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ABSTRACT BOOK

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ABSTRACT BOOK

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Oral

Inaugural Conference

T01 Diverse development of grass abscission zones

Elizabeth Kellogg[1]

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Abscission is a process in which plants shed their parts, and is mediated by a particular set of cells, the abscission zone (AZ). In grasses (Poaceae), the position of the AZ differs among species, leading to the hypothesis that AZ formation is controlled by a conserved developmental module whose position is simply shifted in evolutionary time. We have rejected that hypothesis. A combination of light microscopy, transmission electron microscopy, RNA-Seq analyses, and RNA in situ hybridization were used to compare three species, weedy rice, *Brachypodium distachyon*, and *Setaria viridis*. Phylogenetic analysis shows that the AZ in rice and *Brachypodium* is in the ancestral position above the glumes, whereas the AZ in Setaria is in a derived position below the glumes. Rice and *Brachypodium* are more similar anatomically than Setaria. However, the cell wall properties and the transcriptome of rice and *Brachypodium* are no more similar to each other than either is to Setaria. The overall set of genes expressed in the studied tissues is generally conserved across species, but the precise developmental and positional patterns of expression and gene networks are almost entirely different. Transcriptional regulation of AZ development appears to be extensively rewired among the three species, leading to distinct anatomical and morphological outcomes.

Oral

S1 Natural diversity and evolution

T02 Inferring on karyotype structure and evolution in *Brachypodium* using cross-species chromosome barcoding

Robert Hasterok[1]

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Recent synteny-based paleogenomic analyses have identified polyploidisation and dysploidy as the prime mechanisms that are responsible for the diversity in plant karyotypes and indicated that nested chromosome fusions (NCF) are crucial for shaping the chromosome structure in grasses. Although it provided insight into the putative numbers of protochromosomes in the monocot progenitors and permitted the karyotypes of some present-day grasses, including *B. distachyon*, to be connected with their hypothetical ancestral karyotypes, these studies did not involve other *Brachypodium* representatives. We present comparative characteristics of various *Brachypodium* karyotypes using multicolour FISH with chromosome-specific probes. In order to gain the detailed insight into the structure and evolution of individual chromosomes at the cytomolecular level, we conducted cross-species FISH mapping with series of BAC clones that were derived from chromosomes Bd1-Bd5 of *B. distachyon* and ordered on its physical map. Using this approach, we demonstrated the presence of NCFs and other chromosome rearrangements, such as Robertsonian rearrangements, translocations and inversions that are responsible for diverse karyotype structures across the genus; some of them were strictly genome-specific. This provides new data regarding karyotype evolution in several *Brachypodium* species that have various basic chromosome numbers and different ploidy levels. Good examples are prolonged genome stasis found in *B. hybridum* after the formation of this annual allotetraploid and chromosome dysploidy with diverse genome organisation observed in some perennials, for example *B. pinnatum*, *B. phoenicoides* and *B. mexicanum*. Such cytomolecular studies, in particular when combined with the findings of the ongoing whole genome sequencing projects and further molecular phylogenetic analyses, should contribute to resolving the still enigmatic phylogenetic relations within the *Brachypodium* genus.

Oral

S1 Natural diversity and evolution

T03 Flower and grain trait variation in *Brachypodium*

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Domestication of the small grain cereals is believed to be associated with pre-existing large grain size that early humans further selected, along with other traits, resulting in a range of domesticates that modern agriculture currently exploits. To evaluate the extent of variation for grain size, we undertook a survey of flower- and grain-related trait variation in *Brachypodium*. *Brachypodium* is a small wild grass and was chosen for this study because it has no known history of human domestication and little evidence of exploitation. Therefore, variation is likely to denote so-called standing variation and represent adaptation to the grasses native environment.

Examination of accessions collected from across the Mediterranean region revealed substantial natural variation in floral size (width and length) traits that provide a proxy for grain size. To investigate the genetic architecture underlying grain size and shape variation, we characterized 2 distinct sets of Recombinant Inbred Lines (RILs). One set of RILs was derived from a cross between the large flowered *B. distachyon* accession, Bd21, from Iraq with the small flowered western Mediterranean accession, ABR6. The other set was derived from crossing two Iraqi accessions, Bd21 and Bd3-1, both of which have relatively large grains. Grain characteristics were evaluated in F4:5 and F7:8 Bd21xABR6 families under different environmental conditions while the F9:10 Bd21xBd3-1 family was evaluated under a single condition. To discriminate between variation in flower and kernel traits, we also compared husked and de-husked grains. Major quantitative trait loci (QTL) were found to govern flower size (width, length and weight), inflorescence shattering and trichomes as well as grain size traits. At least one flower size locus co-located with QTL affecting size of other organs.

The identification of different QTLs in the two populations suggests that a complex genetic architecture underlies grain related traits in this non-domesticated grass and may provide insight into the selective processes acting on grain morphology in different environments. The study also indicates the degree and types of natural variation likely to have been present in grasses from this region prior to domestication.

Oral

S1 Natural diversity and evolution

T04 *Brachypodium distachyon* grains in a comparative and evolutionary context

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In a general context our group studies fruit and seed evolution and development – more specifically, we focus on the development of the caryopsis of grasses and cereals. We use *Brachypodium* as our point of reference, as a sister group to the core pooids and the temperate cereal crops (wheat, barley, oats, rye). As a wild and uncultivated, but relatively closely related grass species, we have found it enormously informative in characterizing many aspects of grain development and evolution.

We combine classic morphological and histological approaches with bioinformatics and molecular phylogenetics/ gene expression of candidate developmental regulators (transcription factors; TFs) to understand grain structure and development in *Brachypodium* and its related cultivated/domesticated cereals. Through work on YABBY, MADS-box, AP2-like and ARF TF families we have been characterizing aspects of conservation and diversification in expression and function of fundamental regulators in the development of temperate grains. We can then determine how these findings might help explain the differences in grain structure and development between the species.

Focusing on the YABBY family, detailed expression profiles at cell and tissue level were established and bioinformatics used to predict the promoter motifs of genes potentially regulated by YABBYs. Protein-protein and protein-DNA interactions were examined to explore the functions and gene networks involving *YABBY* genes. Through Bayesian Interference construction, we found that the YABBY genes might have originated before the seed plants, challenging previous findings, and that INO, essential for outer integument initiation in Arabidopsis, has interesting differences in wheat relative to *Brachypodium*. The expression of *YABBY* genes in *Brachypodium* shows interesting patterns where some of the genes have similar expression to the eudicots (polar, abaxial) and rice and wheat (non-polar), showing both conservation and diversification of *YABBY* genes through evolution.

We found that BdYABBY6 could be a potential novel candidate to focus in in terms of grain development based on its detailed expression pattern (both transcriptome and mRNA ISH) and capability of forming homo- and hetero-dimers with gene products in the same clade, BdYABBY1 and BdYABBY2. Knocking down using CRISPR/RNAi lines would give an insight of the importance of this gene during grain development.

Oral

S1 Natural diversity and evolution

T05 *Brachypodium distachyon* genetic variability for beneficial interaction with arbuscular mycorrhizal fungi

Camille Ribeyre[1] , Luis Buendía[1] , Fabrice Devoiles[2] , Felicia Maviane-Macia[2] , Nemo Peeters[2] , Thomas Girin[3] , Sylvie Ferrario-Mery[3] , Marie Dufresne[4] , John Vogel[5] , Benoit Lefebvre[1]

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As most plant species, *Brachypodium distachyon* is colonized by arbuscular mycorrhizal fungi (AMF) which provide soil nutrients in exchange of carbohydrates and lipids. In addition to its role in plant nutrition, arbuscular mycorrhiza is also known to improve plant resistance to biotic and abiotic stresses. Although this symbiosis can be highly beneficial for plants, there is a strong variability in the outcome of the interaction depending on the respective partner genotypes and on environmental factors. Whether the various types of AM benefits can be observed in all plant genotypes is unclear. Moreover, the genetic bases underlying the variability of the AM benefits between plant genotypes are unknown. We are currently screening the *B. distachyon* genetic variability for mycorrhizal growth response (MGR) using an automated phenotyping platform. Sixty-six of the accessions sequenced by the JGI have been grown in pots in presence or absence of the AMF species *Rhizophagus irregularis* and with two levels of nitrogen fertilization. Plant growth kinetics has been deduced from images taken all along the plant cycle and spikelet weights have been measured at the end of the cycle. Positive MGR has been observed for accessions with reduced growth in absence of AMF, suggesting strong dependency of theses genotypes on AMF. Decrease in shoot biomass and increase in spikelet weight have been observed for various accessions suggesting an effect of AMF on resource allocation in these genotypes. Accessions with contrasted MGR will be characterized for their nutrient uptake abilities (focusing on nitrogen and phosphorus), resistance to pathogens and to drought. Beside the analysis of MGR, the phenotypic data acquired during this screen can be useful for the *Brachypodium* community.

Oral

S1 Topic: Natural diversity and evolution

T06 Typification of names and their taxonomic assignment within the *Brachypodium distachyon* complex (Poaceae)

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The *Brachypodium distachyon* complex includes three annual species, whose taxonomic identities have recently been demonstrated by cytological, molecular and morphological data. The initial taxonomic studies contained no nomenclatural revision of the twenty or so heterotypic synonyms at species rank, while the authors needed to describe two species as new. Indeed, the impossibility to study molecularly the herbarium type materials of these names precluded the analysis of these samples. An improvement of the morphological methods, including new criteria relevant both in situ and in herbarium, now allows us to do this nomenclatural synthesis, according to the current taxonomic treatment. Fresh French and Algerian materials from the three species have been calibrated with DNA genome size (2C) and molecular barcoding (ITS + tnrLF). We found that several valid previous names are available for *B. stacei* (whose distribution is more widespread than expected), of which *Festuca rigida* Roth is the older and yet has been used to combine *Brachypodium rigidum* (Roth) Link, the priority name of the species. Admissibility to propose a conservation or a rejection of one of these names is in debate. In order to fix their interpretation, nomenclatural types of a dozen other names will be designated at the end of this work, including lectotypes and neotypes. We found that the herbarium lectotype of *Bromus distachyos* L. (LINN93.48) corresponds to *Brachypodium hybridum* according to our current morphological analysis. In virtue of what a proposal to conserve and retypify the name *Bromus distachyos* L. could be a consensual solution in order to conserve the uses of both B. *distachyon* and *B. hybridum* in their current senses. Field investigation also revealed other possible annual species of hybrid origin – at least one sensu *Trachynia platystachya* (Coss.) H. Scholz – but with an inversed parental combination than *B. hybridum*, which are currently investigated also genomically.

Oral

S1 Natural diversity and evolution

T07 How diverse is *Brachypodium?* an updated view of annual and perennial compilospecies complexes

Pilar Catalan[1] , Luis A Inda[2] , Ernesto Pérez-Collazos[3] , Antonio Díaz-Pérez[3] , María Ángeles Decena[3] , Joaquín Ascaso[3] , José Casanova[3] , Rubén Gorgojo[3] , Beatriz Montes[3] , Luis Villar[4]

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Brachypodium has been selected as a model functional system for monocots and is probably one of the most broadly investigated grass genera in the world. Recent works have recognized near 18 species within *Brachypodium*; some of them are useful tractable models for plant allopolyploidy and for annuality/perenniality switches. Pangenomic and phylogenomics approaches have also identified the genomic diversity present within some species and their respective time course histories. Despite the enormous advances acquired in our understanding of the extent and origins of this diversity, new evidences suggest that the substanding taxonomic and evolutionary diversity of *Brachypodium* could be much larger than that known to date. Here we present an updated view of the new discoveries attained in both annual and perennial *Brachypodium* species complexes using multidisciplinary research approaches. Dissections of the more deeply studied trio of annual *Brachypodium* species have confirmed the multiple founder origins of allotetraploid *B. hybridum* plants from different bidirectional crosses of its extant diploid progenitor species *B. stacei* and *B. distachyon*. However, infertility barriers detected between *B. hybridum* individuals originated during the last million years span call upon the potential existence of current cryptic species within this taxon and even within their purported progenitor species. Genetic, cytogenetic and phylogenetic analysis of the perennial *Brachypodium* taxa have shown that approximately half of them are diploids and half are allopolyploids, with some of the latter showing a grade of ancestral (*B. mexicanum* 4x), intermediate (*B. boissieri* 6x-8x; *B. retusum* 6x) and recently evolved (*B. pinnatum* 4x; *B. rupestre* 4x; *B. phoenicoides* 4x) taxa. New insights from chromosome and genome size data, together with a broader sampling of germplasm, have detected new cytotypes among these species (e. g., *B. phoenicoides* 6x; *B. retusum* 4x; *B. rupestre* 6x) enlarging the potential number of new cryptic *Brachypodium* perennial species. Phylogenomic analyses of these perennial allopolyploids and of current diploid species, using genome skimming data, are revealing the identities of the homeologous subgenomes participating in the polyploids and their inferred coalescent hybridization times. The recent origins of most of the perennial taxa and cytotypes, their respective intraspecific phenotypic similarities and their interbreeding capability fit well the compilospecies evolutionary model. The advent of new genomic, cytomolecular and phenotyping tools is refining our knowledge on the speciation mechanisms and the taxonomy of the annual and perennial *Brachypodium* taxa.

Oral

S2 Comparative genomics and transcriptomics

T08 Life with 1000 genomes: Defining the pan-genome in maize

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One of the major discoveries in plant genomes in the last ten years has been the extent of genome plasticity due to structural variation in the form of copy number variation and presence/absence variation. This genome plasticity leads to the pan-genome which represents all the genes within a species and includes the core genes present in all accessions and dispensable genes that are restricted to a subset of accessions. Large genome resources for maize have been developed including a reference genome with a robust gene atlas, *de novo* assemblies of multiple inbred genotypes, and seedling transcriptomes for ~1,000 genotypes. These datasets have permitted robust characterization of the maize pan-genome and characterization of core genes present in all genotypes and dispensable genes which are variable among genotypes.

Oral

S2 Comparative genomics and transcriptomics

T09 Transcriptome analysis of gene regulatory network reveals differential organization of rhythmic transcriptome between sub-genomes in the allopolyploid grass *Brachypodium hybridum*

Komaki Inoue[1] , Kotaro Takahagi[2] , Yusuke Kouzai[1] , Satoru Koda[3] , Shun Takaya[2] , Minami Shimizu[1] , Yukiko Uehara-Yamaguchi[1] , Yoshihiko Onda[1] , Toshihisa Nomura[1] , Ryuei Nishii[3] , Keiichi Mochida[4]

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Polyploidy, the condition of possessing multiple complete sets of chromosomes, is a widespread phenomenon that has played a significant role in the evolution of plants originating from intraspecies genome duplication (“autopolyploids”) or interspecies hybridization and subsequent genome duplication (“allopolyploids”). These polyploids often exhibit vigorous morphological, physiological, and growth traits, as well as altered gene expression from that of their parental or ancestral species. Although several transcriptome analyses in various allopolyploid species have provided insights into the transcriptional alterations associated with genomic hybridization in such plant species, transcriptional dissection impacts of chromosome doubling and interplaying diverged sub-genomes into alteration in gene regulatory networks (GRNs) in polyploid plants remain largely unaddressed.

In this study, we aimed to demonstrate the impacts of chromosome doubling and interplay between homologous GRNs in the polyploidy grass *Brachypodium hybridum* using homoeolog-transcriptome analysis. We performed a comparative transcriptome analysis with a ploidy series of the genus *Brachypodium: B. distachyon* (Bd21, a natural diploid accession); its artificially induced autotetraploid (4x Bd21); *B. hybridum* (Bd14-1, an allopolyploid accession from natural interspecific hybridization between *B. distachyon* and *B. stacei*); and *B. stacei* (ABR114, a natural diploid accession). Based on the transcriptome analysis results, we elucidated the landscape of transcriptional differentiation in the diurnal transcriptome across the *Brachypodium* ploidy series and identified homologs regulated in response to chromosome doubling in autotetraploid *B. distachyon* and allopolyploid *B. hybridu*. Moreover, we identified GRNs based on the diurnal transcriptome in the *Brachypodium* ploidy series as well as the homeo-transcriptome in *B. hybridum*, and demonstrated differential organization of the network modules and interplay between homeologs in the *B. hybridum* transcriptome. We further demonstrated additive and non-additive expression as well as expression bias between the homeologs in *B. hybridum* GRNs, illustrating the differentiation of the transcriptional network evolved through its allopolyploidization.

These findings highlight the alterations in GRNs through polyploidization and provide novel insights into the nexus of homeologs in GRNs in allopolyploid plants.

Oral

S2 Comparative genomics and transcriptomics

T10 CRISPR/Cas9-based targeted mutagenesis in *Brachypodium* distachyon and B. hybridum and its application to study the model grass genome organisation

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Precise effective tools for genome manipulation are indispensable in dissecting and manipulating the molecular mechanisms responsible for plant genome organisation. Until recently, such manipulations were either not feasible or imprecise. However, development of targeted mutagenesis systems, such as CRISPR/Cas9, has opened up new approaches to understanding genome organisation by means of altering specific functions.

In this work we focused on optimisation of the pipeline for CRISPR-based inactivation of selected genes in diploid *Brachypodium distachyon* and the related allopolyploid, *B. hybridum*. We targeted three genes, encoding *cyclin-dependent kinase G1* (*CDKG1*), *cyclin dependent kinase G2* (*CDKG2*), and *pectin methylesterase* (*PME*). *Cyclin-dependent kinases-like* (*CDKs*) genes have been identified in the Ph1 locus of *Triticum aestivum* that is responsible for the homoeologous chromosome pairing control (Nature, 2006, 439, 749). In *Arabidopsis thaliana*, *CDKG1* is involved in recombination and chromosome pairing during meiosis (PNAS, 2014, 111, 2182). Correct pairing and recombination of homologues is critical for nuclear genome stability and plant fertility, so this prompted us to test whether genome stability in the model small-genome allotetraploid grass is linked with these CDKs. The *PME* gene was targeted due to its involvement into various processes *via* pectin demethylestrification. The involvement of *PME* in the resistance to abiotic stresses and in interactions with beneficial endophytic bacteria is particularly intriguing.

The transient expression in protoplasts allowed identification of functional gRNAs, which were then used for *Agrobacterium*-mediated transformation of the embryogenic callus. Regenerated plants were characterised for the transformation and modification efficiency. Transformation efficiency was 5% and 20% for *CDKGs* and *PME*, respectively, and yielded mutants in all copies of targeted genes in both *B. distachyon* and *B. hybridum*. The activity of Cas9 predominantly results in single nucleotide insertions and deletions in the targeted sequence, although more extensive deletions were also detected in some plants. We demonstrate the effectiveness of the CRISPR/Cas9 system in precise modification of the *Brachypodium* genomes, paving the way for other specific applications. In the future, we aim to characterise the genome stability in the CDKG mutants through the cytomolecular analysis of chromosome pairing at meiosis. We will also investigate the cell wall dynamics in CDKG mutants in response to selected abiotic stresses and the inoculation with endophytic bacteria through immunohistochemical and RT-qPCR analysis.
This research was funded by the National Science Centre Poland (grant DEC-2014/14/M/NZ2/00519).

Oral

S2 Comparative genomics and transcriptomics

T11 LOB-domain transcription factor gene family of *Brachypodium* distachyon

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We study LOB-domain transcription factor family (LBDs) in *Brachypodium distachyon*. These LBDs are plant specific proteins involved in diverse developmental processes from floral development to emergence of lateral roots therefore important regulators of plant architecture.

Twenty-eight genes code for LBDs in *B. distachyon*. Various plant part/organ specificities were found in the expression of these genes, one of them possesses exceptionally high root-specificity. Ectopic expression of two of them resulted in severe developmental phenotypes including altered inflorescence development and reduced fertility. Some of these proteins may interact with cyclins, components of the central regulatory complex of the cell cycle and the analysis of the protein sequence of LOB-domain proteins revealed several putative serine-threonine phosphorylation sites. In vitro phosphorylation confirmed that at least two members of the LOB-domain protein family can be substrate of cdk-cyclin complexes. We hypothesize that some members of this transcription factor family may play a key role in the decision of the plant cells “to divide or to differentiate”.

Root development is studied because root architecture is based on the delicate balance of cell division and differentiation and a strong and efficient root system is also extremely important for the survival of our crop plants under stressful environmental conditions.

Oral

S2 Comparative genomics and transcriptomics

T12 Genome-wide analysis of floral genes in perennial temperate grasses: is there a common genomic toolbox to cope with extreme environmental conditions?

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The grass subfamily Pooideae dominates landscapes in cold temperate regions of the Northern Hemisphere. This group contains a number of economically and ecologically important cereal and forage crops, including wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), oats (*Avena sativa*) and ryegrass (*Lolium perenne*). They have apparently developed complex physiological adaptations to cope with extreme environmental conditions and, at the same time, evolved different floral structures. Understanding how these traits would change with upcoming climatic stresses remains a major challenge in evolutionary biology. Here, we sequenced the transcriptomes of the floral stage of nine distantly related cold-seasonal pooid perennial grasses (*Nardus stricta*, *Lygeum spartium*, *Melica ciliata*, *Stipa offneri*, *Brachypodium sylvaticum*, *Bromus tectorum*, *Hordeum murinum*, *Avenula bromoides*, *Festuca lasto*). These species represent eight tribes within the Pooideae and the temperate BOP clade, in which *Brachypodium* and rice share the most recent common ancestor (40–54 Ma). Through comparative transcriptomic analyses, we identified co-expression patterns that are shared among these Pooideae species and estimated the contribution of lineage-specific gene expression to reproductive phenotypes. Our co-expression analyses with a focus on reproductive processes in these temperate perennial cold-seasonal grass species (together with the integration of expression and genomic data of other Poaceae species) provide insights into transcriptome evolution in the context of reproductive development in grasses. In our current project, we have stressed four selected grass species (water stress, cold stress, salinity stress) to understand if there is a common response of protein-coding genes involved in stress responsiveness contributing towards the evolution of a common response in Pooideae.

Oral

S2 Comparative genomics and transcriptomics

T13 Programmed Cell Death in Developing *Brachypodium* distachyon Caryopsis

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Normal developmental sequence in grass caryopsis entails the death of several maternal and filial tissues in a genetically regulated process termed programmed cell death (PCD). The progression and molecular aspects of PCD in developing caryopses have been reported for domesticated species like barley, rice, maize and wheat. Here, we report a detailed investigation of PCD in developing caryopsis of a wild model species, *Brachypodium distachyon*. *Brachypodium* nucellus degenerates by PCD in a centrifugal pattern following anthesis although at a slower rate compared to cereals. TUNEL and vital staining showed PCD in maternal mesocarp cells at anthesis. The entire mesocarp cells appeared to undergo PCD uniformly but cell disintegration happened more rapidly in lateral mesocarp cells. PCD in *Brachypodium* starchy endosperm cells was detected at 15DPA. To gain insight on the molecular mechanisms of PCD in developing Brachypodium endosperm, we surveyed RNA-Seq expression profile of *Brachypodium* protease genes belonging to A1, C1, C13 and C14 families, in vegetative and reproductive tissues. We identified 11, 10, 4 and 4 genes in A1, C1, C13 and C14 families respectively, that are highly expressed in grain tissues and may be part of the molecular machinery for PCD execution in *Brachypodium* grains. In addition, we examined the in-situ mRNA expression of BdMADS23 an ortholog of OsMADS29, a rice nucellus PCD regulator. Our results suggest that BdMADS23 regulates PCD of nucellus, pericarp and endosperm of *Brachypodium* caryopsis. Previous works have shown the importance PCD for proper grain filling in cereals. Altogether, our study demonstrates similarities, and more importantly differences in the pattern and progression of PCD in cereals and *Brachypodium* grains that may contribute to slow grain filling and low starch status of *Brachypodium*.

Oral

S3 Development and growth

T14 Natural diversity in patterns of biomass allocation in annual and perennial *Brachypodium*

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All plants must allocate limited resources to survival, growth, and reproduction. Remarkable variation in relative allocation to these three areas is observed within and between species, and in response to environmental factors. Decisions about allocation represent trade-offs between survivorship risk and subsequent fitness benefits with two extremes: *annual species*, which reproduce once and then die, and *perennial species*, which reproduce over multiple seasons, often with interim periods of quiescence. I present our work addressing the topics of life history and allocation from three perspectives. First, I assess the current state of the field and propose that perenniality is usefully considered as a syndrome of complex interacting traits, encompassing growth rate, defense strategy, nutrient allocation, phenology and metabolism. Second, I present results from a meta-analysis with the aim of describing climatic parameters associated with the distribution of annual and perennial grass species. Finally, I describe a comprehensive set of experiments linking natural genetic diversity in growth rate in several annual and perennial species to leaf-level and whole-plant ecophysiology. In these experiments we measured components of growth rate under several CO_2regimes that manipulate source/sink dynamics in four *Brachypodium* species. I conclude that *Brachypodium*, with its wealth of genomic tools and diverse life history types, is an ideal system for studying the genomics, development and ecophysiology of resource allocation in grasses.

Oral

S3 Development and growth

T15 Mutations in a predicted DNA polymerase subunit Cdc-27 (ZAO1) result in more rapid flowering of *Brachypodium* distachyon

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A requirement for vernalization, the process by which prolonged cold exposure provides competence to flower, is an important adaptation to temperate climates that ensures flowering does not occur before the onset of winter. In temperate grasses, vernalization results in the up-regulation of *VERNALIZATION1* (*VRN1*) to establish competence to flower; however, little is known about the mechanism underlying repression of *VRN1* in the fall season, which is necessary to establish a vernalization requirement. Here, we report the results from forward genetic screens to identify mutants that are rapid flowering without vernalization and identify novel repressors of flowering time. For example, we identified a previously uncharacterized gene we have named *ZAO1*. This gene is partly involved in repressing *VRN1* before vernalization in *Brachypodium distachyon*. The precocious *VRN1* expression in *zao1* is associated with reduced levels of the repressive chromatin modification H3K27me3 at *VRN1*, which is similar to the reduced *VRN1* H3K27me3 in vernalized plants. This study provides an example of a role for this uncharacterized class of plant-specific genes. Additional rapid flowering mutants will also be discussed.

Oral

S3 Development and growth

T16 Divergent roles of FTs in flowering control in *Brachypodium* distachyon

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The transition from vegetative to generative phase is a decisive time point in plant life cycle. Florigen, the flowering signal molecule, is encoded by FLOWERING LOCUS T (FT) and functions at the core nodes in diverse flowering pathways. Generally, FT and FT-like proteins interact with 14-3-3 proteins and the bZIP transcription factor FD, positively regulate flowering onset in plants. There are six *FT* othologous genes in *Brachypodium distachyon*, two of them, namely FT1 and FT2, are modulated by a *Pooideae*-specific microRNA, miR5200, in a mRNA cleavage manner. Some FTs in *B. distachyon* have been shown to play bilateral roles in flowering initiation, which is different from those in Arabidopsis. For example, because of an alternative splicing (AS) regulation, *FT2* in *B. distachyon* generates two proteins, FT2a and FT2b, promoting and repressing reproductive transition, respectively. More interestingly, we recently find another FT-like protein, FTL9, that plays an antagonistic role in flowering dependent on the day-length environments in *B. distachyon*. Mechanistically, like photoperiod-inductive FT1, FTL9 can interact with FD1 to form a flowering activation complex (FAC), but the floral initiation efficiency of FTL9-FAC is much lower than that of FT1-FAC, thereby resulting in a positive role for FTL9 in promoting floral transition when FT1 is not expressed, versus a dominant-negative role when FT1 accumulates significantly. Accordingly, the divergent roles of FTs suggest a complicated but fine-tune modulation of flowering control in *B. distachyon*.

Reference:

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Oral

S3 Development and growth

T17 Developmental innovations of stomatal form and function in *Brachypodium*

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Plants optimize carbon assimilation while limiting water loss by adjusting stomatal aperture. In grasses, a developmental innovation—the addition of subsidiary cells (SCs) flanking two dumbbell-shaped guard cells (GCs)—is supposedly linked to the grass family's improved stomatal gas exchange efficiency. A mutant screen in the wheat relative and model grass *Brachypodium distachyon* identified a transcription factor necessary and sufficient for SC formation. Unexpectedly, the transcription factor is an ortholog of the stomatal regulator *AtMUTE*, which defines GC precursor fate in *Arabidopsis*. The novel role of *BdMUTE* in specifying lateral SCs appears linked to its acquisition of cell-to-cell mobility in *Brachypodium*. Physiological analyses on *bdmute* mutant plants lacking SCs experimentally support classic hypotheses that SCs permit greater stomatal responsiveness to enhance water use efficiency and larger range of pore apertures to increase photosynthetic capacity. Recently, we profiled the transcriptome of developing and mature leaf regions of both wild-type and SC-less *bdmute* plants to identify novel factors regulating SC development and SC function, respectively. Discovering genes required for SC function will help understand how SCs actually improve stomatal gas exchange dynamics in grasses. Understanding how SCs are formed and enable grasses to breathe more efficiently might allow engineering of stomatal properties in many different crops to improve water use efficiency and plant performance.

Oral

S3 Development and growth

T18 A Leucine Rich Repeat Receptor Kinase regulates vasculature patterning, phloem-xylem polarity and cell wall composition in *Brachypodium*

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Grasses cover about 20% of the Earth's surface. They are facing many climate changes that are putting increased pressure on their development and water management. The plant vascular tissues transport water, nutrients and signaling molecules at long distance and form a complex network through the whole plant. However, the formation of vascular tissues and the differentiation of specialized vascular cells remain poorly understood in grasses. In this study, we re-constructed the vascular network of internodes and nodes of Brachypodium plantlets in three-dimension and show that the vasculature is organized in a similar pattern to the rice vascular network. In order to decipher the underlying molecular mechanisms of vascularization in grasses during development and identify key genes, we conducted a forward genetic screen for abnormal vasculature pattern in a chemically induced-mutant collection of Brachypodium. We identified a mutant severely affected in the organization of vascular tissues. This mutant showed defects in anastomosis of vascular bundles and a typical amphivasal vasculature. The causing gene is an allele of an LRR receptor-like serine/threonine-protein kinase harboring a premature stop codon. This gene participates in pleiotropic mechanisms during plant development and stress resistance. Interestingly, this mutant also showed changes in cell wall composition. All phenotypes were confirmed in a line harboring a T-DNA inserted in the same gene. In order to study expression level in young meristems, we produced transcriptomic data at three developmental stages of vascular bundles by laser microdissection. We propose a major role of this LRR receptor-like receptor kinase during vascularization and identify specific groups of genes acting together during vascular cells differentiation.

Oral

S3 Development and growth

T19 Molecular and cell biological characterization of the BUZZ cell division kinase involved in root hair development

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Plant root systems play a dual role in plant growth by providing both mechanical support and serving as the interface for uptake of essential resources. Most root systems contain root hairs that are tubular extensions of specialized epidermal cells that aid the root in water and nutrient acquisition. Mutants lacking root hairs often appear dwarfed and have decreased plant biomass and yield, but can often be rescued by exogenous treatments of auxin or ethylene. Here we describe the root hairless mutant *buzz* in *Brachypodium distachyon*, which initiates root hairs, but fails to transition to tip growth. However, unlike other root hair mutants, *buzz* exhibits no loss in yield or above ground height and displays 2-fold increased primary root growth rate. Using next generation sequencing (NGS), we identified the causal single nucleotide polymorphism (SNP) resulting in the *buzz* root hairless phenotype. Complementation of the *buzz* mutant with the wild type *BUZZ* gene rescued tip growth of developing root hairs supporting the hypothesis that loss of function of *BUZZ* results in the root hairless phenotype. As auxin and ethylene crosstalk promotes root hair growth and development, we tested exogenous auxin and ethylene treatments, both of which fail to rescue the *buzz* phenotype. *In silico* analysis predicted BUZZ to be a nuclear-localized kinase. By transiently expressing GFP-tagged BUZZ protein in *Nicotiana benthamiana*, we determined BUZZ localizes either to the nucleus in the N-terminal fusion or to the plasma membrane in the C-terminal fusion. Additionally, we examined the expression of the class I basic helix loop helix *(bHLH) ROOT HAIRLESS LIKE SIX -1, -2, -3 (BdRSL1, 2, 3)* genes involved in root hair growth. Revealing that *BdRSL1* and *BdRSL2* were overexpressed in *buzz* mutant roots via qRT-PCR. In addition, we performed RNAseq to compare *buzz* root tips with wild type to examine the developmental pathways impacted in *buzz*. We have also begun biochemical characterization of the BUZZ kinase to identify putative substrates. Overall, these data demonstrate that the BUZZ protein functions early in root hair development and is essential for the root hair initiation to tip growth transition to occur.

Oral

S4 Tolerance and adaptation to abiotic stresses

T20 Genotypic and phenotypic diversity for drought tolerance in *Brachypodium distachyon*

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Drought is arguably the single most climate-related threat to crop yield worldwide and the severity and frequency of drought and heatwaves is expected to increase with continued global warming. Understanding the molecular and biochemical responses to fresh water limitation in plants will help us better prepare for the adverse consequences of future climate scenarios. In this study we used metabolomic and proteomic profiling to gain a mechanistic understanding of plant responses to drought and recovery in diverse *Brachypodium* genotypes selected from a large-scale drought screen. Although the predominant response was a redirection of photosynthate from shoots to roots, a few genotypes exhibited no or opposite effects in biomass distribution following a six-day drought treatment. Six genotypes were sub-selected for further studies due to their divergent drought-induced shifts in biomass partitioning. iTRAQ-based global proteomics survey of aboveground and belowground tissues revealed significant differences in protein profiles depending on freshwater availability and genotype. GC-MS and LC-MS metabolomics platforms were utilized to capture changes in primary and secondary metabolites. Similar to the observations for proteomics, the overall metabolite fingerprints varied significantly between freshwater treatment and genotype. Linking molecular profiles to genetic information will allow us to better understand phenotypic plasticity for drought responses in *Brachypodium*.

Oral

S4 Tolerance and adaptation to abiotic stresses

T21 Screening of *Brachypodium hybridum* genotypes for drought tolerance

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Decrease in water availability is currently considered as an increasing problem which limits plant growth and development, especially in the world's Mediterranean climate zones. Drought is thus considered as a significant constraint leading to oxidative stress damages and reactive oxygen species (ROS) are generally observed as good markers of drought stress response in plants. This study aimed to determine the level of drought tolerance among sixteen genotypes selected from 165 lines of Tunisian *Brachypodium hybridum* species grown under drought stress during 13 days, using H_2O_2 accumulation, lipid peroxidation and antioxidant enzyme activities (SOD, CAT, and GPX) in leaves and roots. The results revealed that drought stress caused significant increase in oxidative stress injuries in all genotypes. Interestingly, the genotype K14 exhibited the highest leaf H_2O_2 content (164.5%), resulting in higher leaf MDA accumulation (232%). Consistent with this, no significant changes were observed in SOD, CAT and GPX activities in both leaves and roots of K14, relatively to unstressed state. Different profile was recorded in R9 genotype in which the most pronounced levels of H_2O_2 (78%) and MDA (500%) were observed in drought stressed roots. In parallel with this effect, root CAT activity increased by 22-fold relatively to unstressed plants. Drought stress resulted in a decrease in H_2O_2 content, mitigation of lipid peroxidation in both leaves and roots of R5 genotype, with a simultaneous increase in leaf and root SOD activity (62% and 94%, respectively). This was associated with enhanced GPX activity where 75 and 40% of increase were recorded in R5 leaves and roots, respectively. In conclusion, we show that *B. hybridum* response to severe drought stress greatly depends not only to genotype effect but also to drought stress distribution between leaves and roots. It is clear from our results that K14 and R9 genotypes are the most sensitive to drought stress as evidenced by the highest level of H_2O_2 and MDA. We also suggest that K14 sensitivity is strictly associated with its inability to minimize ROS accumulation in the leaves, while sensitivity of R9 seems to be related to the preferential production of ROS in the roots. Reduced oxidative stress damages (H_2O_2 and MDA) and enhanced protective antioxidant systems (SOD and GPX) in leaves and roots, together, are efficient mechanisms of enhanced tolerance in R5 genotype to drought stress.

Oral

S4 Tolerance and adaptation to abiotic stresses

T22 Adaptation to stress in *Brachypodium* along the aridity gradient in Israel

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Brachypodium is a crop model plant related to important cereals like wheat and barley. It is naturally distributed around the Mediterranean basin, and especially along the aridity gradient in Israel. The annual *Brachypodium* species provide an excellent system for studying the effects of climate change on plants. These *Brachypodium* species differ in ploidy levels and chromosome numbers. Previous studies suggested an association of polyploidy with aridity in *Brachypodium* in stressful environments, and that changes in life-history traits confer phenotypic adaptation to aridity. We tested for the association between ploidy level and aridity in *Brachypodium* plants from 29 populations along the Israeli aridity gradient, from mesic Mediterranean to extreme desert. Surprisingly, ploidy level was not associated with increased aridity, and fraction of polyploids within and among populations varied quite randomly. Furthermore, plants from 20 populations along the full aridity gradient were grown in a common garden and showed heritable phenotypic variation in flowering time depending strongly on the plants' origin along the gradient, regardless of ploidy level. Notably, plants from arid climates initiated flowering earlier, suggesting that flowering time is a key trait in adaptation to aridity, and that populations under long-term drought are putatively pre-adapted to future desertification.

In order to directly test adaptation to stresses in annual *Brachypodium* spp., we grew plants from 10 populations in a controlled watering experiment in Tel-Aviv University Botanical Garden, and plants from 20 populations in two common gardens in Mediterranean and arid sites. In the controlled watering experiment only the effect of drought stress was tested, while in the two common gardens we tested for the effect of both drought and neighbor's competition stresses combined. In both experiments the results were similar, showing that the origin of the plant, rather than its ploidy, affected fitness and phenology. These findings reinforce our previous results, suggesting no association of ploidy level and phenotype across the gradient, and supporting the hypothesis of pre-adaptation to climate change.

After rejecting the hypothesis of polyploidy as a mechanism underlying aridity adaptation in Israeli *Brachypodium*, we tested the hypothesis that symbiotic endophytic fungi are involved in stress tolerance in arid environments. We isolated and identified several endophytic fungi from *Brachypodium* plants from arid, Mediterranean and mesic Mediterranean sites. Some endophytes were unique to the arid site, suggesting local endophyte-host interaction, enabling plants to cope with aridity.

Overall, this study suggests that phenotypic adaptation to (abiotic) stress through life-history variation and symbiotic endophytes, rather than genome doubling, enables annual *Brachypodium* spp. to grow in arid habitats, and may explain its wide distribution along the Israeli aridity gradient. Uncovering mechanisms of *Brachypodium* adaptation to a changing world may assist in coping future effects on agricultural crops.

Oral

S4 Tolerance and adaptation to abiotic stresses

T23 Ecology of annual *Brachypodium* in Israel: Unexpected ways of adaptation to drier climates at two spatial scales

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Brachypodium comprises three annual species in Israel (diploid *B. distachyon* and *B. stacei*, tetraploid *B. hybridum*) that partly co-occur along a steep natural aridity gradient from *c.* 1.000mm – 100mm annual rainfall. The gradient approximates conditions of ongoing climate change where lower rainfall with greater unpredictability characterizes the arid end of the gradient, while the rainier end holds stronger competition with neighboring plants.

Here, I summarize three recent papers[1,2,3] and new results from a series of experiments on how *Brachypodium* adapts to increasing aridity. We assessed cytotype distribution, various plant traits, germination behavior and competitive ability at two spatial scales of increasing aridity. At large scale, we tested plants from 14 populations along the natural aridity gradient (114–954 mm annual rainfall). At small scale, we tested the microclimatic contrast between plants originating from corresponding north (more mesic) and south (more arid) exposed hillslopes.

Tetraploid *B. hybridum* was the dominant species and comprised 90% of specimens, but no clear trend was found for cytotype distribution with rainfall. Strong trait shifts indicated substantial ecotypic differentiation along the large-scale gradient: earlier phenology, higher reproductive allocation and reduced root investment characterized arid populations. Surprisingly though, none of the classic traits SLA, plant height and seed mass shifted with aridity, and root responses were opposite to theory. Moreover, germination behavior was remarkably stable along the gradient and indicated that increased risk-spreading germination is not an essential strategy to persist in increasingly dry, unpredictable environments. Differences between north and south exposed slopes were small, often inconsistent between sites, and poorly matched the trends across the large-scale gradient. South exposures thus appeared unlikely to harbor distinct ecotypes better adapted to aridity.

These findings highlight that ecotypic differentiation is a crucial way how *Brachypodium* adapts to environmental gradients; yet, this appears restricted at small spatial scales. They draw attention to often understudied traits and question the applicability of some common adaptation theories for intraspecific adaptation to aridity.

[1] *Bareither, Scheffel & Metz (2017) Distribution of polyploid plants in the common annual Brachypodium distachyon (s.l.) in Israel is not linearly correlated with aridity. Israel Journal of Plant Science. 1–10.*

[2] *Kurze, Bareither & Metz (2017) Phenology, roots and reproductive allocation, but not the LHS scheme, shape ecotypes along an aridity gradient. Perspectives in Plant Ecology, Evolution and Systematics, 29, 20–29.*

[3] *Metz, Freundt & Jeltsch (2018) Stable germination behavior but partly changing seed–seed interactions along a steep rainfall gradient. Basic and Applied Ecology 28, 5-16*

Oral

S4 Tolerance and adaptation to abiotic stresses

T24 A Pan-genome perspective on the co-expression response of genes to drought in the model grass *Brachypodium distachyon*

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Recent work has shown that different populations of *Brachypodium distachyon* contribute a significant number of accessory genes to the gene pool. As a consequence, the emerging pan-genome contains nearly twice the number of genes encoded in any individual. Here we analysed the osmotic stress responses of 33 *B. distachyon* ecotypes under two conditions, mild drought (D) and well-watered (control, C). Leaf transcripts were sequenced and pseudo-aligned to the Bd21 reference genome with a double purpose: i) to estimate their abundance and, ii) to assess the proportions of core and accessory genes expressed in response to drought. We built weighted co-expression networks from the transcriptome data and were able to dissect two distinct network topologies in drought and control conditions, respectively. While transcripts of control plants could be clustered in 30 modules with 839 hub genes, plants under mild drought yielded 38 modules with 628 genes annotated as hubs. By comparing the resulting modules, we identified five exclusive D modules containing over-expressed genes enriched in Gene Ontology terms such as proline synthesis, response to water deprivation, phosphate starvation and temperature stimulus. In those exclusive D modules, we performed de novo DNA motif discovery in upstream sequences and identified putative cis-regulatory elements of an ABA inducible leucine zipper transcription factor. In addition, we observed that some genes in these modules display different expression levels among tolerant and susceptible ecotypes. Finally, pan-genome analysis indicated that most expressed transcripts are encoded by core genes, present in all ecotypes, while 14%, including some hubs, correspond to accessory genes.

Oral

Keynote conference

T25 Evolution Canyon: sympatric speciation through niche adaptation across the tree of life

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"Evolution Canyons" display sharp divergences in microsites (free breeding populations living in contrasting *micro*climates or edaphic ecologies), and in adaptive evolution and sympatric speciation across the tree of life. Evolution Canyon I (ECI), in Mount Carmel, explored since 1990, consists of two abutting and microclimatically contrasting slopes. Dry-hot tropical savannoid African slope (AS) is at 250 meters distance from the humid-cool temperate forested European slope (ES). In an area of ~7000 m2, representing a transect of increasing aridity from ES to AS, we identified 2500 species from bacteria to mammals. Adaptive evolution was detected in 16 model organisms growing in *tropical* AS and *temperate* ES, using allozymes and DNA markers. Among the species that incipiently speciated ecologically and sympatrically are soil bacteria (*Bacillus simplex*), wild barley (*Hordeum spontaneum*), wild emmer wheat (*Triticum dicoccoides*), fruit fly (*Drosophila melanogaster*), grain beetle (*Oryzaephilus surinamensis*) and spiny mouse (*Acomys cahirinus*). At ECI only *Brachypodium stacei* grows on temperate ES. By contrast, 62 *B. hybridum* and only 8 *B. stacei* individuals were recorded on tropical AS. We have sequenced the genomes of two *B. stacei* ecotypes, one from each slope, and the transcriptomes (from third generation inbred lines) of 6 *B. stacei* ecotypes from AS and 11 *B. stacei* ecotypes from ES to explore if this taxon also speciates sympatrically in ECI.

Oral

S5 Regulatory elements, networks and epigenomics

T26 Transposable element evolution in *Brachypodium distachyon*: what can we learn from population genomics?

Anne Roulin[1]

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Transposable elements (TEs) are mobile DNA sequences which have the capacity to increase their copy number and/or to move from one location to another in their host genome. Because of their dynamics of transposition, TEs constitute the main component of most eukaryotic genomes and the main cause of genome size increase besides whole genome duplication. With the advent of genome sequencing, the last two decades lead to a deeper understanding of the functional role of TEs and it is now well established that TEs can also produce diverse functional changes, from the disruption of coding sequences to the fine-tuning of gene expression through epigenetic mutations. They may therefore constitute a driving force of evolution. However, little is known about their role as source of genetic variation in natural populations, particularly in plants, where research on TEs largely focused on species of agronomical interest.

Brachypodium distachyon has been developed as a powerful model for research on temperate grass species as it is closely related to major crop cereals and to some of the grasses used for biofuel production. In addition, this species is broadly distributed around the Mediterranean rim, providing access to natural populations from contrasting habitats for which a large collection has been collected and sequenced. I will discuss how my group uses these prime resources to investigate the impact of TEs on genetic diversity and evolution in a natural plant system.

Oral

S5 Regulatory elements, networks and epigenomics

T27 RNA interactome capture in Brachypodium reveals a flowering plant core RBPome

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RNA binding proteins regulate gene expression at the post-transcriptional level through controlling the fate of RNA, such as mRNA localization, translation, splicing and stability. The annotation of RNA binding proteins (RBP) is mainly based on classic RNA binding domains, while RBPs without conventional domains have been identified in different species using RNA interactome capture in vivo. Here, we identify 405 RBPs from leaf mesophyll protoplasts and seedlings in the monocot model Brachypodium using a modified RNA interactome capture employing different UV dosages. Based on the known RNA binding domains, 231 RBPs were classified as classic RNA binding proteins and 174 are candidate RBPs. 50% of classic RBPs are involved in RNA binding, while only a single RBP from the candidate RBP group is involved in RNA binding. The many newly identified RBPs illustrate that the application of this method to Brachypodium helps to annotate gene function and future characterisation of these proteins. Comparing the RBPs previously identified in Arabidopsis with Brachypodium allowed to define a conserved core RBPome for flowering plants. Interestingly, gene ontology analysis of the core RBPome revealed that, apart from ribosomal proteins, the most enriched RBPs are involved in plant specific processes of photosynthesis and carbon fixation. Future analysis should point out what the significance of RNA binding is for the function of these proteins.

Oral

S5 Regulatory elements, networks and epigenomics

T28 SECONDARY WALL INTERACTING bZIP (SWIZ) regulates secondary cell wall biosynthesis in Brachypodium distachyon in response to mechanical stress

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Secondary cell wall biogenesis is a highly regulated spatio-temporal event that vascular plants coordinate through the integration of multiple internal and external cues. These walls provide mechanical strength and hydrophobicity that allows plants to stand upright, resist pathogen invasion, and effectively transport water. One such external cue influencing plant development is mechanical stress, such as wind or physical contact. Mechanical stress induces a general phenomenon across the plant kingdom termed thigmomorphogenesis, which typically results in reduced height and increased secondary growth. We have identified a bZIP transcription factor in *Brachypodium* distachyon that appears to control cell wall thickening in response to mechanical stress. SECONDARY WALL INTERACTING bZIP (SWIZ) mutants have exaggerated thigmomorphogenic phenotypes, with severe dwarfing as well as significantly thickened interfascicular fiber cell walls. Furthermore, SWIZ protein translocates from cytosol to nucleus in response to physical touch, and in yeast-one hybrid screens SWIZ has beens show in interact with the promoters of several cell wall biosynthetic enzymes. Taken together, these data indicate that SWIZ plays a role in the transduction of mechanical stress sensing to elicit increased secondary cell wall biosynthesis.

Oral

S5 Regulatory elements, networks and epigenomics

T29 Stress-induced mobilization of transposable elements in natural accessions of the model plant *Brachypodium distachyon*

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Transposable elements (TEs) are important genetic components of plant genomes. Interestingly, it was shown that their transcriptional activation and mobilization is often triggered by environmental stresses. Due to their ability to either change their location or to generate new stable copies of themselves these elements are able to induce (epi)genetic and phenotypic diversity in plants. In the context of evolution this rises the question if the stress-induced mobilization of TEs plays a substantial role in adaptation to different habitats. Thanks to its compact genome with a reasonable fraction of TEs and the availability of a multitude of sequenced natural accessions, *Brachypodium distachyon* is an excellent model to study the importance of TE-mediated evolution in plants.

Here I present our latest findings that aim to elucidate how environmental stresses can trigger TE-induced (epi)genetic diversity in *B. distachyon*. By applying mobilome-sequencing to 12 accessions from different natural habitats and genetic clusters we are able to link real-time TE mobility to evolutionary history and local adaptation in plants. Besides the contribution to the understanding of plant genome dynamics and plasticity in response to external stimuli these findings will also have direct implications for breeding of stress-tolerant closely related monocotyledonous crops.

Oral

S5 Regulatory elements, networks and epigenomics

T30 High sequence turnover and GC bias in the grass *Brachypodium distachyon* indicate frequent ectopic recombination between retrotransposon copies

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Transposable elements are stretches of DNA which can replicate within genomes. They are a main reason for the high plasticity of plant genomes, where they occur as a diverse community of different families and ancient evolutionary lineages. Despite their importance, little is known about how these lineages differ in activity and their effect on the genome, as most research has focused on single abundant families or summarized TEs at a coarse taxonomic level. Here we investigate the community composition and dynamics of 40 long terminal repeat retrotransposon (LTR-RT) families in two divergent accessions of the wild Mediterranean grass *Brachypodium distachyon*. Our results show, first, that much of the ongoing transpositional activity in the *B. distachyon* genome is due to a non-autonomous centromeric *Gypsy* family and three *Copia* families belonging to the Angela lineage. The latter show evidence of an extremely fast turnover due to high rates of solo LTR formation, with half lifes as short as 63 ky. Second, we found that LTR-RTs also have a long-lasting effect on genomic base composition. *Gypsy* elements of the Retand lineage are old and have GC contents between 50 and 60 percent, compared to a genome-wide average of 45. Because they are the most abundant TEs in the genome, overall GC content shows a strong positive correlation to the presence of this lineage. We present evidence that the high GC content in these elements is due to ectopic GC-biased gene conversion between the numerous paralogous copies. In summary, our study shows how TEs generate variation at a microevolutionary time scale as well as how they have changed genomic base composition the nucleotide landscape over longer time-scales. Both effects suggest an important role of ectopic recombination, a poorly understood process in molecular evolution.

Oral

S5 Regulatory elements, networks and epigenomics

T31 Insertional mutagenesis in *Brachypodium distachyon* using the Tnt1 retrotransposon and its potential use to identify novel sources of disease resistance

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Brachypodium distachyon is an annual C3 grass that has been used as a model system for functional genomics research. Insertion mutagenesis is a powerful tool to create a population of cloning friendly mutants for both forward and reverse genetics studies. Currently, the publicly available T-DNA based insertion mutant population of *B. distachyon* is limited. Therefore, we explored the possibility of using tobacco retrotransposon *Tnt1* to create a transposon-based insertion mutant population in *B. distachyon*. A transgenic *B. distachyon* expressing *Tnt1* was developed and in the subsequent regenerants we observed that *Tnt1* actively transposed during somatic embryogenesis generating an average of 10-15 insertions per line in a population of 22 plants analyzed. FST analyses showed a uniform pattern of insertion in all the chromosomes. Considering the average length of a gene transcript of 3.5 kb, we estimated that ~20,000 lines are required to achieve 90% probability of tagging a given gene in the *B. distachyon* genome using the *Tnt1* based mutagenesis approach. Our results show the possibility of using *Tnt1* to achieve near-saturation mutagenesis in *B. distachyon* that will aid in functional genomic studies of other C3 grasses. The potential use of *Tnt1*-tagged *B. distachyon* lines in identifying novel sources of resistance against fungal pathogens will also be presented.

Oral

S6 Polyploidy and perenniality

T32 Polyploidy as integrator across levels of biological organization: from cells to ecosystems

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Polyploidy, or whole-genome duplication (WGD), has long been recognized as an important speciation mechanism in plants. However, WGD has biological effects that extend far beyond the generation of new species. WGD is a key integrator across levels of biological organization, with effects that range from the molecular and subcellular levels to those of the ecosystem and Tree of Life. The immediate impact of WGD is duplication of all nuclear genetic material, but over time, the component subgenomes become fractionated to yield a composite of duplicated and unduplicated loci. This loss of duplicate genes can begin to occur surprisingly quickly, in perhaps only a few generations. Through gene loss and shifts in gene expression, polyploid individuals originating from a single polyploidization event may become genetically and phenotypically unique, together forming a morphologically, physiologically, and/or ecologically polymorphic population, in contrast to classical views of allopolyploids as genetically identical and chromosomally fixed F1 hybrids. This array of genetic and phenotypic novelty may provide new variants that can potentially drive evolution in new directions, with consequences for the tempo of diversification at macroevolutionary scales. Case studies in grasses (Poaceae) and *Tragopogon* (Compositae) will illustrate patterns of duplicate gene loss and shifts in gene expression in synthetic and natural allopolyploids of recent origin. On longer timescales, signatures of ancient WGDs across angiosperms are often associated with accelerated rates of species diversification, suggesting a causal role of WGD in the diversification of these clades. Although statistical support for co-localized WGD events and diversification rate shifts is low across all angiosperms, many individual WGDs appear to be associated with the origins of novel features and increased diversification, suggesting that features that arise via microevolutionary processes may translate into key innovations on macroevolutionary timescales.

Oral

S6 Polyploidy and perenniality

T33 Reference-genome syntenic mapping and multigene-based phylogenomics reveal the ancestry of homeologous subgenomes in grass *Brachypodium* allopolyploids

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Phylogenomic analyses of a 505,512 RNA-seq SNP data set mapped against the syntenic *Brachypodium distachyon*, *B. stacei* and *B. sylvaticum* reference genomes, and of 268 transcripts from orthologous genes obtained from 12 *Brachypodium* taxa and ecotypes (figure 1) allowed us to reconstruct and date the splits of the dysploid *Brachypodium* diploid backbone species tree and of its allopolyploid sublineages. The nuclear and plastid transcriptome phylogenetic framework together with genome size data contributed to elucidate complex hybridization scenarios for the homeologous subgenomes participating in six *Brachypodium* allopolyploid species. Interspecific hybridization followed by whole genome duplication was the predominant scenario inferred for most the genome mergings, as illustrated by the recent allotetraploid *B. hybridum* derived from *B. stacei*- and *B. distachyon*-type parents.

B. mexicanum emerges as the oldest polyploid species, having ancestral (A) and stacei-like (B) subgenomes, and the largest genome size reported in the genus. The purported high polyploids *B. boissieri* and *B. retusum* show three (A, B, E) and two (A, E-core) subgenomes, respectively. The recently evolved allotetraploids *B. rupestre* and *B. phoenicoides* show two (E, H) subgenomes. A potential chromosome base number of x=10 is inferred for the most ancestral subgenomes A and B, x=5 for the intermediately evolved E and E-core subgenomes, and x=9 for the recently evolved H subgenome (figure 2). Coalescence-based and cross bracing dating analyses have elucidated the ages of the potential progenitor genomes and their derived subgenomes and those of the potential hybridization events, respectively, spanning the late Miocene-late Quaternary period.

Pan-transcriptome analysis detected 5,202 transcript clusters across the studied *Brachypodium* samples, with a number of exclusive genes annotated in annual, perennial and ancestral *Brachypodium* lineages.

Oral

S6 Polyploidy and perenniality

T34 Transposon traps and kissing chromosomes: 3D chromatin interactions in a hybrid nucleus

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It is well-established that chromosomes are not randomly organized in the interphase nucleus. Each chromosome occupies a distinct territory, and the relative positions of these territories are often consistent within a tissue, and sometimes they can even be conserved across species. Hybridization is extremely common in plants, and occasionally even very distinct species successfully hybridize. In these wide hybrids, two very different genomes suddenly occupy the same nucleus. How does hybridization affect chromosome territories? And to what extent the two distinct subgenomes of a hybrid interact in 3D space?

We are using sequencing technology to address these questions in the allotetraploid *Brachypodium hybridum*. Using the Hi-C technique, we can quantify the frequency of physical interactions between loci, as well as interactions between chromosomes. Preliminary results indicate that the two subgenomes exhibit distinct chromosomal behavior, and the two subgenomes do not avoid each other in the hybrid nucleus. On the contrary, the two subgenomes appear to work together to form 3D chromatin structures that bear similarity to a previously described structure thought to be involved in transposon defense.

Oral

S6 Polyploidy and perenniality

T35 Homoeologous gene expression and subgenome contributions in response to water stress in the allotetraploid *Brachypodium hybridum* (Poaceae)

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Speciation processes driven by allopolyploidy (hybridation and whole genome duplication) are linked to the colonization of new ecological niches. These new species often present an enhanced abiotic stress resistance compared with its diploid ancestors. Polyploidy involves massive genetic changes in the newly formed, including nonadditive expression of homoeologous genes that could increase phenotypic plasticity and the potential for adaptation to new environments. However, our knowledge about the effects of abiotic stresses on nonadditive expression patterns is still limited.

In the *Brachypodium distachyon* species complex, the allotetraploid *Brachypodium hybridum* is derived from bidirectional crosses of its parents *B. distachyon* and *B. stacei*. These species have been proposed as a polyploid system to investigate the effects of subgenome dominance and origin in the phenotypic, physiological, and adaptive responses of allotetraploid hybrids.

Here, we conduct the first comparative drought transcriptome analysis of *B. hybridum* and its closer ancestors. We evaluate whether homeolog gene expression changes in the *B. hybridum* drought-transcriptome contributes to phenotypic variation and differentiation of this species in drier environments. More specifically, we investigate: (1) whole-genome differential expression pattern (transcriptome) between *B. hybridum* and its diploid progenitors in response to drought; and (2) the legacy of diploid progenitors in allopolyploid gene expression. Because the drought-functional response of *B. hybridum* is correlated to *B. stacei*one, we hypothesize that homoeolog inherited from *B. stacei* may have contributed in a large extent to the transcriptional level drought-response in *B. hybridum*.

After 21 days of progressive soil drying, we found that *B. hybridum* exhibiteda significantly stronger gene expression in response to drought compared to their diploid progenitors (1.38-fold and 4.92-fold higher number of differentially expressed genes – DEGs – than *B. distachyon* and *B. stacei* respectively). Noticeably, 51.29 % of DEGs*in B. hybridum* were unique. Gene Set Enrichment Analyses (GSEA) show that genes underlying molecule transport, response to heat and catabolic processes are significantly up-regulated, whereas genes related to plastid organization are significantly down-regulated by drought. Only 5.4 % of the total of genes expressed were shared between species. Interestingly, in the three species, genes controlling the proline biosynthesis process were significantly up-regulated, whereas, photosynthetic genes were significantly down-regulated.

Under drought, 56.8 % of expressed homoeolog pairs show a non-additive expression pattern: 24.35 % of expressed gene pairs exhibited *B. distachyon* expression-level dominance, 15.22 % *B. stacei* expression-level dominance, and 17.24 % transgressive expression. Only 0.84 % of expressed gene pairs exhibited additivity.

Our results indicate that (i) the relatively stronger drought-response at transcriptomic level of *B. hybridum* is concordant with the superior physiological drought-response observed for this species; (ii) contrarily to our expectations, homoeolog inherited from *B. distachyon* may have contributed in a larger extent to the transcriptional level drought-response in *B. hybridum*.

Oral

S6 Polyploidy and perenniality

T36 The fate of 35S rRNA genes in allotetraploid *Brachypodium hybridum* - an evolutionary point of view

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Allopolyploidy consists of interspecific hybridisation that is followed by chromosome doubling and has long been considered as a major driving force in the angiosperm evolution. However, the merger of two or more divergent genomes within one nucleus presents challenges to long term viability including: deletions, translocations, transposon activation and meiotic irregularities. In addition to these structural changes, the expression of duplicated genes can result in silencing of one of the homoeologues. One of the first examples of interaction between ancestral genomes that result in altered gene expression in allopolyploids is nucleolar dominance (ND). This enigmatic phenomenon consists of reversible silencing of 35/45S rDNA loci inherited from only one of the ancestors. Although our understanding of the involvement of epigenetic modifications in ND establishment and maintenance has advanced significantly in the last decade, the mechanisms by which one ancestral rDNA set is chosen for silencing remain unclear.
Brachypodium hybridum is a natural allotetraploid (DDSS) with its genomes most likely originating from two diploid species: *B. distachyon* (DD) and *B. stacei* (SS). Preferential silencing of *B. stacei*-like rDNA loci via ND was confirmed in different tissues of *B. hybridum*, including both meristematic and differentiated root cells, prophase I meiocytes, microspores, and in different tissues of immature and imbibed embryos. In order to shed more light on the molecular mechanisms that lay behind ND we applied a combination of molecular and cytogenetic approaches to study the organisation, expression and epigenetic landscape of 35S rDNA in 16 *B. hybridum* genotypes. Southern blot hybridisation on genomic DNA was used to determine each ancestral 35S rDNA contribution. In all studied accessions we observed rDNA units derived from both progenitors, however, a significant reduction in the copy number of Bs-homeologues was noted. The percentage of S-genome rDNA copies varied from 7% in ABR100 to 39% in ABR101. This trend was also confirmed using available genomic approaches where *B. hybridum* genotype ABR113 had only about 25% rDNA inherited from S genome while the majority (75%) of rDNA was dominated by highly homogenous *B. distachyon*-inherited units. The expression of rDNA homeologues was analysed using RT-CAPS approach based on the polymorphisms in the ITS region. In all studied genotypes we revealed only *B. distachyon*-specific bands indicating the expression dominance of D-genome loci. Additionally, we used a methylation-sensitive enzyme to assess global differences in DNA methylation. In all studied genotypes of *B. hybridum* we did not reveal unmethylated *Pst*I (enzyme sensitive to CWG methylation) bands of the *B. stacei*-origin. This supports the involvement of CWG methylation in the maintenance of ND in this allotetraploid.
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Oral

S6 Polyploidy and perenniality

T37 The perennial model grass *Brachypodium sylvaticum* -stress tolerance of two accessions growing in contrasting climates

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Perennial grasses are widely grown in different parts of the world as an important feedstock for renewable energy. Their perennial nature that reduces management practices and use of energy and agrochemicals give these biomass crops advantages when dealing with modern agriculture challenges such as soil erosion, increase in salinized marginal lands and the runoff of nutrients. *Brachypodium sylvaticum* is a perennial grass that was recently suggested as a suitable model for the study of biomass plant production and renewable energy. However, its plasticity to abiotic stress is not yet clear. We studied the salinity and freezing tolerance of two accessions isolated from two different areas of the world and characterized the mechanism(s) regulating stress tolerance in *B. sylvaticum* Osl1, originated from Oslo, Norway and Ain1, originated from Ain-Durham, Tunisia. Our results indicated that Osl 1 accession displayed higher salt tolerance than Ain 1. The enhanced salt tolerance was characterized by a better ion homeostasis with higher Na^+ compartmentalization and lower Na^+ allocation to the leaves. Osl1 limited sodium transport from root to shoot, preventing toxicity damage in the shoot. Interestingly, Osl1 exhibit a strong freezing tolerance and enhanced winter hardiness. A system biology approach (combining whole plant physiology, transcriptomics, metabolomics and lipidomics) revealed enhanced membrane stability and reduced oxidation damage in Osl1 under freezing. A HDZIP-I group transcription factors appeared to regulate freezing tolerance in Osl1 by specifically activating the synthesis of osmoprotectants. All together, the phenotypic variation between different *B. sylvaticum* accessions provides an excellent experimental system to characterize the functional significance of genes and metabolic pathways associated with abiotic stress tolerance of perennials grasses.

Oral

S7 Ecology and environment

T38 The genetic and physiological basis of local adaptation along environmental gradients

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Local adaptation is a fundamental driver of biodiversity on planet Earth. While recent experiments have begun to dissect the genetic basis of local adaptation, we still have a very poor understanding of how individual genetic loci contribute to local adaptation over large-scale environmental gradients. To understand local adaptation at a continental scale, we are conducting a long-term 13 field site study, spanning 24 degrees of latitude from central Mexico to the northern United States, in the major bioenergy crop switchgrass (*Panicum virgatum*). Much of the functional genetic variation in switchgrass is distributed clinally with latitude as well as between upland and lowland ecotypes.

Southern lowland populations are generally much more tolerant to heat, drought, and pathogens, while northern upland populations are more freezing tolerant. To understand genetic basis of local adaptation across central North America, we conducted a multi-site quantitative trait locus (QTL) study with a northern upland X southern lowland four-way, pseudo-testcross F2 tetraploid mapping population. We have now identified numerous QTLs contributing to variation in biomass, flowering time, plant height, and resistance to pathogens. The vast majority of these QTLs had strong genotype x environment interactions, with additive effects varying greatly among field sites. Overall, many of these loci have major positive benefits with minimal fitness trade-offs across field sites.

To understand how individual environmental factors contribute to local adaptation, we are conducting in-depth laboratory and field studies of fungal pathogen resistance and freezing/chilling tolerance. In addition, we recently planted a GWAS population of switchgrass at all of these field sites, which should allow us identify the genes underlying adaptation at a continental scale.

Oral

S7 Ecology and environment

T39 Facilitation as a driver of within population variation in *Brachypodium*

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Global climate change has a large impact on ecosystems worldwide. Terrestrial ecosystems found in harsh environments, such as semi-arid steppes, are especially vulnerable. Amplification of drought severity places added stress on plant communities in landscapes already susceptible to water shortages. One means of coping with changing conditions may be interspecific plant facilitation, in which nurse plants positively influence spatially associated species. A more thorough understanding of the interaction between local microhabitat and plant responses is key to elucidating and predicting a plant population's capacity to cope with a variety of environmental stressors. The *Brachypodium distachyon* complex (*B.distachyon*, *B. hybridum*, *B. stacei*) is an ideal study system to investigate adaptive capacity owing to its rapid incorporation as a model for plant biology and the prevalence of wild populations worldwide, especially in the Mediterranean basin. We first examine how the direction and strength of spatial association to nurse shrubs varies between 13 *Brachypodium* populations along an aridity gradient. Second, we assess within population responses to microclimate variability, a result of the amelioration of the harsh landscape by neighboring plant species (interspecific interactions). More specifically, we study differences in morphological characteristics and flowering times between plants found underneath nurse shrubs in contrast to plants found outside of nurse shrubs in natural populations and in the growth chamber. Preliminary analysis indicates an unexpected direction of spatial association between nurse shrubs and *Brachypodium* abundance between the two most extreme sites. In addition, in a subset of the data, we see a trend towards significant differences in morphological characteristics (stem height, spikelet length, number of seeds) between the plants found outside of nurse shrubs and those found underneath nurse shrubs at our most xeric site: ongoing genotyping indicates either spatial microclimate sorting or very high plasticity within species. Facilitative interactions may be an important driver for phenotypic responses which have been rarely incorporated in the ecological work conducted on *Brachypodium*.

Oral

S7 Ecology and environment

T40 Influence of leaf functional trait variation on the response to insect herbivory in the *Brachypodium distachyon* species complex

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For plants, the amount of herbivory received depends largely on the balance between their ability to produce defensive traits (i.e., resistance) and their nutritional quality. Once herbivore damage occurs, its impact on plant fitness is essentially determined by the plant's ability to regrow and reproduce after herbivory (i.e., tolerance). Together, resistance to herbivory, tolerance to damage and plant nutritional status comprise the plant response to herbivory which pivots on a set of several functional traits of complex nature (e.g., SLA, dry matter and water content, C:N ratio, concentration of secondary compounds in tissues, silicon, etc.). Although intra and interspecific variation in plant response is dependent upon the ecological context, plant response variation to herbivory hinges basically on the natural variation and plasticity of these functional traits.Among all the evolutionary processes, polyploidy (whole-genome duplication) typically induces dramatic phenotypic and genotypic changes that may impact on species interactions, including herbivory. Theory predicts that whole-genome duplication may enhance resistance and tolerance to herbivory, yet whether polyploid lineages are more resistant and tolerant to herbivory than diploid ones is still unclear. Here, we present results from a laboratory bioassay by using natural insect herbivores (grasshoppers) to analyze the resistance and tolerance to insect herbivory in the *Brachypodium distachyon* species complex. This complex comprises three species, the natural allotetraploid *Brachypodium hybridum* (2n = 4x = 30) which is derived from bidirectional crosses of its diploid parents *Brachypodium distachyon* (2n= 2x= 10) and*Brachypodium stacei* (2n= 2x = 20). These species vary in key leaf functional traits and the amount of herbivory received in natural conditions (*B. hybridum* polyploids are more damaged in nature). Thus, at species level, we investigate whether these three species differ in their plant response to herbivory, and if damage is associated to interspecific variation in constitutive silicon (herbivore deterrent in grasses) and/or to variation in nutritional quality. In addition, we explore inter-populational plant response to herbivory. We predict that, because *B. hybridum* plants have lower C:N than their species ancestors they will receive higher plant damage.

Our results indicate, however, that *B. stacei*is the species less resistant to herbivory, whereas plant damage recorded in *B. hybridum* and *B. distachyon* was significantly lower. *B. distachyon* genotypes showed higher tolerance to herbivory than *B. stacei* and *B. hybridum* tetraploids. Levels of plant damage seems to be associated to modulable leaf nutritional quality rather than to constitutive leaf silico content. Therefore, our results do not ascertain in *Brachypodium* species complex that whole-genome duplication enhances resistance and tolerance to herbivory.

Oral

S7 Ecology and environment

T41 The value of regional collections of natural populations to unravel the ecological and genetic basis of adaptive variation in Arabidopsis thaliana

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Adaptive variation in phenotypic traits enhances the viability of populations in changing environments and accounts for the establishment of new populations in novel environments. In the long run, natural selection determines the distribution range of any organism whose populations may eventually occur in contrasting environments and displaying broad phenotypic variation. Acquiring an in-depth understanding of the ecological and genetic basis underlying adaptive variation is not a straightforward task, given the multiple combinations of environmental cues and genetic regulatory pathways that plants can use to adjust their phenotypes to the environment. *Arabidopsis thaliana* represents an outstanding model plant to conduct evolutionary studies and disentangle the process of adaptive variation in plants. The reason is the powerful combination of dense regional collections of natural populations from diverse environments across the distribution range, detailed phenotypic characterisation of life-history traits in controlled conditions but also in field experiments, the detailed functional knowledge of several key genes, and in the last few years the availability of complete genome sequences.

We present some major findings from a long-term research conducted on *A. thaliana* from SW Mediterranean Basin (Spain, Portugal and Morocco) to unravel the evolutionary ecology of the species across the region and the genetic basis of adaptive variation in life-cycle traits. We focus on three major aspects of our research that overall highlight the relevance of dense regional collections of natural populations to achieve our goals. First, we stress the fact that adaptive variation shapes the life cycle by acting on traits in a hierarchical manner, although the intensity and sign of natural variation on the same traits need not to be constant over space and time. Second, we show that dense collections of natural populations also allow the detection of low-frequency functional alleles of key flowering time genes from different genetic regulatory pathways and their relationship with environmental variables, illustrating the spatial complexity of the genetic basis of adaptive variation at a regional scale. And third, we demonstrate the importance of phenotyping a large number of naturally occurring accessions in field experiments to better depict genome-wide signatures of adaptive variation in flowering time. Finally, we wrap up the communication by outlining ongoing and future research lines, identifying the potentiality but also the caveats of the approach, and providing hints on the importance of model systems, such as *A. thaliana*, to better understand the response of plants to global climate change in these challenging times.

Main references: Plant Physiol, 2011, 157, 1942; Evolution, 2012, 66, 2287; Plant Cell Environ, 2012, 35, 1672; PLoS ONE, 2014, 9, e87836; Ecol Evol, 2016, 6, 2084; Plant Cell Environ, 2016, 39, 1737; Evolution, 2018, 72, 1570; Plant Biol, 2018, 20, 148; Plant Cell Environ, 2018, 4, 1806.

Oral

S7 Ecology and environment

T42 The impact of wind stress and mechanical stimulation on the growth and composition of *Brachypodium distachyon* stems

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The wind is important abiotic stress from an agronomic and economic point of view. While the response of plants to various abiotic stresses is intensively studied, there is relatively little research on wind stress (WS) and mechanical stress (MS) in plants, especially in the grasses. Our study aims to provide information on how wind stress and mechanical stimulation affect the growth and development of the model grass *Brachypodium distachyon*.

In particular, we have focussed on the consequences of WS and MS on cell wall composition and architectural features of the stems, as well as phenotypic responses. Two *Brachypodium distachyon* ecotypes, Bd21 and ABR6, were exposed to wind stress (2-3m/s, 8h/day) and mechanical stimulation (brushing - 80 flexures/day) for two weeks. Both treatments resulted in reduced main stem length and delayed flowering for the two ecotypes (Fig 1A-C). Furthermore, reduction in seed yield and aboveground biomass was observed after stress treatments (Fig. 1C). More detailed analysis including histology, anatomy, and composition analysis of stem cell walls revealed differences in response to WS and MS and between both genotypes Bd21 and ABR6. Histological staining and scanning electron microscopy showed that both stresses altered anatomical characteristics when compared with control plants and resulted in cell wall thickening of particular stem tissues. Moreover, the overall organisation of stem tissues was affected by both stresses, resulting from significant changes in the area of vascular bundles, interfascicular region, epidermis and pith (Fig 2A). Immunolocalisation using a range of monoclonal antibodies against non-cellulosic cell wall glycans, revealed differences in the labelling pattern obtained with pectin-related antibodies between treatments and ecotypes. Using the same antibodies, ELISA confirmed alterations in pectin characteristics (Fig 2B). Moreover, a gel diffusion assay showed increased pectin methylesterase activity after both stress treatments (Fig 2C). Cell wall composition analysis revealed that treatments induced an increase in lignin content (Fig 2D) localised mostly in the cortex and interfascicular tissue. Moreover, differences in cell wall monosaccharide content were also observed. Sugar release after enzymatic hydrolysis was significantly reduced after both stress treatments. Furthermore, three-point-bending tests showed differences in the mechanical properties of stems exposed to WS/MS compared to controls.

Our results show that wind and mechanical stress induce significant architectural changes across multiple scales, from the whole plant to organ, tissue and cellular level highlighting the complex nature of how plants respond to wind stress and mechanical stimulation.

Oral

S7 Ecology and environment

T43 Genetic, epigenetic and metabolomic differentiation of Turkish *Brachypodium distachyon* accessions into two geographically distinct populations

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As an annual species, growing on broken ground *Brachypodium distachyon* (*Brachypodium*), represents an ideal model with which to suggest the molecular drivers of environmental adaptation. We have initiated a project where we are assessing the relative contribution of genomic, epigenetic, transcriptional and metabolomic variation to environmental adaptation. Turkey represents one site of *Brachypodium* diversification and can be broadly sub-divided into five distinctive climato-environmental regions; representing different rainfalls, temperature ranges, altitudes, and soil types. A new collection of Brachypodium accessions was established by sampling at 12 sites within each of the regions. Seed (T_o) was taken from each accession and grown under uniform controlled environmental conditions at Aberystwyth University. Thus, any variation reflected innate differences arising from environmental adaptation. At 6 weeks following germination, T_o leaves were sampled and assessed for genomic (WGS), epigenetic (BS-Seq), transcriptional (RNA-seq) and metabolite (by high resolution Flow-Infusion Electospray - Mass Spectroscopy; FIE-MS) variation. Phenotypic analyses indicated that the T_o population could be broadly differentiated into two subpopulation based on seed dimensions, plant height, period of vernalisation, flowering time and drought tolerance. Genome sequencing (10 fold coverage) also indicated that variation in single nucleotide polymorphisms was linked to a north and southern Turkish population; broadly delineated by the Anatolian plateau. Further assessment indicated that epigenetic, transcriptional and metabolomic analyses also aligned with the Northern-Southern split in *Brachypodium* populations. Examination of the molecular drivers for this split are indicating that; for example, epigenetic-regulatory genes, drought-linked transcriptional regulators, TCA (tricarboxylic acid) and glyoxylate cycles were differentially active in the two populations. We suggest that our data indicated that natural selection through both genetic and epigenetic variation to drive adaptive changes in the transcriptome and metabolome.

Oral

S8 Adaptation to abiotic and biotic constrains

T44 Use of non-invasive phenotyping, molecular approaches and beneficial microbes in the understanding and improvement of *Brachypodium* nutrient uptake with focus on Nitrogen, Phosphorus and Zinc

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In times of increasing global population and decreasing arable land per capita, the understanding of plant nutrient uptake and possible nutrient uptake improvement strategies are of utmost importance. Our work focuses on Nitrogen (N) – the second most abundant nutrient in plants, Phosphate (P) – whose resources are limited on global level, and the micronutrient Zinc whose deficiency affects the health of over 20% or the world population.

We present studies where the use of plant growth promoting rhizobacteria (PGPR) has resulted in improved plant performance under limited N or P. Here, plant growth has been analyzed by non-invasive root phenotyping techniques GrowScreen Page (Gioia *et al*., 2017) Fig. 1A, and/or via EcoFab (Sasse *et al*., 2019) Fig. 1B. The latter has been used in combination with Plant Screen Mobile (Muller-Linow *et al*., 2019), for non-invasive shoot leaf area estimation.

Increase of plant biomass has been seen in P deficient plants inoculated with a PGPR. A time series analysis of root phenotype, allowed not only visualization of increased root length and changes in root architecture, but also to pin-point the time when growth promotion takes effect after inoculation. A sand experiment confirmed these results and found biomass increase in the inoculated plants. Study of the molecular mechanisms behind this phenotype is ongoing.

In the case of limiting N where plants were inoculated with N-fixing PGPR, an end-point harvest showed a change in the ratio of primary to lateral roots, but here the most important finding was an increase of N concentration in root and shoot, increase of shoot biomass and leaf area. We complemented this destructive harvest with proteomics to investigate the systemic response of *Brachypodium* constitutively grown under limiting N, to the interaction with the respective PGPR. Data analysis revealed that these N-fixing bacteria impact central Nitrogen metabolism in *Brachypodium*, and indicate a mode of action that upregulates specific N transporters on the root plasma-membrane.

Finally, in a transfer of knowledge approach, we are non-invasively determining the base-line plant phenotype of Zinc deficient *Brachypodium* (Nagel, 2009) and carrying-over novel candidates originating from an Arabidopsis proteomics study (Arsova *et al*., in preparation), through transcript profiling experiments. In this manner we hope to pre-select candidates that could be used as breeding targets in crop plants.

Figure 1: Representative root image of *Brachypodium distachyon* 21-3, grown and phenotyped using GrowScreen Page and custom-made software (A). *Brachypodium distachyon* 21-3 grown in an EcoFab under sterile environment (B). Green–primary root, Red –lateral roots.

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Plant Methods, 2019, **15:** 2
Funct Plant Biol, 2009, **36:** 947
New Phytol, 2019, **222:** 1149-1160

Oral

S8 Adaptation to abiotic and biotic constrains

T45 Rapid activation of WRKY-dependent immunity facilitates native resistance against the sheath blight pathogen, Rhizoctonia solani, in *Brachypodium distachyon*

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Rhizoctonia solani is a soil-borne plant pathogenic fungus that causes disease in a wide range of plant species including commercially important crops. In particular, the subgroup AG-1 1A is the causal agent of sheath blight, which severely decreases yield in cereals such as rice. Many approaches have been attempted to improve plant resistance to sheath blight through the ectopic introduction of defense-associated genes such as fungal cell-wall lytic enzymes or transcription factors (TFs). We recently demonstrated that exogenous application of the plant immune hormone salicylic acid (SA) confers resistance against sheath blight in rice and *Brachypodium distachyon*. However, the molecular mechanisms underlying plant native resistance against *R. solani* remain largely unknown because no rice plants including both cultivated and wild species exhibit complete resistance to *R. solani* in spite of its population-wide exploration.

In the present study, we report the time-series comparative transcriptome analysis of three natural accessions of *B. distachyon*, including Bd21, Bd3-1, and Tek-3, following inoculation with *R. solani*. From the screening of *R. solan*-resistant *B. distachyon* accessions, we identified that Tek-3, as well as Bd3-1, exhibited considerably less symptoms than Bd21 after inoculated with *R. solani*. Our transcriptome analysis revealed dynamically expressed genes (DYGs) in *B. distachyon* during *R. solani* infection. Functional enrichment analyses of the DYGs revealed that Bd3-1 and Tek-3 commonly induced defense-associated genes that were potentially regulated by WRKY TFs at 8–16 hours post inoculation (hpi), whereas Bd21 upregulated a similar set of genes at 16–24 hpi, suggesting that rapid induction of WRKY-dependent defense genes facilitates native *R. solani* resistance in *B. distachyon*. To further dissect the WRKY transcriptional regulatory networks associated with *R. solani* resistance in *B. distachyon*, we inferred gene regulatory networks (GRNs) based on the time-series transcriptome dataset and identified WRKYs that constitute hubs in the GRNs of both susceptible and resistant accessions. We assessed phylogenetic distribution and SA-responsiveness of the hub WRKYs and found that SA-inducible WRKYs that include putative homologs of OsWRKY45, the master regulator of SA-dependent blast resistance in rice, may constitute hubs in the GRN of Bd3-1 and Tek-3. Moreover, RNAi-based silencing of the SA-inducible WRKYs, as well as overexpression of a bacterial SA degrading enzyme, led to enhanced susceptibility to *R. solani* in Bd3-1, which suggest that the WRKYs may function in SA-dependent immunity contribute to the native *R. solani* resistance in Bd3-1 and Tek-3. Our findings provide new insights into the molecular mechanisms and their diversity of the native resistance against *R. solani* in grass plants.

Oral

S8 Adaptation to abiotic and biotic constrains

T46 The SNF2 family of chromatin remodelers is conserved in *Brachypodium distachyon* and involved in the response to combinatorial abiotic stresses

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Chromatin structure plays an essential role in the regulation of nuclear processes. To regulate the access to DNA, different chromatin remodeling complexes appeared early during evolution and are well conserved in plants, animals and fungi. Among them, SNF2 helicases are DNA-dependent ATPases able to dynamically alter DNA-histone interactions to overcome the nucleosome barrier and increase accessibility to DNA. In plants, this family has been described in different species and at least some of its members have been shown to be important regulators of development, genome structure and stability and the response to biotic and abiotic stresses. However, we are still far to understand the complexity of this family and their specific roles in regulating plant adaptability under different environments. Here we present the description of the SNF2 family of *Brachypodium distachyon* (BdSNF2) and the evolutionary relationship of the family in different model plant species to provide a platform for future functional analysis of the family. The high conservation of the BdSNF2 family suggests that these proteins may be key regulators of plant and embryonic development, defense against pathogens, hormone signaling and response to abiotic stresses. The analyses of transcriptomic data for the members of the BdSNF2 family revealed that half of them were differentially expressed in response to the combination of different abiotic stresses, but not by single ones. This result indicates that SNF2 ATPases may be good targets to modify plant adaptation in response to multi-dimensional environments mimicking on-field conditions. Hence, our phylogenetic and expression analyses may contribute to establish the foundation for further analyses of the SNF2 family in temperate cereals.

Oral

S8 Adaptation to abiotic and biotic constrains

T47 *Brachypodium distachyon* and B. hybridum root, rhizosphere and bulk soil bacterial communities differ between native and invaded ranges

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Invasive species are eroding the native biodiversity and associated ecosystem services of natural areas in California and much of the US. Although plant trait and abiotic controls on invasions are well-studied, there is not yet a clear understanding of the rhizosphere microbial mechanisms contributing to plant invasions. Greater insight into these mechanisms may allow us to better prevent and control invasions. During the last decade *Brachypodium distachyon* (Poaceae) has emerged as one of the most important model systems for functional genomic studies of temperate cereals and forage grasses and for bioenergy, and recently its derived allotetraploid *B. hybridum* has been proposed as a model for allopolyploidy. The invasion of *Brachypodium* (*B. hybridum*) species in California is in an incipient stage, threatening natural and agricultural systems as it actively spreads across much of the western half of California. Herbarium specimens held at the University of California, Berkeley, identify *B. hybridum* as early as 1921 in Alameda County. Sampling of *B. distachyon* and *B. hybridum* occurred in 9 sites in Spain and 8 sites in California, respectively, in 2016. During each field collection, five replicate *Brachypodium* plants and five replicate invasive co-occurring (non-*Brachypodium*) grasses were collected (usually *Bromus* or *Avena* spp). From each plant we collected a shoot, root, rhizosphere, and bulk soil samples. We extracted rhizosphere DNA using the MoBio PowerSoil DNA Isolation Kit. The 16S ribosomal RNA gene (16S rRNA) V3 and V4 regions were analyzed to classify the diversity of bacteria and archaea in the soil. All samples were pooled together in equimolar concentrations then sequenced with an Illumina MiSeq instrument. Sequence data was analyzed using Quantitative Insights into Microbial Ecology (QIIME) against the Greengenes reference databases using 97% similarity. Analysis of similarity (ANOSIM) was performed in QIIME using a Unifrac index to statistically compare community similarity among treatments. We performed alpha diversity analyses and generated PCoA plots using QIIME. Beta diversity analyses were performed using MicrobiomeAnalyst, JMP13 was used to perform a two-way ANOVA, and the vegan and ggpubr packages in R to perform a PERMANOVA and generate figures, respectively. The bacterial community composition differs by species and country ($p < 0.05$; Figure 1). The rhizosphere, root, cores, and bulk soils between *B. distachyon*-Spain and *B .hybridum*-USA also differ from each other. In addition, there are several *B. hybridum* sites in the US that cluster more strongly with the *B. distachyon* Spanish sites. Putative pathogenic bacteria were more abundant in the *B. distachyon* Spanish rhizosphere samples than any other sample set from either country. These analyses show that it is likely that allotetraploid *B. hybridum* is a successful invader due to enemy release from its *B. distachyon* progenitor species home range.

Oral

S8 Adaptation to abiotic and biotic constrains

T48 Molecular characterization of SPL/miR156 regulatory component in *Brachypodium* spp.

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The genus *Brachypodium* has emerged as a model system to advance our knowledge about the biology of agriculturally important traits in small grain cereals. Here, we studied the role of*SQUAMOSA*-Promoter Binding like Transcription Factor Proteins (SPLs) in *Brachypodium* growth and development, and its regulation at the post-transcription level by alternative splicing and miR156. The *Brachypodium* genome contains 18 *SPL* genes, which were classified into eight phylogenetic groups. Gene structure and motif composition of *BdSPLs*were found to be similar within their own group. Our data indicates that *SPL*gene family in *Brachypodium* expanded through large scale duplication events through segmental (41%) and tandem duplications (23%). Over 50% of *BdSPL*genes (*BdSPL1/-3/-11/-13/-13A/-14/-16/-17/-18/-23)* were targeted by *Bd-miR156*, elevated expression of these genes were observed during spikelet initiation as compared to the maturation stage. Alternative splicing was the key process involved in the *BdSPLs*and miR156 interaction. RNA-seq analysis revealed that *BdSPL*genes were differentially expressed at different stages of plant development especially during the transition from juvenile phase to the reproductive phase. *In-silico* expression analysis of *BdSPL*genes observed at different stages of plant development and reproductive process were validated by RT-PCR and qRT-PCR. Their expression was monitored further under abiotic stresses, especially in relation to high temperature, highlight their central role in these processes. Co-expression network and protein-protein interaction analyses indicate that *BdSPL* genes are associated with transcription, hormone metabolism, RNA and transport pathways. Taken together, this first ever study of *SPL* gene family in *Brachypodium* provides new paradigms for the development of future generation of cereals in the changing climate scenario.

Oral

SS8 Adaptation to abiotic and biotic constrains

T49 *Brachypodium distachyon*: a good model to study ammonium assimilation, nutrition and stress in cereals

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Nitrogen (N) is a key mineral nutrient demanded by plants and its limiting availability in agronomic soils is one of the main factors restraining the crop yield. The use of fertilizers provokes N losses that provoke detrimental effects on the environment such as water contamination or contributing to global warming. At present, ammonium-based nutrition is gaining interest because it mitigates the environmental pollution associated with nitric fertilization. However, the main constraint to an extended use is the overall sensitivity of crop plants to ammonium nutrition. The main symptom of ammonium stress is a decrease in plant growth, but may even provoke plant death. The causes of ammonium stress remain to be completely elucidated but are related to pH homeostasis, cation imbalance and an excessive energetic cost associated with maintaining cytosolic NH_3/NH_4^+ homeostasis. Interestingly, Poaceae is considered as a rather tolerant plant family towards ammonium nutrition, although there exists a great variability in higher plants sensitivities when it is the only N source, even within a given species. The knowledge about the molecular mechanisms governing this differential sensitivity is still in its infancy.

Despite *Brachypodium distachyon* has been proposed as a model plant for C3 cereals, the comprehension on N signalling and metabolism in this species is still scarce and no knowledge is available regarding its capacity to use ammonium as N source. In this sense, our objective was to characterize the physiological and molecular traits involved in its response to ammonium nutrition. Plants were grown with the exclusive supply of nitrate or ammonium (1 and 2.5 mM) for 24 days on hydroponic culture. *B. distachyon* revealed as a good model to study ammonium nutrition since it responded similarly to other monocot crops. The plants increased the storage of NH_4^+ in roots, as well as the synthesis of amino acids and proteins. This was possible thanks to the induction in the root of NH_4^+ assimilatory machinery including enzymes associated to TCA cycle. To further understand *B. distachyon* response upon ammonium nutrition, we engaged two strategies: a quantitative proteomic study in the roots and an approach based on natural variation. For the second one, we characterized 52 natural accessions of *B. distachyon* (Gordon *et al*., 2017) grown under ammonium and nitrate nutrition and we quantified a panel of metabolic traits related to nitrogen and carbon metabolism. We expect that the data obtained will be useful to get more insight into the natural genetic variability associated with ammonium tolerance with, among others, the use of genome-wide association analysis.

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Reference: Gordon *et al*., Nature Commun, 2017, 8:2184

Oral

S9 Crop and biomass crop translation

T50 From genome to genomes. Charting the genome landscape(s) of western civilisation

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Cereals are the most important crops worldwide. Until recently access to the di-/tetra- and hexaploid cereal genomes was hampered by the enormous size, high repeat content and polyploidy. 17 years after the completion of the human genome these limitations have been circumvented by making use of different complementary approaches. After a decade of technology development and implementation and the decoding of large – in part – polyploid cereal genomes now allow to address genome questions, biological questions and to start to address systems biology and comparative genomic questions. Using the emerging genome sequences as an information backbone, in depth transcriptional profiling of the developing grain in context of a hexaploid genome is undertaken and used to gain insights into the transcriptional coordination among three homeologous subgenomes. Deep profiling of the global transcriptome of single endosperm cell types (starchy endosperm, aleurone and transfer cells, respectively) was performed to test for the effects of polyploidy on gene expression of homeologous, duplicated genes in bread wheat. Expressed genes were subject to network-based co-expression analysis revealing significant cell-type and time point specific gene expression. The presentation will aim to review important steps in the unlocking of large and complex cereal genomes, highlight progress in cereal genome sequencing and a systems biology use case in the multidimensional transcriptional analysis of the developing grain. Also, a “sneak preview” of forthcoming data and the analytical challenges and future in cereal genomics will be addressed.

Oral

S9 Crop and biomass crop translation

T51 Hemoglobins of vascular plants: from model plants to crops (and way back)

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The first hemoglobin discovered in plants was the leghemoglobin of soybean root nodules. This finding was extended to other symbiotic hemoglobins in nodules of legumes, *Parasponia* and actinorhizal plants such as *Casuarina* and *Myrica*. These hemoglobins provide O_2 to N_2-fixing bacteria within nodules. With the advent of technology for massive genome sequencing and wide-scale transcriptomics, it has become clear that hemoglobins are ubiquitously expressed in plant organs, from seeds to flowers. Based on phylogenetic and biochemical analyses, three classes of Glbs can be distinguished in vascular plants: Glb1s have extreme O_2 affinity and are induced by hypoxia; Glb2s have moderate O_2 affinity and, with certain exceptions, are the evolutionary precursors of symbiotic hemoglobins; and Glb3s have low O_2 affinity and high sequence homology to bacterial truncated hemoglobins. The proteins also differ structurally. For example, Glb1s and Glb2s are typically hexacoordinate (two histidines coordinate heme iron) whereas Glb3s and leghemoglobins are pentacoordinate (only one histidine is involved in iron coordination), which in part explains the differences in O_2 affinity and reactivity towards physiological ligands. However, the functions of Glbs are not well defined. Several Glb1s are involved in modulating the level of nitric oxide (NO), a key signal molecule in plant biology, and there is evidence that some Glb2s and/or Glb3s can scavenge NO *in vivo* or act as O_2 transporters, perhaps in tissues with high metabolic activity.

Here we present data on the phylogeny and some possible functions of Glbs in model plants and representative crops, both monocots and dicots. Interestingly, monocots such as barley, rice and corn lack Glb2s, suggesting that this class of proteins appeared, probably as a result of gene duplication, after the early divergence of angiosperms. Monocots and dicots may contain multiple Glb1s that in some cases (barley, rice, soybean) have been biochemically characterized. Model plants for genetic and molecular biology studies are boosting the search for Glb functions. We have identified two genes in the model small grass *Brachypodium distachyon* (Bradi1g69320, Bradi2g19690) and in switchgrass (J01114, Ia04000). All these genes presumably encode Glb1s. In dicots there are ample differences in the number of Glbs even within model plants: *Arabidopsis thaliana* contains one Glb of each class, whereas the model legumes *Lotus japonicus* and *Medicago truncatula* have at least two Glb1s, probably one Glb2, two Glb3s and three (*Lotus*) or twelve (*Medicago*) leghemoglobins. We have used lines that overexpress or are knockout for specific Glbs of the dicot model plants to show a triple interaction among Glbs, NO and phytohormones. With the help of specific mutants, we are pursuing now the study of Glb functions in model legumes and expect to tackle soon similar studies of this challenging issue in *B. distachyon*.

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Oral

S9 Crop and biomass crop translation

T52 Functional analysis of fatty acid elongase TaFAE1 gene from biofuel feedstock Thlaspi arvense reveals differences in seed-oil biosynthesis among Brassicaceae

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Pennycress (*Thlaspi arvense* belongs to the Brassicaceae family and is closely related to the model plant *Arabidopsis thaliana* as well as other agronomical important plants such as rapeseed (Brassica napus), camelina (*Camelina sativa*) or lepidium (*Lepidium campestris*). In addition to its extreme cold tolerance or over-wintering growth, Pennycress seeds have unique characteristics in terms of seed-oil content (32-36% w) and composition, with a high content (38-40%) of erucic acid (22:1). This has attracted the interest of Pennycress as biofuel feedstock because of the exceptional low-temperature behaviour and energetic capacity characteristics of its seed-oil. However, although Pennycress could be an attractive alternative feedstock in Europe, particularly in the Mediterranean region, it is not cultivated in the EU territory. In our Group we have characterized Pennycress germplasm of European origin and some interesting agronomical traits were detected. Genes involved in seed-oil, erucic acid biosynthesis in Pennycress were identified, and their regulation was studied during Pennycress seed maturation. Question arised of the molecular and biochemical elements responsible of the great heterogeneity in seed-oil content and fatty acid composition among Brassicaceae. As a first step to answer to this question, we have performed a functional analysis of the *TaFAE1*, encoding the fatty acid elongase responsible of erucic acid biosynthesis in Arabidopsis. This enzyme catalyses the two-step elongation of oleic acid (18:1) to produce eicosenoic acid (20:1) and erucic acid. Two genetic backgrounds were used, Col-0 plants that accumulate very low levels (less than 2,5 %) of erucic acid and other Very Long Chain Fatty Acids (VLCFAs) and a *fae1-1* mutant, deficient in FAE1 activity that does not accumulate any VLCFAs. A seed-specific promoter was used for the transgene analysis to avoid over-expression or tissue-unspecific artefact results in our analysis. Our results showed that expression of the Pennycress *TaFAE1* gene in Arabidopsis resulted in a 3 to 4-fold increase of the erucic acid content in the seed-oil from Arabidopsis. Our data also suggests that the Pennycress *Ta*FAE1 enzyme has higher affinity for 20:1 substrates than the endogenous *At*FAE1 enzyme. The phylogenetic analysis suggested that this could be correlated with some specific domains of the FAE1 protein. Furthermore, 22:1-CoA substrates were efficiently incorporated to the triacyl-glycerol (TAG) oil fraction in the Arabidopsis transgenic lines expressing the *TaFAE1* gene, indicating that DGAT1 was capable to use 22:1-CoA substrates efficiently, contrasting to previous hypotheses. All these data can be useful for the manipulation of the erucic acid trait with the objective of the sustainable production of high quality biofuel from oil-seed plants.

Oral

S9 Crop and biomass crop translation

T53 Diversification of cultivated barley and selection footprints in the landraces of the Iberian Peninsula

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Landraces are populations of crop plants adapted to a particular environment. Extant landraces are surviving genetic archives, keeping information about the selection processes experienced by them until settling in their current niches. Barley landraces were abundant in Spain and well collected in germplasm banks over the last century.

Barley landraces in Spain represent the adaptation and partial admixture of several germplasm groups that arrived in the Iberian Peninsula probably in different waves starting around 7,000 years ago. This process resulted in the occurrence of at least four germplasm groups (Fig. 1) still recognizable in current landraces. These groups show different allelic combinations at key genes of the vernalization and photoperiod pathways, which differentiate them from varieties grown in latitudes that are more northern.

These groups also show differential responses to environmental features that determine the adaptation of crops, like vernalization potential or occurrence of frost. Besides those well-known genes, the barley genetic groups differ in several genomic regions whose possible role in adaptation is not known yet.

We performed an association of genetic and agroclimatic data to find out the main factors driving the landrace distribution over the Iberian Peninsula. For this purpose, a set of high-resolution maps of climatic variables, computed from over 2,000 temperature and 7,000 precipitation stations across peninsular Spain was built, and specific agroclimatic variables that could affect barley growth were derived. An initial association was carried out with 7,479 SNP markers, combining Illumina Infinium assays and genotyping-by-sequencing. Further association with a subset of genotypes and exome-capture data helped to narrow down the search of possible candidate genes (Fig. 2). We report a number of associations that suggest that differential adaptation of the germplasm groups identified are dominated by responses to winter temperatures and frost, followed by response to water availability. Several candidate genes underlying some of the main regions are proposed.

LEGEND Figure 1 (taken from Contreras-Moreira et al. 2019, Mol. Ecol.). Top: group membership probabilities resulting from a Structure cluster analysis. Bottom: geographic distribution of the four subpopulations over elevation in mainland Spain (colour coded as in the top graph), represented in a UTM-30N projection, axes in meters.

LEGEND Figure 2. Chromosome segment of 1 Mb (7H) showing the position of SNP markers and their association (Bayes factor, Y-axis) with a frost probability variable. Blue symbols, first analysis with 7,479 SNPs. Red symbols, markers derived from exome capture data. Triangles, positions of known genes.

Oral

S9 Crop and biomass crop translation

T54 Molecular passport of a new Zea weed emerged in European maize fields

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Maize is the cereal with the highest production worldwide for the last decade. This explains the considerable concern caused by the discovery of a new weed in European maize fields. Its focal points are mainly in the Northeast of Spain, and on a smaller scale, in the Southwest of France. Early identification of crop-related weeds becomes crucial to determine the success of their control. As the origin of this new weed is uncertain, our objective was to characterize it using microsatellite markers. Maize-like weeds, putative hybrids between commercial maize and the weed, teosinte (*Zea mays* ssp. *mexicana* and *Zea mays* ssp. *parviglumis*) and commercial maize varieties were genotyped with 17 microsatellites and the data used to: explore the genetic relationships among them by constructing a dendrogram; determine their associations by Principal Component Analysis; and study the population genetic structure. All samples were distinguished using only six microsatellites. All analyses showed that the samples mainly grouped according to the type they belonged to, though a close genetic relationship between the crop and the weeds became clear. In terms of genetic structure, the highest levels of admixture were observed in the weeds and the teosintes, and the lowest in the commercial varieties. The weeds shared most of their genetic background with the commercial varieties, what reveals their high degree of hybridization. Consistently, the genetic variation (F_{ST}), that is a measurement of the genetic differentiation among groups, was negligible in all cases except when the teosintes were compared to the commercial group. In agreement with this, most of the molecular variance occurred within populations (51.83%) and not among populations (10.09%). All the commercial varieties had a high membership in their own group and seem to have originated in a small portion of the huge genetic variability present in teosinte. This, together with the lower levels of gene diversity found in maize compared to teosinte, supports the occurrence of a bottleneck during maize domestication. The weeds genetically resemble most the commercial maize cultivars grown nowadays at the infested regions, though they still maintain some genetic similarity with maize putative wild ancestor (*Z. mays* ssp. *parviglumis*). Our findings also evidence the gene flow between weed and cultivated maize. Natural spontaneous hybridizations between the crop and the weeds have been found and the genetic proximity between the modern maize varieties and the hybrids has been verified. The most similar weed to the cultivated maize seems to be the closest to the original cross between them. On-going pollinations by the weeds (favoured crossing direction) explain the generation of more and more weedy plants.

Finally, the vague separation between the two teosinte subspecies could be due to misclassification and/or hybridization between them.

Oral

S9 Crop and biomass crop translation

T55 From models to crops: using *Brachypodium* toward an improvement of Nitrogen Use Efficiency in cereals

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New cereal varieties with improved Nitrogen Use Efficiency (NUE) need to be developed to increase grain yield while reducing the usage of fertilizers. This can be achieved by genetically enhancing the plant capacity to uptake and assimilate nitrate from the soil and its capacity to remobilize nitrogen (N) from the leaves. Many molecular and genomic data have been acquired in the model dicot *Arabidopsis thaliana*, whereas many physiological and agronomical studies have been done using Pooideae species (wheat and barley) and other cereals (rice, maize). We are using *Brachypodium distachyon* to transfer molecular knowledge from *Arabidopsis* to cereals (Girin *et al.*, J. Exp. Bot. 2014). We first characterised the response of *Brachypodium* to variations of N availability, validating the species as a monocot model to decipher cereal N nutrition. Pre- and post-anthesis nitrate (NO_3^-) availability affects characteristics of agronomical importance such as vegetative growth, tillering, grain yield and grain N content. At grain filling stage, NO_3^- uptake is largely affected by the ion availability, whereas N remobilisation from source tissues is more stable. Constitutive and NO_3^--inducible High Affinity and Low Affinity Transport Systems (HATS and LATS, respectively) for root nitrate uptake have been characterised. Apparent specificities such as high grain N content, strong post-anthesis NO_3 uptake and efficient constitutive HATS, further identify Brachypodium as a direct source of knowledge for crop improvement. Functions of *BdNRT* and *BdNLP* family members are currently being investigated, based on the roles of their orthologs in NO_3^- uptake and in regulating N nutrition (respectively) in other species.

Oral

S9 Closing Conference

T56 More, more, more, the genus *Brachypodium* as a sequence-enabled functional genomics model

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The development and acceptance of *Brachypodium distachyon* as a model system was propelled by whole genome sequencing. Since the sequencing of the initial reference genome in 2008 sequencing has continued to push the entire genus into a role as a model for both functional and evolutionary genomics. Functional studies are being enabled by: the sequencing and assembly of over 100 natural accessions, the creation of a pan-genome that showed nearly half of the high-confidence genes are missing from some lines, the creation of reference quality assemblies for three commonly used lines, surveys of the epigenetic landscape, and mutant sequencing that has identified over 1 million mutations. Evolutionary and functional studies have been enabled by the sequencing of three additional species: *B. stacei*, *B. hybridum* and *B. sylvaticum*. The first two together with *B. distachyon* serve as a tractable model to study polyploid genome evolution and regulation and the latter serves as a model for perenniality. Additional species are being sequenced now that will further serve to understand the evolution of the genus and the molecular basis of perenniality. A brief overview of how various sequencing projects enabled the development of powerful resources with an emphasis on the most recent projects will be presented.

Poster

S1 Natural diversity and evolution

P01 Multiple founder events explain the genetic diversity and structure of the model allopolyploid grass *Brachypodium hybridum* in the Iberian Peninsula hotspot

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It is accepted that contemporary allopolyploid species have originated recurrently, but very few cases have been documented using multiple natural formations of the same species. To extend our knowledge, we have investigated the multiple origins, genetic variation, and structure of the allotetraploid grass *Brachypodium hybridum* with respect to its progenitor diploid species *B. distachyon* (D genome) and *B. stacei* (S genome). For this, our primary focus is the Iberian Peninsula, an evolutionary hotspot for the genus *Brachypodium*. We analysed 342 *B. hybridum* individuals from 36 populations using 10 nuclear SSR loci and two plastid loci. The *B. hybridum* genetic profiles were compared with those previously reported for *B. stacei* and *B. distachyon*. In addition, phylogenetic analysis of the plastid data was performed for a reduced subset of individuals. The nuclear SSR genetic analysis detected medium to high genetic diversity, with a strong south-to-north genetic structure cline, and a high selfing rate in *B. hybridum*. Comparative genetic analysis showed a close relatedness of current *B. hybridum* D allelic profiles with those of *B. distachyon*, but a lack of similarity of *B. hybridum* S allelic profiles with those of *B. stacei*. Plastid analysis detected three different bidirectional allopolyploidization events: two involved distinct *B. distachyon*-like maternal ancestors and one involved a *B. stacei*-like maternal ancestor. The Southeastern (SE) Iberian Peninsula *B. hybridum* populations were more genetically diverse and could have originated from at least two hybridization events whereas Northeaster-Northwestern (NE-NW) Iberian Peninsula *B. hybridum* populations were less diverse and may have derived from at least one hybridization event. The genetic and evolutionary evidence support the plausible *in situ* origin of the SE and northern Iberian Peninsula *B. hybridum* allopolyploids from their respective local *B. distachyon* and unknown *B. stacei* ancestors. The untapped multiple origins and genetic variation detected in these *B. hybridum* populations opens the way to future evolutionary analysis of allopolyploid formation and genomic dominance and expression in the *B. hybridum* – *B. distachyon* – *B. stacei* grass model complex.

Poster

S1 Natural diversity and evolution

P02 Exhaustive cytogenetic search within perennial Mediterranean and Eurasian Brachypodium taxa untapped new cytotypes and potential new species

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In contrast to the annual lineages that show mainly ancestral phylogenetic splits, the perennial lineages span all levels of phylogenetic depth in the *Brachypodium* tree. Two allopolyploid Mediterranean species, *B. boissieri* and *B. retusum*, have shown to have up to three ancestral, intermediate and recently evolved subgenomes, whereas the remaining Mediterranean and Eurasian species belong to a recently evolved "core perennial" clade that contains both diploid and allopolyploid taxa and cytotypes. All the studied Mediterranean and Eurasian allopolyploid perennial cytotypes possess dysploid heterologous genomes, similar to those present in currently extant perennial diploids, and close but different to those in currently annual diploids. In this study we have conducted a large cytogenetic survey of perennial *Brachypodium* taxa in the western Mediterranean region and in some Eurasian localities. Through combined genome size and chromosome count analyses, we have confirmed the chromosome base numbers, genomic composition and ploidies of some taxa, have untapped some neglected cytotypes and have discovered new cytotypes. Our results indicate that: i) the widespread mesic Eurasian *B. sylvaticum* (19 populations surveyed) is exclusively a diploid showing 2n=2x=18 and a mean genome size (GS) of 0.92 pg/2C; ii) the widespread continental Eurasian *B. pinnatum* (10 populations) includes two diploids of 2n=2x=16, 0.96 pg/2C, and 2n=2x=18 , 0.86pg/2C, and an allotetraploid of 2n=4x=28, 1.48pg/2C; iii) the S Spain endemic and strict dolomitic *B. boissieri* (5 populations) is a putative hexaploid of 2n=6x=46 with a large GS of 3.1pg/2C; iv) the xeric Mediterranean *B. retusum* (19 populations) shows putative allotetraploid 2n=4x=32, 1.71pg/2C and allohexaploid 2n=6x=42, 2.37pg/2C genotypes across Spain and Morocco, and putative allotetraploid 2n=4x=32, 1.68pg/2C genotypes in S France; v) the mesic western European *B. rupestre* (16 populations) comprises diploid 2n=2x=18, 0.9pg/2C genotypes in the Caucasus, allotetraploid 2n=4x=28 1.51pg/2C genotypes in N Spain, and newly found putative allohexaploid 2n=6x=38, 2.26pg/2C genotypes in S France; vi) the xeric western Mediterranean *B. phoenicoides* (14 populations) includes allotetraploid 2n=4x=28, 1.46pg/2C genotypes, and allohexaploid 2n=6x 1.92pg/2C and 2.18pg/2C genotypes across France, Spain and Morocco. Crossing experiments (Khan and Stace, 1999) corroborated the capability of these species to interbreed. Our data suggest that each separate cytotype could correspond to a different cryptic species.

Poster

S1 Natural diversity and evolution

P03 Genomics and phylogeograhy of Brachypodium sylvaticum in western Eurasia

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Brachypodium sylvaticum has been recently selected as a model grass system for perenniality and biofuel plants. The species is broadly distributed in oceanic places across Eurasia, and is also present in humid and shady Mediterranean forests. Despite its wide geographical distribution range, its genetic diversity, structure and phylogeography have not been studied in deep along its entire native region nor with genomic data. New methodologies based on high-throughput sequencing have facilitated the investigation of taxa using large sets of genetic markers and to infer their evolutionary history. Here we have applied Genome Skimming approaches to analyse 20 populations of *B. sylvaticum* from western Eurasia, including France, Spain and Morocco. Complete sequences of the chloroplast genome (plastome) and of the nuclear ribosomal cistron and several single copy genes (Beta-amylase, DGAT, Calmodulin, Gigantea, Topoisomerase VI, Waxy) were assembled and aligned. The plastome data set was much conserved across most studied populations. The nuclear Beta-amylase, DGAT and Waxy data sets were uninformative and did not show phylogeographic signal. By contrast, Maximum Likelihood (ML) analysis of the Gigantea data set identified three diverging lineagess from Morocco, the Atlantic Pyrenees, and a mixed lineage that included populations from South of Spain and South of France. Phylogenetic results based on the rDNA cistron showed a similar topology to that of Gigantea: most of the Atlantic Pyrenean, and Middle Atlas (Rif) samples were separated into two clear clades. Calmodulin and Topoisomerase VI based analyses also corroborated those divergences. Haplotype network analyses showed two unlinked networks revealing two possible colonization routes of the western Eurasian area. The first route implies a migration from Morocco towards Northeast of the Iberian Peninsula, and the second involves unclear migration patterns between South France, central and western France, and North and South of Spain. Our preliminary results evidence the complexity of the phylogeography and populations dynamics of the recently evolved *B. sylvaticum*. More samples from eastern Eurasia and new genes will be included in future on-going studies in order to clarify the unexplored evolution of its populations.

Poster

S1 Natural diversity and evolution

P04 A RADseq phylogeography of the model circum-Mediterranean grass Brachypodium stacei

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Brachypodium stacei is the single extant diploid species representative of the basal lineages of the genus. Previous phylogenetic and biogeographic data indicated that it probably originated in the circum-Mediterranean region during the late Miocene. The species is currently distributed in a wide circum-Mediterranean range, from Macaronesia in the West to Iran in the East. Characterizing and dating the genetic structure of *B. stacei* could help to disentangle the evolutionary processes that shaped its current distribution and to identify the sources of the main lineages that hybridized to *B. distachyon* giving rise to the allotetraploid *B. hybridum*, a species model involved in thoroughly studies to uncover the evolution of polyploidy. The aim of this study was to reconstruct the phylogeography of *B. stacei* in its native range using a large number of SNPs from RADseq sequences. SNPs were called using the *denovo* option of the program iPyrad. Genomic structure was assessed with the Bayesian STRUCTURE program, bar plots of individual admixture coefficients, and Principal Component Analysis (PCA) using the R package LEA, and population relationships were reconstructed with GENEPOP, building a Fst-based NJ tree. Hierarchical allelic fixation and diversity indices were computed using the R package HIERFSTAT. Ages of divergence were estimated using SNAPP and secondary calibrations. The structure analyses suggest the existence of two main genetic groups. The first group contains eastern Mediterranean and SW Asian populations from Iran, Israel and Greece, and one western population from Menorca, whereas the second group is represented by circum-Mediterranean and Canary Isles populations. One Israelian population and one Mallorcan population contain mixed individuals from both groups. According to fixation indices, 36 % and 28% of the total genetic diversity was ascribed to significant differences among genetic groups and among populations within groups, respectively. Populations from the eastern Mediterranean + SW Asia genetic group showed higher levels of gene diversity (0.33), followed by those of a mixed group (0.28) and the circum-Mediterranean + Canarian populations (0.08). Coalescent-based dating analysis detected an early divergent event between the eastern vs circum-Mediterranean groups ~1.3 Ma, followed by a recent radiation of close Mediterranean lineages that separated from each other ~ 0.3Ma. These results point towards to the current existence of highly divergent lineages within the species.

Keywords: *Brachypodium stacei*, fixation indices, Genomic Structure, phylogeography, RADseq- SNPs, SNAPP dating analysis.

Poster

S1 Natural diversity and evolution

P05 Underscoring fungal endophytes of temperate Brachypodium and Loliinae grasses through NGS techniques: genomic diversity and host distribution

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Cold seasonal pooid grasses of subtribe Loliinae and the model genus *Brachypodium* maintain considerable systematic symbiosis with endophytic fungi of the genus *Epichlöe* (Clavicipitaceae, Ascomycota) and their asexual state (*Neotyphodium).* These fungal organisms can have mutualistic or pathogenic interactions with their hosts, according to their reproduction form. However, these interactions are often mutualistic, due to the predominant asexual state of the endophyte, and are vertically transmitted through the host seeds. Systemic endophytes contribute to the growth of plant, reduce the affection caused by pathogens and herbivores, and provide tolerance to drought and other stresses, offering better adaptation capacity for the plants to the abiotic and biotic environment. In this study, a low coverage genomic analysis was developed from *Festuca* and *Brachypodium* leaf tissue samples freshly collected in the field, dried in silica gel or taken from herbarium specimens aiming to detect and identify their endophytes and to analyse their phylogenetic relationships. Representative species from both hemispheres of the main Loliinae and *Brachypodium* lineages were selected. The sequencing was carried out through low coverage *Genome skimming* sequencing using the Illumina platform. Nine out of 46 analysed grass species (*Brachypodium sylvaticum*, *F. caldasii*, *F. camusiana*, *F. fontqueri*, *F. nigrescens*, *F. parvigluma*, *Festuca spectabilis*, *Festuca superba*, *Festuca triflora*) contained fungal sequences among their respective plant paired-end (PE) sequence reads. The mitochondrial genome and the nuclear ITS region of the endophytes were assembled from the fungal PE reads mapped to reference *Epichlöe festucae* molecules, and used in the molecular identification of each organism and in subsequent phylogenomic analysis. We used Blastn to search for the taxonomic identity of the assembled endophytic ITS sequences. We identified 7 species of *Epichlöe* and 2 species of *Neotyphodium*. *Epichlöe festucae* was present in European *Festuca nigrescens*, *F. spectabilis* and *F. triflora*, *E. elymi* in South American *F. caldasii*, *E. typhina* in NW Moroccan *F. fontqueri* and its asexual form *Neotyphodium occultans* in tropical mountain *F. camusiana* and *F. parvigluma*, and *E. sylvatica* in Eurasian *Brachypodium sylvaticum*. Phylogenetic analyses using maximum likelihood (IQTREE) and Bayesian (BEAST) methods were performed with the aligned mitochondrial and ITS data sets and a collection of ingroup and outgroup (ITS) sequences downloaded from Genbank. The best resolved ITS trees showed a separate clade of *Epichloe typhina* and *E. sylvatica* sequences, including those obtained from *Brachypodium sylvaticum*, and a clade of *E. festuca* sequences, including those obtained from the European fescues. Endophytic sequences from palaeo and Neotropical fescues fell within a clade of *E. bromicola* strains. Our restricted phylogenies do not support a clear plant-endophyte co-evolutionary pattern though some fungal clades are grass lineage-specific.

Poster

S1 Natural diversity and evolution

P06 Phylogenetic reconstruction of Brachypodium using genome skimming data

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The model system *Brachypodium* is a worldwide distributed genus nested in an intermediate evolutionary position within the pooid clade. *Brachypodium* contains ca. 20 species (3 annuals, 17 perennials) that show a complex evolutionary reticulate history of isolated and interbreeding diploid and allopolyploid lineages. The annual and short-rhizomatose perennial species correspond to the most ancestral diverging lineages whereas the long and strong-rhizomatose perennial species lineages belong to more recently evolved lineages. The phylogenetic relations of the *Brachypodium* species and the identities of the subgenomes of the allopolyploid taxa are not well known, especially within the perennials. Consecutive hybridization and genome doubling events between diploids and lower allopolyploids have been hypothetised to have originated the higher allopolyploids. Different ploidies have been detected in several perennial *Brachypodium* species, like in *B. boissieri* (*6x-8x*), *B. hybridum (4x), B. mexicanum (4x), B.phoenicoides* (*4x, 6x*), *B. pinnatum* (*2x, 4x*), *B. retusum* (*4x, 6x*), and *B.rupestre* (*2x, 4x, 6x*). We have used new high-throughput genome skimming sequencing methods to elucidate the evolutionary history of the perennial *Brachypodium* tree. The genomes of six diploid and eight polyploid *Brachypodium* samples, corresponding to 10 species and 4 intraspecific cytotypes, were sequenced in the Illumina platform. Paired-end sequence reads from these samples were used to assemble their respective plastomes, nuclear ribosomal cistrons and six nuclear single-copy genes (B-amylase, CAL, DGAT, GI, TOPOVI, Waxy). The assembly and alignment process included the mapping of the allopolyploid reads to a concatenated sequence created with the sequences of the diploid species followed by syntenic multiple alignments, aiming to retrieve the potential heterologous copies of the allopolyploids. Exploratory phylogenetic analysis performed through maximum likelihood (IQTREE) and Bayesian (Beast) searches recovered the same diploid skeleton tree in most cases. The plastid tree identified the maternal subgenomes of the allopolyploids *B. boissieri* (*B.distachyon*-type) and *B. phoenicoides* 554 (*B. arbuscula*-type) and other intermediate cases. The rDNA cistron tree showed the ancestry of the *B. mexicanum* and *B. boissieri* subgenomes and the recent origin of those of *B. retusum* (*B. arbuscula*-type) and *B. phoenicoides* (*B. sylvaticum*-type). Alternative but complementary resolutions were obtained from the single-copy nuclear trees. Most of them recovered ancestral copies for *B. mexicanum*, ancestral, intermediate and recently evolved copies for *B. boissieri* and *B. retusum*, and majoritarily recently evolved copies for tetraploid and hexaploid *B. phoenicoides* and tetraploid *B. pinnatum*.

Attendant

S1 Natural diversity and evolution

P39 Brachypodium distachyon root architecture in relation to 18 grass family members: variation to exploit with non-destructive phenotyping of allocation to seed and stem borne roots

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Grasses have high flexibility in the timing and location of root system architecture, possibly contributing to their global geoclimatic success. Grass root systems have roots borne from the seed (seminal roots), and roots borne from the stem (nodal roots, crown roots or adventitious roots). Allocation among seminal and nodal root types controls root system architecture macrostructure. It shifts with age as nodal roots emerge, but also strongly with environment because nodal roots generally are suppressed or promoted with soil resources. Dicotyledons in contrast, form one main seed borne root (tap root or primary root), and very few stem-borne roots. Brachypodium is a therefore a valuable model for root architecture for the major crops that are Grasses, like the cereal maize, rice, wheat, sorghum and millet; filling a gap beside Arabidopsis and Medicago as small, genetically-tractable, model plants (1).

We established the extent of variation across Grasses for allocation among seed and stem borne roots, to exploit in genetic improvement beyond Bd-21. Nineteen Grasses were grown in long tubes of soil, and shoot parameters, tillering, and seminal and nodal root number and length were measured at three time points. Four-fold variation in the ratio of nodal to seminal root number exists across Grass species, with Bd-21 approximately at the midpoint of the variation. The warm climate Grasses had the highest ratio (rice highest); and Triticum spp. the lowest (*T. urat*ulowest). Higher ratio of nodal roots is partly driven by: i. the development of a singular primary, seminal root (for rice, millets, sorghum, maize, ryegrass, tall fescue and Brachypodium); ii. smaller seed size (not for maize, sorghum and rice). A phylogenetic tree was drawn using all root phenotypes. This shows pan-genomic targets for shifting root architecture through seed versus nodal roots, which seem to be associated in part with climatic origins.

Brachypodium is an excellent model for phenotyping seminal and nodal root allocation because it is small, and plants can be grown to the adult stage in manageable pot sizes (2). Non-destructive rhizotron phenotyping in large boxes of soil quantifies the root architecture of Brachypodium and other grass genotypes past nodal root stages (3). This platform is being upgraded to automatically image up to 800 rhizoboxes, along with shoot growth. It will be extremely interesting to contrast Foxtail millet and Brachypodium sources as a starting point for designing root system architecture in cereals crops based on seed and stem borne root allocation.

(1) Chochois V et al. 2012. Journal of Experimental Botany63: 3467

(2) Chochois V et al. 2015. Plant Physiology168: 953

(3) Nagel KA et al. 2012. Functional Plant Biology39:891-904

Poster

S2 Comparative genomics and transcriptomics

P07 The SNF2 family of chromatin remodelers is conserved in *Brachypodium distachyon* and involved in the response to combinatorial abiotic stresses

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Chromatin structure plays an essential role in the regulation of nuclear processes. To regulate the access to DNA, different chromatin remodeling complexes appeared early during evolution and are well conserved in plants, animals and fungi. Among them, SNF2 helicases are DNA-dependent ATPases able to dynamically alter DNA-histone interactions to overcome the nucleosome barrier and increase accessibility to DNA. In plants, this family has been described in different species and at least some of its members have been shown to be important regulators of development, genome structure and stability and the response to biotic and abiotic stresses. However, we are still far to understand the complexity of this family and their specific roles in regulating plant adaptability under different environments. Here we present the description of the SNF2 family of *Brachypodium distachyon* (BdSNF2) and the evolutionary relationship of the family in different model plant species to provide a platform for future functional analysis of the family. The high conservation of the BdSNF2 family suggests that these proteins may be key regulators of plant and embryonic development, defense against pathogens, hormone signaling and response to abiotic stresses. The analyses of transcriptomic data for the members of the BdSNF2 family revealed that half of them were differentially expressed in response to the combination of different abiotic stresses, but not by single ones. This result indicates that SNF2 ATPases may be good targets to modify plant adaptation in response to multi-dimensional environments mimicking on-field conditions. Hence, our phylogenetic and expression analyses may contribute to establish the foundation for further analyses of the SNF2 family in temperate cereals.

Poster

S2 Comparative genomics and transcriptomics

P08 Optimisation of an *Agrobacterium-delivered* CRISPR/Cas9 system for targeted mutagenesis in Brachypodium species

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The CRISPR/Cas9 system enables precise genome editing and thus it is important in many functional genomic studies. However, the *Agrobacterium*-mediated transformation process of *Brachypodium* species is laborious and time-consuming. Because some of the gRNA, which should recognise the target site in a genome, may be non-functional, it is important to check their functionality using protoplasts assay prior to any experiment.

In our research, we used the CRISPR/Cas9 technology for a targeted mutagenesis of the genomes of *Brachypodium* species. We targeted three genes encoding *cyclin-dependent kinase G1* (*CDKG1*), *cyclin-dependent kinase G2* (*CDKG2*), and *pectin methylesterase* (*PME*). For each gene, gRNAs were designed that target unique sequences within the genome. Bioinformatics tools were used to assess the likelihood of an off-target mutation (http://www.rgenome.net/cas-offinder/). For ease of analysis, each gRNA had a restriction enzyme target site at the Cas9 cleavage site. We used vectors that had previously been optimised for monocots with codon-optimised Cas9 and the relevant gene promoters (Cell Res, 2013, 23, 1233). The gRNA sequence and the gene encoding endonuclease were introduced to the destination vector using Gateway Recombination Cloning Technology (Invitrogen, Carlsbad, CA, USA). The vectors that were prepared were initially tested using a protoplast transient assay. The protoplasts from *Brachypodium* leaf mesophyll cells were isolated using the protocol described by Jung et al. (Methods Mol Biol, 2015, 1284, 433) with minor modifications. Then, polyethylene glycol (PEG) mediated transfection experiments were performed. After two days of incubation, the genomic DNA from the protoplasts was isolated using the CTAB method. Subsequently, the extracted DNA was cleaved with a suitable restriction enzyme and then an enrichment PCR reaction, which preferentially amplifies mutated (i.e. not digested) DNA sequences, was performed. The PCR products that were obtained were then cloned and verified using DNA sequencing. The selected vectors, which were capable of editing the protoplast genome, were used to transform a *Brachypodium* callus. The *Agrobacterium*-mediated *Brachypodium* transformation was performed according to the protocol published by Alves et al. (Nat Protoc, 2009, 4, 638). Because the hygromycin resistance gene was present within the T-DNA part of the vector, the transformed callus was selected on a medium to which hygromycin had been added. The plants that were obtained were analysed to determine the occurrence of a mutation using PCR/restriction enzyme assays. The *Agrobacterium*-delivered CRISPR/Cas9 system has not only been successfully used for gene editing in the diploid *B. distachyon* but also in *B. hybridum*, which is an allotetraploid species.

This research was funded by the National Science Centre Poland (grant DEC-2014/14/M/NZ2/00519).

Poster

S3 Development and growth

P09 Biosynthesis of Brassinosteroids in *Brachypodium distachyon*

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GC-MS/SIM analysis revealed the presence for castasterone (CS) and its biosynthetic precursors such as campesterol (CR), campestanol (CN), 6-deoxocathasterone (6-deoxoCT), 6-deoxoteasterone (6-deoxoTE), 6-deoxotyphasterol (6-deoxoTY), 6-deoxocastasterone (6-deoxoCS), teasterone (TE) and typhasterol (TY) in *B. distachyon*. A crude enzyme solution prepared from *B. distachyon* successfully catalyzed almost all biosynthetic reactions involved in CN-dependent and CN-independent pathway for brassinosteroids (BRs) biosynthesis, indicating that both CN-dependent and CN-independent pathway are operant to generate BRs in the plants. Brassinolide (BL) known as the most biologically-active BR was not identified from *B. distachyon*. Additionally, the crude enzyme prepared from *B. distachyon* could not mediate conversion of CS to BL. Further, heterologously expressed Brachypodium Cytochrome P450 85A1 in yeast (BdCYP85A1/V60/WAT21) did not show BL synthase activity for conversion of CS to BL. These demonstrate that the biologically-active BR in *B. distachyon* is CS, but not BL. BdCYP85A1/V60/WAT21 catalyzed conversion of 6-deoxoTE to TE, 6-deoxo-3-dehydroTE to 3-dehydroTE, 6-deoxoTY to TY and 6-deoxoCS to CS, demonstrating that BdCYP85A1 has BR 6-oxidase activity. Phylogenetic analysis revealed that BR biosynthetic genes in Brachypodium is much closer than those in rice than Arabidopsis, suggesting that knowledge on biosynthesis of BRs in Brachypodium can be directly useful in development of high-productive monocotyledonous cereals such as rice, rye and wheat. In this presentation, biochemical function of other BRs biosynthetic genes such as *BdDET2*, *BdCYP90A1*, *BdCYP90B1* and *BdCYP90D1* will be also discussed.

Poster

S3 Development and growth

P10 Optimizing *Brachypodium* growth with light-emitting diodes

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Plants evolved to use light from the sun, yet much plant research relies on artificial lighting supplied by fluorescent bulbs. While sunlight provides a continuous spectrum of visible light (along with UV and infrared), typical fluorescent bulbs only emit several spectral peaks in blue, green, and yellow. Light-emitting diodes (LEDs) have many advantages over fluorescent lighting. Like sunlight, LEDs can emit a continuous spectrum and provide spectral peaks that align with wavelengths most efficient for photosynthesis. Additional technological advantages of LEDs include greater energy efficiency and longer bulb lifespan that maintain a constant spectrum. We sought to find a light composition favorable for *Brachypodium distachyon* growth and cultivation. *Brachypodium* accessions with a range of vernalization requirements were grown under various continuous spectrum and single wavelength commercial LED fixtures. *Brachypodium* displayed a high degree of morphological and developmental plasticity under different light sources. Two LED fixtures with similar spectra were identified that resulted in compactness, increased tillering and spikelet initiation, and more rapid flowering. Importantly, seed count and seed fill were not compromised, and in some accessions even showed improvement. Higher light intensities produced shorter and compact plants, whereas red and blue light influence flowering time. Our results show certain LED lighting can enhance traits desirable for plant genetics such as compactness, fecundity, and generation time. Plants evolved to use light from the sun, yet much plant research relies on artificial lighting supplied by fluorescent bulbs. While sunlight provides a continuous spectrum of visible light (along with UV and infrared), typical fluorescent bulbs only[KM1][KM2] emit several spectral peaks in blue, green, and yellow. Light-emitting diodes (LEDs) have many advantages over fluorescent lighting. Like sunlight, LEDs can emit a continuous spectrum and provide spectral peaks that align with wavelengths most efficient for photosynthesis. Additional technological advantages of LEDs include greater energy efficiency and longer bulb lifespan that maintain a constant spectrum. We sought to find a light composition favorable for *Brachypodium distachyon* growth and cultivation. *Brachypodium* accessions with a range of vernalization requirements were grown under various continuous spectrum and single wavelength commercial LED fixtures. *Brachypodium displayed* a high degree of morphological and developmental plasticity under different light sources. Two LED fixtures with similar spectra were identified that resulted in compactness, increased tillering and spikelet initiation, and more rapid flowering. Importantly, seed count and seed fill were not compromised, and in some accessions even [KM3]showed improvement. Higher light intensities produced shorter and compact plants, whereas red and blue light influence flowering time. Our results show certain LED lighting can enhance traits desirable for plant genetics such as compactness, fecundity, and generation time.

Poster

S3 Development and growth

P11 Comparative analysis of *Brachypodium* distachyon anatomical features with Kazakhstani wheat varieties upon infection with brown leaf rust

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Production of high-quality wheat grain in Kazakhstan is an important strategic direction contributing to the stabilization of agriculture, ensuring the country\'s food security and a decent position in the club of grain exporters on the world market. One of the most frequent sources of its loss is *Puccinia recondita* or brown (leaf) rust, with epiphytoties leading to the loss of up to 60% of crop yield. Our scientific group is the first in Kazakhstan, engaged in detailed studies on the use of *Brachypodium distachyon* as a model object for studying changes caused by the brown rust pathogen. One of the aspects of our study is the comparative analysis of its anatomical features with Kazakhstani wheat varieties.

Seeds of *B. distachyon* (Bd21 line) were obtained from the RIKEN BioResource Center, Japan. In the tillering phase, the plants of the experimental variant were inoculated with urediniospores, while control consisted of untreated plants. SRI of Biological Security Issues, MES RK, provided us with Kazakhstani population of *P. recondita*. Identification of morphometric features was carried out in accordance with Ermakov (2007), anatomical – in accordance with Barykina (2004) conventional methods.

Our experiments have shown that infected flag leaves have a statistically significantly thickened blade in the area of vascular bundles by 23%. The diameter of the conducting bundles of the leaf blade decreases by 13% (control – 88.39±2.00 µm, experiment – 76.19±2.71 µm). Diameter of the xylem vessels increases by 24% compared to the control (control 17.97±3.87 µm, experiment – 22.36±5.25* µm). Thickening of the stem and vessels relatively to control by 1.5 and 1.1 times in infected plants was noted. In the roots of Bd21, two anatomo-topographic zones are distinctly distinguishable: the primary cortex and the central cylinder. The primary root bark of the control plants consists of rounded parenchyma cells with intercellular spaces. In infected plants, the primary cortex is not developed, practically reduced. The inner layer of the cortex is differentiated into densely closed endoderm cells. Primary conducting tissues of the root form a complex bundle in which radial strands of xylem alternate with elements of phloem.

The wheat varieties were picked because of their degree of resistance and susceptibility to *P. recondita*, 15% of infection for Kazakhstanskaya 19, and 40% for Kazakhstanskaya early. On some of the organs noticeable changes were observed. In particular, in the internal structure of the roots of the Kazakhstanskaya 19, as in the infected leaves, the thickness of the primary cortex increased by 1.11 times, while in Kazakhstanskaya early, on the contrary, the size of the primary cortex reduced by 1.36 times.

In addition, 15 potentially informative morpho-anatomical characteristics were identified that can be used to identify Brachypodium distachyon cytotypes. Research is funded by MES RK project AP05134104/SF4

Poster

S3 Development and growth

P12 Using *Brachypodium* as a model to study key genes regulating reproductive development in temperate grasses

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MADS-box transcription factors (TFs) are central to the ABCDE model for flower development where C-lineage genes are involve in stamen and carpel specification and D-lineage genes play essential roles in ovule specification and development. In our project, we are studying two members within each of the C and D classes (BdMADS3, 58, 13 and 21) from *Brachypodium distachyon. B. distachyon* is an annual grass that has been established as a reference model to study temperate grasses and is closely related to economic valuable crop species, including, wheat and barley. The main goal of this project is to investigate the gene expression patterns (temporal and spatial) throughout development and the phylogeny of BdMADS3, 58, 13 and 21 transcription factors in *B. distachyon* in comparison to other cultivated cereals.
Our work provides more details about the expression of C and D class genes during flower and grain development in temperate grasses/cereals and reveals points of similarity and difference between *B. distachyon* and *Triticum aestivum*. The variation between the two members of the family could suggest functional divergence within the *Poaceae*.

Poster

S3 Development and growth

P13 SVP-like genes are responsive to vernalisation and ambient temperature in *Brachypodium distachyon*

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SVP genes are known to regulate the floral transition in *Arabidopsis thaliana* and floral meristem identity in *Arabidopsis* and barley. In this project, we show that an *SVP*-like gene, *BdVRT2*, may also be involved in regulating the floral transition through the vernalisation and ambient temperature-dependent pathway. *BdVRT2* expression is upregulated during vernalisation and phenotyping of T-DNA mutant lines shows that plants with a knock out of *BdVRT2* flower significantly later than null sibling controls indicating a function as a flowering promotor. To test whether *BdVRT2* functions in the ambient temperature-dependent flowering pathway, T-DNA mutant plants were grown under different ambient temperature conditions. Flowering time of the *BdVRT2* mutant plants was extremely delayed under warm temperatures (24°C) and the late flowering phenotype of these plants seems to be more severe with increasing temperature. We hypothesize that *BdVRT2* functions as a promotor of flowering at high temperature however to our knowledge, the accession Bd21-3 has no or a very minor flowering response to increasing temperature. We speculate that this might indicate the existence of a repressive, antagonistic pathway to *BdVRT2* which leads to flowering at an optimum temperature, unlike the linear flowering response of *Arabidopsis* to temperature.

Poster

S3 Development and growth

P14 The conservation of the cell-type-specific communication during lateral root formation from *Arabidopsis* in *Brachypodium distachyon*.

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The challenge to produce more food for a rising global population is becoming an increasing issue. Crop plants, particularly cereals, are a vitally important food supply, both directly and through use in animal feed: an estimated 75% of the human energy demand is fulfilled by cereal starch. Plant root architecture is crucial for plant development, as it provides a mechanism to explore the soil environment for water and nutrients. This critically affects development, biomass and yield. Lateral root (LR) formation is the major determinant of root system architecture. Understanding the regulation of LR development is therefore of vital agronomic importance. Studies in *Arabidopsis thaliana* have revealed that cellular communication is crucial for proper LR development. Especially the interaction between the pericycle and the endodermis appears to be essential. To accommodate the growing LR, the endodermis undergoes a dramatic volume loss, accompanied by altered cell morphology, without losing membrane integrity. Moreover, the plant locally degrades the Casparian strip (CS), a lignified primary cell wall modification. In contrast, the cortex and epidermis are pushed away after their middle lamellae have been degraded by cell wall remodeling enzymes. Cereals such as wheat, rice and maize have much larger roots displaying multiple layers of cortex that provide additional mechanical constraints that need to be overcome during LR development. Although they are well-studied, still little is known about inter cell layer communication and they integrate chemical and mechanical signals during LR development. *Brachypodium distachyon* is a promising alternative for LR studies in monocots due to its relatively rapid life cycle, small stature and simple growth requirements. We are now characterizing LR development in *Brachypodium* to test to what extend the molecular mechnanisms described in *Arabidopsis* are conserved with a particular interest for the pericycle-endodermis interaction. To this end we are combining histology, live-cell imaging and cell type-specific transcriptomics to address these questions. Preliminary results from whole mount studies obtained using the *CLEARSEE* protocol combined with the fluorescent stains such as Calcofluor White, Basic Fuschin and Nile Red coupled with multiphoton excitation microscopy, are being employed to construct a developmental atlas of LR development in *Brachypodium*.

Poster

S3 Development and growth

P15 Contrasting developmental plasticity in response to nitrate availability in two *Brachypodium distachyon* accessions

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Previously, studies in *Arabidopsis thaliana* have shown that nutritional nitrate availability affects the developmental process of axillary shoot branching[1], and furthermore, the degree to which is does so is indicative of the existence of contrasting nitrate sensitive and insensitive accessions in *Arabidopsis*. A phenotypical screen was performed using several accessions at high and low nitrate feeding regimes, to deduce if similar behaviour occurs in different accessions of the model monocot *Brachypodium distachyon*. From this, two accessions (Bd21 and Bd3-1) were identified (Bd21 and Bd3-1) as having differing levels of developmental sensitivity to nitrate, both in terms of shoot branching (tillering) and other phenotypical traits such as height at senescence. Furthermore, N and C content analysis revealed differences in resource allocation between these accessions.

A F6:F7 Bd21 x Bd3-1 recombinant inbred mapping population[2] was screened to identify the genomic loci associated with nitrate plasticity in developmental responses. Approximately 150 lines were screened for flowering time, tillering, greenness of flag leaf (SPAD), senescence time, height at senescence, and seed yield.

Additionally, CRISPR/Cas9 gene editing was used to generate mutants in *Brachypodium* for several targets known to be involved in *Arabidopsis* shoot branching, such as the strigolactone synthesis and signalling pathway components MAX3, MAX4 and D14, to identify whether they similarly control tillering in monocotyledonous systems.

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Poster

S3 Development and growth

P16 Role of the NBCL genes in *Brachypodium distachyon* development

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In cultivated grasses, tillering, inflorescence architecture and abscission are major agronomical traits. In barley and maize, *NOOT-BOP-COCH-LIKE* (*NBCL*) genes have been shown to be involved in vegetative and reproductive development. In comparison to dicots, the role of grass *NBCL* genes is largely under investigated and in barley and maize, *NBCL* loss-of-function mutants present different phenotypes, especially in tillering and in blade-sheath boundary region patterning. To better understand the role of these genes in grasses and to overcome domestication effects that can conceal the original role of some genes, we studied TILLING *nbcl* mutants in the non-domesticated grass *Brachypodium distachyon*. In *Brachypodium*, *BdUNICULME4-LIKE* (*BdCUL4-LIKE*) and *BdLAXATUM-A-LIKE* (*BdLAXA-LIKE*) are orthologous to barley *HvUniculme4* and *HvLaxatum-a*, respectively, and to maize *Zmtassels replace upper ears1* and *Zmtassels replace upper ears2*, respectively. Our results show that *BdCUL4-LIKE* is required for ligule and auricle development, and plays positive roles in tillering and spikelet determinacy. Moreover, *BdLAXA-LIKE* plays a negative role in tillering, promotes spikelet relaxation, and is involved in the control of floral organ number and identity. The characterization of new *nbcl* mutants in a non-domesticated grass context highlights original roles for grass *NBCL* genes related to important agronomic traits.

Poster

S3 Development and growth

P17 The Sowing of *Brachypodium retusum* in quarries restoration

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The final morphology of the areas affected by mining is structured as a sequence of slopes. Herbaceous sowing has been widely used to superficially stabilize these slopes and determine a first phase of landscape and environmental integration for these altered areas.

One of the objectives considered in the selection of herbaceous species is precisely to present fast growth and obtain high cover. In the restoration, it is common to use commercial herbaceous mixtures. However, the persistence of these species is usually brief, giving way to colonization by the local vegetation species. The use of native herbaceous species, with greater persistence, can associate several objectives of ecological restoration: erosion control, control of weeds and promotion of plant succession. This may be the case of *Brachypodium retusum*.

The objective of the study is to know the viability of the introduction by sowing of *B. retusum* as a complement or in substitution of commercial species.

The applied treatments have been: irrigation vs non-irrigation, and sowing of *B. retusum* with commercial sowings vs sowing only with *B. retusum*. In total, 20 plots of 5 m2 were established with 5 replicates for each combination of the two factors.

The vegetation cover has been obtained in 5 m permanent transects for each plot (Gounot, 1969). For the study of survival and development of *B. retusum*, 4 permanent zones of 625 cm2 have been established, where the number of germinations, the number of live seedlings and the number of tillers were counted. The dry aerial biomass was obtained in 5 surfaces of 625 cm2, in the first year and after 5 years.

The irrigation or the presence of other species no affect to the total germination of *B. retusum*. The plots with irrigations show less germination. During the field studies it was observed that the excessive energy of the irrigations applied negatively affected the evolution of the young seedlings.

The cover of *B. retusum* is low during the first months after sowing and there are no differences between sowing compositions. Five months after sowing, the *B. retusum* cover is superior in monospecific crops.

However, the aerial biomass of *B. retusum* has increased significantly after 5 years but there is no clear effect of the presence of commercial species in this evolution.

Poster

S4 Tolerance and adaptation to abiotic stresses

P18 Comparative structural analysis of the drought responsive dehydrin and aquaporin gene families in *Brachypodium* and close grasses

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Dehydrins (DHNs) belong to the group 2 LEA (Late Embryogenesis Abundant) genes and play an important role in the response of plants to abiotic stress, mainly heat, salinity and drought. Under these stresses, DHNs accumulate to a large extent in maturing seeds and in all vegetative tissues. As many studies reveal, there is a positive correlation between DHN gene expression (synthesis of DHN proteins) and plant stress tolerance. Aquaporins (AQPs) belong to the major intrinsic protein (MIP) superfamily of membrane proteins conserved in plants and animals as well as in bacteria. Supporting evidence suggests that AQPs have an important role in stomatal closure and circadian regulation. There are more than 150 MIPs identified and, although some of them are constitutively expressed, others are regulated in response to drought and salinity.

In this study, sequence and annotation data has been retrieved from Phytozome and Ensembl Plants in order to compare DHNs and AQPs in four *Brachypodium* species, 54 *B. distachyon* varieties and five cereals (Zea *mays*, Sorghum *bicolor*, Oryza *sativa*, Hordeum *vulgare* and *Triticum aestivum*).

In the *B. distachyon* intra-species comparison, drought tolerant lines seem to contain slightly shorter aquaporin and dehydrin genes, and some of them are dissimilar to those of the rest. The physical distribution of AQPs includes a cluster of genes that splits the lines into two main groups depending on its location, either in chromosome 3 or in chromosome 4 of this species. However, this does not seem to be related to drought stress susceptibility.

A phylogenetic analysis of the dehydrin gene family shows a close relation between the *B. stacei* and *B. hybridum* – S subgenomes, followed by the *B. distachyon* and B. *hybridum* –D subgenome, and *B. sylvaticum*. However *B. sylvaticum*, typical from humid environments, contains nine DHNs, four of which are not close to any of the nine DHNs present in the other Mediterranean *Brachypodium* species. A deeper analysis of orthologs shows that a DHN in *B. distachyon* chromosome 1 is not related to those of *Sorghum* or *Oryza* and that there are clusters of DHNs in chromosomes 5 and 6 of *Triticum aestivum* related to those of chromosomes 3 and 4 of *B. distachyon*. Only one DHN in *Brachypodium* spp is found in all the analyzed grass species with the exception of *Hordeum vulgare*.

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Poster

S4 Tolerance and adaptation to abiotic stresses

P20 Trials to develop the *Brachypodium* resources in RIKEN BRC

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In Experimental Plant Division, RIKEN BioResource Research Center (BRC), we collect huge number of *Arabidopsis thaliana* resources, such as knockout mutants and EMS mutants. We also collect the overexpressor and natural accessions, which can be used as an individual line and also screening pools. We preserve and distribute these resources to research communities with appropriate quality controls. We also collect the Full-length cDNA clones of many kinds of model plants species, and plant cell cultures (https://plant.rtc.riken.jp/resource/index.html).

From 2013, we started distributing of the *Brachypodium* seeds (*B. distachyon*, Bd21). We also distribute the nearly 40 thousand of *Brachypodium* Full-length cDNA clones (developed by Dr.Mochida in RIKEN CSRS). Recently, we started distributing the embryogenic *Brachypodium* callus for domestic use in Japan to assist the genetic transformation. So far, we have generated several *Brachypodium* transformants by overexpressing *Arabidopsis* drought tolerant-related genes to bridge *Arabidopsis* information to *Brachypodium*. Last year, plant-microbe symbiosis research and development team was newly established in RIKEN BRC to understand regulatory mechanism behind the plant-microbe symbiosis and provide a research platform leading to industrial applications.

In this meeting, we report our recent trials to perform the genome editing using CRISPR/Cas9 system in *Brachypodium*. We would discuss our future targets to knock out such as *Brachypodium* orthologs of functional genes in environmental stress response and plant-microbe interaction, etc. We also report the development of the suspension cell cultures of Bd21 line. We are now in preparation for distributing this cell line to the research community. In addition, we are starting the development of EMS mutagenesis using the inbred *Brachypodium* line with 11 generations of selfing. Finally, we present our future plans including the analysis of the interaction between *Brachypodium* and microbial symbionts. Any suggestions and comments are welcome.

Poster

S4 Tolerance and adaptation to abiotic stresses

P21 Whole plant phenotyping and molecular identification of Zn transporters during Zn deficiency and excess in the monocotyledon plant model *Brachypodium distachyon* at two developmental stages

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Zinc (Zn) is an essential micronutrient for plants and around two billion people are depending on legumes and grains as their main Zn source. This transition metal is however toxic for plants at high concentrations in soils and affects biomass and yield. The eudicot model plant *Arabidopsis thaliana* has already been characterized for Zn homeostasis under various Zn regimes and recently we have investigated the transcriptome, proteome and ionome upon Zn deficiency and re-supply in this species (Arsova, B. *et al*., 2019, *bioRxiv*, 600569). Although some important players of Zn uptake and transport, such as ZRT, IRT-like Protein (ZIP) family genes, have been studied in wheat, rice and barley, it is unclear how different or similar are the responses of monocots and dicots to Zn supply at the molecular level. In the current study, we aim at characterizing Zn regulation in the model plant *Brachypodium distachyon*. For this, 10-day-old *Brachypodium* plants were treated with high- and low-Zn medium for two and three weeks. We measured root length, shoot weight and leaf area to examine how Zn supply influences *Brachypodium* morphology. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analysis was performed to determine Zn and other metal ion distribution in root and shoot under different Zn treatments. Quantitative RT-PCR analysis of 20 genes orthologous to *Arabidopsis* Zn homeostasis genes revealed their expression pattern in response to Zn depletion and surplus. We observed the most commonality between *Brachypodium* and *Arabidopsis*, as well as the highest correlation between Zn content and gene regulation, for ZIP genes. In general, our results show that comparative molecular study can be useful to reveal the Zn regulatory mechanisms in monocots, which can lead to new Zn biofortification and stress resistance strategies in vital grains.

Poster

S5 Regulatory elements, networks and epigenomics

P22 GRASS NAC REPRESSOR OF FLOWERING is a transcriptional repressor that forms a negative feedback loop with SWAM1 to control secondary wall thickening in *Brachypodium distachyon*

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Xylem, fiber, and other cell types are surrounded by a thick secondary cell wall largely comprised of cellulose, hemicelluloses, and lignin. A large network of mostly MYB and NAC transcription factors controls the biosynthesis and deposition of these polymers.We identified *Brachypodium distachyon GRASS NAC REPRESSOR OF FLOWERING* (*GNRF*) as a potential regulator of secondary cell wall biosynthesis based on gene expression, protein-DNA interactions, phylogeny, and transgenic and mutant plant phenotypes. Stable overexpression of *GNRF* in *B. distachyon* and transient expression in rice protoplasts resulted in repression of secondary wall *CELLULOSE SYNTHASE A* (*CESA*) genes. Those same genes were upregulated in *gnrf* mutants, *gnrf-6* with a T-DNA insertion in the 5'UTR and *gnrf-4* which has a point mutation that introduces a stop codon in the first exon. In yeast, GNRF protein interacted upstream of *CESA* genes enriched for the VNS element, C(G/T)TNNNNNNNA(A/C)G, which we identified as a binding motif by DNA affinity purification sequencing. Using similar approaches and datasets we resolved that GNRF and SECONDARY WALL ASSOCIATED MYB1 (SWAM1) form a negative feedback loop where SWAM1 protein activates cell wall genes and *GNRF* expression and in turn GNRF protein represses *SWAM1* and cellulose gene expression. GNRF is an ortholog of *Arabidopsis thaliana SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN2* (*SND2*) and simple diploid eudicots have a single copy. Phylogenetic analyses strongly suggest that *SND2* was duplicated in the lineage of grasses, but there is only one copy in the Pooideae which includes *Hordeum vulgare* and *B. distachyon*. That copy appears to function as a repressor of cell wall thickening, while the other copy has been described as an activator as it has been described in *A. thaliana*.

Poster

S5 Regulatory elements, networks and epigenomics

P23 Progress in the recognition process between chitosan and *Brachypodium* root tissue

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Plants can counter pathogens' attacks thanks to resistance proteins, which function as a *pattern recognition receptor* (PRR), and which are often receptor kinases (Couto and Zipfel, 2016) in plants' immune system. Plants recognize pathogens either through the *pathogen-associated molecular pattern* (PAMPs), or through the molecules that are released during a host tissue damage or DAMP (*danger-associated molecular pattern*). The recognition process activates the resistance mechanisms in plants. From this model, we use chitosan compounds, *inter alia*, to elicit plants' biological control of pathogens. The interaction of this effector with *Brachypodium* triggers a cascade of metabolic reactions and end products, including peroxidase families. These can be easily analysed in the root exudates. If chitosan behaves like PAMP units, elements like the interaction mode of this effector and its hypothetical receptor, or the molecular mechanisms that underlie the recognition process, remain unclear. Similarly, the relationship between recognition and the cascade reaction leading to the expected biological effect is rather vague. Nevertheless, we observe that the concentration of chitosan matters. In practical terms, root architecture varies in a characteristic and durable way according to the initial chitosan content in its medium. From this, scanning electron microscope analyses show that the effector adopts different patterns depending on its concentration. On this basis, *Brachypodium*'s root tissue sections are subjected to different concentrations of effector, and analysed by monoclonal antibodies. These experiments evidence that the effector displays a strong affinity for the galacturonan domain of pectic polysaccharides, which contrasts with the affinity for other epitopes, such as arabinan domains and galactan domains. Root tissue from wheat, rice, and maize corroborate these observations. In order to compare the structure of the epitopes and the differences observed in docking events, we model putative structures of these different glycans using sugar building software (Emsley et al. 2015; Woods Group, 2005-2017). In this work in progress, we analyse all these data, in the aim of deciphering the recognition process between the effector and the root tissue in *Brachypodium*.

Poster

S5 Regulatory elements, networks and epigenomics

P24 Impact of intra-specific TE variations on local epigenetic states and gene expression

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Transposable elements (TEs) are mobile DNA sequences which have the capacity to replicate and/or move from one location to another in their host genome. TEs are the main driver of genome size expansion in Eukaryotes but can also alter gene expression and function. Host genomes have yet developed various mechanisms to silence TE transposition and counteract their potential deleterious effects. These mechanisms include DNA and histone methylation, as well as heterochromatin formation and changes in the spatial organization of the nucleus. TE polymorphisms provide thus a great potential for modulating gene expression and genome organization. Today, bisulfite sequencing is regarded as the gold standard method for DNA methylation studies. We sequenced 11 different accession of the model grass *Brachypodium distachyon* and used publicly available data from the model plant Arabidopsis thaliana. Our results are indicating striking differences in genome organization in regard to TEs localization. Consequently also the DNA methylation silencing differs between the two studied plants. To asses the consequences of these epigenetic differences, we performed in parallel for the two plants a transcriptomic analysis. First results are indicating a diverse impact of TE and methylation status to neighboring genes between the two organisms. Overall, we demonstrate that genome organization, epigenetic status and gene expression are similarly diverse as the TE landscape of the organism.

Poster

S5 Regulatory elements, networks and epigenomics

P25 Regulation of flowering time in *Brachypodium distachyon*

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In higher plants, phase transition from vegetative to reproductive development is tightly coordinated through networks that integrate internal and external stimuli. PANICLE PHYTOMER2 (PAP2) is a SEPALLATA-like protein that plays an important role in regulating rice meristems and floral organ identity (Kobayashi et al., 2012). It promotes the phase changes of the shoot apical meristem from vegetative to inflorescence meristem, acting together with AP1-like proteins to negatively regulate *RCN4* (*TFL1* orthologue) in a conserved pathway between *Arabidopsis thaliana* and rice (Liu et al., 2013). In this work, we study the role of the *Brachypodium distachyon* gene BdPAP2 in regulating flowering time. In order to achieve this goal, we first over-expressed *BdPAP2* in *A. thaliana* plants. Transgenic plants showed an earlier transition from vegetative to reproductive state and, consequently, developed fewer rosette leaves than control plants. This phenotype was correlated with increased expression levels of the floral integrators *SOC1* and *FT*. Interestingly, *TFL1* expression levels were significantly reduced in these plants, suggesting a possible functional conservation in the pathway reported for *A. thaliana* and rice. We also analyzed two independent *BdPAP2* mutant lines of *B. distachyon*. One of the lines carries a T-DNA insertion that introduced a 4xCaMV35S enhancer sequence in an intron of *BdPAP2*, upregulating its expression. This activation tagged line flowered earlier and developed fewer leaves than control plants. The other mutant was a sodium azide mutant that contains a SNP mutation in a splice-site donor that is expected to disrupt *BdPAP2* function. As expected, this mutant showed a delay in flowering time. Our results obtained in *A. thaliana* and *B. distachyon* suggest that BdPAP2 controls flowering time by regulating the *B. distachyon TFL1* orthologue, *BdRCN4*. As a first attempt to test this hypothesis, we studied the 3` distal region of *TFL1* homologues of *B. distachyon*. We found the putative regulatory boxes in all the three genes analyzed, in a similar number and disposition of those found in *A. thaliana*. We are now focusing our efforts in corroborating if BdRCN4 is a direct target of BdPAP2, as well as if this regulation depends on the interaction of BdPAP2 and AP1-like proteins. This research will shed new light on the pathways that regulate flowering time in grasses.

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Poster

S6 Polyploidy and perenniality

P26 The *Brachypodium distachyon* species-complex as a model for dissecting the role of allopolyploidy in plant adaptation

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Interspecific hybridization can lead to increased aggressiveness in the hybrid forms relative to the parental species and this is often associated with invasiveness in new environments. European annual grasses from the *Brachypodium* genus illustrate this phenomenon very well, with extensive post Columbian invasion of Mediterranean areas in the New World. The original *B. distachyon* morphotype has been resolved into three distinct species: two diploids, *B. distachyon* and *B. stacei*, and a derived allotetraploid species, *B. hybridum* (Catalán et al., 2012). The *B. distachyon* complex is an ideal model to study numerous aspects of adaptation, including invasiveness. The *B. distachyon* complex is a well developed model that cycles rapidly and can be grown cheaply and at scale (Opanowicz et al., 2008). High quality annotated genomes from parental and hybrid species are available and large collections of well defined natural accessions have been made from across its native range. We are developing high throughput phenotyping and reverse genetic approaches to measure traits that contribute to reproductive success with the aim of mapping allelic variants in both diploid and polyploidy species. These traits range from the level of chromosome pairing in diploids and polyploids to seed set, seed number and the response to environmental variables such as nutrients, heat and water stress. We will discuss the different approaches and the future prospects for developing this species-complex as a tractable model for studying the role of interspecies hybridization in natural ecosystems using state of the art genomics, phenomics and cell biology.

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Attendant

S6 Polyploidy and perenniality

P40 Does reproductive assurance explain the incidence of polyploidy in plants and animals?

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Polyploidy is generally more common in plants than in animals, but most hypotheses that address this disparity focus on developmental factors that prohibit polyploidy in animals. This review addresses a complementary hypothesis: modes of reproductive assurance that increase the probability of successful polyploid establishment and diversification are common in plants and rare in animals. Recent polyploid lineages in both plants and animals frequently achieve reproductive assurance through asexual reproduction or selfing mating systems. This fact corroborates theoretical models of polyploid establishment, which consistently predict that reproductive assurance will mitigate density-dependent mating disadvantages among incipient polyploid populations. However, plant and animal clades that diversified following ancient genome duplications generally retain sexuality and outcrossing mating systems. This suggests that facultative forms of reproductive assurance, which do not interfere with the ability to outcross, may promote both the establishment and long-term success of polyploid lineages. Facultative reproductive assurance is much more common among plants, where it often occurs parallel to normal outcrossing (e.g., vegetative reproduction, geitonogamy). Furthermore, the prevalence of polyploidy and the prevalence of facultative reproductive assurance are broadly congruent among major plant clades. We discuss the implications of these findings and suggest directions for future research in both plants and animals.

Poster

S7 Ecology and environment

P27 Mycobiome diversity of *Brachypodium rupestre* from high and low diverse grasslands.

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In the last decades, *Brachypodium pinnatum* subsp. *rupestre* (*B. rupestre* according by Schippmann since 1991) has expanded and increased its dominance in calcareous grasslands throughout Europe [1]. In Western Pyrenees, the expansion is related to the relaxation of grazing practices and to an intense use of prescribed winter burnings [2]. Repetitive burnings can be stressful for plants. Fungal endophytes are known to favour the fitness of most host species under stressful conditions [3]. Our objective was to characterize the mycobiome of B. rupestre in order to identify fungal endophytes related to fire regime that could favour the expansion of the species.

For that purpose, ten plants were gathered in two different environments. Five growing in high-diverse grasslands where *B. rupestre* cover is below 40%, the grazing intensity is medium and the burning frequency is low (>10 years), and the other five developing in low-diverse grasslands where *B. rupestre* cover is above 90%, the grazing intensity is low and the burning frequency is intense (each 2-3 years). The five samples were collected along a transect, with a distance of 50m between two sampling points. We extracted DNA from shoots, rhizomes and roots and used high-throughput sequencing (MiSeq PE300, Illumina) to characterize the mycobiome. Additionally, fungal endophytes were isolated following the standard methodology of isolation [4] and identification [5] of cultivable species.

Total readings (OTU's number) of metadata sequences in roots, rhizomes and shoots were 428827, 76960 and 7884, respectively. The fungal endophyte composition of roots and rhizomes were similar between them and different from the shoots. Total readings of sequences were greater in the low-diverse grassland (313621) than in the high-diverse grassland (200050). The grasslands showed different endophyte species composition in roots (Figure). *Lachnum* sp., *Paracamarosporium* sp. or *Hymenoscyphus* sp. tended to appear in low diverse grasslands, whereas *Albotricha* sp. and *Drechslera* sp. were more frequent in high diverse grasslands. In particular, *Lachnum* sp. fructification has also been reported on burned roots of *Ulex europaeus* in the Cantabrian mountains.

The traditional fungal cultivation method was able to isolate and identify well-represented fungi (i.e., endophytes with a high number of OTU's sequenced in metadata, such as *Lachnum* sp., *Albotricha* sp. and *Hymenoscyphus* sp.). Massive sequencing identification is faster and more effective than the traditional isolation cultivation method.

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Poster

S7 Ecology and environment

P28 Metabolomic characterisation of Turkish soils as potential drivers of *Brachypodium distachyon* genotype diversity

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The plant genotype is shaped by numerous factors, clime and soil among others. The comprehension of influence of these factors on genetic diversity is of critical importance. As a well-established model for monocotyledonous plants, *Brachypodium distachyon* in an excellent object for such studies. Thus, in this work we focused on initial characterisation of Turkish soils collected from the collection sites of Turkish populations of *B. distachyon* through pH and electrical conductivity measurements as well as metabolomics analysis. Soils from 57 sites were divided into five groups based on diverse climate regions of Turkey. Turkey was selected as the centre of its natural geographical range, is a great rich source of *B. distachyon* diversity which could be related to environmental variables.

Soil suspensions were used to measure pH and electrical conductivity. The soil pHs were similar in all analysed samples with mean value of 7.5. Likewise, electrical conductivity did not differ between the groups and its values ranged from 0.5 to 15 dS/m with a mean of 2.19 dS/m. The electrical conductivity is an indication of soil salinity, thus such results suggest that collected soil was not highly saline. For metabolomic analysis the sieved soil samples were first extracted with chloroform/methanol/water (1:3:1) mixture and clear supernatant was injected into flow infusion electrospray high-resolution mass spectrometry (FIE-HRMS) utilising an Exactive HCD mass analyser equipped with an Accela UHPLC system (Thermo-Scientific). This allowed generation of metabolite fingerprints of both, positive and negative ionisation mode. The obtained intensity matrices were log-transformed and used for further statistical analysis in MetaboAnalyst (Nucl Acids Res, 2018, 46, 486). The annotations of metabolites significantly differed between analysed regions and were made using MZedDB (BMC Bioinform, 2009, 21, 227). This analysis showed similarities between the south-east and central regions which differ from coastal regions. Considering the biochemical sources of variation, cyclic compounds, including carveol and limonene were more predominant in samples from the Mediterranean coast. Simultaneously potentially harmful chemicals such as catechol, protocatechuic, benzaldehyde were more abundant in the remaining regions.

In the future studies, we aim to combine soil metabolomic data with genomic diversity of *B. distachyon* collected from respective sites to asses selective pressure of soil nutrients on the *B. distachyon* genetics.

This research was funded by the National Science Centre Poland (grant 2015/18/M/NZ2/00394).

Poster

S7 Ecology and environment

P29 Characterization of *Brachypodium* varieties as tree cover crops in Mediterranean conditions.

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Accelerated water erosion is a major environmental problem in tree crops under Mediterranean conditions, which threatens their long term sustainability (Gómez, 2017). Despite of the practical use of cover crops under these conditions, there is limited information on the prediction of the flowering and maturity dates of *Brachypodium* species for different tree growing areas within the Mediterranean. Different areas within this region, despite sharing the same climate type, present significant differences in their temperature regimes. This communication presents the preliminary results of the first year of a study aimed to calibrate a temperature-based phenology model for *Brachypodium* in Southern Spain. The four varieties currently registered in the EU: two *B. hybridum* (Ibros and Iskyri) and two *B. distachyon* (Zulema, and Kypello) were used for the study.

In two different locations in Southern Spain with contrasting temperature regimes, Córdoba (warmer with 17.8 °C average annual temperature) and Lanjarón (colder with 14.5 °C average annual temperature) a controlled experiment was carried out during the agricultural year 2018-19. At each site 8 pots (15 l in volume each) were seeded in late October with two replications of each of the four varieties. Each pod was seeded at a seed density of 1gr m^{-2} and they were irrigated regularly to prevent water stress according to the rainfall distribution of the season. The air temperature and the plants height was recorded automatically at a 30 minutes interval.

The phenological evolution of the plants was assessed regularly during the growing seasons according to Meier (2001). A phenology model based on growing degree days, GDD Eq. 1, was developed using the Richard equation following the procedure described by Gómez and Soriano (2019). Basically the models were calibrated using the experimental data of the season 2016-17 minimizing the root mean square error between predicted and observed days since sowing for each of the registered phenological stages using the solver function of Excel ®. The developed models were validated using the experimental data of season 2017-2018.

This communication presents the preliminary results of the differences in phenology among these four varieties, the calibration of the phenology model and the implications of these differences for their use as cover crops different areas among the region.

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Poster

S7 Ecology and environment

P30 Genetic, epigenetic and metabolomic differentiation of Turkish *Brachypodium distachyon* accessions into two geographically distinct populations

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As an annual species, growing on broken ground *Brachypodium distachyon* (*Brachypodium*), represents an ideal model with which to suggest the molecular drivers of environmental adaptation. We have initiated a project where we are assessing the relative contribution of genomic, epigenetic, transcriptional and metabolomic variation to environmental adaptation. Turkey represents one site of *Brachypodium* diversification and can be broadly sub-divided into five distinctive climato-environmental regions; representing different rainfalls, temperature ranges, altitudes, and soil types. A new collection of *Brachypodium* accessions was established by sampling at 12 sites within each of the regions. Seed (To) was taken from each accession and grown under uniform controlled environmental conditions at Aberystwyth University. Thus, any variation reflected innate differences arising from environmental adaptation. At 6 weeks following germination, Toleaves were sampled and assessed for genomic (WGS), epigenetic (BS-Seq), transcriptional (RNA-seq) and metabolite (by high resolution Flow-Infusion Electospray - Mass Spectroscopy; FIE-MS) variation. Phenotypic analyses indicated that the Topopulation could be broadly differentiated into two subpopulation based on seed dimensions, plant height, period of vernalisation, flowering time and drought tolerance. Genome sequencing (10 fold coverage) also indicated that variation in single nucleotide polymorphisms was linked to a costal and interiorTurkish population. Further assessment indicated that epigenetic, transcriptional and metabolomic analyses also aligned with this split in Brachypodium populations. Examination of the molecular drivers for this split are indicating that, for example, epigenetic-regulatory genes, drought-linked transcriptional regulators, TCA (tricarboxylic acid) and glyoxylate cycles were differentially active in the two populations. We suggest that our data indicated that natural selection through both genetic and epigenetic variation to drive adaptive changes in the transcriptome and metabolome.

Poster

S8 Adaptation to abiotic and biotic constrains

P31 Circadian clock genes under drought stress in *Brachypodium distachyon*

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Circadian rhythms provide temporal organization of biological processes in every living creature ranging from bacteria to mammals. Role of circadian clock in fitness optimization through temporal synchronization of gene expression, metabolism and physiology to predictable, regular changes of the environment has been widely studied in *Arabidopsis*. Approximately one-third of the *Arabidopsis* genes show circadian clock dependent oscillations underlining the importance of circadian rhythm in overall fitness. Although functions and members of interlocking feedback loops through which the plant circadian clock oscillates have been reviewed in detail in *Arabidopsis*, our knowledge on circadian clock of other plant species is still rudimentary.

Here we examined the response of *Brachypodium distachyon* core clock genes (PRR95 - Bd4g36077; LHY/CCA1 - Bd3g16515; TOC1 - Bd3g48880; GI - Bd2g05226; LUX - Bd2g62067; ELF3 - Bd2g14290; ELF4/1 - Bd4g13227; ELF4/2 - Bd1g60090; ELF4/3 -Bd4g29580) to moderate drought stress. We monitored relative transcript amount changes for two days in green plant parts and in root separately during long-day (18:6 light: dark photoperiod), continuous light and continuous dark conditions after two weeks of mild drought stress (40% soil water content).

As results, water limitation induced marked changes in gene expression of several circadian clock components in green plant parts: such as PRR95 and LHY/CCA1, which had reduced expression in stressed plants, while components of evening loop (TOC1, GI and LUX) showed phase advance in their expression under drought condition. In comparison to shoot, amplitude and phase of clock gene expression in root differs markedly from that in the shoot and genes in evening loop except GI did not show rhythmic expression even in well-watered plants indicating that circadian clock runs on a simplified manner in root. Contrary to shoot, most of the clock genes showed slight increase in their relative transcript amount under drought stress.

Based on differences between expression pattern of clock genes in shoot and root we suppose that (i) circadian clock runs on an organ-specific manner (ii) circadian clock responses to drought stress differently in shoot and root. In addition ELF3 and members of ELF4 family did oscillate neither in shoot nor in root implying that there might be differences between clock components of the dicot model *Arabidopsis* and *Brachypodium*, the model plant of monocots.

Poster

S8 Adaptation to abiotic and biotic constrains

P32 P. koreensis: A plant growth-promoting bacterium for *Brachypodium distachyon* growing with limited nitrogen

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Nitrogen is the most abundant and essential mineral for plants. It is in nucleic acids and proteins; notably rubisco for photosynthesis. The world population is growing and available arable land is decreasing. Higher demand for crop production has come with an increased use of chemical fertilizer; of which N is the major component. Use of N fertilizers in high input farming regions such as Europe, China and India, results in emissions and leaching. There is an urgent need to reduce nitrate leaching into groundwater. In contrast, low input farming regions such as in Africa require additional N. To cope with the global N problem, plant growth-promoting rhizobacteria (PGPR) might play a crucial role as a biological fertilizer due to their ability to fix atmospheric nitrogen. Many *Pseudomonas* spp. have been tested as PGPR because of their distinctive traits: production of growth regulators, siderophores, volatile organic compounds (VOCs), protection enzymes, and N-fixation. *Pseudomonas koreensis* has not been tested therefore we grew it with *Brachypodium distachyon* under two different nitrogen conditions and phenotyped whole-plant biomass, nutrient content and protein abundance. Under limiting N, root and shoot biomass increased in plants inoculated with *P. koreensis*. The root architecture was also changed: the primary seminal root decreased significantly while lateral root length increased slightly. Proteomics revealed a higher abundance of proteins associated with central N metabolism in inoculated plants grown with limiting N. A notable example was the high-affinity nitrate transporter NRT3.1 (a member of the protein family NAR2 and a dual component transporter with NRT2.1). It appeared to be a key driving force for the improved biomass under low N. In agreement, elemental analyses revealed a higher N content in root and shoots of plants inoculated with *P. koreensis* under limiting N. As expected, changes on the protein levels of central N-metabolism in Brachypodium were identified as one mode of action behind the plant growth-promotion by *P. koreensis*. Confirmation of phenotypes and proteomic responses remain to be tested in soil and crops. Nevertheless, these findings support the use of *P. koreensis* as a new PGPR to reduce the amount of chemical N fertilizer while maintaining crop biomass or yield for the future global demand.

Poster

S8 Adaptation to abiotic and biotic constrains

P33 Infrared spectromicrosopy of live *Brachypodium* root hair and cap cells to discover specialized environmental monitoring

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All elements for plant life apart from carbon are provided through the root systems. The root cap and epidermis cells are the outermost root cells and the forefront of environmental contact. Covering the root tip, cap cells protect the meristem and function in branch root positioning along the root[(1)]. The epidermis covers the root proper and consists of hairless and neighboring root hair cells. The cellular lineage and genetic basis of root hair formation are well known in the model species *Arabidopsis thaliana* and, to a much lesser degree, in other species[(2)]. Physiological reactions to specific, isolated conditions, e.g. formation of infection threads for nodule formation in legumes or growth of longer root hairs upon phosphate deficiency, are well documented. However intriguing, inconsistencies in root hair phenotypes arise due to soil/solution conditions[(3)] and are dependent on genotypes and root types[(4)]. Almost nothing is known about the interpretation of environmental conditions by root hairs or if they are more specialized for environmental interpretation than other root cells in direct contact with the environment such as cap cells.

In order to address the question of whether the outermost root cells have different responses to soil conditions depending on their developmental type, the responses of root hair and root cap cells to growing conditions were monitored live on young *Brachypodium* seedlings using synchrotron-based Fourier Transformation Infrared (sFTIR) spectromicroscopy. sFTIR spectromicroscopy allows spectral monitoring of living cells without cellular damage over several hours. The generated absorbance spectra are specific for chemical bonds and have been used repeatedly in the past to identify specific molecules[(5)]. We germinated Brachypodium seeds on nutrient-rich media, nutrient-free media or on moist filter paper. After two to four days the primary roots were detached, mounted on silicon wafers, and monitored using sFTIR spectromicroscopy. Even after several hours of measurements we detected absorption spectra specific for living cells for both root cap and root hair cells. These spectra included high absorption representing water and DNA, indicating the non-destructive nature of this technique. Currently the analyses of root cap and root hair cells germinated on different media are ongoing. We hypothesise that the two cell types will be distinct regardless of growing conditions, and that spectra within cell types will depend on the growing conditions. If the growing conditions lead to different absorbance spectra within a cell type, the result will be highly novel and open interesting opportunities to understand root sensing of its environment.

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Poster

S8 Adaptation to abiotic and biotic constrains

P34 Greater N capture by *Brachypodium* roots promoted by beneficial bacteria: A whole plant phenotyping approach advancing EcoFabs with non-invasive imaging and dynamic analyses

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Grasses provide 50% of human calories, and wheat 20% of human calories. Since grasses cannot fix atmospheric nitrogen like legumes, chemical N fertilisers must be applied to meet global food demand. However, only ca. 50% nitrogen fertilizer is taken up by the roots of crops such as wheat. Over-fertilisation of N is leading to water and air pollution, with political and environmental consequences in Europe, China, India, and other high-input, high demand regions. Improving crop nitrogen use efficiency is one of the main objectives in sustainable agriculture. A large aspect in this effort is improving crop root system efficiency for nitrogen uptake.

Brachypodium is a robust model of the roots and root-associated microorganisms of grasses; notably wheat[(1)]. Roots and shoots of *Brachypodium* have been phenotyped, revealing variations in root architecture and root-shoot allocation in response to water[(2)], nitrogen[(3)] and phosphorus[(3)]. Further, certain root microbiome members and root pathogen are similar in *Brachypodium* and wheat[(4),(5)]. The 3D-printed microcosm, Ecofab, was recently used in four international laboratories to demonstrate repeatable shoot traits, root morphologies, and root exudates in Brachypodium distachyon Bd21-3 at different P levels[(6)]. Ecofabs can be maintained sterile to test Brachypodium growth in response to beneficial microorganisms and chemical exudates and signals.

We aim to identify microorganisms that stimulate *Brachypodium* N capture, notably under the replete N conditions of polluting farming systems. We recognize that the N capture phenotype involves shoots and roots and may be expressed dynamically. We are therefore modifying the current EcoFab[(6)] to: i. supply a relatively steady N concentration to the roots; ii. allow non-destructive leaf and colour phenotyping through time using an adaptation of Plant Screen Mobile[(7)]; iii. improve non-invasive root phenotyping using camera and stereomicroscope; and iv. discover inoculation dynamics in root hairs using an inverted microscope.

We found that *Brachypodium distachyon* Bd21-3 expressed the classic whole-plant phenotype in response to N in sterile conditions in the EcoFab and in a hydroponics system. It developed longer roots and longer root hairs under lower nitrogen levels (0.3 mM, 3 mM N) than in higher nitrogen levels (15 mM, 30 mM N), and root allocation increased compared to the shoot. Preliminary inoculant tests showed: *Sinorhizobium meliloti* promoted root length and shoot growth at 3 mM N, and *Herbaspirillum seropedicae* promoted root length at 0.3 mM N. *Herbasprillum* and *Sinorhizobium* genera are known beneficial bacteria that produce plant hormones, 1-aminocyclopropane-1-carboxylate, and N-Acyl homoserine lactones which can impact root system architecture and improve nitrogen use efficiency. Experiments are continuing to repeat EcoFab dynamic phenotypes and resolve their timing, spatially localize the innoculants outside and inside the roots over time, and test signal modes of action.

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Poster

S8 Adaptation to abiotic and biotic constrains

P35 Characterization of a nitrate high-affinity transport system in *Brachypodium distachyon*

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Nitrogen is an essential element for growth and plant development. Improving the nitrogen use efficiency of crops is a priority for the future in order to limit the fertilizer use and environmental impacts. An efficient nitrate uptake system contributes to improve the nitrogen use efficiency of crops in conditions of low N availability. The High-Affinity nitrate Transport System (HATS) in plants is active in low range of external nitrate and is mediated by a two-component system (the high affinity transporters NRT2 associated to a partner protein NRT3).

In *Brachypodium*, the model plant for C3 cereals, seven *BdNRT2* and two *BdNRT3* have been identified (Girin *et al.* 2014). Expression of the *BdNRT2* and *BdNRT3* genes in response to nitrate availability fits with the characteristics of the HATS components already described in other species (David, Girin *et al.* 2019). Likewise, co-expression of *BdNRT2A* and *BdNRT3.2* is required for an effective nitrate transport in the heterologous expression system *Xenopus* oocytes. Functional interaction between BdNRT2A-GFP and BdNRT3.2-RFP has been observed at the plasma membrane in *Arabidopsis* protoplasts transiently expressing both constructs, while a cytosolic localization was observed when *BdNRT2A-GFP* or *BdNRT3.2-RFP* were transiently expressed separately, suggesting a monocotyledonous/dicotyledonous specificity for the functional interaction. Site directed mutagenesis on a Ser residue of the C-terminal extension of BdNRT2A specifically conserved in monocotyledonous plants and mimicking a constitutive phosphorylation status of this Ser was used to study the species specificity of the NRT2/NRT3 interaction. Finally, $^{15}NO3$ influx measurements with *Brachypodium* mutants, defective in *BdNRT2A* expression, confirmed that BdNRT2A is a major contributor of the HATS in *Brachypodium*.

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Poster

S8 Adaptation to abiotic and biotic constrains

P36 Using Brachypodium as a tool for screening resistance against wheat root diseases

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Deoxynivalenol (DON) is an important toxin produced by certain *Fusarium* species, mainly *Fusarium graminearum* and *Fusarium culmorum*. One of the major roles of DON is facilitating the infection and spread of the fungus in a variety of cereals, especially in wheat. Strains of the fungi containing a disruption in the biosynthesis of DON are severely impaired in their ability to infect cereals, and it has been shown that the presence of DON is essential for effective colonization of the roots by *Fusarium*.

Studying the role of DON in the plant is then an essential part in understanding the infection mechanism of *Fusarium* inside the cell, and subsequently, leading to new ways to minimize or disrupt disease spread. To understand this effect, an RNA-sequence experiment was performed using seedlings from both the crop model *Brachypodium distachyon* and wheat (*Triticum aestivum*), inoculated with a fixed concentration of DON at two different time points. Due to the high complexity in working with a hexaploid genome, the use of a diploid model like *Brachypodium* is important in understanding and correlating the different responses by both species in response to DON. Upon analysing the differentially expressed genes obtained from the experiment, a significant deregulation of the ROS pathway was observed upon DON inoculation and was consistent between both time points as well as both species.

In a separate experiment, similar phenotypes in root development and growth, following an independent DON and ROS inoculation where observed, establishing the potential for DON regulation of ROS in the plant during root development. Along with ROS, genes involved in stress homeostasis by the plant also show a significant differential expression in the presence of DON, leading to a complex cross-talk between stress-induced, DON-induced and ROS-induced genes.

To study and characterize this cross-talk further, a new RNA-sequence experiment was performed in *Brachypodium* using independent DON and ROS treatments. Additional susceptible and resistant lines, obtained from the Joint Genome Institute's *Brachypodium* pangenome, were included in this experiment, making it possible to study the DON-ROS cross-talk in different genetic backgrounds.

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S8 Adaptation to abiotic and biotic constrains

P37 Modifying *Brachypodium distachyon* resistance to Fusarium head blight and Fusarium root rot with Plant hormones

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Fusarium head blight (FHB) is an economically important fungal disease of cereals caused by several species of *Fusarium*. *Fusarium* species can also cause Fusarium root rot (FRR) which is not a well characterised disease. Genetic control and fungicides resistance are partially effective thus novel control methods are required to improve FHB resistance in many susceptible wheat (*Triticium aestivum*) varieties. Plant hormones have an important impact on resistance to plant pathogens aside from development and growth. Here I have identified the effects of a range of hormones on resistance to FHB and FRR caused by *F. graminearum* using the model cereal *Brachypodium* distachyon (Bd), accession line Bd3-1. *B. distachyon* is a good model system for investigating *F. graminearum* pathogenesis in floral and root tissues. Importantly, all Bd tissues can be infected by *F. graminearum* and Bd has conserved hormone signalling pathways with other cereals [1,2]. Jasmonic acid (JA), Indole-3-acetic acid (IAA), and the ethylene precursor 1-Aminocyclopropanecarboxylic acid (ACC) improved resistance to FRR whereas the hormone salicylic acid (SA) and the cytokinin trans-Zeatin (Tz) increased susceptibility to FRR. Additionally, a less well-known plant signalling molecule 3-aminobutanoic acid (BABA) conferred an increase in susceptibility to FRR whereas BABA's isomer 4-aminobutanoic acid (GABA) conferred an increase in FRR resistance. Interestingly the hormones JA and ACC showed an opposite effect to FRR by increasing FHB susceptibility. Furthermore, the hormone Tz was the only hormone to have substantially increased Bd susceptibility to both FHB and FRR. The hormones SA and IAA showed no significant effect on FHB resistance.

There were considerable differences in resistance in a given tissue depending on the hormone exogenously applied. The effect that specific hormones have on pathogen resistance partly depends on the trophic life-style of the pathogen. Considering JA and ethylene is associated with necrotroph resistance (kills host cells and feeds on dead plant tissue) and SA is associated with biotroph resistance (feeds on live plant tissue), the results suggest that *Fusarium graminearum* behaves primarily as a necrotroph in Bd3-1 roots. Furthermore, there were contrasting effects between FHB and FRR resistance for a most hormones. It is thought that *Fusarium graminearum* adopts a hemibiotrophic life-style (starts as a biotrophic and switches to necrotroph feeding) in Bd head tissues and therefore I hypothesise that there are a different suite of plant hormone signalling pathways activated during FHB compared to FRR. Current research is under way to understand this difference in resistance between Bd tissues.

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Poster

S3 Development and growth

P38 Thermocycles are the prevailing cue in determining *Brachypodium distachyon* diurnal gene regulation

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Plants are continuously exposed to varying environmental conditions; some anticipated diurnal changes in light and temperature and others far less predictable. Key to adaptation to a diurnal environment is the anticipation provided by the circadian clock, which coordinates broad changes in gene expression with a period of about 24 h. We measured transcript abundance over time in *Brachypodium distachyon* entrained in photo- and thermocycles then transferred to photocycles or thermocycles alone, or constant light and temperature conditions. Approximately 3% of the transcriptome remained rythmic under constant conditions, far fewer transcripts than what has been reported in comparable datasets from *Arabidopsis thaliana*, maize, rice, poplar, and photocycle-entrained *Setaria viridis*. The peak time, phase, of expression for a majority of the cycling transcripts occired just prior to dawn or dusk, with the exception of free-run where we observed a lengthening of period, from 24 h to 27 h, and a resulting shift in phase. We found conserved gene ontology terms associated with particular times of the day. Many of the known circadian clock genes were rhythmic under all four conditions, with the majority also demonstrating a period change under constant conditions including*LUX*, *LHY*, *GI*, and *TOC1*. Furthermore, we identified sequence motifs enriched in the promoters of similarly phased genes and used DNA affinity purified sequencing datasets to associate the motifs with potential upstream transcription factors influencing rhythmic expression. In doing so we elaborate on *B. distachyon* diurnal and circadian regulated gene expression.

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