DNA-Methylation levels.

**Perturbation of Recombination observed in the mapping of *Calcaroides-C***

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As part of ongoing research into the control of recombination in barley and its application, F1 plants derived from a cross between barley cultivar *Morex* and BW105(*Cal-c*) were subjected to a sustained period of elevated temperature of 30ºC/25ºC to analyse the effect of the heat stress on the patterns of recombination. The Bowman near-isogenic line BW105 contains the *Calcaroides-C* mutation that causes an ectopic sac-like structure to form at the transition between the lemma and awn (Franckowiak & Lundqvist 2002). The mutation has been mapped to chromosome 5H on the proximal region of the long arm (Pozzi et al., 2000) and was chosen as a potential mapping target that would benefit from increased recombination in the peri-centromeric regions of the genome.

The genetic maps derived from the progeny of the heat stressed F1 plants did indeed show significant increases in recombination in the peri-centromeric regions in comparison to that found in the progeny of unstressed control plants confirming the reported temperature effect in barley on the distribution of crossovers and recombination (Higgins et al., 2012; Phillips et al., 2015).

However, despite the mutation successfully mapping to 5HL, finer mapping was compromised by an unexpected change in recombination pattern found in both treated and control populations. Compared to consensus maps, there was a very strong suppression of recombination from ~50 cM to <4 cM in a region on 5HL that could be indicative of an inversion associated with *Cal-c* potentially induced by the causal neutron treatment.

Interestingly the reduction of recombination in the long arm was associated with an increase in a proximal region of the short arm of 5H where markers normally 9 cM apart become effectively unlinked. These associated effects potentially indicate that the inversion would have an intra-chromosomal effect on recombination that works across the centromere. The potential mechanisms and applications of the observed effects are discussed.

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**Mutant panel of diploid einkorn wheat developed by heavy-ion beam mutagenesis and screeing of early-flowering mutants**

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Mutation analysis is a powerful tool for investigation of gene function. Heavy-ion beam mutagenesis has been recognized to be an effective method of producing mutations because of its high linear energy transfer (LET). High-LET radiation effectively induces DNA double-strand breaks than other mutagenic methods. We have been constructing a large scale mutant panel of diploid einkorn wheat (*Triticum monococcum*) using heavy-ion beam mutagenesis. Seeds of the wheat strains were treated with C ion beam, and sown in the field. The harvested selfed seeds of each spike of M1 plants were used to produce the M2 lines. To determine the optimal conditions for mutant generations, we examined the effect of dose (Gy) and LET on plant viability and mutation (albino) rate. We concluded that the optimal condition was 50Gy of C ion beam with LET of 50keVµm-1. We are now keeping about 10,000 M2 lines. We are using the mutant panel for screening mutation of reproductive growth, especially for flowering-time mutants. Several flowering-time mutants of great interest have been identified; non-flowering mutants (*mvp*: *maintained vegetative phase*), late-flowering mutants, and early-flowering mutants. The maintained vegetative phase mutant lacks flowering promoter gene *VRN1*. Recently, we identified four extra early-flowering mutants, named *extra early-flowering1* (*exe1*), *exe2*, *exe3* and *exe4*. Among them, *exe1* and *exe3* have the deletion mutation of a circadian clock regulator gene *Wheat PHYTOCLOCK 1* (*WPCL1*). The *exe* mutants have a decreased photoperiod sensitivity compared to WT plants indicating that biological clock functions in photoperiod sensitivity.

**Genomic selection for accelerated barley breeding**

*Ruth Hamilton*

*Claire Halpin, Robbie Waugh, Helena Oakey, Bill Thomas, Hazel Bull*

Increasing biofuel production will be advantageous in the future as we move away from coal based fuel, however first generation biofuels (from food sources) will lead to land competition with food crops. This “Food vs Fuel” struggle will have long term impacts on input resources for farming, levels of crop production and food prices. This problem can in part be resolved by increasing the production of second generation biofuels, which is biofuel produced from plant material that is not a food source e.g. straw.

Currently the levels of second generation biofuel production is not high: there are larger production costs in comparison to first generation biofuels. There has been little breeding that improves straw saccharification yield (i.e. the yield of sugars released from biomass that can be converted to biofuel).

Genomic selection (GS) is a new method of breeding that takes into account genome wide genetic marker information. GS uses both phenotypic and genotypic data to assign breeding values (GEBVs) for a specific trait to cultivars in a breeding programme.

It has been demonstrated that saccharification can be improved in crop species- without altering traits that are agronomically important. Using a GS supported breeding program to improve straw saccharification yield would be advantageous as the trait is most likely under the control of multiple genes with small effects and is also affected by environmental conditions. This project utilizes a multi-environment GS model (Oakey et al., 2016) to breed for spring barley with high straw saccharification, while maintaining agronomically important traits.

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**Identification and application of *TFA* genomic regions that confer transformation amenability in barley**

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Different plant cultivars of the same genus and species can exhibit vastly different genetic transformation efficiencies. However, the genetic factors underlying these differences in transformation rate remain largely unknown. In barley, cv. ‘Golden Promise’ is the most useful and well-studied cultivar for genetic transformation. By contrast, cv. ‘Haruna Nijo’ is recalcitrant to genetic manipulation, although numerous genomic resources have been developed for this haplotype. Recently, we identified genomic regions of barley important for successful transformation with *Agrobacterium*,utilizing the ‘Haruna Nijo’ × ‘Golden Promise’ F2 generation. A total of 3,013 ‘Haruna Nijo’ × ‘Golden Promise’ F2 immature embryos were inoculated with *Agrobacterium*, and 60 transformants were obtained. These plants were genotyped using 1,131 genome-wide SNP markers. We observed significant segregation distortions from the expected 1:2:1 ratio toward the ‘Golden Promise’ in regions of chromosomes 2H and 3H, indicating that the alleles of ‘Golden Promise’ in these regions might contribute to transformation efficiency. We termed this region as *Transformation Amenability* (*TFA*) loci responsible for *Agrobacterium-*mediated transformation. The genomic regions identified herein likely include necessary factors (i.e. regeneration from callus) for *Agrobacterium*-mediated transformation in barley. The potential to introduce these loci into any haplotype of barley opens the door to increasing the efficiency of transformation for target alleles into any haplotype of barley by the *TFA*-based methods proposed in this presentation.

Pyramiding of the row-type locus *VRS3*

*Hazel Bull, Sarah McKim, Andy Flavell, Robbie Waugh, Bill Thomas*

Genetic characterization of the barley row-type mutant *vrs3* has identified it to be a putative JmjC histone demethylase; in a two-rowed genetic background (*Vrs1*.b, *int-c*.b) *vrs3* is characterized by an intermediate phenotype, two-rowed at the base of the spike and six-rowed at the top. However, when *VRS3* is pyramided with other row-type loci (*VRS1*, *INT-C*) a more regular six-rowed phenotype is formed. Here we explore the effect of pyramiding *vrs3* with varying allelic combinations of natural six-rowed alleles *vrs1*.a and *Int-c*.a on row-type and grain dimensions.

# **Genome Editing in Barley: Targeting (1,3;1,4)-β-Glucan Synthases**

Guillermo G. Gimenez, Abdellah Barakate, Joanne Russell, Jennifer Stephens, Edwin R. Lampugnani, Monika S. Doblin, Geoffrey B. Fincher, Rachel A. Burton, Robbie Waugh, Matthew Tucker and Kelly Houston.

(1,3;1,4)-β-Glucan is one of the most abundant non-cellulosic polysaccharides of primary cell walls in grasses, and is found at high levels in barley endosperm compared with other cereals. (1,3;1,4)-β-Glucan in barley grain has a negative impact on brewing and distilling filtration processes. Conversely, (1,3;1,4)-β-glucan has beneficial effects on human health, where it acts as soluble dietary fibre in the small intestine. The genes encoding (1,3;1,4)-β-glucan synthases, namely *HvCslF* and *HvCslH* (Cellulose synthase-like) gene super-family have been identified1,2. *HvCslF6* has been shown to play a key role in the synthesis of grain (1,3;1,4)-β-glucan. However, other members of this gene family are expressed in different tissues: *HvCslF3* in roots; *HvCslF9* in early grain stages and *HvCslH* in leaves2,3. These 4 genes were targeted using CRISPR/Cas9-based technology and a wide range of mutations (indels) leading to frameshift changes in T0 plants was obtained. A total of 44 T1 genotypes were confirmed to have CRISPR/Cas9-induced mutation*.* For each gene, multiple unique independent CRISPR/Cas9 mutated lines were selected for analysis in subsequent generations. We isolated homozygous and *Cas9*-free (stable) genotypes carrying non-synonymous mutations likely to affect protein function. Our work currently focuses on characterising stable lines using immunocytochemistry techniques to assess grain (1,3;1,4)-β-glucan content and other cell wall changes. A comprehensive phenotypic characterisation will complement this study, quantifying (1,3;1,4)-β-glucan and analysing cell wall composition in general. These mutants represent a valuable genetic resource for studying barley cell walls, and demonstrate the effectiveness of the CRISPR/Cas9-based gene editing technology.

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**What story can a single barley anther tell? A micro-proteomics approach to study protein diversity in developing anther.**

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Micro-proteomics is a method that extracts, separates, detects and identifies proteins from extremely small amounts of sample. Unlike macro-proteomics studies, which analyse proteins at a population level by using extracts from millions of cells, the micro-proteomics approach allows for precise characterization of the proteome of specific cells or tissues.

Here, we report the development of the micro-proteomics pipeline for analysing barley anther proteome at a single anther level to a depth of more than 4000 proteins. The method is rapid, reproducible, robust and sensitive enough to detect low abundant proteins, including chromatin-associated factors involved in chromosome structure and gene regulation. The micro-proteomics approach promises to be an excellent tool to study anther proteome of barley mutants, which are often semi-sterile and thus offer limited amounts of inflorescence tissue.

We applied our single anther proteomics approach to characterize proteome of barley anthers at early stages of meiosis (leptotene and zygotene). Protein extracts from single staged barley (Hordeum vulgare cv. Golden Promise) anthers were analysed by Nano Liquid chromatography-tandem mass spectrometry (nLC-MS/MS). Up to ~34 thousands of peptides, representing 4307 proteins have been identified by searching the MS/MS data against Uniprot Hordeum vulgare database.

**The International Database for Barley Genes and Barley Genetic Stocks.**

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The demand for an International Database for Barley Genes and Barley Genetic Stocks began already in the 1980s. Compiling data started in 1971 in Barley Genetics Newsletters first issue, but took new turn during the 1990s. The main part of the database shows an overall structure of descriptions with special paragraphs dedicated to cover previous nomenclature, inheritance, locus location, descriptions of morphological and physiological characters, first detected mutant, all mutant events, mutants used for description, parent germplasm and references. It includes for the locus name the use of the three-letter symbols. Each mutant is associated with a stock number, this barley genetic stock (BGS) number corresponds to an accession (GSHO or NGB) number kept in the Barley Genetic Stock Collection, Aberdeen, Idaho, USA, or at NordGen, Sweden. Only one allele of each description is kept at the Main Stock Center but all alleles are stored at NordGen. The BGS database comprises about 750 descriptions with more than 4000 alleles and about 2000 references, and many of the genes are illustrated with images.

The second part of the database comprises basic information of all 10000 Swedish barley mutants to find their original stock accession for degeneration, mutagens used for induction, inheritance and their relation to BGS descriptions.

The database is easy to be used, by names, symbols and other objects you are looking for. It is available at http://www.nordgen.org/bgs

**Characterisation of pollen development genes in barley using CRISPR**

Johanna Astrand, Zoe Wilson, Jose Fernandez

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Pollen development is essential for reproduction of plants and is controlled by an intricate gene network. Failure to form viable pollen leads to male sterility and forces outcrossing to produce seeds, which may cause yield losses. Traits resulting in male sterility have for a long time been of interest to plant breeders for the generation of hybrid varieties with increased yield, decease resistance, or improved grain quality. The gene network controlling pollen development in *Arabidopsis thaliana* (*A. thaliana*) has been partly elucidated but little knowledge has been transferred to crops such as barley, maize and wheat. In the current project the CRISPR-Cas9 gene editing system is being applied to study the genes underlying pollen development in barley. Directed mutagenesis via the CRISPR-Cas9 system is being used to introduce mutations in gene sequences encoding putative pollen development genes derived from *A. thaliana* homologs. The impact of these on growth and fertility is being assessed.

**An open-flowering mutant obtained from the cleistogamous cultivar in barley**

Katsuyuki Kakeda

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Fertilization in closed flowers, termed cleistogamy, is associated with improvements in the level of resistance against Fusarium head blight (FHB) in barley. Natural variants of barley carrying a single recessive gene at the *cleistogamy 1* (*cly1*) locus have the cleistogamous flower, in which the palea and lemma remain tightly closed throughout the pollination period. The cleistogamy in barley is due to the failure of lodicules to develop and swell sufficiently for flower opening at anthesis. The *Cly1* gene was identified by a map-based cloning and found to encode an AP2 transcription factor, HvAP2 (Nair et al. 2010). The cleistogamous *cly1* allele contains a synonymous nucleotide polymorphism (SNP) at the microRNA (miR172) targeting site. The SNP mutation would inhibit the binding of miR172 with *cly1* mRNA and thereby cause the expression of HvAP2 protein, which is normally suppressed in the wild type by the miR172-mediated pathway. The HvAP2 protein is assumed to negatively regulate the development of lodicules, resulting in floret closing during anthesis. Recently, an open-flowering mutant (designated MGC) was found in an EMS mutagenized population from a cleistogamous barley cultivar ‘Misato Golden(MG)’. The MGC mutation was shown to cosegregate with the *cly1* locus, where the mutant (open) allele was recessive to cleistogamous (*cly1.b*) allele. This study reports molecular characterization of the newly induced *cly1* mutant allele and the functional implications for flower opening.

**Water-use via the barley circadian clock: Adaptation potential by “breaking” the clock.**

Kayla McCarthy, Sue Hartley, Seth J Davis

Future climate scenarios predict that weather dynamics will become more extreme and less predictable,threatening food security. Seasonal and diurnal physiological synchrony, achieved through the circadian system, has been linked to fitness advantages in plants, such as improving stress regulation. Daily rhythms of co-ordinated gene expression, fine tuned by sensitivity to environmental influences, balance the predictable with inclement change. This is via an interconnection of a variety of signalling networks by the clock. Light and temperature cycles strongly influence circadian growth in a number of studied plant species. Water availability is another vital determinant for plant growth. Abscisic acid signalling pathways connecting stomata closure with the circadian gene network and rhythms are linked to active control of water regulation in the Arabidopsis circadian system. In barley the genetic components of the clock are largely conserved. Following evidence of greater drought tolerance in barley (Hordeum vulgare) circadian mutants, our project investigates coinciding changes in water-regulation physiology to daily temperature. We use evening-complex mutants in Spring barley (c. Bowman) to examine water-stress traits. Work presented examines water-uptake and growth of hydroponically grown, young plants in controlled growth conditions, with and without strong temperature oscillations and osmotic stress. Understanding how diurnal temperature drives clock rhythms, to appropriately coordinate water-use regulation can provide important information in understanding plant fitness especially in an agriculturally valuable plant such as barley.

**Mapping and Characterisation of Barley Desynaptic Mutants**

Malcolm Macaulay, Abdellah Barakate, Miriam Schreiber, Jennifer Stephens, Nicola Uzrek, Isabelle Colas, Dominika Lewandowska, Adeline Sourdille, Mikel Arrieta, Sybille Mittmann, Luke Ramsay and Robbie Waugh.

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Large genome crops such as wheat or barley, display a skewed distribution of chiasmata towards the telomere, resulting in the peri-centronomic regions which contain approximately a third of barley genes displaying little or no recombination. An ability to modify the patterns of recombination in these species could therefore have significant impact on crop breeding. During meiosis, homologous chromosomes recognize each other, align (synapse) and pair via chiasmata, ensuring correct segregation at metaphase and thus avoiding aberrant chromosome numbers within gametes.

To gain a better understanding of underlying meiotic mechanisms we are characterising a collection of 14 desynaptic (des) mutants. Using the semi sterile and wild type phenotype, candidate genes for *des5*, *des8*, *des9*, *des10* and *des12* have been identified and the effect of the mutant alleles on the genetic distribution of recombination events established alongside cytological characterisation of meiosis.  A new bulked segregant approach using pools of semi-sterile and WT F2 individuals and a 50K SNP array has been developed and employed. We will report on the success of this approach and its use for the low and high resolution mapping of additional targeted desynaptic mutants.

**Field multi-omics approaches in barley toward robust crop development**  
  
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Abstract:  
Many agronomic traits are controlled by quantitative inheritance under   
the influence of genotype / environment interaction. In order to   
understand the interaction and to design robust crops against various   
environmental changes under practical field environment, we have been   
attempting to establish a methodology that will allow us to design crops   
to fit any field environments with a data driven research approach.   
Time-series data of morphological and physiological status of field   
barley along its life cycle are obtained to deduce the key physiological   
changes (named “state traits”) associated with the objective trait   
(Mochida, Saisho and Hirayama 2015 Front. Plant Sci. 6:740. doi:   
10.3389/fpls.2015.00740). To examine the relevance to heading time and   
field growth profiles in two different locations (Kurashiki and   
Yokohama), chronological omics data including transcriptome, hormonome,   
and epigenome for a large number of samples along the life cycle are   
collected using multiple barley varieties. In this presentation, we will   
show the outline of the omics datasets, and discuss phenological   
plasticity in two barley accessions (domesticated barley [OUJ064] and a   
wild barley [OUH602]).

**Gene editing using CRISPR/Cas9 to induce new mutations in the**

**SYP121 gene in Arabidopsis and barley**

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**Summary**

The SNARE protein equivalent to the Arabidopsis SYP121 (also known as PEN1 and SYR1) is a member of the SNARE superfamily of proteins that are involved in cell signalling, vesicle traffic, growth and development. Key residues are F9-x-R11-F12, that form the core binding site for SM and Kv channel proteins. Eliminating this site dramatically alters plant growth under limiting potassium, it also affects stomatal response to the hormone abscisic acid and drought stress. These are straightforward phenotypes that can be tested. Also, the SYP121 gene has other functions including protein interaction and trafficking phenotypes and non-host resistance against barley powdery mildew, *Blumeria graminis* sp. *hordei*. SYP121 is a nonessential component of the preinvasive resistance against Colletotrichum fungus and is also required for mlo resistance in barley. The SYP121 mutation results in stomatal phenotypes that reduce CO2 assimilation, slow vegetative growth and increase water use efficiency in the whole plant, conditional upon high light intensities and low relative humidity.

We used the SYP121 gene as a target for CRISPR/Cas 9 based genome editing in both Arabidopsis and barley. We identified the barley SNARE with the FxRF motif in the first 15 residues of the protein in the target genotype Golden Promise. We then designed constructs that were expected to knock-out this part of the gene in Arabidopsis and in barley.

The opportunity to use CRISPR/Cas9 to induce mutations in the same target gene in both Arabidopsis and barley underlines the value of this technology for analysis of this important protein in both a model and crop plant.

**Key words:** CRISPR/Cas9, Barley, Arabidopsis, Off-target, SYP121 gene.

**Developing of a barley cv. Golden Promise TILLING population and different mutation detection methods**

1Miriam Schreiber and 1Nicola Uzrek, 1Abdellah Barakate, 1Adeline Sourdille, 1Malcolm Macaulay, 2Bernardo Clavijo, 2 Jon Wright, 1Luke Ramsay and 1Robbie Waugh

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In barley (*Hordeum vulgare*) meiotic recombination shows a non-random pattern of recombination which is skewed towards the ends of the chromosomes. This results in around 30% of the genes in the centromeric region not recombining; a big restriction for geneticists and breeders alike. Here we are highlighting a barley EMS (ethyl methanesulfonate) TILLING (Targeting Induced Local Lesions in Genomes) population in Golden Promise (GP) as a resource for mutations and the potential methods of screening for those. For this we developed a double mutagenised GP population initially with 25mM EMS in 2014. Seeds were harvested and combined for a subsequent mutagenesis with 30mM EMS in 2015. Alongside this we built a GP genome assembly in collaboration with Earlham. A first analysis of the TILLING population using whole barley exome capture on 20 plants from the M2 population showed a mutation frequency of approximately 5.9 mutations per Mb and highlighted big differences between the individual plants. The population has and is currently being used to screen for mutations in meiotic genes. In addition to the whole barley exome capture we have used two further methods to identify mutations. One is a target specific capture using MYbaits from Arbor Biosience and the other is amplicon sequencing. The different analysis strategies and some results will be shown. As GP is one of the few barley cultivars that can be used for genetic transformation we will be able to complement any identified mutant alleles and see if it rescues any observed phenotype.

**Untitled**

Jennifer Shoesmith

The APETALA2 (AP2)-like transcription factors, first characterised in Arabidopsis as key regulators of floral development, also play developmental roles in cereal crops such as maize, rice and barley. Our previous work showed that the Zeo1.b semi-dwarf dense spike mutant has a SNP in the HvAP2 gene making its transcript to resistant to miR172-guided cleavage, resulting in over expression of AP2 (Houston et al., 2013). Florets in Zeo1.b also have small lodicules leading to cleistogamy. This project characterises an x-ray induced deletion mutant called gigas1.a which has opposite phenotypes to Zeo1.b, including a lax spike, elongated spikelets and enlarged lodicules. Using the 50K iSelect SNP Array (Bayer et al., 2017), this narrowed the size of the deletion down to a one million base pair region that contains 27 genes, six of which are high confidence. We subsequently generated CRISPR-Cas9 lines targeting our best candidate. Lines containing a frame shift mutation in the first exon of our candidate gene phenocopies all gigas1.a phenotypes. We are now investigating the developmental and molecular mechanisms of our candidate gene including its direct targets and interactors.

**LUSH SPIKE – towards the genetics and mechanism of spikelet survival in barley**

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Improving grain yield is a major objective of crop breeding, and a promising avenue for maximizing yield is through enhanced spikelet survival during pre-anthesis development. However, little is known about spikelet survival and its impact on grain yield in cereals. In barley, the growth period awn primordium to tipping is considered as the most critical pre-anthesis phase determining spikelet/spikelet primordia abortion, and grain yield since ~70% of spikelet abortion occurs during this phase, regardless of growth conditions and row-type. Mortality of spikelet primordia may begin with the onset of rapid stem and spike growth and lasts until heading. Interestingly, the high broad-sense heritability (>80%) of spikelet survival in barley underscores that there are major genetic players regulating this trait. Therefore, we aim to discover QTL for spikelet survival in a GWAS panel and validate them in bi-parental double haploid (DH) mapping populations. Furthermore, interesting QTL will be mendelized, functionally characterized, and the underlying gene will be identified using a map-based approach. Histological analysis of spikelet/spikelet primordia during pre-anthesis phase will be performed to describe the developmental process of spikelet survival/abortion in barley. Additionally, the histologic studies will be complemented with the tissue-specific transcriptome, metabolome and phytohormone analysis. Finally, the spatio-temporal patterns of transcript, metabolite and phytohormone distribution/modulation in the spike may illustrate the mechanistical regulation of spikelet survival.