2015 International Brachypodium Conference

16-19 June 2015
Amherst Massachusetts

Scientific Organizing Committee
Pilar Catalan (University of Zaragoza, Spain)
David Des Marais (Harvard University, USA)
Sinead Drea (University of Leicester, UK)
Robert Hasterok (University of Silesia, Poland)
Zhiyong Liu (China Agricultural University, PRC)
Todd Mockler (Donald Danforth Plant Science Center, USA)
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Ludmila Tyler (Dept. of Biochemistry and Mol. Biology, University of Massachusetts)
Elsbeth Walker (Biology Department, University of Massachusetts)
Program

**Tuesday, June 16, 2015**
**Marriott Room, 11th floor Campus Center**

3:00-5:00 Registration

5:00 Welcome and Introduction

5:15 OPENING KEYNOTE ADDRESS
Rick Amasino - University of Wisconsin, Madison
Environmental control of the timing of flowering

6:15 Opening reception

**Wednesday, June 17, 2015**
**Auditorium, 1st floor Campus Center**

8:00-9:00 Registration, poster set-up

8:30-10:20 PLENARY SESSION: Plant-microbe interactions
Chairperson: Nicola Pecchioni, CRA-CER Cereal Research Centre, Italy

8:30 Keynote Speaker: Karen-Beth Scholthof -- Texas A&M University
A systems biology approach to study the tug-of-war in grass-virus interactions using Brachypodium

9:15 Zhiyong Liu -- China Agricultural University
Allelic variation and domain functional analysis of the BSMV resistance gene Bsr1 in Brachypodium

9:35 Jan Bettgenhaeusser -- The Sainsbury Laboratory
Novel and classical resistance genes provide nonhost resistance to wheat stripe rust in *Brachypodium distachyon*

9:55 Ariane Girardin -- INRA-CNRS, Castanet-Tolosan
*A Brachypodium distachyon* LysM-RLK is involved in perception of arbuscular mycorrhizal symbiotic signals
10:15 Coffee break

10:45-12:30 PLENARY SESSION: Novel tools and techniques
Chairperson: Robert Hasterok, University of Silesia, Poland

10:45 Keynote Speaker: Olga Zabotina -- Iowa State University
A set of Brachypodium transgenic lines with post-synthetically modified cell walls as a tool to study cell wall integrity control

11:30 Robert Hasterok -- University of Silesia in Katowice
Cytogenetics of Brachypodium -- current status and future prospects

11:50 Kirankumar S. Mysore -- The Samuel Roberts Noble Foundation
Insertional mutagenesis of Brachypodium distachyon using the Tnt1 retrotransposon and its use to study nonhost disease resistance

12:10 Steven Eichten -- Australian National University
DNA methylation variation across Brachypodium distachyon highlights genetic diversity and frames adaptation via the ‘extended genotype’

12:30-2:00 Lunch Blue Wall - various dining options, on your own

2:00-3:45 PLENARY SESSION: Development and growth -- vegetative
Chairperson: Karen Sanguinet, Washington State University, USA

2:00 Keynote Speaker: Liam Dolan -- University of Oxford
Root epidermal development in Brachypodium distachyon

2:45 Pubudu Handakumbura -- University of Massachusetts
GNRF suppresses floral transition and secondary wall synthesis in Brachypodium distachyon

3:05 Agnieszka Zienkiewicz -- Michigan State University
Enhancing storage lipid synthesis in vegetative tissues of Brachypodium distachyon by overexpressing WRINKLED1

3:25 Michael T. Raissig -- Stanford University
Stomata development in Brachypodium – Rewiring the bHLH transcription factor network
3:45 Coffee & Poster Session. Authors of odd numbered posters present.

5:30 Banquet at the Naismith Memorial Basketball Hall of Fame
Board buses west of campus center

Thursday, June 18, 2015
Marriott Room, 11th floor Campus Center

8:30-10:20 PLENARY SESSION: Natural variation, ecology, evolution
Chairperson: Pilar Catalan, University of Zaragoza, Spain

8:30 Keynote Speaker: Antonio Manzane--Universidad de Jaén
Adaptive differentiation in the Brachypodium distachyon species complex

9:15 Pilar Catalán--Universidad de Zaragoza
Phylogeny and biogeography of Brachypodium: perennial/annual evolutionary switches

9:35 Ludmila Tyler--University of Massachusetts
Building tools for genome-wide association studies: Flowering time helps explain population structure in Brachypodium distachyon

9:55 Jared Streich--Australian National University
A landscape genomics approach to elucidate the genetic and climatic diversity of Brachypodium species

10:15 Coffee break

10:45-12:30 PLENARY SESSION: Development and growth -- reproductive
Chairperson: Sinead Drea, University of Leicester, UK

10:45 Keynote Speaker: Cristina Barrero-Sicilia--Rothamsted Research
Germination in domesticated and wild Triticeae seeds: comparison between Brachypodium distachyon and Hordeum vulgare

11:30 Koen Geuten--University of Leuven
FLOWERING LOCUS C in Brachypodium distachyon

11:50 Syabira Yusoff--University of Leicester
Phylogeny and expression profiling of YABBY genes in Brachypodium distachyon
12:10 Tadamasa Sasaki -- RIKEN
Analysis of the role of Brachypodium ADP-glucose pyrophosphorylase in storage starch biosynthesis

12:30-1:30 Lunch Blue Wall - various dining options, on your own

1:30-3:15 PLENARY SESSION: Comparative genomics and crop translation
Chairperson: Zhiyong Liu, Chinese Agricultural University, PRC

1:30 Keynote Speaker: Rob Alba, Donald Danforth Plant Science Center
Systems-level analysis of drought and heat responses in the model C3 grass *Brachypodium distachyon*

2:15 Bruno Contreras-Moreira -- Fundación ARAID
The pangenome of *Brachypodium distachyon*: estimating the true genomic diversity of a species

2:35 Sofia Kourmpetli -- University of Leicester
Comparative grain transcriptomics of Brachypodium and temperate crops

2:55 John Vogel -- DOE Joint Genome Institute
Brachypodium projects at the DOE Joint Genome Institute: expanding the model

3:15 Coffee break

3:45-5:30 PLENARY SESSION: Environmental control of plant development
Chairperson: Rick Amasino, University of Wisconsin, Madison, USA

3:45 Keynote Speaker: Long Mao - Chinese Academy of Agricultural Sciences
Posttranscriptional Regulation of *FLOWERING LOCUS T* in *Brachypodium distachyon*

4:30 Pip Wilson -- Australian National University
Investigating changes in the genetic architecture of agriculturally relevant traits in Brachypodium in response to variable climates using genome wide association studies

4:50 Daniel P. Woods -- University of Wisconsin, Madison
Molecular basis of the ability for short days to substitute for vernalization in the temperate grass, *Brachypodium distachyon*
5:10 Jill C. Preston -- University of Vermont
Evolutionary genetic origin(s) of vernalization responsiveness in temperate pooid grasses

5:30 Poster session, Authors of even numbered posters present

6:30 Buffet Dinner, Cape Cod Lounge, Campus Center

8:00 Poster session

Friday, June 19, 2015
Marriott Room, 11th floor Campus Center

8:30-10:15 PLENARY SESSION: Abiotic stress
Chairperson: David Des Marais, Harvard University, USA

8:30 Keynote Speaker: Jean-Benoit Charron, McGill University
Improving freezing tolerance in grasses: what can we learn from Brachypodium?

9:15 Edoardo Bertolini -- Scuola Superiore Sant’Anna
Transcriptional and post-transcriptional regulatory networks controlling leaf cell differentiation during drought stress in Brachypodium distachyon

9:35 David Chan-Rodriguez -- University of Massachusetts
The mechanism of phytosiderophore secretion mediated by BdTOM1 transporters

9:55 Keiichi Mochida -- RIKEN Center for Sustainable Resource Science
Comparison of metabolic profiles across different growth stages and across different stress conditions between Brachypodium distachyon and wheat

10:15 Coffee Break

10:45 CLOSING KEYNOTE ADDRESS
Richard Sibout -- Institut National de la Recherche Agronomique
Crossing forward and reverse genetic screens to decipher lignification in Brachypodium distachyon

11:30 Closing address
P1
Phylogeny and biogeography of *Brachypodium*: perennial/annual evolutionary switches

Pilar Catalán¹,², Diana López-Álvarez¹, Rubén Sancho¹, María Luisa López-Herráñz¹, Antonio Díaz-Pérez¹,³

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Abstract
We present an exhaustive phylogenetic and biogeographic study of the 20 worldwide distributed taxa (17 species, 1 variety, 2 undescribed cytotypes) of *Brachypodium*. Haplotypic statistical-parsimony networks, multigenic Minimum-Evolution gene tree discordances, Bayesian dating analysis and maximum likelihood biogeographic approaches provided a testable hypothesis for the reconstruction of the *Brachypodium* species tree, for the estimation of its nodal divergence times and for the inference of the macroevolutionary processes that led to the current distributions of its lineages. Our results support the early splits of the annual and short-rhizomatose lineages (*B. stacei*, *B. mexicanum*, *B. distachyon*) in the Holarctic region during the early-Middle Miocene (and *B. hybridum* in the Pleistocene), and a profusion of rapid splits with vicariances and long distance dispersals for the perennial lineages since the late Miocene to the Pleistocene in the Mediterranean and Eurasian regions, with sporadic colonizations of more remote areas. Several perennial allopolyploid species (*B. boissieri*, *B. retusum*, *B. phoenicoides*, *B. rupestre* 4x, *B. pinnatum* 4x) showed homeologous copies from both ancestral and recent genome donors. Our comprehensive molecular phylogenetic survey of all the currently known *Brachypodium* illustrates a complex reticulate scenario of recently evolved diploid and allopolyploid lineages, and all the transitional life-cycle forms from perenniality to annuality. The *Brachypodium* species also show different adaptations to different climatic conditions and could therefore be used as model plants to investigate the genomic evolutionary switches between different life-cycle traits and abiotic stress tolerances that are critical for engineering and breeding proposed perennial grass biomass crops.
P2

Competition contributes to explain coexistence in mixed populations and climatic segregation of diploids and allotetraploids of the Brachypodium distachyon species complex

Pedro J. Rey\textsuperscript{1}, Ana Fernández-Ocaña and Antonio J. Manzaneda\textsuperscript{1}

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Abstract

Polyploids of heteroploids plant species have often been regarded as better colonizers as well as superior competitors than its diploids progenitors, which may explain habitat and geographic segregation of diploid and polyploids. Diploids and allotetraploids forms of the \textit{B. distachyon} complex are known to be segregated geographically and climatically. Such segregation seems to be mediated by drought-related trait phenotypic differentiation: tetraploids develop a drought escape strategy, what afford them to inhabit dryer environments by performing higher water use efficiency and flowering earlier than diploids. Here we experimentally examined competitive ability and exclusion between diploids and allopolyploids in dry and humid environments of southern Spain where both forms coexist both regionally and in sympatry. We hypothesized that competitive exclusion of diploids by allopolyploids will occur only at severe levels of aridity stress, while it will be weaker or lacking under humid environments. We conducted two field experiments in each type of environment, one involving competence with other species, and other comparing performance of both cytotypes when planted in pure and mixed stands. Results confirmed the expectation that tetraploids have superior ability than diploids to compete in saturated multispecific environments, especially in arid zones. However, we only partially confirmed the necessary conditions for competitive exclusion of diploids by tetraploids in arid environments: while diploids are weak competitors for tetraploids they do not suffer stronger negative effects from tetraploids than from themselves. Competition and competitive exclusion became weaker at humid zones. Results help to explain coexistence of both cytotypes in some localities.
P3
Chloroplast genome assembly, annotation and phylogenomics of 56 Brachypodium distachyon complex accessions

Rubén Sancho\textsuperscript{1,5}, Carlos P. Cantalapiedra\textsuperscript{2}, Diana López-Álvarez\textsuperscript{1}, Pilar Catalán\textsuperscript{1,3,5} and Bruno Contreras-Moreira\textsuperscript{2,4,5}

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Abstract
Assembly, annotation, alignment and phylogenomic analyses were conducted with 56 chloroplast genomes of accessions belonging to the three annual species of the Brachypodium distachyon complex (\textit{B. distachyon}, \textit{B. stacei} and \textit{B. hybridum}). Genomic rearrangements were detected between the \textit{B. distachyon} and \textit{B. stacei} genomes, with a major 1160 bp insert in LSC and deletion of one copy of \textit{rps19} gene in IRb of the \textit{B. stacei} and \textit{B. hybridum} genomes, respectively. Phylogenomic analyses of several grass cpDNA genomes were conducted with RAxML and MrBayes, using Anomochloa marantoidea to root the trees. \textit{Brachypodium} is placed in an intermediate diverging position within the Pooideae clade. The three studied \textit{B. hybridum} plastid genomes were inherited from \textit{B. stacei}-type maternal parents. Intraspecific genealogical relationships within \textit{B. distachyon} detected two main diverging lineages, one corresponding to the EDF (extremely delayed flowering) - miscellaneous clade and the other to the remaining accessions. Within the late group, a paraphyletic eastern Mediterranean-SW Asian group and a monophyletic western-central Mediterranean group could be differentiated, both showing consistent geographical structuring. Noticeably, the EDF-miscellaneous lineage included accessions from different Mediterranean localities (Turkey, Croatia) that are biologically (e. g. flowering time) structured, paralleling the results from nuclear SNP-based reconstructions. Our plastid tree also retrieved 9 cases of potential chloroplast capture, 7 within the EDF-miscellaneous clade and 2 within the E.Med-SW.Asian and WC.Med clades, suggesting the existence of gene flow between the most diverged \textit{B. distachyon} lineages.
P4

A Landscape Genomics Approach to Illicit the Genetic and Climatic Diversity of Brachypodium Species

Jared Streich\(^1\), Pip Wilson\(^1\), Shuangshuang Liu\(^2\), Steve Eichten\(^1\), Kevin Murray\(^1\), Niccy Aitken\(^1\), Aaron Chuah\(^1\), Pilar Catalan\(^3\), Luis Mur\(^4\), Kent Bradford\(^2\), Justin Borevitz\(^1\)

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\(^4\) University of Aberystwyth, Aberystwyth, Wales

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Abstract

The model species *Brachypodium distachyon* and its sister species *B. stacei* and *B. hybridum* compose the nearly cosmopolitan *Brachypodium distachyon* species complex whose native range spans the greater Mediterranean region, but now present in North and South America, and Australia. We genotyped by sequencing >23k SNPs from more than 1,100 accessions from 803 populations composing 17 climate types to explore genetic and climate diversity using reference genome and non-reference genome pipelines. We correlate genomic data to locally extracted climate data and MaxEnt models to describe the niche breadth and range limits of native and colonizing genotypes. This work has identified genetically diverse sites to scan for allele by climate associations. We have selected a globally diverse subset for genome wide association studies and selected contrasting regional climates and growing seasons for phenotypic evaluation of adaptive traits. This will allow complex trait dissection analysis to candidate genes, further develop invasion biology, and promote Brachypodium species as model organisms.
**P5**

**Transcriptome analysis of allopolyploid *Brachypodium***

Kotaro Takahagi\(^1,3\), Komaki Inoue\(^2,3\), Minami Shimizu\(^1,3\), Yukiko Uehara-Yamaguchi\(^3\), Yoshihiko Onda\(^1,3\), Kazuo Shinozaki\(^4,5\) and Keiichi Mochida\(^1,3\)

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**Abstract**

Whole-genome duplications have played important role for generating new species in plants. However, regulations that control interaction of duplicated genomes remain largely unknown. To understand evolitional and functional roles of duplicated genomes, it should be significant to figure out transcriptome in polyploidy plants. In this study, we established a new analytical pipeline to compare the compositions and expression patterns of transcripts derived from each homoeolog, and performed transcriptome analysis using an allopolyploid plant in genus *Brachypodium*. First, we compared genome re-sequencing data between three *Brachypodium* species of allopolyploid *Brachypodium* (*B. hybridum*) and two ancestors (*B. distachyon* and *B. stacei*), then constructed virtual genomes that resembled each ancestral genome sequence. Next, we identified homoeo-loci corresponding to each RNA-seq reads from *B. hybridum* leaf tissues, by a computational approach based on their sequence similarities. As the result, 85–88% of RNA reads were classified to homoeo-loci. It was estimated that in the *B. hybridum* transcriptome, RNAs were almost equally transcribed. We then performed expression analysis of homoeologous genes using RNA reads that are successfully classified to their homoeo-loci, and found 4034 genes with expression bias in between homoeologs. With an enrichment analysis based on the gene ontology terms, for example, genes related to a function such as cellular process significantly over-represented in these genes. The transcriptome analytical pipeline established in this study should be applicable to other plant species and would contribute to accumulate knowledge on regulatory mechanisms of gene expression or functional differences between homoeolog genes in allopolyploid plants.
P6
Building tools for genome-wide association studies: Flowering time helps explain population structure in *Brachypodium*

**Ludmila Tyler**, Scott J. Lee, Nelson D. Young, Gregory A. Delulio, Elena Benavente, Michael Reagon, Jessica Sysopha, Riccardo M. Baldini, Angelo Troia, Samuel P. Hazen, and Ana L. Caicedo

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**Abstract**

*Brachypodium distachyon* is a powerful model system for cereals and bioenergy grasses. Genome-wide association studies (GWAS) of natural variation can elucidate the genetic basis of complex traits, but have been hindered in *B. distachyon* by the lack of sufficient numbers of well-characterized accessions. We will report on genotyping-by-sequencing of 84 *B. distachyon*, seven *B. hybridum*, and three *B. stacei* accessions with diverse geographic origins, including Albania, Armenia, Georgia, Italy, Spain, and Turkey. Over 90,000 high-quality single-nucleotide polymorphisms (SNPs) distributed across the Bd21 reference genome were identified. Our results confirm the hybrid nature of the *B. hybridum* genome, which appears as a mosaic of *B. distachyon*-like and *B. stacei*-like sequences. Analysis of more than 50,000 SNPs for the *B. distachyon* accessions has revealed three distinct, genetically defined populations. Surprisingly, these genomic profiles are more readily explained by differences in flowering time than by geographic origin. High levels of differentiation in loci associated with floral development support the differences in flowering time between *B. distachyon* populations. GWAS investigations combining genotypic and phenotypic data also suggest the presence of one or more photoperiodism, circadian clock, and vernalization genes in loci associated with flowering time variation within *B. distachyon* populations. Our characterization expands the germplasm resources available for *Brachypodium* and illustrates the feasibility of GWAS in this model grass.
P7

Transcriptional and post-transcriptional regulatory networks controlling leaf cell differentiation during drought stress in *Brachypodium distachyon*

Edoardo Bertolini¹, Alessandra Mingrino¹, Concetta De Quattro¹, Mario Enrico Pè¹, Erica Mica¹²

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Abstract

Drought stress response is specific to developmental stages and cell types and involves complex interplay of a multitude of regulatory pathways, leading to rapid reprogramming of plant growth in order to cope with a sub-optimal environment. Subjecting *Brachypodium distachyon* (Bd) to severe and prolonged drought treatment, leads to a dramatic reduction in leaf size (46%), mostly due to a decrease in cell expansion, whereas cell division rate remains largely unaffected. To shed light on the regulatory networks that guide cell differentiation during drought stress condition, here we present a genome-wide characterization of the transcriptional and post-transcriptional molecular pathways activated in response to drought, focusing on three different developmental zones (proliferation, expansion, and mature cells) of young Bd leaves. To investigate the mechanisms controlling cell division and expansion during drought, we dissected the third emerging leaf to perform whole transcriptome and small RNAs sequencing (Illumina). Small RNA-Seq data allowed the identification of 188 new miRNA genes, divided into known and novel families, and a large number of small interfering RNA-producing loci. RNA-Seq data, produced on the same biological material, allowed us to better define the transcriptional profiles of coding and long noncoding genes, highlighting new regulatory networks and the cross-talk between different RNA molecules. This large data set provides novel evidence for a regulatory network where long noncoding RNAs, miRNAs and their target coding genes are deeply interconnected to orchestrate and reprogramming leaf development from undifferentiated to differentiated mature cells.
P8

The mechanism of phytosiderophore secretion mediated by BdTOM1 transporters

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Abstract

Present in insoluble ferric oxides under aerobic conditions, iron is not readily available for plant use. To overcome this limitation, grasses release phytosiderophores (PS) to the soil, where they bind and solubilize iron. The membrane transporter YS1 takes up the solubilized Fe(III)-PS complexes. In many grasses, PS secretion follows a diurnal pattern with a peak 4-6 hours after sunrise. Exocytosis of PS-containing vesicles or direct transport through the plasma membrane may explain PS secretion. Published work indicates that large vesicles accumulate at the periphery of barley root cells just prior to PS release. Also, Transporter Of Mugineic Acid1 (TOM1) was identified as a potential plasma-membrane-localized effluxer of PS. We aim to determine the mechanism of PS secretion by studying expression patterns and localization of \textit{Brachypodium distachyon} TOM1 transporters. \textit{Brachypodium} has two TOM1 genes (\textit{BdTOM1.1} and \textit{BdTOM1.2}) that are highly up-regulated in roots during iron deficiency. \textit{BdTOM1.1} and \textit{BdTOM1.2} have 62\% amino acid identity and belong to the Major Facilitator Superfamily of secondary transport carriers. Transgenic plants over-expressing \textit{BdTOM1.1-GFP} showed \textit{BdTOM1.1} in large dot-like structures moving with the cytoplasmic stream in root hairs. Interestingly, we observed many dot-like structures during peak PS secretion, but a few of these structures later in the day, when PS secretion is low or non-existent. These results support the idea that PS secretion involves filling vesicles with PS and then releasing them to the plasma membrane by exocytosis. Moreover, over-expressing \textit{BdTOM1.1-GFP} up-regulates \textit{BdTOM1.2} in the roots regardless of iron status, suggesting interaction between these two genes.
The cleavage function of miRNAs associated with abiotic stress responses in Brachypodium

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Abstract
Substantial progress has been made in the study of the miRNAs which aid in the post-transcriptional gene regulation of Brachypodium. To further enhance our knowledge of the role these small RNAs play in the regulation of the abiotic stress responses, ten PARE (Parallel Analysis of RNA Ends) libraries were constructed from two biological replicates of plants subjected to cold, drought, heat, and submergence stresses as well as control conditions. Sequencing these libraries resulted in a total of nearly 700 million raw sequences. Two miRNA target prediction algorithms were used to predict over 6000 target sites and a computational pipeline was developed to compare these predictions with PARE data and identify instances of potential stress-preferential miRNA guided post-transcriptional gene regulation. Over 150 target sites were identified for which a corresponding PARE sequence exhibited an over two-fold change in abundance between control and stress condition. To better understand the relationship between changes observed in PARE sequences at the predicted target sites and changes in target mRNA levels, RNAseq libraries were constructed, sequenced, and analyzed. Characterization of the miRNA/mRNA-target pairs based these changes revealed over 40 miRNA target sites which showed changes in PARE sequence abundance under stress conditions that cannot be explained by changes in mRNA abundance and indicate regulation of the stress responses may occur via miRNA-guided cleavage. Further characterization of how these miRNA and target mRNA levels change throughout the stress responses will give us a deeper understanding of the regulatory network needed for these plants to adapt to abiotic stress. Funded by the DOE.
P10

Functional characterization of AtNCED3 homologous candidate genes in Brachypodium distachyon

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Abstract
Growth, development and productivity of plant are affected by abiotic stress conditions. Abscisic acid (ABA) is involved in responses to environmental stresses, such as drought, salinity and high temperatures. 9-cis-epoxycarotenoid dioxygenase (NCED) is thought to be a key enzyme in ABA biosynthesis. AtNCED3 is induces and controls the level of ABA under drought stress in Arabidopsis. However, the ABA regulatory system of NCED in Brachypodium is not clear. In this study, we demonstrate that the expression of AtNCED3 homologous candidate gene, Bradi1g13760, is strongly induced by drought stress in Brachypodium. In addition, Bradi1g13760 gene provided higher drought tolerance to Arabidopsis. Overexpression of Bradi1g13760 gene in transgenic Arabidopsis promoted transcription of drought-inducible genes and caused an increase in endogenous ABA level. We introduced the Bradi1g13760 gene into Brachypodium and generated transgenic plants. The transgenic Brachypodium overexpression Bradi1g13760 gene are now exposing to dehydration for evaluating drought stress tolerance. The research infrastructure for environmental stress response in Brachypodium will provide benefits not only for improving cereal crops and pasture grasses but also for breeding biomass crops.
P11

**Functional response to drought and genetic expression in the Brachypodium distachyon species complex.**

Luisa M. Martínez¹, Aureliano Bombarely ², Francisco Amil-Ruiz³, Teresa Salido¹, Ana Fernández-Ocaña¹, Pedro J.Rey¹ and Antonio J.Manzaneda¹.

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**Abstract**

Polyploidy is a major force in plant speciation and is key in the evolutionary history of some *Brachypodium* lineages. Additionally, polyploids are often more tolerant than their lower-ploidy ancestors to drought stress, which might have contributed to adaptive differentiation of polyploid *Brachypodium* lineages in dry habitats. To test this, we first conducted an experiment involving the three species of the *B. distachyon* complex (*B. distachyon* 2n=2x=10, *B. stacei* 2n=2x=20 and its derived allotetraploid *B. hybridum* 2n=4x=30) to investigate their functional response to water stress. We applied a drought treatment to 119 inbred lines from these and recorded physiological measurements related to drought-tolerance. Second, we sequenced the transcriptome of 5 *B. distachyon*, 1 *B. stacei* and 1 *B. hybridum* accessions in response to drought. To analyze the differential expression of the homoeologs (duplicated genes in the polyploid) compared with its parental species, we used the diploid progenitor genomes as a references to identify and separate the different homeologs for each subgenome in the allopolyploid. We mapped and counted the reads to quantify gene expression using Tophat2/cufflinks and HyLite. Finally, gene expression of selected genes that were induced by drought was quantified by RTq-PCR in 15 selected genotypes with different tolerances to drought (five lines from each species). Our results show that *B. hybridum* response to drought is essentially correlated to *B. stacei* one, whereas *B. distachyon* functional response to drought is differentiated from *B. hybridum* and *B. stacei* responses. Our study suggest that specific physiological differentiation may underlie observed ecological divergence.
P13
Comparison of metabolic profiles across different growth stages and across different stress conditions between *Brachypodium distachyon* and wheat.

Yoshihiko Onda\(^1,2\), Kei Hashimoto\(^3\), Takuhiro Yoshida\(^4\), Tetsuya Sakurai\(^4\), Yuji Sawada\(^5\), Masami Yokota Hirai\(^3\), Kiminori Toyooka\(^3\), Keiichi Mochida\(^1,2,3\), and Kazuo Shinozaki\(^1,3\).

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Abstract

*Brachypodium distachyon* has become a model plant for studying biological phenomena observed in temperate grass plants. As a model species, growth scale is essential to analyse any changes in the molecular factors in a spatiotemporal context throughout its life cycle. Here, we demonstrated the availability of BBCH (Biologische Bundesanstalt, Bundessortenamt and CHeMical industry) scale, which is comparable with the Zadoks scale conventionally used for Triticeae crops, for sensitive and robust staging based on morphology in *B. distachyon*. We compared the chronological progression of *B. distachyon* accessions, Bd21 and Bd3-1, as well as that of Chinese Spring wheat. The comparison of growth stage illustrates the morphological similarities and the differences in timing of lifecycle events. Furthermore, we compared metabolite accumulation patterns across different growth stages or across different stress conditions by a widely targeted metabolome analysis. The metabolic profiling determined commonalities and specificities in chemical properties that were dependent on organisms, growth stages and/or stress conditions. From a qualitative standpoint, majority of the metabolites accumulated equivalently in *B. distachyon* and wheat. This qualitative similarity indicated the superiority of *B. distachyon* as a model for Triticeae crops.
P14
An Fe-deficient Brachypodium distachyon mutant defective in phytosiderophore release

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Abstract
While non-grass species use a combination of soil acidification and iron reduction to obtain sparingly soluble iron from the soil, grasses synthesize and secrete Fe(III) chelating agents called phytosiderophores (PS). When chelated by PS, Fe(III) solubility in the rhizosphere increases and Fe(III)-PS complexes are taken up into the root via specific transporters of the Yellow Stripe1 family at the plasma membrane. How PS are released from the root cells after synthesis remains unknown. We have investigated PS secretion in the model grass, Brachypodium distachyon (brachypodium), and find that 3-hydroxy-2'deoxyxymugine acid (HDMA) is the dominant type of PS secreted by brachypodium, and that secretion of HDMA is diurnally regulated. In our EMS-mutagenized brachypodium population, we identified a mutant line with leaf interveinal chlorosis, which we named interveinal yellow1 (ivy1). Foliar Fe application rescued the chlorotic phenotype. Metal content analyses revealed that mutant plants are low in Fe, Zn and Cu. HPLC analyses of PS in the roots and root exudates show that mutant plants are capable of synthesizing PS in normal amounts, but they do not release the PS from roots into the rhizosphere. We will present our progress toward cloning the ivy1 gene, which is likely to reveal novel aspects of the PS secretion mechanism.
P15

**GNRF suppresses floral transition and secondary wall synthesis in Brachypodium distachyon**

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**Abstract**

Several NAC transcription factors have been shown to play crucial roles in secondary cell wall biosynthesis and overall plant growth and development. *GRASS NAC REPRESSOR OF FLOWERING (GNRF)* was selected for functional characterization as it is highly expressed in stems, mirroring the expression pattern of characterized secondary cell wall regulators in *Arabidopsis thaliana*, including NST1, SND1 and SND2. Moreover its expression is co-regulated with cell wall biosynthesis genes. Over expression mutants (*GNRF-OE*) were generated by constitutively over expressing *GNRF* full-length coding region under the maize ubiquitin promoter. A homozygous mutant allele (*gnrf-1*) harboring a non-synonymous point mutation was isolated from a TILLING mutant collection. Surprisingly, over-expression mutants failed to flower and could not transition into the reproductive stage. Conversely the *gnrf-1* mutants flowered significantly earlier than control plants. Transcription profiling using a microarray revealed a fifty-fold reduction in two flowering time pathway genes *BdFUL1* and *BdFUL2*, consistent with the persistent vegetative growth phenotype. Three genes involved in cellulose biosynthesis, *BdCESA4/7/8*, two genes associated with lignin biosynthesis, *BdCAD1* and *BdCOMT4*, and a gene with a predicted role in xylan biosynthesis, *BdGT47-1*, were analyzed for changes in transcript abundance. In most cases, these genes were significantly down-regulated in *GNRF-OE* and up-regulated in *gnrf-1* stems. Chemical and histological analysis of *GNRF-OE* stems revealed reduced lignin content compared to the controls where as no significant change was observed between the *gnrf-1* and control samples. Taken together, these data suggest that *GNRF* functions as a pleiotropic repressor regulating cell walls and flowering in *Brachypodium distachyon*. 
P16

Brachypodium distachyon SWAM1 is a positive regulator of secondary cell wall synthesis and biofuel feedstock attributes and is not found in the Brassicaceae

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Abstract
Several MYB proteins have been reported to regulate plant cell wall biosynthesis, but no activators have been identified in the grasses. Here, we identified Brachypodium distachyon SECONDARY WALL ASSOCIATED MYB1 (SWAM1) as a potential regulator of secondary cell wall biosynthesis based on gene expression, phylogeny, and transgenic plant phenotypes. SWAM1 interacts with cellulose and lignin gene promoters in vitro and in vivo with preferential binding to AC-rich sequence motifs commonly found in the promoters of cell wall-related genes. SWAM1 over-expression (SWAM-OE) lines had greater above-ground biomass with only a slight change in flowering time while SWAM1 dominant repressor (SWAM1-DR) plants were severely dwarfed with a striking reduction in lignin of sclerenchyma fibers. Cellulose, hemicellulose, and lignin genes were significantly down-regulated in SWAM1-DR plants and up-regulated in SWAM1-OE plants. Considering lignin is inversely correlated with bioconversion efficiency phenotypes, ethanol yield was measured after culturing stems with Clostridium phytofermentans. There was no reduction in ethanol yield in SWAM1-OE lines; however, yield was significantly increased for SWAM1-DR samples. Phylogenetic and syntenic analyses strongly suggest that the SWAM1 clade was present in the last common ancestor between eudicots and grasses, but was lost from the Brassicaceae. Collectively, these data suggest that SWAM1 is a transcriptional activator of secondary cell wall thickening and biomass accumulation in B. distachyon and potentially in food and energy crop species, as well
P17

Chromosome territory arrangement in *Brachypodium*: FISH and computational modelling analysis

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Abstract
At interphase, chromosomes are highly decondensed and occupy distinct 3-D areas within the nucleus, known as chromosome territories (CTs). CT arrangement can be directly visualised using *in situ* hybridisation with fluorescent DNA probes that paint specifically individual chromosomes or mark specific chromosomal domains such as centromeres and telomeres. This approach facilitates the studies on the relationship between the internal architecture of the nucleus and such key intranuclear processes as regulation of gene expression or DNA repair. In this work, we present the results of studies on different aspects of nucleus architecture in *Brachypodium distachyon* and its close relatives. The distribution of centromeres and telomeres in the interphase nuclei from different organs was analysed using FISH. We observed that the majority of *B. distachyon* nuclei in the root tip cells displayed the Rabl configuration while both *B. stacei* and *B. hybridum* mostly lacked the centromere-telomere polarisation. Moreover, a chromosome painting approach was used to visualise the arrangement of individual CTs and associations between homologous chromosomes. In order to assess whether CTs in *B. distachyon* are distributed randomly or are subjected to certain patterns, the experimental data need to be complemented by a computer simulation. The model proposed in this study reflects the decondensation process of the post-mitotic chromosomes within the given sphere. It takes into account such parameters as the number, size, and morphology of chromosomes and allows to adjust the size and shape of nuclei. This work was supported by the Polish National Science Centre [grant no. DEC-2012/04/A/NZ3/00572].
P18
Characterization of the recessive root hairless mutant buzz

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Abstract
Plants respond to nutrient heterogeneity by the spatial deployment of the root system for the acquisition of soil resources. Root development and architecture is important for plant adaptation and reproductive success in different environments. To gain insight into broader questions of genetic cues involved in modulating the root development and architecture in temperate grasses we performed a forward genetic screen in \textit{Brachypodium distachyon} and identified a root hairless mutant termed \textit{buzz}. The \textit{buzz} mutant is a powerful tool in understanding root development in monocots at the molecular, genetic and developmental level. The expression of several candidate genes has been tested based on known putative orthologs from maize, rice and \textit{Arabidopsis thaliana}, however no change in gene expression between \textit{buzz} and wild-type roots was observed. Hormone treatments are not able to rescue the \textit{buzz} mutant phenotype, however the roots of the \textit{buzz} mutant respond to auxin as shown by the reduction in the primary root growth rate and the swelling of the root tip. Our general strategy involves (1) utilizing the MutMap\textsuperscript{+} technique to identify the unique genomic region that harbors the \textit{buzz} mutation; (2) performing expression analyses by qRT-PCR and RNA Seq; (3) determining the implications of root hairs in pathogenesis and nutrient depleted soils. Identifying the causal gene responsible for the root hairless mutant phenotype will have profound implications for our understanding of root development in temperate grasses and other agronomically important crop plants such as wheat and barley.
Stomata Development in Brachypodium – Rewiring the bHLH Transcription Factor Network

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Abstract
Stomata are pores in the plant epidermis that regulate carbon dioxide and water vapor exchange between the environment and the plant. Production of mature stomata involves a series of cell divisions and cell fate transitions, and the molecular genetic regulation of this process has been extensively studied in Arabidopsis. Less attention, however, has been paid to stomatal development in grasses, where stomatal shape has changed, stomata are flanked by subsidiary cells, and stomata develop in specific cell files aligned along the leaf axis. To uncover genetic networks controlling stomatal development in grasses, we screened an EMS-mutagenized Brachypodium population and identified several stomatal mutants, some of which we found to be orthologues of stomatal regulators from Arabidopsis. Molecular and genetic analysis of these Brachypodium genes, however, showed that they can adopt novel functions. For example, stomataless, a mutant that fails to produce stomata, bears a mutation in BdICE1. Although Arabidopsis ICE1 is also implicated in stomatal formation, it is redundant with its sister gene SCR2 and both genes must be inactivated to eliminate stomata. Moreover, BdICE1 appears to act at a different developmental stage than the Arabidopsis homologues. This prompted us to investigate the putative “stomatal initiation” module (SPCH and ICE1 families) in Brachypodium through analysis of transcriptional and translational reporters and targeted loss- (CRISPR/Cas9) and gain-of-function lines. Our results suggest that, although individual genes are conserved, the regulatory relationships among these genes have changed. This highlights ways in which gene regulatory networks might be customized to produce diverse developmental patterns.
P20
Enhancing storage lipid synthesis in vegetative tissues of *Brachypodium distachyon* by overexpressing *WRINKLED1*

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Abstract
The use of plant vegetable oils for production of biodiesel has an enormous potential to revolutionize the global bioenergy outlook. Plants normally accumulate oil in seeds in the form of triacylglycerols (TAGs) as storage reserves. Recent studies have demonstrated that a variety of molecular/genetics strategies can lead to TAGs accumulation in leaves. Examples include the overexpression of *DGAT* or *MGAT* genes, ectopic expression of transcription factors encoded by genes such as *LEAFY COTYLEDON 1 (LEC1), LEC2* and *WRINKLED1*. The ectopic expression of the WRI1 ortholog of the monocotyledonous model plant *Brachypodium distachyon* induces TAGs accumulation in Brachypodium leaf tissues. In addition to TAGs accumulation, browning of leaf blades due to premature cell death was observed in the leaf tissues. To minimize spotty necrosis in leaf tissues we developed stem-specific expression vectors containing promising promoters with high internode expression. Here, we report the enhanced accumulation of TAGs in stems of transgenic *Brachypodium distachyon* plants expressing the Brachypodium *Wrinkled1* gene under *BdTIFY 3A-like* promoter.
The B-class genes: targets and timing in floral development

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Abstract
Although the molecular genetics of floral development has been intensely studied in the eudicots, many of the genes underlying floral development in the grasses remain uncharacterized. The ABC(D)E-class MADS box transcription factors are core regulators of floral organ identity in the eudicots. A number of core MADS box transcription factors have been cloned in maize and rice, and B- and C-class MADS box function, in particular, seems to be conserved. As in the eudicots, B-class MADS box genes control second whorl organ (lodicule) and stamen development in both maize and rice. This conservation in function stands in stark contrast to the extreme morphological differences between eudicot flowers and highly derived grass florets. Thus, understanding not only of the MADS box genes, but also of their downstream targets, is of key importance in elucidating the full gene regulatory network that mediates floral development. We present our work on finding the targets of a B-class MADS box gene in maize, sterile tassel silky ear1 (sts1). We have identified the genome-wide targets of STS1 using RNA-Seq and ChIP-Seq. Our experiments have begun to reveal a complex transcriptional regulatory network centered around the B-class MADS box genes in maize.
P22
Examining the Ability of an Obligate Heterodimer to Bind DNA in an Homodimeric System

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Abstract
The ABC(D)E MADS box proteins are a family of DNA-binding transcription factors that regulate floral development. In all investigated flowering plants, B-class MADS box proteins regulate second whorl floral organ development, and stamen development. In planta, the B-class MADS box proteins APETALA3 (AP3) and PISTILLATA (PI) cannot substitute for one another, despite their close sequence similarity. This result is explained, in part, by DNA-binding assays: most AP3-like and PI-like proteins bind DNA as obligate heterodimers. However, in the order that contains the grasses, both obligate B-class heterodimerization and PI-like homodimerization has evolved in several species independently. For example, in Brachypodium distachyon, one PI-like protein (BD16) can homodimerize, while the other PI homolog forms obligate heterodimers with the Brachypodium AP3 homolog. In order to investigate the function of B-class hetero- vs homodimers, we have synthesized extant versions of an ancestral obligate heterodimer. We also induced point mutations on BD16 that may confer the ability to act as an obligate heterodimer. We will use gel shifts to assess the ability of these proteins to bind DNA. Future experiments will characterize hetero- vs homodimer function in B. distachyon. These experiments will provide insight into how and why obligate heterodimerization arises, and its phenotypic consequences.
P23

FLOWERING LOCUS C in Brachypodium distachyon

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Abstract

After our discovery of FLOWERING LOCUS C (FLC) genes in cereals, we analyzed their function in Brachypodium distachyon. Because the genes have diverged significantly in sequence, our initial aim in these studies was to provide a comparison to FLC in Arabidopsis thaliana to determine the extent of functional conservation. We generated transgenic lines and analyzed their flowering time, we investigated gene expression in response to prolonged winter cold, we analyzed gene expression in multiple accessions before, during and after cold to understand how this correlates with vernalization requirement, we investigated whether chromatin marks change at the Brachypodium FLC locus during cold and whether this explains epigenetic silencing using chromatin immuno precipitation. In this presentation, we will report the results of these experiments.
P24
Analysis of the role of Brachypodium ADP-glucose pyrophosphorylase in storage starch biosynthesis

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Abstract
In most plants, ADP-glucose is produced in plastids by ADP-glucose pyrophosphorylase (AGPase). However, in the endosperm of grass species, ADP-glucose is produced in the cytosol and imported into plastids via ADP-glucose transporter (BT1). Therefore, the coordinated expression of AGPase and BT1 in the cytosol is necessary for storage starch biosynthesis in the endosperm of grasses, including economically important cereals. Hence, we investigated the expression patterns of AGPase and BT1 genes during plant growth and seed development in Brachypodium. Results from phylogenetic analysis followed by a sequence database search revealed there were three genes encoding the large subunit (APL), two genes encoding the small subunit (APS) of AGPase, and one gene encoding BT1 in Brachypodium. One APS had two types of transcripts derived from alternative splicing (E1a and E1b type). Interestingly, the gene corresponding to APL2 was absent in Brachypodium. In rice and maize, APL2 functions as the cytosolic APL. Quantitative gene expression analyses of AGPase revealed that APS1 E1a and APL3 were mainly expressed in seeds. Moreover, the BT1 was also concurrently expressed in seeds. Intracellular localisation analyses using Brachypodium protoplasts showed the cytosolic localisation of Brachypodium APL3 and rice APL2. These results indicated duplicate copies of APS1 E1a and APL3 functioned as the cytosolic AGPase in the Pooideae endosperm. These results also suggested that gain of the ability of APL3 to localise to the cytosol with the disappearance of APL2 during Pooideae evolution may have allowed for the maintenance of ADP-glucose production in the cytosol for storage starch synthesis.
P25
Phylogeny and Expression Profiling of YABBY Genes in Brachypodium distachyon

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Abstract
Members of the YABBY gene family have a role in promoting abaxial cell fate in eudicots, while their involvement in the polar regulation of lateral organ development in monocot plants is less clear. To shed light on the YABBY family evolution and function in plants, we have compared the sequences from representatives of major plants lineage and generated several phylogenetic trees using Bayesian Inference (BI) analyses to investigate the evolution of this gene family and how Brachypodium fits into the system. In addition, the expression patterns of all YABBY family members in Brachypodium were mapped and quantified, spatially and temporally, throughout plant development using RT-PCR, mRNA in situ hybridization and transcriptomics. Special attention was paid to expression patterns in the developing caryopsis or grain, the distinctive fruit of the grass family, to provide more insights into the roles of the YABBY family members in fruit evolution specifically and grass development more generally.
P26
Creating a multi-functional library of grass transcription factors for the energy crop model system Brachypodium distachyon

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Abstract
Comprehensive collections of full-length transcription factor cDNAs (fl-cDNA) have proven to be an extraordinary reagent for model systems research. We are constructing a complete Brachypodium distachyon transcription factor collection in an entry vector that will be of value for numerous functional studies. Through the analysis of several types of expression profiling data sets, we identified high priority candidates for the regulation of biofuel feedstock relevant traits, such as growth and cell wall biosynthesis and abiotic stress tolerance. We synthesized 372 unique transcription factors from families that include bHLH, bZIP, CCAAT, GRAS, Homeodomain, HSF, MADS box, MYB, NAM, WRKY, and several classes of zinc fingers. The collection will be transferred into multiple destination vectors for downstream applications including protein-DNA and protein-protein interaction platforms in yeast. Genes that yield positive interactions can then be shuttled from their pENTR vector to a variety of other constructs for various purposes including expression in planta to further characterize their functions. We are presently evaluating B. distachyon protein-DNA interactions in yeast using two approaches. The first is “gene-centric” where a promoter is tested for interactions with all transcription factor proteins in the library. The second approach is “protein-centric” and interrogates the capacity of each transcription factor protein to interact with a collection of 768 synthetic 250 bp promoters. This collection of synthetic promoters was designed to maximize potential binding motif sequence diversity and all possible 8 nt DNA motifs occur in at least 4 independent promoters. Proof-of-concept experiments demonstrate the utility of this approach and we are currently expanding the analysis to infer the binding specificities for all of the transcription factors synthesized in this project.
P27
DNA methylation variation across *Brachypodium distachyon* highlights genetic diversity and frames adaptation via the ‘extended genotype’

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Abstract
Excitement has grown in the last decade regarding the role of DNA methylation as a possible epigenetic regulator of phenotype. As a chromatin mark, DNA methylation is known to suppress the activity of repetitive genomic elements and, in some cases, genes. Beyond this, the role of DNA methylation may range from transient regulation of genes to mitotic and/or transgenerational sources of adaptive variation. Although an important chromatin mark, DNA methylation can be thought of as one portion of an organism ‘extended genotype’ in which a variety of non-traditional sources of heritable variation (chromatin variation, transposable element polymorphism, and polyploidization) may act to provide additional diversity for rapid adaptation. *Brachypodium distachyon* provides a robust system with diverse germplasm and genomic resources to investigate the extended genotype. Profiles of DNA methylation across seven diverse reference inbred lines highlight a largely conserved methylome. However, thousands of regions displaying differential methylation can be identified. Methylation diversity appears largely associated with genetic divergence. From this, the role of the extended genotype may be more pronounced in situations where genetic (allelic) diversity is severely reduced. An examination of clonal *Brachypodium* populations provides a unique opportunity to study the possible role of extended genotype adaptation across natural populations.
P28

Cytogenetics of *Brachypodium* – current status and future prospects

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Abstract

Cytogenetics focuses on the study of nuclear genomes at the microscopic level. In its modern incarnation, known as molecular cytogenetics, it represents a multidiscipline that amalgamates various methodological approaches of cytology and molecular genetics as well as advanced microscopy, flow cytometry and digital image processing. Techniques such as FISH with increasingly sophisticated probes and fluorescent antibodies targeting specific nuclear components, empowered by cutting-edge microscopes and digital image acquisition and processing systems, offer unprecedented insights into various aspects of genome organisation both at interphase and during mitosis and meiosis. In contrast to that of animals, the cytomolecular organisation of plant nuclear genomes is still relatively poorly studied and understood. The genus *Brachypodium* now represents a well-established and exceptionally good system for advanced molecular cytogenetic studies, due in part to highly desirable ‘model’ biological features of its representatives but also because of the rapidly growing repertoire of various experimental tools. In this presentation, we outline the current status of our investigations into various aspects of grass genome organisation, such as (i) karyotype structure and evolution, (ii) distribution of chromosome territories within the nucleus, (iii) dynamics of epigenetic modifications of chromatin during embryo development and cell differentiation, (iv) true nature of selective silencing of rRNA genes in some *Brachypodium* allopolyploids, (v) instability of a small grass genome subjected to a mutagenic treatment. The authors acknowledge financial support from the Polish National Science Centre (grants no. 2012/04/A/NZ3/00572 and 2014/14/M/NZ2/00519).
P29
Assessment of the *Brachypodium distachyon* genome assembly with the whole-genome BioNano map

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Abstract
The widely recognized model plant *Brachypodium distachyon* was the first sequenced species of the grass subfamily Pooideae. Though it is generally accepted that the current assembly for *B. distachyon* line Bd21 is one of the best assemblies for grass genomes, there is no independent and direct assessment of its quality. We employed a sequence-independent technology, BioNano genome (BNG) map, which is an optical restriction map of megabase-size DNA contigs built by analyzing genomic DNA fragments with BioNano Genomics’ Irys™ platform. We generated a whole-genome consensus BNG map of *B. distachyon* line Bd21 that contains 233 BNG contigs and spans 284 Mb, with N50 of 1.79 Mb. Comparison of the BNG map with the *B. distachyon* genome sequence assembly (Bdistachyon.MIPS_1_2) validated 98% of the sequence assembly; no large misplaced or misoriented sequence fragments were observed, although some misassembled regions were detected. We were able to place four unassigned scaffolds of a total length of 155,061 bp in the pseudomolecules. We were unable to align 20 BNG contigs with the current assembly; they contain only repeats representing 10.4 Mb in length. These results confirmed that the current release of the *B. distachyon* genome assembly is of good quality, yet it can be further improved with the help of BNG map. The potential application of this sequence-independent technology to facilitate natural variation analysis will be discussed.
P30

Insertional mutagenesis of *Brachypodium distachyon* using the Tnt1 retrotransposon and its use to study nonhost disease resistance

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Abstract

*Brachypodium distachyon* is an annual C3 grass that recently 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 23 insertions per line in a population of 163 plants analyzed. A total of 3,793 flanking sequence tags (FSTs) were recovered from the transgenic lines. FST analyses showed a uniform pattern of insertion in all the chromosomes. Southern blot analysis supported the occurrence of the transposition events confirming the mobility of the transposon during embryogenesis and its stability during sexual reproduction. Considering the average length of a gene transcript of 3.5 kb, we estimated that 11,909 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 rust pathogens will also be presented.
P31
Novel and Classical Resistance Genes Provide Nonhost Resistance to Wheat Stripe Rust in *Brachypodium distachyon*

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Abstract
The plant pathogen *Puccinia striiformis* f. sp. *tritici* (PST), commonly known as wheat stripe rust, is an obligate biotroph with a wide host range. In addition to the agronomically important grass species wheat, barley and rye, it can also infect the taxonomically distant *Brachypodium distachyon*. To obtain an understanding of the genetic components determining host range in this plant-pathogen system, it will be essential to explore the interaction of PST with taxonomically distant hosts. As *B. distachyon* is a model grass species with a sequenced genome, a high rate of recombination and advantageous morphological characteristics, it serves as a good system to study the genetic basis of PST resistance in a distant host. We found that several *B. distachyon* accessions were completely resistant to various UK and Australian PST isolates, whereas other accessions showed a range of susceptibility symptoms. Three populations were used to establish the genetic architecture of this resistance, including ABR6 x Bd21, BdTR10H x Tek-4 and BdTR13K x Bd21. Resistance to three UK and one Australian PST isolates was rough-mapped to three major QTLs, designated *Yrr1* to *Yrr3*. Interestingly, the gain of function interval of *Yrr1* does not contain any known resistance gene homologs, whereas classical resistance genes seem to underlie *Yrr2* and *Yrr3*. Moreover, isolate specificity was observed to *Yrr2* and to additional minor effect QTLs. Transformation studies are underway to confirm the identity of the resistance genes at these loci.
A 
*Brachypodium distachyon* LysM-RLK is involved in perception of arbuscular mycorrhizal symbiotic signals

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Abstract

Root endosymbioses allow better access for plants to nutrients such as nitrogen, phosphor or water. Therefore they are of agronomical and ecological importance. Among endosymbionts, the arbuscular mycorrhizal (AM) fungi are able to colonize roots of most of land plants including those of *Brachypodium distachyon*, and exchange nutrients for carbohydrates. AM fungi secrete lipo-chitooligosaccharides (LCOs) and chitoooligosaccharides (COs) called Myc-factors, which are signal molecules. LCOs, called Nod-factors, are also secreted by rhizobial bacteria forming another endosymbiosis restricted to the legume family. Nod-factors are essential for establishment of this symbiosis. In contrast, the exact role of Myc-factors in the AM symbiosis is not yet known but their applications on different species induce molecular and developmental responses. Our project aims to better understand the roles of Myc-factors. To do this, we are searching for their receptors. Proteins encoded by the *Lysin-Motif Receptor-Like Kinase (LysM-RLK)* multigenic family are candidates to be LCO and CO receptors. *B. distachyon* appears to be a promising model for this study since it possesses the smallest LysM-RLK family among plants with sequenced genomes that are able to establish the AM symbiosis. CO and LCO receptors will be identified using a combination of biochemical and reverse genetic approaches on the *B. distachyon* LysM-RLK family. We found that a LysM-RLK has high affinity for LCOs. Reverse genetics is in progress to determine its role in LCO responses and in the AM symbiosis. For this, a chemically mutated *B. distachyon* population has been screened by TILLING for mutations in this *LysM-RLK*. 
P33
Allelic Variation and Domain Functional Analysis of the BSMV Resistance Gene Bsr1 in Brachypodium

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Abstract
We found Brachypodium distachyon inbred line Bd21 can be infected by barley stripe mosaic virus (BSMV) strain ND18, whereas Bd3-1 showed highly resistant to ND18. Bsr1, a CC-NBS-LRR gene, was isolated from Bd3-1 by map-based clone approach. Natural variations in resistance to BSMV ND18 and allelic variations of Bsr1 in 46 B. distachyon, 15 B. stacei and 227 B. hybridum accessions were characterized. The results showed the Bsr1 alleles of the Brachypodium could be classified as four Clades. The B. distachyon accessions contain either Clade I and Clade II and B. stacei accessions contain either Clade III or Clade IV, while the B. hybridum lines contain four kinds of combination, indicating independent evolution of B. hybridum from natural hybridizations between B. distachyon and B. stacei.

The N terminal (CC-NB1-451) of Bsr1 had a 94.0% of amino acids similarity with that of bsr1. However, bsr1 has a 96 amino acids unique region at the C terminal. Bsr1 co-expressed with ND18 amplicon induced necrosis by agro-infiltration in Nicotina benthamiana leaves, whereas bsr1 did not. To characterize the function of the Bsr1 domains, four fragments including the CC-NB1-451, LRR domain, the bsr1 unique region and the C terminal fragment were swapped, achieving 9 constructs in binary vector pMDC32. Transient expression in N. benthamiana showed that the LRR domain and the C terminal are critical region for the BSMV resistance.
P34

Virus-induced Alternative Splicing Landscapes in *Brachypodium distachyon*

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Abstract

Alternative splicing (AS) influences diverse biological processes including growth, development and response to stress. We performed RNA-sequencing of the model monocot, *Brachypodium distachyon*, infected with *Panicum mosaic virus* (PMV). We identified ~44,443 transcripts. Expression of ~28,900 transcripts in ~18,800 gene loci was ≥2 FPKM, and ~42% of multi-exonic genes were alternatively-spliced. Among the major AS types, intron-retentions predominated, followed by alternate acceptor, alternative donor and exon-skipping. Comparative analysis of AS landscapes in *Brachypodium*, rice, maize, *Sorghum*, *Arabidopsis*, potato, *Medicago* and poplar further revealed conserved AS patterns among monocots and dicots. We identified previously unknown AS events in ~100 immune-related genes encoding receptor-like kinases, NB-LRR resistance proteins, transcription factors, RNA-silencing and splicing-associated proteins. Cloning and molecular characterization of Bd-SCL33, a serine/arginine-rich (SR) spliciing factor, uncovered multiple novel intron-retaining splice-variants that are developmentally regulated and modulated during virus infection. Strikingly, Bd-SCL33 splicing patterns are conserved among six additional grass-infecting viruses including *Brome mosaic virus*, *Barley stripe mosaic virus*, *Maize mild mottle virus*, *Sorghum yellow banding virus*, *Wheat streak mosaic virus* and *Foxtail mosaic virus*. Together, our analysis provides novel insights into virus-triggered AS landscapes conserved among monocots and dicots, and uncovered previously unknown AS events in plant defense-related genes.
P35
Genomic and genetic dissection of quantitative resistance of Brachypodium distachyon to Puccinia brachypodii

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Abstract
Genomic regions associated with quantitative resistance to the leaf rust Puccinia brachypodii were found on chromosomes 2, 3 and 4 of Brachypodium distachyon and three QTLs (Rpbq1, Rpbq2 and Rpbq3) were mapped in a segregating population derived from the resistant Bd3-1 × susceptible Bd1-1. After a validation experiment, Rpbq2 and Rpbq3 were confirmed and prioritized for further research. To finely map Rpbq2 and Rpbq3, large segregating populations have been developed for each QTL separately, by selfing heterozygous plants for one QTL, with the remaining QTL in susceptible homozygous phase. The segregating populations were genotyped to obtain homozygous recombinants, that were then phenotyped with the H-Ki isolate of P. brachypodii. Rpbq3 interval was reduced, and the chromosome segment comprised between Bradi4g10017 and Bradi4g100345 was the most linked with the resistance. Rpbq2 results were less clear and a second experiment of phenotyping at seedling stage is needed to validate a reduced interval. Moreover, the response of Brachypodium transcriptome induced by the infection with H-Ki isolate at 18hpi was investigated using the Brachypodium Tiling Array BradiAR1b520742. A total of one-hundred genes were modulated by infection, more frequently in Bd3-1 than in Bd1-1. A set of putatively candidate genes involved in defense network like NB-ARC/NBS-LRR, and genes participating in the detoxification process were identified and compared with the fine mapping results. The first set of candidate genes derived by the functional genomics and genetic approaches provide a first characterization of the molecular basis of resistance to the leaf rust in the model grass Brachypodium.
P36
How useful is the model? Comparative analysis of Brachypodium and wheat responses to infection with a necrotrophic fungal pathogen.

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Abstract
Brachypodium distachyon has been widely adopted as a pathosystem for study of cereal pathogens. Fusarium crown rot (FCR), primarily caused by the necrotrophic fungal pathogen Fusarium pseudogrominearum, constitutes a major disease risk for the Australian wheat industry. The aim of this work was to assess whether species in the genus Brachypodium will be a useful model for characterizing the molecular basis for resistance to F. pseudogrominearum. Two Brachypodium species were found to be readily infected by F. pseudogrominearum producing similar symptoms and disease progression to that observed in wheat and barley. A large number of Turkish natural accessions were screened for FCR susceptibility and several ecotypes were found to differ significantly in disease severity. Brachypodium hybridium accessions exhibited significantly greater quantitative resistance compared with B. distachyon accessions. RNAseq was utilized to observe the host molecular response during FCR infection in wheat and B. distachyon. Comparing induction of homologs in wheat and Brachypodium revealed a high degree of similarity in response between crop and model. However, significant differences for induction of defence related phytohormones and other defence related metabolites were observed using liquid chromatography – mass spectrometry (LC-MS). B. distachyon transformants constitutively expressing a salicylate hydroxylase (nahG) from Pseudomonas putida were developed (Bd21-3 background) to assess the importance of salicylic acid mediated defence responses in promoting resistance against necrotrophic pathogens. The potential utility and limitations for Brachypodium as a molecular model for studying cereal-pathogen interactions are discussed in light of observed similarities and differences.
Evolutionary genetic origin(s) of vernalization responsiveness in temperate pooid grasses

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Abstract
The ability of plants to match their reproductive output with favorable environmental conditions has major consequences for individual lifetime fitness and geographic patterns of diversity. In temperate ecosystems, some plant species have evolved the ability to use winter chilling (vernalization) as a cue to ready them for spring flowering, a trait known as vernalization responsiveness. However, it is unknown how important the evolution of vernalization responsiveness has been for the colonization and subsequent diversification of taxa within the northern and southern temperate zones. Grasses of subfamily Pooideae, including several important crops such as wheat, barley, and oats, and the model species Brachypodium distachyon, predominate in the northern temperate zone, and it is hypothesized that their radiation was facilitated by the early evolution of vernalization responsiveness. Predictions of this single early origin hypothesis are that a response to vernalization is widespread within the subfamily, and that the genetic basis of this trait is conserved. To test these predictions, we are comparing flowering time and genome-wide gene expression of phylogenetically representative pooids under chilling and control conditions. Our data demonstrate that vernalization responsive pooids are phylogenetically widespread, and at least part of the vernalization gene network is conserved throughout the subfamily. These results thus far support the idea that the evolution of vernalization responsiveness was important for the initial transition of pooids out of the tropics into the temperate zone.
Investigating changes in the genetic architecture of agriculturally relevant traits in *Brachypodium* in response to variable climates using Genome Wide Association Studies.

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**Abstract**

The geographic range of *Brachypodium distachyon* covers a broad spectrum of climatic regions; including hot arid deserts, alpine and temperate climates. As such, a wide diversity of life strategies, plant architecture and stress tolerance mechanisms have evolved and been selected for over time. Genome wide association studies (GWAS) allow us to use this diversity to investigate the genetic architecture of traits of interest. Once causative loci are identified, candidate genes can be further investigate or QTLs used for breeding programs. However population structure must be taken into account when selecting a natural diversity set for GWAS to reduce the occurrence of false positive results. By performing GBS on a large number of accessions from around the world we have selected a balanced subset for GWAS analysis. We phenotyped this GWAS set for agriculturally relevant traits such as early vigour components, flowering time and yield components across contrasting variable climatic conditions using our dynamic climate chambers to mimic the circannual and diurnal patterns in temperature, humidity, light intensity and light spectrum of individual climatic regions. This allowed us to investigate how the genetic architecture of traits changes in response to the growth environment. We can then identify causative alleles that are important across environments and ones that are key to trait expression in a specific environment, allowing trait plasticity.
P39

Molecular basis of the ability for short days to substitute for vernalization in the temperate grass, Brachypodium distachyon

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Abstract

Proper timing of flowering is essential for reproductive success in plants and is a major determinant of biomass yield. Vernalization is the process by which competence to flower is achieved after prolonged exposure to winter cold. In addition to vernalization prolonged exposure to non-inductive short days (SD) followed by a shift into inductive long days (LD) has the ability to substitute for the vernalization response in a range of flowering plant families. However, unlike vernalization, little is known about the molecular nature of the SD substitution for vernalization phenomenon. Here, we report the characterization of the SD substitution response across 60 diverse Brachypodium accessions, and assess the genetic basis of flowering in populations derived from crosses between accessions that vary in their ability for SD to substitute for vernalization. An F2 cross between a SD responsive and SD non-responsive accession reveal that the ability for SD to substitute for vernalization is controlled by a single large effect locus which we have fine mapped to an FT-like (FTL) paralog. Remarkably, all SD non-responsive accessions share the same non-synonomous change within a conserved residue in the 3rd exon that is not present in any of the SD responsive accessions. FTL is dynamically regulated by the photoperiod and is expressed only in SD. Furthermore, our genetic analyses reveal a key vernalization gene VERNALIZATION2, is responsible for the LD repression of FTL. The cloning of FTL provides the first molecular insight into the SD substitution for vernalization phenomenon in plants.
P40

Natural variation and functional genomics: *Brachypodium distachyon* as an emerging system for studying grass cell walls and cereal stress tolerance.

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Abstract
Grasses are major sources of food and, potentially, lignocellulosic biofuels. *Brachypodium distachyon* is related to several economically important crop species and permits researchers to rapidly perform developmental and functional genomics studies. We used the lignin-detecting fluorogenic dye, Basic Fuchsin, to analyze the development of secondary cell walls in stems of three *B. distachyon* ecotypes at high spatio-temporal resolution. We detected differences in the timing and extent of both lignification and biomass accumulation between each ecotype. Basic Fuchsin is thus a useful comparative tool for imaging lignin. We have also recently launched a new project, which we call “Fast Farming”, to screen for and characterize cell wall-related genes that confer tolerance to abiotic stresses such as drought when these genes are over-expressed in *B. distachyon*. This project uses activation tagging to screen several thousand lines (provided by Dr. John Vogel, JGI) in which the expression of endogenous genes is amplified. We have identified several initial hits with enhanced drought tolerance, and are performing secondary screening of these lines. Once candidate genes for stress tolerance have been identified, this information can be applied to modify or select for the expression of orthologous genes in related cereal crops, potentially increasing grain yields under the adverse conditions that are increasingly prevalent around the world as a result of climate change.
P41
Effects of PHENYLALANINE AMMONIA LYASE (PAL) knockdown on cell wall composition, biomass digestibility, and biotic and abiotic stress responses in Brachypodium

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Abstract
The phenylpropanoid pathway in plants synthesizes a variety of structural and defence compounds, and is an important target in efforts to reduce cell wall lignin for improved biomass conversion to biofuels. Little is known as to what trade-offs exist in grasses when reducing function of the first gene family in the pathway, PHENYLALANINE AMMONIA LYASE (PAL). Therefore, we targeted by RNA interference (RNAi) PAL isoforms in the model grass Brachypodium distachyon, identifying large reductions (up to 85%) in stem tissue transcript abundance for two of the eight putative BdPAL genes. BdPAL-knockdown culm cell walls had 43% and 57% reductions in lignin and ferulic acid, respectively, and a nearly two-fold increase in the amounts of polysaccharide-derived carbohydrates released by thermochemical and hydrolytic enzymic partial digestion. PAL-knockdown plants exhibited delayed development and severely reduced root growth, along with increased susceptibilities to the fungal pathogen Fusarium culmorum. Surprisingly, these plants generally had wild-type (WT) resistances to caterpillar herbivory, drought, and ultraviolet light. RNA-seq analyses revealed that the expression of genes associated with stress responses including ethylene biosynthesis and signalling were significantly altered in PAL knockdown plants under non-challenging conditions. These data reveal that, although an attenuation of the phenylpropanoid pathway increases carbohydrate availability for biofuel, it can have an adverse effect on plant growth and disease resistance to fungal pathogens. Our data identify notable differences from how Arabidopsis pal mutants respond to stresses and provide insights into what challenges may arise when deploying phenylpropanoid pathway-altered grasses as dedicated bioenergy crops.
P42

The pangenome of *Brachypodium distachyon*: estimating the true genomic diversity of a species

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Abstract

The genetic diversity of a species is the sum of the diversity found in all individuals of that species. Many studies have attempted to estimate the diversity of a species by resequencing diverse accessions and aligning the reads to a reference genome. While this approach readily identifies SNPs and small indels, it underestimates total genomic diversity because highly divergent regions align poorly to the reference and, of course, any sequence not found in the reference will be missed entirely. Thus, the true extent of diversity within a species is largely unknown. De-novo genome assemblies and independent annotation can be used to more accurately estimate the true genomic diversity within a species. We applied this approach using 54 Brachypodium distachyon accessions to create a pan-genome that contains all the diversity found in the accessions sequenced. Our results indicate that the pan-genome is substantially larger than the genome of any individual accession, twice as large by some measures. Systematic comparison of the individual genomes identified a set of core-genes found in all sequenced lines and a larger set of shell genes present only in some accessions. Our results also characterize the variability of conserved non-coding sequences among individuals of this species, which we are correlating with genome-wide expression patterns and gene variability. Together, the core genes, shell genes and all the non-coding sequences constitute the pan-genome of *B. distachyon*. Overall, this work supports a dynamic view of genomes within a species, which is updated with every new accession studied.
P43
Comparative grain transcriptomics of *Brachypodium* and temperate crops

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Abstract

*Brachypodium*, as a sister to the core pooids that include wheat, barley and rye, represents a good model for the study of grain development and evolution of grain form in the temperate cereals. Using a comparative cellular developmental and transcriptomic approach, we aim to gain insights into the transcriptional regulation of key stages in grain development.

We have sequenced the transcriptome of one vegetative and several distinct developmental stages of *Brachypodium* grains; pre-anthesis ovaries, young grain (1-3 DAA), mid-length grain (3-8 DAA), full-length (8-15 DAA) and mature grain (15-24 DAA), as well as mature grain without the developing embryo and germinating grains. By looking at the differential expression of genes through grain development we are starting to identify clusters that coincide with the initiation of key developmental stages, such as the initiation of endosperm proliferation, cellularisation and aleurone layer differentiation, as well as the activation of specific metabolic pathways, such as starch and protein biosynthesis. Using RT-PCR/qPCR and mRNA *in situ* hybridisation analyses, we are undertaking a more detailed cross-species comparison of the temporal and spatial expression of selected transcription factors identified from our RNAseq experiment, in order to fully characterise key regulators of grain development in the temperate cereals.
P44

Uncovering the genetic basis for biofuel feedstock traits in *Brachypodium distachyon*

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Abstract

Biofuels derived from plant biomass present a promising avenue to address the negative aspects of fossil-fuel dependence. The efficiency of biofuel production relies in part on the lignocellulosic feedstock hydrolysis efficiency. In order to capitalize on the potential of lignocellulosic biofuels, the genes underlying natural genetic variation for conversion efficiency must be determined. We utilized a recombinant inbred mapping population to map biofuel-related traits in the model grass species *Brachypodium distachyon*. We detected a bioconversion quantitative trait locus (QTL) using our *Clostridium phytofermentans* bioassay that resulted in an 8% increase in ethanol yield. We are currently using next-generation sequencing to identify recombination breakpoints for three pairs of near isogenic lines segregating within the *BIOFUEL1 (BFL1)* QTL. Additionally, examination of genes within *BFL1* based on gene expression, polymorphisms, and available gene annotation revealed a putative glycosyltransferase as an excellent candidate gene. We are currently utilizing available T-DNA collections as well as the CRISPR/CAS9 system to study the effect of perturbing this glycosyltransferase. Identification and characterization of *BFL1* should enhance our ability to modify current and future biofuel crops for higher conversion rates thus increasing the efficiency of the biofuel production process overall.
P45

*Brachypodium* projects at the DOE Joint Genome Institute: expanding the model

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**Abstract**

The widespread adoption of *Brachypodium distachyon* as a model system was greatly accelerated by the reference genome sequence produced by the DOE Joint Genome Institute (JGI). Several projects at JGI are continuing this tradition by producing sequence resources that will vastly expand the utility of four *Brachypodium* species as research tools. For the first project we sequenced, assembled and annotated 54 *B. distachyon* genomes to define the *B. distachyon* pan-genome. Initial analysis indicates that the pan-genome may be twice the size of the genome of any single accession. The goal of the recently initiated second project is to sequence thousands of *B. distachyon* chemical and radiation mutants to create a comprehensive mutant resource. The first 100 mutants have been sequenced to determine the spectrum and frequency of mutations induced by three mutagens. The third project seeks to establish a model system to study polyploid genome evolution and regulation by sequencing the diploid species *B. stacei* and the allopolyploid species *B. hybridum*. The *B. stacei* assembly has been completed and the *B. hybridum* assembly is currently being ordered using a genetic map. The fourth project is the sequencing of the perennial species *B. sylvaticum* to develop a perennial model grass. A *B. sylvaticum* assembly was created using only PacBio sequence. The fifth and final project is the improvement of the *B. distachyon* reference genome to essentially ‘finished’ genome quality. An overview of these new resources and how they are supported by other experimental tools will be presented.
The Brachypodium ENCODE Project

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Abstract

ENCODE (Encyclopedia of DNA Elements) projects in human, mouse, C. elegans and Drosophila have collectively demonstrated that integrated genome feature maps enable advanced modeling and understanding of complex pathways. The ENCODE project summarized here will identify and characterize numerous functional elements associated with progressive drought responses in the genome of the model grass Brachypodium distachyon. Our effort will develop deep knowledge of chromatin dynamics and gene expression networks related to abiotic stress responses in temperate grasses. This knowledge will contribute to the improvement of wheat, barley, oats, and switchgrass, which are pivotal to food and biofuel production. To accomplish this goal, the Brachypodium ENCODE project will: (1) collect fundamental information about the Brachypodium transcriptome, epigenome, chromatin dynamics, and gene regulatory networks, and (2) elucidate regulatory dynamics of gene networks that can be manipulated to increase productivity in temperate grass crops, especially under drought conditions. Moreover, this ENCODE project represents a unique opportunity to expand the functional genomic resources for Brachypodium, map drought-related phenotypes to small active genomic regions, and identify correlations between genome dynamics and drought avoidance or tolerance mechanisms in temperate grasses. Progress towards these goals will be reported.
P47
Drought Avoidance Mechanisms in *Brachypodium distachyon*

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Abstract
*Brachypodium distachyon* is a temperate monocot species that is related to wheat, barley, oats, rye, and switchgrass. It is an excellent model for investigating the molecular mechanisms, speciation, and evolution of drought avoidance in temperate crops because different accessions of this species exhibit significant phenotypic variation for drought-related traits, it is extremely well suited for high-throughput phenotyping, it has not been domesticated, and extensive germplasm and functional genomics resources exist. However, the anatomical, morphological, and physiological mechanisms of drought avoidance in *B. distachyon* remain largely uncharacterized. The objectives of this study are to (1) select a core collection of 24 *Brachypodium* accessions that are genetically diverse and exhibit notable variation in drought-related traits, (2) characterize anatomical, morphological, or physiological mechanisms of drought avoidance in these 24 accessions, and (3) determine the heritability (or plasticity) of these putative drought-avoidance mechanisms. Here, we present the DroughtCore24, which is a diverse collection of 24 *Brachypodium* accessions that (together) are particularly well suited for investigating drought-avoidance mechanisms in this temperate grass. Anatomical characterization via scanning electron microscopy indicates that stomatal density (and associated transpiration rates) is one component of drought avoidance in this species. Although counter-intuitive, leaf hair density is positively correlated with drought sensitivity in *Brachypodium*. The phenotypic plasticity of stomatal and leaf hair density are currently under investigation.
P48

Comparative study of proline and soluble protein in *Brachypodium distachyon* L.

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Abstract

*Brachypodium distachyon* L. (Bd) as model object is studied in Kazakhstan for the first time with the purpose of its further use in breeding and genetic purposes. Bd has small, in comparison with the major food and fodder cereals, genome size that makes it a convenient object for the molecular and genetic analysis, as well as agricultural genetics, including study of the structure of a cellular wall, seeds development and answers to adverse effects of environmental factors. 14 day old seedlings of Bd and two wheat varieties Kazakhstanskaya-19 and Kazakhstanskaya early ripe (drought-resistant, struck by a brown rust up to 40%) served as research material. Bd (Bd-21 line) seeds were kindly provided by the RIKEN BioResource Center in Japan. The Bailey method was used in order to estimate the content of soluble protein in Bd and wheat leaves (mg/ml) at a wavelength of 330 nm. Optical density was measured on Ultrospec 1100 pro “Amersham BioSciences” spectrophotometer at the wavelength of 522 nm. Calibration curve in the range of 0.01 - 0.2 mm of pure Ajinomoto proline was made for estimation of proline content. Comparative study of the quantitative content of soluble protein in leaves of seedlings of Bd and wheat showed that its content in Bd leaves (0.464±0.03 mg/ml) statistically exceeds its content in soft wheat by 40-42%. According to experimental results content of proline in Bd is almost twice lower, than in studied varieties of soft wheat.
K1

Environmental control of the timing of flowering

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Abstract
Proper timing of flowering is essential for reproductive success in plants, and is a major determinant of biomass yield. To adapt to seasonal variation in temperate climates, many plant lineages have evolved systems that sense seasonal cues and respond to the cues with developmental transitions such as flowering. An example is the vernalization requirement. Vernalization is the process by which competence to flower is achieved only after prolonged exposure to winter cold. A vernalization requirement is found in a range of flowering plant families, yet relatively little is known about the molecular nature of this phenomenon outside the eudicot model Arabidopsis thaliana. Moreover, what we have learned in Arabidopsis is not informative about vernalization in grasses or other families of plants as these systems evolved independently. We are characterizing the vernalization response across diverse Brachypodium accessions, and have begun to assess the genetic basis of the impressive range of variation in flowering. In parallel, screens for vernalization-insensitive and rapid-flowering mutants have revealed several novel flowering-time genes, some of which appear to be negative upstream regulators of the vernalization gene in grasses, VERNALIZATION1. The flowering pathways in Arabidopsis and Brachypodium will be compared and contrasted.


K2

A systems biology approach to study the tug-of-war in grass-virus interactions using Brachypodium

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Abstract

Viruses cause significant losses in global agricultural production, yet little is known about antiviral defense mechanisms in grasses. Brachypodium has become a viable tool for advancing pathosystems biology work in the field and laboratory. An exemplar of the field research is our recent study of endemic infections of Panicum mosaic virus (PMV) and its satellite virus (SPMV) of switchgrass (Panicum virgatum) and St. Augustinegrass (Stenotaphrum secundatum). In the laboratory, Brachypodium and Setaria viridis (Setaria) are used to study PMV and SPMV, and six other monocot-infecting viruses. From this, we have discovered multiple conserved and unique virus-specific and host-dependent defense-signaling responses triggered during compatible grass virus infections. This includes the first comparative framework of SA, JA and ET signaling responses modulated during diverse C_3 and C_4 grass:virus interactions. Using the PMV-Brachypodium pathosystem we identified global virus-induced alternative splicing (AS) landscapes and identified AS events in certain defense genes (e.g., NB-LRR and RLKs) and splicing regulators (e.g., SCL33). The changes in the levels of defense signaling hormones and molecules using targeted metabolic profiling tools correlated with the transcriptional responses. Another interaction in this host:virus system includes extensive alteration of the 3’-termini of the PMV and SPMV genomic RNAs, a strategy that may be used by the virus as a decoy towards host defenses or, alternatively, a host-dependent mechanism to restrict virus accumulation. Together, these findings have extended our understanding of monocot antiviral defenses and provide the promise of developing tools and strategies to abrogate crop losses due to virus infections.
K3

A set of Brachypodium transgenic lines with post-synthetically modified cell wall as a tool to study cell wall integrity control.

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Abstract

The cell wall represents the plant interface where interactions with pathogens and perception of other environmental cues take place. Plant pathogens produce an arsenal of cell wall degrading enzymes (CWDEs) targeted to specific cell wall components to penetrate the host tissue. Emerging evidence indicates that plants have developed systems for sensing biotic and abiotic stresses by monitoring the cell wall damage/integrity. We used well characterized microbial enzymes with diverse specificities and created a set of transgenic Brachypodium plants with specific cell wall modifications to investigate their impact on plant stress responses. We also developed a new approach for fast and easy transient expression of the genes of interest using matured seeds, which allowed quicker assessment of the expressed gene impact on the plant phenotype. By challenging the transgenic plants with necrotrophic fungus B. sorokiniana, we revealed that two cell wall degrading enzymes, acetyl esterase and ferruloyl esterase expressed in plant apoplast have opposite effect on plant susceptibility to biotic stress. Thus, reduction of xylan acetylation by expressing acetyl esterase increases Brachypodium resistance to pathogen. Transgenic plants showed constitutive upregulation of several defense related genes, which suggests the constitutive induction of the PAMP Triggered Immune (PTI) response initiated by introduced cell wall modification. In contrast, reduction of coumaric and ferulic acids, which are involved in polysaccharide-lignin cross-linking, by expressing feruloyl esterase increases the cell wall digestibility in transgenic plants and their susceptibility to B. sorokiniana. Transcriptome analysis of these plants is in progress to elucidate the effect of cell wall perturbation. We propose that post-synthetic modification of cell walls using well characterized degrading enzymes is a promising approach to investigate the impacts of cell wall modifications on plant fitness. In our studies, the transgenic plants expressing a single or combination of enzymes serve as a toolset for studies of the specific signaling networks initiated by cell wall perturbations caused by microbial CWDEs during penetration and disease development.
K4

Root epidermal development in *Brachypodium distachyon*

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Abstract

Most of the chemical elements that constitute land plants are absorbed from the soil through root systems and associated symbionts. Increasing nutrient root uptake capacity is crucial for the globally recognized goal of raising yields while decreasing fertilizer use. Root hairs are filamentous extensions of epidermal cells that extend the absorbing surface of roots into the surrounding soil. They play essential functions in nutrient acquisition and are particularly important for the uptake of nutrients with limited soil mobility such as phosphate. We previously described the development of root hairs on wild type *Brachypodium distachyon*. Root hairs develop from the smaller daughter cell of an asymmetric cell division resulting from an asymmetric mitosis: the rootward daughter cell develops as a root hair cell and the shootward daughter cell develops as a hairless epidermal cell. Consequently hair and non-hair cells alternate along cell files in the root epidermis, and root hairs form in every epidermal root file. *RSL* genes positively regulate root hair development in *Arabidopsis thaliana*. We show that *RSL* genes positively regulate root hair development in *B. distachyon* and act after the formative asymmetric cell division that give rise to root hair cells and hairless epidermal cells. Our latest results demonstrating the role of *RSL* genes in *B. distachyon* root hair development will be presented.
Adaptive differentiation in the \textit{Brachypodium distachyon} species complex

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Abstract

The \textit{Brachypodium distachyon} species complex comprises three closed-related species, \textit{B. distachyon}, \textit{B. stacei} and their derived allotetraploid \textit{B. hybridum}. These species are ecological and geographically differentiated across their range of distribution. Although all species grow in Mediterranean-type climate, which is typically characterized by a relative long estival drought, \textit{B. distachyon} diploids occur frequently in wet habitats where summer drought is attenuated. Contrastingly, \textit{B. stacei} and \textit{B. hybridum} allotetraploids inhabit mainly dry environments where a predictable summer drought period exists. My research focus on the role of adaptation to water stress as a key ecological determinant underlying ecological divergence and differentiation of these species. Here, I present ecological and physiological data that support the hypothesis that differences in tolerance to water stress may underlie the ecological divergence of these closely-related species growing under contrasting environments. Thus, our results show that \textit{B. distachyon} functional response to drought appear differentiated from \textit{B. hybridum} and \textit{B. stacei} responses, whereas \textit{B. hybridum} response to drought is essentially correlated to \textit{B. stacei} one, suggesting a common adaptive response to water stress in these two later species, and/or parental dominance of \textit{B. stacei} on the functional response to drought of the derived allotetraploid \textit{B. hybridum}. 
K6
Germination in domesticated and wild Triticeae seeds: comparison between Brachypodium distachyon and Hordeum vulgare

Cristina Barrero-Sicilia

Dedicated to Professor Pilar Carbonero upon retirement

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Abstract

During the maturation phase of development, the cultivated Triticeae seeds (barley, wheat, etc.) accumulate abundant reserves (mainly proteins and starch) in the endosperm that will be hydrolised upon germination. Although phylogenetically related, Brachypodium distachyon seeds are characterized by low levels of starch and high levels of $\beta$-1,3-1,4-glucans in the endosperm cells, and thick mannan-rich cell walls in the coleorhiza and root. The germination process, both in barley and Brachypodium, can be separated into two phases: 1) germination sensu stricto, when the coleorhiza is the first structure that protrudes after the pericarp and testa rupture (coleorhiza emergence), followed by the root emergence when the coleorhiza ruptures, and 2) post-germination reserve mobilization, when proteins and other reserves are hydrolised. Immunolocalization of mannans indicate their presence in the root embryo and in the coleorhiza in the early stages of germination, decreasing thereafter; at the same time, a pick of endo-$\beta$-mannanase activity, and the expression of mannanase encoding genes are observed. If the coleorhiza in monocots and the micropylar endosperm in dicots have similar functions and the importance of BdMAN genes in both type of seeds during germination sensu-stricto is discussed. In post-germinating reserve mobilization, water imbibed seeds synthesize GA in the embryo which diffuses to the aleurone layer where it triggers the expression of hydrolase genes, such as BdCathB, encoding a CathepsinB-like protease, that is regulated by the Transcription Factors (TF) BdDOF24 and BdGAMYB. The structure and function of these Brachypodium TFs and their orthologs in barley HvDOF24 (BPBF) and HvGAMYB are compared.
K7

Systems-level analysis of drought and heat responses in the model C3 grass *Brachypodium distachyon*

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Abstract

Sustainable increases in crop productivity are a critical challenge for 21st-Century plant scientists. Towards this goal we are integrating large-scale phenotypic, genetics, and genomics datasets to elucidate the molecular basis of traits that improve stress tolerance in grass crops. Our approach leverages the LemnaTec Scanalyzer3D Platform, weighted gene correlation network analysis (WGCNA), yeast one-hybrid (Y1H) screens, and a variety of Next-Gen sequencing platforms. Together, these tools will allow heritable drought-related phenotypes to be mapped to small tractable regions of the *Brachypodium* genome. Two high-throughput image-based phenotyping datasets of *Brachypodium* accessions have been collected from the LemnaTec Scanalyzer3D Platform at the Danforth Plant Science Center. These two datasets comprise over 400,000 images from 143 *Brachypodium* accessions that capture growth and development under high temperature, progressive drought, and combined high temperature/drought conditions. Trait data were obtained from digitized images using PlantCV, which is a Python-based, open-source software suite, built for high-throughput image processing and trait data extraction. Mapping of drought-related phenotypic variation to genome coordinates using temporal GWAS is underway. An ENCODE approach is being used to identify virtually all drought-related functional DNA elements across the *Brachypodium* genome, with the hope that drought phenotypes can soon be mapped to these drought-related functional DNA elements. Data from this *Brachypodium* ENCODE Project, combined with WGCNA and Y1H screens, will also provide novel insights about the *Brachypodium* transcriptome, epigenome, chromatin dynamics, and gene regulatory networks under well-watered and drought-stress conditions. Gene regulatory networks elucidated by high-throughput Y1H screens serve as logical targets to increase abiotic stress tolerance in temperate monocot crops. We are also sequencing non-model genomes from grasses that are known to be extremely drought-tolerant (*e.g.*, *Oropetium*) and these data reveal neofunctionalization of LEA proteins as one strategy for adaptation to extremely dry habitats.
Improving freezing tolerance in grasses: what can we learn from Brachypodium?

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Abstract
To alleviate the adverse effects of abiotic stresses, plants rely on an array of cellular events that allow them to adapt and survive. While it is well established that these response mechanisms are multigenic, far less is known regarding how the dynamic tuning of the plant’s chromatin structure translates the incoming stress signals into orchestrated responses from the DNA. My laboratory, in conjunction with others, has begun the characterization of the cold acclimation and freezing tolerance mechanisms of a number of Brachypodium accessions. So far, our work has revealed that the commonly used Brachypodium accessions exhibit a clear cold acclimation response and have a measurable ability to develop freezing tolerance. However, little difference in terms of survival was observed amongst the tested accessions, despite their previous classification as either spring or winter genotypes. In this talk, I will first describe our recent attempt at expanding the known range of phenotypic variations of freezing tolerance in Brachypodium. Then I will discuss our latest progress aimed at understanding the epigenetic mechanisms that underlie cold acclimation in grasses by describing our work regarding the characterization of the SAGA coactivator complex and its impact on chromatin dynamics in response to low temperatures. Finally, I will outline strategies to improve stress tolerance in grasses by harnessing stress responsive components such as lipocalins and endophytes.
K9

Posttranscriptional Regulation of *FLOWERING LOCUS T* in *Brachypodium distachyon*

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Abstract

The florigen gene *FLOWERING LOCUS T* (*FT*) plays a key role in plant flowering. Numerous works have been conducted on *FT* regulation at the transcriptional regulation level, but not much is known about other regulatory modes for this important gene. In this talk, I will present two posttranscriptional regulatory modes of the Brachypodium *FTs*. Firstly, I show that mRNAs of the two Brachypodium *FT* homologs *BdFT1* and *BdFT2* are targets of a Pooideae-specific microRNA miR5200 which is induced under short-days (SD) conditions but repressed in long-days (LD), suggesting that miR5200 functions in photoperiod-dependent manner. Further study reveals a correlation between histone methylation at miR5200 loci and its expression suggesting the involvement of epigenetic regulation for miR5200. Second, I show our recent observation of an alternatively spliced variant of *BdFT1* (*BdFT1.2*) that may be implicated in *FT* regulation. I will present evidence that *FT1.2* may function by competing with the major splicing form (*FT1.1*) in protein-protein interaction and is involved in the age regulatory pathway.
K10
Crossing forward and reverse genetic screens to decipher lignification in *Brachypodium distachyon*

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**Abstract**

*Brachypodium distachyon* is currently used as a model system in several plant biology fields. We produced a chemically-induced mutant population at INRA with the goal to accelerate the research in biofuel production. The collection was intensively used for reverse and forward genetic screens. It allowed us to identify mutants with modified cell wall composition. As a model plant for the production and use of lignocellulosic biomass, deciphering the phenolic pathway leading to lignin biosynthesis is a prerequisite. Moreover and as compared to dicots, grass cell walls show some specific features such as lignin acylation by *p*-coumaric acid and arabinoxylan acylation by ferulic and *p*-coumaric acids. We thus focused our activities on the identification of mutants affected in different steps of *Brachypodium* lignification. Most mutants displaying improved saccharification also displayed altered growth or development. This presentation will highlight not only the many advantages, but also the limits of working with chemically-induced mutant lines.
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