***Super-open flowering 1* mutant generated by chronic irradiation by gamma ray to wild barley**

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Plant domestication is a consequence of gradual accumulation of natural mutations in wild plants. As wild barley (*Hordeum vulgare* subsp. *spontaneum*) is an immediate ancestor of cultivated barley (*H. vulgare* subsp. *vulgare*), gene function presumably is better retained in wild barley than in cultivated plants. Here we mutagenized a wild barley strain OUH602 originated from the Caspian See region. Plants were grown in the “Gamma field “of the Institute of Radiation Breeding and irradiated chronically from germination to maturation by gamma ray dose at 0.24 – 0.77 Gy/day and 5 days irradiation/week from a cobalt 60 (60Co) source. Generation of the irradiated plants was defined as M1. M3 lines derived from 1,600 M2 plants were cultivated in an ordinal experimental field and 10 M3 plants from each M2 plant were forward-screened by eyes for morphological mutation. Line #44205 segregated a single plant showing a mutant with lemma and palea gaped in wider angle than that of the wild type. Lodicule is a floral organ of grass species and functionally related and ontogenically orthologous to the petal of the hermaphroditic angiosperm flower. Lodicule of this mutant was roughly 1.5 times larger in depth and length than that of the wild type. As a single recessive gene controlled the phenotype, the gene was named “*Super-open flowering 1”* and gene symbol *sof1*. Characterization of this mutant and genetic mapping of *sof1* will be presented.

Presenter of this paper is Takao Komatsuda <takao@affrc.go.jp>

**Genetic characterization of the *albostrians* barley provides new insights on underlying mechanism of leaf variegation**

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The barley *albostrians* mutant represents one classical type of variegation resulting from the absence of 70S ribosomes in plastids of *albino* leaf sectors. It served as a model system to study the cross-talk between nucleus and the other DNA-containing organelles and greatly extended the field of chloroplast biology. So far the *albostrians* gene was unknown, complicating further the elucidation of molecular mechanisms underlying variegation. Here, map-based cloning revealed one single gene of which the first exon carries a 4bp deletion in the *albostrians* mutant compared to wild type. Further, the biological function of the cloned *albostrians* gene was confirmed by site-directed mutagenesis through RNA-guided endonuclease. A genetic model for the typical variegation of *albostrians* mutant was proposed on the basis of the identified localization to plastids and known regulations from the homologous gene in Arabidopsis.

Key words: *albostrians* gene; chloroplast development; barley genome editing

**Title: Semi-dwarf barley mutants defective in the brassinosteroid metabolism – a promising**

**alternative for breeding and a useful tool for analysis of physiological reaction to drought**

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Brassinosteroids (BRs) are a class of steroid phytohormones, which regulate various

processes of plant morphogenesis and physiology – from seed development to regulation of

flowering and senescence. BR-deficient or BR-insensitive mutants of various plant species

show growth reduction of a various extent. Especially in cereal crops, the semi-dwarf mutants

are of great value for potential application in breeding, as semi-dwarf varieties are more

resistant to lodging under unfavorable weather conditions, thus assuring higher yield, and in the

past few decades significantly contributed to the success of the ‘Green Revolution’.

In our research a collection of the semi-dwarf ‘Bowman’ Near-Isogenic Lines (NILs),

which represent mutants defective in the BR metabolism was applied. Uniform genetic

background of this collection allows an efficient functional analysis of genes, as well as

comparative physiological and phenotypic analyses of the genotypes. This approach led to

identification of more than 20 mutations in four barley genes of the BR biosynthesis and

signaling pathways. Recently, the characterized NILs deficient in BR metabolism were

employed in a project aimed at analysis of the role of endogenous BRs in a regulation of plant

reactions to drought. Interestingly, the semi-dwarf NILs show enhanced tolerance to drought

when compared with the ‘Bowman’ cultivar. The research provided also an insight into the BR

homeostasis under the control and drought conditions and indicated that the endogenous BRs

influence accumulations of other phytohormones. We also showed that the endogenous BRs

regulate homeostasis of various non-enzymatic antioxidants under the control and drought

conditions.

**Identification and characterisation of barley *broad leaf* mutants and the affected genes.**

**Moritz Jost**

Leaves are major photosynthetic organs that convert the sun’s energy into biomass. As such, the performance of cereal crops is substantially influenced by both the size and shape of their leaves. While the genetic control of leaf size and shape is very well understood for dicotyledonous species like *Arabidopsis thaliana*, our understanding in the monocotyledonous grasses is still fragmentary. In previous work, we have identified the barley protein BROAD LEAF1 (BLF1), a novel negative regulator of cell proliferation, which mainly limits leaf growth in the width direction. Plants with a loss-of-function allele of BLF1 develop wider leaves due to increased cell division during primordia outgrowth. We are currently investigating its molecular function and are identifying potential interactors and downstream targets. Being a member of the INDETERMINATE DOMAIN protein family, BLF1 has both a putative DNA-binding site as well as a protein-interaction domain. We are currently using a yeast two-hybrid assay to screen for potential interactors of BLF1. To elucidate its putative role as transcription factor, we are deploying a DAP-seq approach to identity possible BLF1 target sequences.

In parallel we are exploiting the collection of *broad leaf* mutants from the NordGen Barley Mutant collection to identify additional leaf size regulators using an exome-capture based mapping by sequencing approach.

**The Cys-Arg/N-End Rule Pathway Is a General Sensor of Abiotic Stress in barley.**

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Abiotic stresses such as drought, salinity and waterlogging are major constraints to yield in global agriculture. The N-end rule pathway of ubiquitin-mediated proteolysis has been shown to be a molecular mechanism that controls plant responses to multiple stresses. We have previously shown that a reduced oxygen level (i.e. waterlogging) is sensed in barley through this pathway [1]. In addition, that this pathway controls the barley response to drought and salinity [2]. Plants that have a reduction of *PRT6* in barley are hypersensitive to the stress hormone ABA, with to stomatal aperture. Consequently, these plants have enhanced water retention and survivability under water deficit. The mechanism involves the controlled degradation of key regulatory proteins Group VII ERFs. The structure of the start amino acid of these proteins (Met-Cys-, MC-) destines them for destruction under normal oxygen and nitric oxide (NO) levels, but when oxygen and NO levels decline, they become stable. In summary, the manipulating of the N-end rule pathway by reducing its function (and stabilising its protein targets) enhances waterlogging tolerance in barley as well as tolerance to drought and salinity stress, with great potential for breeding programs. Currently we are also investigating how the N-end rule pathway regulation regulates the plant immune system. N-end rule mutants of barley showed enhanced resistance to infection by *P.japónica* and *P.Mildew,* which suggests that the N-end rule modulation of the immune response by this mechanism is conserved in flowering plants.

[1] Mendiondo *et al.****Plant Biotechnology Journal*** 2016.

[2] Vicente/Mendiondo *et al.****Current Biology*** 2017.

***Lys3* encodes a DOF transcription factor required for embryo-size determination in barley.**

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Barley grains are composed of hull, starchy endosperm and embryo, the latter being a concentrated source of several essential nutrients, vitamins and amino acids. Embryo size is therefore a potential target for optimising barley grain quality for feed and food. Mutants with large embryos have been described in other cereal species. However, the molecular mechanisms underlying the control of embryo-size in barley remain to be elucidated.

Previous work on numerous shrunken (low-starch) mutants of barley has suggested that some have larger-than-normal embryos. We compared the grain/embryo sizes of several shrunken barley mutants and found that the larger-than-normal-embryo phenotype is largely restricted to four mutants affected at the *lys3* locus. To discover the genetic basis of the *lys3* mutation, we used a combination of fine-mapping, chromosome flow-sorting and DNA sequencing of all four *lys3* mutants and their wild-type controls. The sequencing data were analysed using a variation of the MutChromSeq approach (Sánchez-Martín et al., Genome Biology 2016). We discovered one *Lys3* candidate gene that encodes a transcription factor of the DOF (DNA-binding with one zinc finger) class. One *lys3* mutant has a deletion of the entire *Lys3* gene and the others have amino-acid substitutions in the highly-conserved DNA-binding domain.

Expression analysis suggests that *Lys3* is expressed in the endosperm but not in the embryo. We conclude that *Lys3* is a part of a regulatory network that affects both endosperm starch synthesis and embryo growth. We are currently attempting to investigate further the targets of the *Lys3* transcription factor.

**Genetic regulation of lateral spikelet size in barley**

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Directional selection for increased grain/seed size is one of the key events during the process of domestication and establishment of agriculture practice (Fuller et al. 2014). The barley (*Hordeum vulgare* L.) inflorescence known as spike, has three uni-floretted spikelets, one central (CS) and two lateral spikelets (LSs) along the rachis (inflorescence axis). So-called two-rowed barleys have fertile grain bearing CSs, sterile LSs whereas six-rowed barleys have all three fertile grain bearing spikelets (Komatsuda et al. 2007). In the majority of six-rowed barleys, the grain bearing LSs are smaller than their CS counterparts. Having uniformly sized CSs and LSs is one critical factor for industrial processing in barley (Bull et al. 2013). Towards understanding the genetic variation in LS size in barley we have characterized a mutant called *small lateral spikelets1.a* (*sls1.a*) by a map-based approach. *sls1.a* is a recessive loss-of-function mutant, where upon mutation in the respective locus, the length, width, and consequently area of LSs is reduced. Thus, the mutant phenotype gives a clear indication that the wild type allele of this locus promotes LS size. By using mapping populations generated from Bowman × BM-NIL(*sls1.a*), BM-NIL(*sls1.a*) × Betzes, and BM-NIL(*sls1.a*) × Harrington, we delimited the *sls1.a* on the long arm of chromosome 1H. Within the *sls1.a* mapping interval, a putative candidate gene mutation was identified. In an identical phenotypic mutant series (*syn.* to *sls1.a* LSs) obtained from Dr. Udda Lundqvist, we identified five independent mutants carrying mutation in the *sls1.a* candidate gene. Currently we are exploring barley genotypes having allelic variation at *sls1.a*, *vrs1*, and *Int-c* loci for their effect on lateral grain size.

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**HUNTING GENES FOR BARLEY SHOOT ARCHITECTURE THROUGH MUTANT AND GERMPLASM COLLECTIONS**

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In cereals, shoot architecture largely determines light interception, biomass and grain yield, other than playing a major role in plant adaptation to environmental conditions. Advances in genomic resources provide new opportunities for the identification of genes and pathways controlling shoot architecture in barley to inform breeding of new ideotypes. Current research in our group takes advantage of both mutant and germplasm collections to identify genes involved in canopy architecture traits such as tillering, leaf size and angle, as well as culm morphology traits related to lodging resistance. In the context of the BarPLUS project (<https://barplus.wordpress.com/>), genes involved in tillering and leaf inclination angle are under analysis through forward and reverse genetic screenings of the HorTILLUS TILLING population in collaboration with the University of Silesia. Among classical tillering mutants, we focused on positional cloning and functional characterization of *uniculme4 (cul4)*, a recessive locus showing pleiotropic effects on shoot and spike development and encoding a barley BTB-ankyrin transcriptional co-factor. Comparison of *cul4* and wild-type transcriptome and hormonal profiles highlighted regulatory genes and specific metabolic and developmental pathways as targets of *Cul4* regulation, providing new insights into molecular mechanisms of shoot development.

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**Are tiller and leaf development controlled by the same genetic modules?**

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Tillers and leaves largely define barley shoot architecture. Tiller development is defined by three stages including: (1) axillary meristem initiation in the leaf axil of the crown; (2) leaf primordial development on the axillary meristem; and (3) elongation of internodes into a tiller. Leaves are divided into three primary regions: the proximal sheath that wraps around the culm and provides support to the plant, the distal blade that is the primary photosynthetic organ, and the blade-sheath boundary that is composed of a ligule and two auricles. Recently, there have been reports that branch and leaf development share some of the same genes. Some of these genes are referred to as boundary genes because they mark the boundary of a new organ. For example, mutations in *UNICULM4* (*CUL4*) reduce tiller development and disrupt the blade-sheath boundary, and CUL4 transcripts are detected in the leaf axil and ligule, indicating that CUL4 expression is marking a boundary for the establishment of tiller and ligule development. To further examine the question of shared genes and to better understand the genetic control of leaf and tiller development, my lab has been studying three classes of mutants including: (1) those that exhibit few tillers and a disrupted blade-sheath boundary (*CUL4* and *ELIGULUM-A*), (2) those that exhibit few tillers and a wildtype blade-sheath boundary (*UNICULM2*, *LOW NUMBERS OF TILLERS1*), and (3) those that exhibit wildtype tiller number and a disrupted blade-sheath boundary (*HvLIGULESS1* and *HvLIGULESS2*). In this talk I will discuss the isolation of *ELIGULUM-A* and *HvLIGULESS1* and their role in establishing boundaries, our current understanding of the genes that are shared between tiller and ligule/auricle development, and what we know about gene interactions during tiller and ligule/auricle development.

**Mutation identification by direct comparison of whole-genome sequencing data of mutant and wild type individuals: the example of *zero-rowed spike 1***

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At the first International Barley Mutants Workshop, we reported (separately) about (i) the next-generation sequencing methods for the isolation underlying barley mutant phenotypes and (ii) the identification of mutant with infertile central florets (*zero-rowed spike 1, zrs1*) that was created by field irradiation of the wild barley accession OUH602. We have since joined forces to isolate the gene underlying the *zrs1* phenotype. Mutant and wildtype individuals were sequenced to 6-fold whole-genome coverage with short Illumina reads. Alignment of the sequences to the Morex reference assembly, followed by automated variant discovery and functional effect prediction in context of the most recent barley gene annotation resulted in a short list of candidate genes harboring large-effect variants present only in the mutant. One of these disrupted a splice-site in a gene for whose homologs in *Arabidopsis thaliana* and *Brachypodium distachyon* highly similar morphological mutant phenotypes had been reported. The candidate mutation localized to the genetic centromere of 4H, where *zrs1* had also been mapped genetically. RNA sequencing of mutant and wildtype spikes confirmed the presence of mis-spliced transcripts of the candidate gene only in the mutant samples. Further validation through transgenic approaches is on-going. Our strategy for rapid mutation identification will be applicable to other induced mutants of barley if isogenic wildtype plants are available. In this case, a direct whole-genome comparison of mutant and wildtype samples will reveal only a handful of candidates and enable gene discovery in low-recombining regions of genome.

**Functional characterisation of high tillering mutants in barley**

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Shoot architecture is fundamentally important for growth and yield in temperate cereals like barley. It is determined by the activity of the shoot apical meristem which gives rise to leaves, axillary meristems, stems, tillers and inflorescences. However, little is known about the genes and regulatory pathways that control shoot architecture in barley.

Here, we provide new insights into the genetic control of shoot architecture through barley mutant analysis as a tool to dissect major regulators controlling plant morphology. We analysed allelic high tillering mutants designated as *many noded dwarf* (*mnd*) by phenotypic analysis and transcriptomics. Detailed phenotyping of the *mnd* mutants revealed pleiotropic developmental defects in both vegetative and reproductive traits. Vegetative growth was increased in *mnd* mutants including excessive development of leaves that subsequently led to higher numbers of axillary buds and tillers. Interestingly, *mnd* mutants were able to produce tillers at elongated nodes close to the shoot apical meristem and at the base of the inflorescence. Scanning electron micrographs uncovered that *mnd* mutants failed to suppress bract growth and spikelet meristems reversed into vegetative branches. Reproductive development was impaired resulting in a reduction of spikelets and seeds per spike. Using mapping by RNA sequencing, we were able to identify the causative gene underlying the *mnd* locus on chromosome 7HL. Expression analysis of shoot apical meristems of *mnd* mutants revealed the mis-expression of known barley floral regulators and suggested a major role of this gene in barley gene transcription and regulation of development.

**VRS3 encodes a chromatin state regulator represses lateral spikelet fertility and modifies the function of other row-type genes**

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Barley has two inflorescence forms: a ‘two-row’ spike, where the fertile central spikelet is flanked by two infertile lateral spikelets, and a ‘six-row’ spike where both central and lateral spikelets are fertile. Induced loss-of-function alleles of any of five *VRS* genes (*VRS1*,*2*,*3*,*4* or *5*) induce lateral spikelet fertility to varying degrees. Using a combination of mapping and synteny approaches, we identified VRS3 as encoding a putative Jumonji C-type H3K9me2/me3 demethylase. Comparative transcriptomics revealed VRS3-dependent control of VRS gene expression and of genes involved in stress, hormone and sugar metabolism*.* In addition, combining a *vrs3* mutant allele with natural six-rowed alleles of *VRS1* and *VRS5* led to increased lateral grain size and greater grain uniformity.

We next assembled a near-isogenic panel of single and double *vrs* mutants introgressed into the two-rowed Bowman cultivar to directly compare their control of spike architecture and explore their potential for developing a new six-row genetic model. Comparative ontogeny, genetics and gene expression across the panel suggested row-type genes act to prevent carpel emergence in lateral spikelets via a shared node of *VRS1* induction by *VRS4* and *VRS3*, while *VRS5* likely regulates additional targets. Combining weakly impaired function in these upstream regulators generated fully six-row phenotypes in the presence of functional *VRS1* alleles, highlighting avenues to alter row-type without influencing other traits under *VRS1* control. *vrs3* also synergistically increased reproductive branching, spikelet number, tillering and improved grain homogeniety when combined with *vrs4*, while when combined with vrs5, led to increased spikelet number, decreased tiller number and increased grain weight. Taken together, we reveal the identity of VRS3 and interactions which confer lateral spikelet infertility, meristem determinacy and divergence in tillering regulation, which may provide novel routes to improving six-row barley grain quality.

**Characterization of leaf rust resistance genes and their introgression into Bowman barley.**

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Leaf rust of barley, caused by *Puccinia hordei*, is a widely distributed and important disease. Yield losses up to 30% are most common in moderate epidemics, but losses as high as 62% have been recorded in severe cases. Breeding resistant cultivars is the preferred method of controlling leaf rust because fungicide applications are costly and raise concerns about environmental contamination. To date, 25 major effect resistance genes, referred to as Reaction to *Puccinia hordei* (*Rph*) genes, have been mapped to positions across the genome. To develop a set of differential lines in a common genetic background for characterizing the virulence phenotypes of *P*. *hordei* isolates, a backcrossing program was initiated to transfer 15 leaf rust genes (*Rph1*-*Rph15*) into the susceptible cultivar Bowman. From 4 to 10 backcrosses were made to introgress the individual *Rph* genes into Bowman. To verify the resistance phenotypes of the introgression lines with the original *Rph* donor sources, inoculations were made with 12 diverse races of *P*. *hordei*. In all cases, the infection types of the introgression lines matched closely with those of the respective donor sources. Introgression lines and donor sources were genotyped for single nucleotide polymorphisms (SNPs) by the barley oligonucleotide pooled assay (BOPA1) and genotyping-by-sequencing (GBS) in order to define the introgressions containing the *Rph* genes. The identified introgressions confirmed previously reported chromosomal positions for the respective *Rph* genes. BLAST searches of the BOPA SNP markers in the 15 introgression lines were made against the reference barley genome (cv. Morex). These queries identified several annotated genes involved in disease resistance that co-localize on the same contigs as many of the BOPA SNP markers within the *Rph* introgressions. These introgression lines will be useful tools for characterizing the virulence diversity of *P*. *hordei* and cloning leaf rust resistance genes.

**Environmentally sensitive control of male fertility in barley**

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The barley *HvMALE STERILITY1 (HvMS1)* geneencodes for a PHD-finger transcription factor that is orthologous to the *Arabidopsis* *MS1* gene*.* It is expressed specifically in the anther tapetum and is essential for pollen development. Overexpression of *HvMS1* in barley results in complete male sterility. These *HvMS1* over-expression lines show indehiscent anthers and sticky pollen; the pollen is viable but non-functional due to failure in dehiscence.

Double transformants, containing *HvMS1* over-expression and *HvMS1* RNAi-silencing constructs, recovered fertility due to the reduction in *HvMS1* expression. Interestingly, male sterility caused by *HvMS1* over-expression was sensitive to temperature, with anther opening and restoration of fertility observed when grown at >20˚C. The cause of this reversion is being investigated. This temperature sensitivity makes the maintenance of the sterile lines feasible by growing them at >20˚C, enabling their use as pollen donors for selfing and sterile line maintenance. Such reversibility is key for hybrid system development. This also provides a valuable tool for breeding, in the form of a male sterile line that does not need emasculation and thus avoids undesirable contamination by self-fertilisation.

**Molecular mechanisms of anther opening in barley**

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Abstract

The control of male fertility is a basic requirement for cost-efficient hybrid seed

production but it remains an unfulfilled need of wheat and barley breeding. To identify

novel targets for male fertility control in crop cereals, we investigate the molecular

mechanisms of pollen release during anther dehiscence using barley as a model

system. From the historic barley collection of male sterile genetic mutants, we are

currently working on msg36 and msg38, which display normal plant and stamen

morphology but impaired anther dehiscence. Histological analysis indicates that this

is caused by a failure of cell separation in the septum and stomium regions.

Additionally, msg38 shows reduced secondary thickenings in the endothecium and

lower starch accumulation in pollen. The candidate genes for MSG36 and MSG38

putatively encode a pectin-degrading enzyme and an auxin biosynthesis enzyme,

respectively. MSG36 and MSG38 transcripts accumulate precisely at the stages

where the mutant phenotypes are first observed and only in mature stamens,

suggesting that they function specifically in late stamen development. Moreover,

MSG36 transcripts accumulated at lower levels in the MSG38 mutant. We

hypothesize that auxin is the signal that activates the anther dehiscence program in

barley, which includes pectin degradation at the cell wall of septum and/or stomium

cells to facilitate their separation. We propose that modulating the activity of the

MSG36 or MSG38 enzymes may provide a means to control the timing of anther

opening and, therefore, male fertility in temperate cereals.

**Abstract- Changing plant architecture to improve crop yield**

**Wilma van Esse**

Cereal crops such as wheat, rye and barley are important for food and feed supply. Crop yield in temperate cereals depends on the number of seed per seed head (spike), number of side shoots (tillers), and the seed weight. Improvement of crop yield is limited by negative correlations between these yield components, and these correlations are hard to break using conventional breeding methods. Optimizing the balance between the growth of vegetative (shoots) and generative (seeds) organs is an effective strategy to increase cereal crop yield. At present, breeding strategies that focus on fine tuning this balance as means to improve yield are limited due to a lack of knowledge on the factors that control the development of the harvested organs. Therefore, a thorough fundamental understanding of the underlying molecular mechanisms is essential to develop novel breeding strategies for yield improvement. The overall aim of my research is to identify and characterize the genes that affect seed weight as well as seed and tiller number in barley, an emerging model system for temperate cereals. Therefore, detailed phenotypical analysis of X-ray and neutron mediated mutations in barley cultivars with altered seed tiller number are performed. In addition, we make use of *in vitro* and *in planta* techniques like RNA-Seq, yeast-n-hybrid and ChIP-Seq, to shed light on the molecular mode of action of key regulatory branching genes.

**Walking a Waxy Path: Molecular Characterisation of Barley *Eceriferum-yy***

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The cuticle is a waterproof barrier that covers aerial parts of land plants contributing to the reduction of non-stomatal water loss and conferring protection to many biotic and abiotic stresses. The cuticle is mainly composed by cutin and cuticular waxes, a complex mixture of aliphatic compounds derived from the lipid biosynthetic pathway. The composition of cuticular waxes is species- and organ-specific and can be modulated by environmental factors. Cuticular waxes form crystal structures on plant surfaces that confer a bluish-white (glaucous) or a glossy-green (non-glaucous) colour depending on its composition. In barley 1,580 *eceriferum* (*cer*) loci have been identified and described based on the glaucous appearance of the leaves, leaf-sheaths and spikes. Here I describe the characterisation and fine mapping of *Cer-yy*, a dominant, organ-specific suppressor of wax accumulation in barley.

We analysed the composition and crystal structure of cuticular waxes of leaves, leaf-sheaths and spikes of wild type and *Cer-yy* mutants; this 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 and revealed that in *Cer-yy* mutants the expression of the *Cer-cqu* gene cluster, a key element in the synthesis of β-diketones, is strongly down-regulated. We mapped *Cer-yy* to a sub-centimorgan region on the top of barley chromosome 1H, allowing the identification of candidate genes. I will conclude by discussing the major findings of this research and outlining future developments aimed at identifying *Cer-yy* and understanding its contribution to the regulation of wax biosynthesis in barley.

**Understanding cell wall biosynthesis and properties in barley**

Claire Halpin

Grass cell walls have several unique properties that influence crop agronomic performance, digestibility, and the use of biomass as an industrial feedstock for energy and chemical production. Primary among these properties are the structure and composition of lignin, and the content of cell wall-bound phenolic acids. We have been using Genome Wide Association studies (GWAS) to investigate the genomic loci that influence these properties, as well as agnostically investigating the loci that influence stem strength, lodging, digestibility and saccharification in barley. Several excellent candidate genes have been identified and are being validated using CRISPR-mediated targeted mutagenesis. CRISPR has also been used for validation in wheat, along with TILLING mutants.

**The use of natural variation to identify genes controlling differentiation during ovule and seed development in barley**

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Barley is a diploid cereal crop used in the feed and brewing industries, as well as a niche functional food for humans due to its high nutritional qualities. The benefits of the barley grain are derived mainly from the endosperm, which along with the embryo, is produced after fertilisation of the embryo sac within the ovule. During seed development, nutrients are released from maternal ovule tissues and transferred into the endosperm, which concurrently differentiates on a radial axis to form two prominent cell types; the peripheral aleurone and the inner starchy endosperm. We have been studying ovary and grain development in barley with a view to understanding how different sub-epidermal cell types are specified and contribute to downstream grain development. We have developed several microscopic assays to quantify sub-epidermal details of ovule and grain development, and applied these in a panel of 190 barley cultivars. Association mapping identified multiple genomic regions that contribute to variation in cell identity. To identify candidate genes contributing to the phenotypic variation we have used a combination of GWAS, laser capture microdissection, RNAseq profiling and plant transformation. By comparing these different approaches we hope to deliver fundamental genetic knowledge regarding cell-type specific mechanisms that contribute to grain development. This knowledge may be applied in future to tailor specific reproductive traits for improvements in grain yield and composition.

**ERASysAPP Crop Clock (CCP)**

**Improving crops biomass by uncovering the circadian clock network using dynamical models**

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Lennart Ljung, Linköping University, Sweden

**Monika Spiller**, Syngenta Seeds GmbH, Germany

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The circadian clock is an internal timing system that allows plants to predict daily and seasonal changes in light and temperature and thus to adapt photosynthesis, growth, and development to external conditions. The core oscillator is well understood in the model plant Arabidopsis, however, relatively little is known about the dynamic effects of the clock on agronomic behavior of crop plants. We therefore model the circadian clock of the important crop barley and its effects on the transcriptome, metabolome and phenotypic performance. To this end, we have adapted tools from the fields of Control Systems and Machine Learning to learn how species in complex networks regulate each other and how these regulations vary in response to genetic or environmental changes.

In parallel we perform RNAseq analyses of known mutants in genes for regulation of circadian clock genes and candidate genes to be involved in the regulation of the circadian clock in barley. These findings will be implemented in the modelling approach together with agronomic data from field trials in different environments.

Understanding the circadian clock of the model crop barley and its effects on important agronomic traits may have great impact on precision breeding of barley and related cereals.

The results of the field trials together with relevant insights to the circadian clock network will be presented.

**Dosage of duplicated and antifunctionalized homeobox proteins influences leaf and spikelet development in barley**

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Spike architecture is one of the major domestication traits in barley (*Hordeum vulgare* ssp*. vulgare*), which also influences grain yield. Barley has two principal spike types: two-rowed forms and six-rowed forms governed by a single gene, *Six-rowed Spike 1* (*Vrs1*, syn. *HvHox1*) which belongs to homeodomain leucine zipper class I protein family. It was proposed that the *HvHox1* is only expressed in lateral spikelet meristems and act as a negative regulator of its development. It was also shown that the gene is the resultant of a recent gene duplication occurred only in tribe Triticeae, and its paralogous gene is known as *HvHox2.* Here, we show *HvHox1* expression in central and lateral spikelet meristems but with a different dosage level. We also found that *HvHox1* and *HvHox2* have similar spatio-temporal but different dosage-dependent expression during spikelet and leaf development. Both proteins have similar dimerization and DNA binding properties, but exhibit differences in their transactivation property. Expression of these genes in barley leaves is the prime evidence of the wide leaf and narrow leaf phenotypes observed in six- and two-rowed barley plants, respectively. Overexpression of *HvHox2* under *HvHox1* promoter recovers (partially) the fertility of lateral spikelets in transgenic two-rowed barley, indicating that *HvHox2,* is a positive regulator of spikelet development. Transcriptome analysis during early leaf development in six-rowed and two-rowed showed that HvHOX1 suppresses cell proliferation in leaf cells. We exemplify that dosage of duplicated and antifunctionalized homeobox proteins influences leaf and spikelet development in barley.

**Breeding barley grains with improved nutrition and health benefits.**

Dr Steve Jobling, CSIRO Agriculture & Food, Canberra Australia

This talk will describe work done in CSIRO to develop novel cereal grains with unique fibre and nutritional profiles.

Research into cereal carbohydrates and nutrition over the last 15 years has led to the development of BARLEYmaxTM, a natural wholegrain with enhanced nutritional benefits. This barley grain contains twice the dietary fibre of regular grains, four times the resistant starch and has a low glycaemic index. BARLEYmax is a hulless barley with a novel allele of starch synthase II gene leading to higher amylose, betaglucan and fructan. Nutritional substantiation trials have shown benefits for gut health increasing short chain fatty acids and altering the microbial profile. With significant health benefits to consumers it is being commercialised through The Healthy Grain Company and has been on the market in Australia since 2008 and is now on sale in Japan and the US.

In a similar time frame, CSIRO has also developed KebariTM an ultra-low gluten barley that can be used for brewing and food uses. This high fibre wholegrain will be of benefit for people with coeliac disease whose diet is normally fibre deficient. A gluten free beer “Pioneer” is currently on sale in Germany.

Barley also contains high levels of soluble betaglucan an important factor in the cholesterol lowering properties of this grain but which has negative effects on barley used for malting and brewing purposes. Work on understanding how the *CslF6* gene controls betaglucan structure and solubility will be described with the ultimate aim of creating better malting barleys and ultimately cholesterol lowering wheats.

**Genome editing using CRISPR-Cas9: generating mutants to characterise putative cell wall genes.**

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CRISPR-Cas9 is a popular genome editing tool due to its comparative ease of use and effectiveness in producing targeted mutations. We have applied this technology to several members of the *Cellulose Synthase Like* (*Csl*) gene family to generate CRISPR-Cas9 induced mutant allelic series for these genes. While the function of, *CslF6* and *CslH* have been characterised previously as synthesising (1,3;1,4)-β-glucan in the grain and leaves of barley respectively 1,2, the generation of mutants will provide genetic resources to facilitate the further study of these important genes. Grain (1,3;1,4)-β-glucan content influences the use of barley; high levels of this polysaccharide have a negative impact on brewing and distilling filtration processes but have a positive impact on health, as supported by statements by FDA and EFSA 3,4, due to reducing the risk of developing cardiovascular diseases and type II diabetes. Additionally the allelic series produced by CRISPR-Cas9 targeting of *CslF3* and *CslF9* could aid the determination of their function. We will provide an overview of how we generated these lines, and an update on their phenotypic and genetic characterisation.

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**Cloning root mutants in barley**

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In the Triticeae, only a limited number of root mutants have been described despite their importance in plant biology and breeding studies. Recently, the interest in mutant collections has increased due to the availability of new genomics-assisted mapping and cloning approaches (eg. mapping by sequencing) enabling the exploitation of mutant collections in a forward-genetics fashion. In this context, we screened a barley mutant population (approx. 3,000 M5 lines in the cv. Morex background, induced using NaN3) formerly produced for TILLING purposes (Talamè et al. 2008, PBJ 6:477-485), for root morphology mutants. By means of a paper-roll method, several tens of lines showing root phenotypic aberrations at the seedling stage were identified. Mutants were grouped in three categories: root growth rate/length (short and long, 77%), root morphology (gravitropism, etc., 15%) and root hairs (hairless, shorthairs, etc., 8%). Six out of seven root mutants confirmed to be under simple Mendelian, single locus genetic control based on segregation in F2 cross populations. SNP array-based bulk-segregant analysis allowed us to quickly map mutations at cM-range interval. NGS-based approaches are being tested both on phenotypic bulks and single mutant lines in order to streamline the process of linking a mutant with the underlying gene. With this approach, a candidate gene involved in auxin membrane trafficking has already been strongly associated to a short-root mutant.

**Title: Using Barley mutants to understand meiosis and recombination in cereals.**

**Speaker: Isabelle Colas**

**Abstract:**

A greater understanding of the control of recombination in crop plants would be particularly useful for crop species such as barley (and wheat) where a highly skewed distribution of meiotic crossover events means that up to half of the genes rarely, if ever, recombine. In these crops, substantial proportions of the chromosomes are inherited together as a large linkage block, preventing the generation of novel gene combinations and useful variation that could be exploited in breeding programmes. In order to improve our understanding of recombination in barley, and ultimately to be able to modulate recombination in barley, we are characterizing a collection of 14 non-allelic desynaptic (*des)* mutants that exhibit perturbed meiosis and semi-sterility compared to wild type. A number of these mutants have now been genetically mapped using the semi-sterility phenotype and cytologically characterized. In particular, 3D-SIM microscopy analysis reveals that each mutant is differently affected for synapsis, crossing over formation and meiosis progression. We will discuss results of some of these mutants, showing the importance of the interplay between synapsis and recombination and the implication for the modulation of recombination for breeding purpose.

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***HvRaw1* – a major gene controlling trichome formation on barley awns**

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Long awns are characteristic for barley. They are photosynthetically active and contribute assimilates during the grain filling stage which can be the reason why commercial varieties predominantly carry awns. Awnless or hooded varieties which are either lacking awns completely or where awns are converted into a cap structure are the exception and are mainly found among landraces. *Hordeum spontaneum* carries long awns coated with highly silicified barbs whose function is not completely known. Barbed awns represent also the wildtype condition in cultivated barley. Barbs on awns may help to bury seeds under natural conditions, or protect barley spikes from foraging animals. Some barley have barb-less awns, so-called smooth awns. Those were preferred when barley harvesting and threshing was still a predominantly manual labor and farm workers often suffered from asthma caused by the barb dusts. It is unclear why barbs were not removed during barley domestication and breeding when the obvious advantage of barbed awns is elusive. Some reports correlated smooth awns with reduced fertility because stigma hairiness can be dramatically reduced in genotypes carrying smooth awns. To address these open questions we isolated the underlying gene *HvRaw1*. Access to the gene sequence will help now tracing sequence diversity and selection patterns in natural genetic resources and wild barley and it will provide the basis to test linkage between fertility and awn roughness.

**Mutations in barley genes encoding negative regulators of ABA signaling as a promise for drought-resistant crops**

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The negative regulators of ABA signaling serve as promising candidates for studies focused on decoding the ABA-dependent drought stress response, since mutations in their genes often cause a drought-tolerant phenotype. Among these genes is *CBP20* (*Cap-Binding Protein 20*), which encodes a small subunit of the cap-binding complex (CBC) and *ERA1* (*Enhanced Response to ABA 1*) encoding β-subunit of farnesyltransferase.

Here, we report identification and characterization of unique plant material –barley mutants *hvcbp20.ab* and *hvera1.b* identified in our laboratory in barley TILLING population (*Hor*TILLUS)*,*. We performed a wide spectrum of analyses that comprised physiological, morphological and transcriptome studies of both mutants under drought stress.

Mutation in each of described genes, *HvCBP20* and *HvERA1*, confers semi-dwarf phenotype, ABA-sensitivity during seed germination and drought tolerance. Transcriptome analysis integrated with observed phenotypic traits allowed to conclude that the *hvcbp20.ab* mutant exhibited better fitness to stress conditions by its much more efficient and earlier activation of stress-preventing mechanisms. The network hubs involved in the adjustment of *hvcbp20.ab* mutant to the drought conditions were proposed. The analysis of *hvera1.b* response to prolonged drought stress linked *HvERA1* to the metabolism of galactolipids, that build the chloroplast membranes. We hypothesized that it might result in the protection of *hvera1.b* photosystem and thus, in its better photosynthesis performance under water stress.

These results enabled to make a significant progress in understanding the role of barley *CBP20* and *ERA1* genes in the drought stress response.

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**The barley circadian clock is profoundly different than Arabidopsis**

Seth Davies

Plant growth and stress resistance are coordinated outputs that respond to predictable environmental variation that results from the earth's rotation. Here I will discuss the interplay between exogenous environmental sensing and endogenous responses through a comparison of the profound differences of the clock of barley to Arabidopsis. It was reported that the Arabidopsis circadian clock times metabolic homeostasis and this will be considered for barley, where key differences are noted. Genetics of the barley clock has revealed interesting associations between fitness and growth not seen in Arabidopsis. The circadian clock itself is a multiple transcriptional-translational feedback system and in this talk I will overview the clock mechanism and compare that to current understanding in barley, with a highlight of network differences. Taken together the barley oscillator has inherent interest to breeding and is an exciting comparative clock system.  
  
  
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