The 3rd International Brachypodium Conference

Beijing ○ China

July 30-31, 2017

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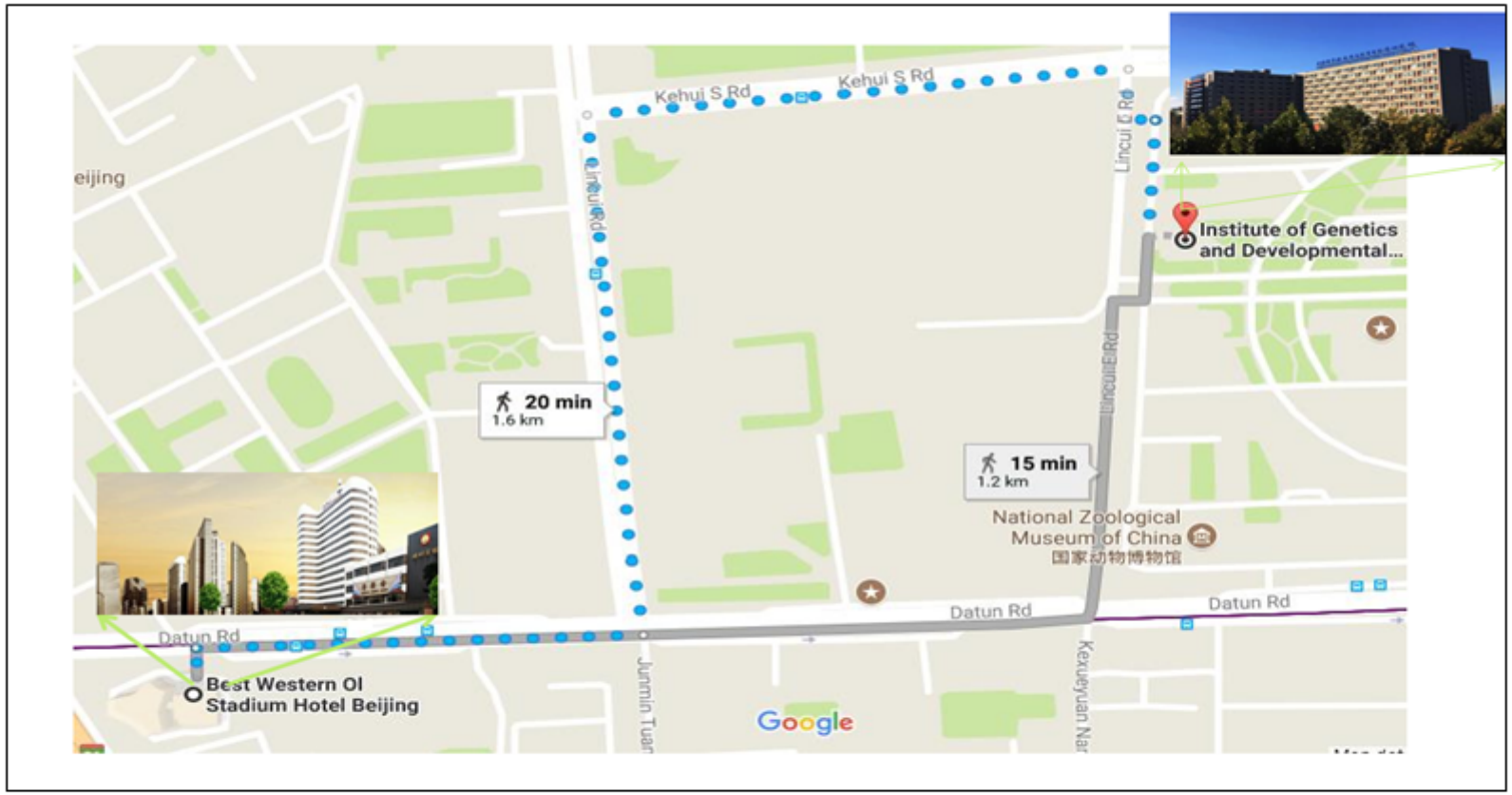
The Conference Roadmap



- **When you arrive at the Institute of Genetics & Development Biology, you may follow the signs in the hall to the meeting room.**
- **If you have any problems, you can contact our faculty:**

  Guanghao Guo (15600912996) Qiuhong Wu (15600910387)

Conference Agenda

July 30, 2017

Conference attendees entry to the IGDB meeting room (Room 215-216)

8:20-8:30 Opening remarks

Session 1: Novel tools and resources. Chair: Zhiyong Liu, IGDB, CAS

Time	Speaker	Institute	Title
8:30-9:00	John Vogel	DOE Joint Genome Institute, USA	What can you learn from 1,019 genomes? Pan-genomics, polyploidy, epigenetics and a cast of mutants
9:00-9:20	Robert Hasterok	University of Silesia in Katowice, Poland	Dissecting grass genome organisation at the cytomolecular level using the model genus Brachypodium
9:20-9:40	Pilar Catalan	Universidad de Zaragoza, Huesca, Spain	Phylogenomics and gene evolution in model annual and perennial Brachypodium species
9:40-10:00	Hailong An	Shandong Agricultural University, China	Ds tagging: a gateway for gene discovery in *Brachypodium distachyon*
10:00-10:30	Coffee break & Poster		
Session 1: Novel tools and resources. Chair: Yongqiang Gu, USDA-ARS			
10:30-10:50	Ming Cheng Luo	University of California, Davis, USA	Revisit polyploidization of Brachypodium taxa, evidence from analyses of whole-genome optical maps
10:50-11:10	Cecilie S. L. Christensen	University of Copenhagen, Danmark	Altering lignin composition in Brachypodium using CRISPR/Cas9
11:10-11:30	Yoshihiko Onda	RIKEN Center for Sustainable Resource Science, Japan	A simple and versatile genome-wide SNP genotyping by multiplex PCR targeted amplicon sequencing in *Brachypodium distachyon*
11:30-11:50	Xiaoquan Qi	Institue of Botany, Chinese Academy of Sciences, China	Generation of *Brachypodium distachyon* T-DNA mutant population for studying nonhost resistance to wheat stripe rust
12:00-14:00	Lunch		
13:00-14:00	International Brachypodium Steering Committee Meeting		

Session 2: Development, epigenetics and growth. Chair: Yuling Jiao, IGDB, CAS

14:00-14:20	Sam Hazen	University of Massachusetts, USA	Transcriptional regulation of biomass accumulation in *Brachypodium distachyon*
14:20-14:40	Karen A. Sanguinet	Washington State University, USA	Identification of the BUZZ kinase involved in root and root hair development
14:40-15:00	Liang Wu	Zhejiang University, China	Gene silencing by endogenous and exogenous miRNAs in flowering-time control in *Brachypodium distachyon*
15:00-15:20	Alexander Betekhtin	University of Silesia in Katowice	Brachypodium tissue culture as a model system to reveal the functions of the components of the cell wall
15:20-15:40	Koen Geuten	KU Leuven, Belgium	A FLOWERING LOCUS C homolog is a vernalization regulated repressor in Brachypodium and is cold-regulated in wheat
15:40-16:10	Coffee break & Poster		

Session 2: Development, epigenetics and growth. Chair: Karen Sanguinet, Washington State University

16:10-16:30	NataliaBorowska-Zuchowska	University of Silesia in Katowice, Poland	The preferential silencing of *B. stacei*-inherited rRNA genes in *Brachypodium hybridum* - an epigenetic point of view
16:30-16:50	Zhongjuan Zhang	Max Planck Institute for Plant Breeding Research, Germany	How does CUC2 regulate leaf serration development in Arabidopsis?

Session 3: Natural variation and evolution

16:50-17:10	Justin Borevitz	Australian National University, Australia	Population structure of theBrachypodium species complex and genome wide dissection of agronomic traits in response to climate
17:10-17:30	Weining Song	North West Agriculture and Forestry University	Brachypodium SPP in Israel likely a hexaploid and containing low genetic diversity
17:30-17:50	Zujun Yang	University of Electronic Science and Technology of China	Diversity of Brachypodium samples in Israel revealed by molecular and cytogenetic methods
18:00-20:30	Dinner		

July 31, 2017

8:00-8:20 Conference attendees entry to the meeting room

Session 4: Plant-Biotic & Abiotic interactions. Chair: Dawei Li, China Agricultural University

8:20-8:40	Kemal Kazan	Queensland Bioscience Precinct, Australia	Brachypodium: A useful model host for cereal-fungal pathogen interactions
8:40-9:00	Pubudu P. Handakumbura	Pacific Northwest National Laboratory, USA	Linking phenotype to genotype: A metabolomics approach to build trait association network models for Brachypodium
9:00-9:20	Yusuke Kouzai	RIKEN Center for Sustainable Resource Science, Japan	Expression profiling of marker genes for defense-associated phytohormones in *Brachypodium distachyon* highlights its similar immune systems to rice
9:20-9:40	Qiuhong Wu	Institute of Genetics & Developmental Biology, CAS, China	Interaction of Bsr1 and TGB1 confers Barley Stripe Mosaic Virus resistance in Brachypodium, barley and wheat

9:40-10:10 Coffee break & Poster

Session 5: Plant-Abiotic Stress Interactions. Chair: Justin Borevitz, Australian National University

10:10-10:30	YongqiangGu	USDA-ARS, Western Regional Research Center Albany	Using the JGI Brachypodium T-DNA collection to reveal novel transcription factor roles in abiotic stress responses
10:30-10:50	Zhaoqing Chu	Shanghai Chenshan Plant Science Research Center, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences	Cool season turf grass heat tolerance study through genomic and genetic analyses with *Brachypodium distachyon*
10:50-11:10	Keiichi Mochida	RIKEN Center for Sustainable Resource Science, Japan	Homoeolog-specific activation for heat acclimation in the allopolyploid grass *Brachypodium hybridum*
11:10-11:30	AritaKus	University of Silesia in Katowice, Poland	Establishing *Brachypodium distachyon* as a model in analyses of plant genome stability after mutagenic treatment
11:30-11:50	CAI Jinshu	ShezhenWenke Landscape Corp., Ltd, China	Influence of Supplemental Lighting with different light quality on the turf growth of *Festuca Arundinacea*
11:50-12:05	Pilar Catalan	Universidad de Zaragoza, Huesca, Spain	Brachypodium Conference 2019

12:05 Meeting Conclusion Remarks: Long Mao, Institute of Crop Sciences, CAAS

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Session 1: Novel tools and resources

What can you learn from 1,019 genomes? Pan-genomics, polyploidy, epigenetics and a cast of mutants

John Vogel

Abstract

The sequencing of a single reference genome played a pivotal role in establishing *Brachypodium distachyon* as a model system. Since the production of that initial genome assembly, technological innovations in DNA sequencing have decreased costs to the point where it is now feasible to sequence many Brachypodium genomes. The DOE Joint Genome Institute has sequenced over 1,000 genomes from four Brachypodium species. An overview of the lessons learned from these sequences will be presented including: A comparison of 54 *B. distachyon* genomes that revealed a pan-genome is considerably larger than the genome of any individual plant. A comparison of the genomes of several *B. hybridum* lines with their diploid progenitors *B. distachyon* and *B. stacei* that revealed multiple origins of *B. hybridum*. An exploration of epigenetic dynamics in *B. distachyon* in response to cold.And, finally, the cataloging of hundreds of thousands of mutations to create a new resource for functional genomic studies.

Session 1: Novel tools and resources

Dissecting grass genome organisation at the cytomolecular level using the model genus Brachypodium

Robert Hasterok[1], Alexander Betekhtin[1], Natalia Borowska-Zuchowska[1], Agnieszka Braszewska-Zalewska[1], Karolina Chwialkowska[2], Marta Hosiawa-Baranska[1], Dominika Idziak-Helmcke[1], Arita Kus[1], Jolanta Kwasniewska[1], Miroslaw Kwasniewski[2], Joanna Lusinska[1], Ewa Robaszkiewicz[1], Magdalena Rojek[1], Rakesh Sinha[1], Aleksandra Skalska[1], Marta Sowa[1], Elzbieta Wolny[1], Karolina Zubrzycka[1]

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Abstract

Modern molecular cytogenetics combines various methodological approaches of cytology, molecular genetics and advanced digital image analysis. It focuses on the study of nuclear genomes at the microscopic level. The cytomolecular organisation of plant genomes is still rather poorly investigated, compared to that of animals. Most plant genomes, including those of economically and ecologically crucial cereals and forage grasses, are usually large and saturated with repetitive DNA, which hampers detailed molecular cytogenetic analyses.

Model organisms possess a combination of features, which makes them more amenable to scientific investigation than others. One of the most recent and rapidly developing model systems are representatives of the Brachypodium genus, particularly *B. distachyon*. They possess small, and in some cases, already sequenced genomes with a low repeat content, diverse basic chromosome numbers and ploidy levels. They also have an interesting phylogeny, short life cycles and simple growth requirements, complemented by a rapidly and continuously growing repertoire of various experimental tools.

This presentation outlines and discusses our current projects and their future prospects, using Brachypodium species for research on various aspects of grass genome organisation, e.g. (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 and (v) instability of a small grass genome induced via mutagenic treatments.

This work is supported by the National Science Centre Poland (grants no. 2012/04/A/NZ3/00572, 2014/14/M/NZ2/00519 and 2015/18/M/NZ2/00394

Keywords: Brachypodium, chromosomes, grass nuclear genome organisation, model organism, molecular cytogenetics

Session 1: Novel tools and resources

Phylogenomics and gene evolution in model annual and perennial Brachypodium species

Rubén Sancho[1], Bruno Contreras-Moreira[2], David Des Marais[3], Sean Gordon[4], John Vogel[4], Pilar Catalan[1]

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[4]DOE Joint Genome Institute, Walnut Creek, CA, USA

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Abstract

We have reconstructed a historical scenario for the diverging and merging genomes inherited by diploid and allopolyploid species of Brachypodium, using a set of selected loci, Genotyping-By-Sequencing and RNA-seq data. We built a comprehensive phylogeny of Brachypodium from five neutral genes using Species-Tree Minimum-Evolution and species-network analyses, and Maximum Likelihood reconstructions of genomic and transcriptomic reads mapped to the three available 2x Brachypodium reference genomes. Gene content and dosage in annual-*vs*-perennial, and in diploid-*vs*-polyploid species was estimated using GET_HOMOLOGUES-EST. Our 5-gene data support Mid-Miocene splits of ancestral genomes that preceded Late-Miocene to Quaternary origins of extant diploid species' genomes (*B.stacei*, *B.distachyon*, core perennials). Ancestral Mediterranean genomes presumably merged with recent perennial genomes generating the West-Palaearctic perennial allopolyploids (*B.boissieri, B.retusum, B.phoenicoides*). Close homeologous American genomes plausibly evolved *in situ*, originating *B.mexicanum*. Quaternary *B.hybridum* resulted from reciprocal *B.stacei*x *B.distachyon* WGD crosses. Core perennial diploids (*B.arbuscula, B.sylvaticum, B.rupestre, B.pinnatum*) evolved in Eurasia from Upper Pleistocene genomes. Karyologically unknown African, Malagasy and Taiwanese species were reconstructed as polyploids. GBS/RNAseq-based phylogenies supported this scenario and further detected ancestral and recent subgenomes in *B. retusum*. Gene content

analysis detected 5202 (ingroup) core genes, 49 present in annuals and absent in perennials, 30 present in perennials and absent in annuals, and 14 present in polyploids and absent in diploids. 143 core genes were private to *B. boissieri, B. retusum* and *B. mexicanum*. Ongoing gene-evolution analysis is being performed for selected groups of core (flowering time) and shell (drought and cold tolerance) genes across the Brachypodium taxa.

Keywords:annual and perennial Brachypodium taxa, GBS and RNAseq data, diplo/allopolyploids, gene content-dosage-evolution, phylogenomics

Session 1: Novel tools and resources

Brachypodium grain transcriptome: a new tool for the identification of potential regulators of key developmental transitions in cereals

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Abstract

The caryopsis of temperate cereals is a unique type of fruit with great economical value. From a developmental point of view though, very few genetic regulators have been identified to date, and most of the relevant research is undertaken in systems such as maize and rice - which are considerably different in many aspects from the grains of the temperate cereals.

Brachypodium, as a sister to the core pooids that include wheat, barley and rye, represents a good model for the study of grain development. We have selected eight stages of grain development that encompass key transition points in *Brachypodium distachyon* and conducted a comprehensive transcriptomic analysis. We have generated a new valuable resource for the investigation of gene expression patterns throughout grain development and germination that could also be used for comparison purposes with other important crop species in a gene function and evolutionary context. We particularly focused on transcription factors, as they often act as master regulators of developmental processes and we have therefore suggested candidate regulators of developmental transitions and distinctive biological processes they are involved in.

Keywords: Brachypodium, transcriptomics, grain development, transcription factors

Session 1: Novel tools and resources

Revisit polyploidization of Brachypodium taxa, evidence from analyses of whole-genome optical maps

Tingting Zhu[1], Zhaorong Hu[1,2], Juan C. Rodriguez[1], Karin R. Deal[1], Jan Dvorak[1], John P. Vogel[3], Zhiyong Liu[4], and Ming-Cheng Luo[1]

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Abstract

There are three recorded Brachypodium taxa, the diploid *B. distachyon* (2n=10) and *B. stacei* (2n=20), and the allotetraploid *B. hybridum* (2n=30). *Brachypodium* has been widely used as genomic and genetic model; it is of importance to shed light on the structure and evolutionary relationships among genomes of the three taxa of Brachypodium. We applied BioNano genome (BNG) mapping technology to construct whole-genome optical maps for the three taxa of Brachypodium, and performed multiple comparisons. Our results shows that *B. stacei* (2n=20) is indeed diploid and diverged greatly from the other diploid *B. distachyon* (2n=10), while *B. hybridum* (2n=30) is an allotetraploid that originated via hybridization of the two diploids. Structural variations between the polyploid *B. hybridum* and its two diploid progenitors indicated the in-del events distributed unevenly across the chromosomes but agrees with the pattern of chromosomal distribution of retro transposons. We demonstrated a great utility of BNG maps for polyploid genome analysis and confirmed the origin of *B. hybridum* via hybridization between *B. distachyon* and *B. stacei*, however, little divergence between the genome of *B. hybridum* and those of the diploids progenitors has taken place.

Keywords: Optical mapping; polyploidization; structural variation

Session 1: Novel tools and resources

Ds tagging: a gateway for gene discovery in *Brachypodium distachyon*

Hongyu Wu[1], Caihua Qin[1], Xiaodong Xue[1], Yi Xu[1], Qinxia Li[1], Xiaoquan Qi[2]* and Hailong An[1]*

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Abstract

Transposon tagging is a powerful tool for gene discovery in various species. Recently, *Brachypodium distachyon* L. is presented as a model plant for cereals especially the temperate cereals like wheat and barley. So far there is no research work published against successful transposon tagging in Brachypodium. Here we described an efficient Ds transposon tagging system in *Brachypodium distachyon* and its potential for gene discovery in Brachypodium and wheat. The Ac transposase driven by a 1X 35S promoter and Ds element with a *ZmUbi1s*GFP cassette inside were constructed within the same T-DNA, then transformed into Brachypodium via Agrobacterium-mediated transformation. It's easy to detect the somatic transposition events from the leaves of the T0 plants. After selfing, Ds insertional lines without the T-DNA insert were identified from the T1 progeny at a highest frequency about 10%. Using this system, more than 3,000 Ds insertional lines were generated and near 600 Ds flanking sequences were isolated. From the population hundreds of mutants with visible phenotypes were identified. So the system is efficient enough to produce Ds insertional lines in a large scale and to identify genes with new functions. Due to the fact that Brachypodium is close to wheat, so we have good chance to imagine that those genes play similar roles in temperate cereals such as wheat and barley.

Keywords: *B.distachyon*, *Ac/Ds* tagging system, transposants, insertion sites, mutants

Session 1: Novel tools and resources

Altering lignin composition in Brachypodium using CRISPR/Cas9

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Abstract

The aim of this project is to reduce recalcitrance of bioconversion of the ligno-cellulosic material to increase bioethanol production yield by altering the composition of lignin in Brachypodium. CRISPR/Cas9 genome editing method will be used to target two of the genes coding for cinnamyl alcohol dehydrogenase (CAD4 & CAD5). This project will investigate the function of these two genes coding (CAD4 & CAD5) controlling the last step in the lignin biosynthesis. This will be done by knocking-out or alter the function of the genes.

Waste materials from cereals are a great source for bioethanol production. However lignin is highly recalcitrance to degradation and reduces the hydrolysis of cellulose to fermentable sugars. Cinnamyl alcohol dehydrogenase (CAD) catalyses the last step in the monolignol biosynthesis and mutants with reduced CAD activity results in higher bioethanol production without a growth penalty. Seven CAD genes have been identified in Brachypodium and BdCAD5 was identified as *bona fide* with highest expression rate in all tissue. All BdCAD genes are cytosolic except BdCAD4, which is located in the chloroplasts and the function of this gene is still unclear. In this study the function of BdCAD4 and BdCAD5 will be investigated by using the genome editing tool CRISPR/Cas9 to alter the reading frame by induced mutations. Three individual sgRNA targets for each gene were selected and the activities were tested in protoplast, followed by callus transformation.

Keywords: Brachypodium, CRISPR/Cas9, cinnamyl alcohol dehydrogenase (CAD), lignin, bioethanol

Session 1: Novel tools and resources

A simple and versatile genome-wide SNP genotyping by multiplex PCR targeted amplicon sequencing in *Brachypodium distachyon*

Yoshihiko Onda[1,2], Kotaro Takahagi[1,2,3], Minami Shimizu[1], Komaki Inoue[1], Keiichi Mochida[1,2,4,5]

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Abstract:

Next-generation sequencing technologies have enabled genome re-sequencing for exploring genome-wide polymorphisms among individuals as well as targeted re-sequencing for rapid and simultaneous detection of polymorphisms in genes associated with various biological functions. Therefore, a simple and robust method for targeted re-sequencing should facilitate genotyping in a wide range of biological fields. In this study, we developed a simple, custom targeted re-sequencing method, designated 'multiplex PCR targeted amplicon sequencing' ('MTA-seq'), and applied it to genotyping of the *Brachypodium distachyon*. To assess the practical usability of MTA-seq, we applied it to genotyping of genome-wide single nucleotide polymorphisms (SNPs) identified in natural accessions by comparing the re-sequencing data to the reference accession Bd21. Examination of the SNP genotyping accuracy from eight parental accessions and an F_1 progeny revealed that approximately 95% of the SNPs were correctly called. The assessment suggested that the method provides an efficient framework for accurate and robust SNP genotyping. The method described here enables easy design of custom target SNP-marker panels in various organisms, facilitating a wide range of high-throughput genetic applications such as genetic mapping, population analysis, and molecular breeding.

Keywords:SNP, genotyping, marker panel, amplicon sequence, natural accession

Session 2: Development, epigenetics and growth

Transcriptional regulation of biomass accumulation in *Brachypodium distachyon*

Sam Hazen .

University of Massachusetts, USA

Session 2: Development, epigenetics and growth

Identification of the BUZZ kinase involved in root and root hair development

Rachel Dannay[1], Thiel A. Lehman[1], Ying Wu[1,2], Rhoda Brew-Appiah[1], Tobias I. Basin[3], Karen A. Sanguinet[1]

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Abstract:

Root development and architecture is crucial for plant adaptation and reproductive success in diverse environments. To gain insight into the molecular and genetic cues involved in modulating root development and architecture in temperate grasses, we identified a roothairless mutant in *Brachypodium distachyon* termed *buzz*. The *buzz* mutant displays a root hairless phenotype with a dramatic increase in root growth rate. We used an NGS approach to identify SNPs associated with the *Bdbuzz* mutant phenotype. We identified a SNP in a previously uncharacterized kinase, which leads to an amino acid substitution in a highly conserved *Gly* residue in the kinase domain. We describe characterization of the *Bdbuzz* mutant. *Bdbuzz* is root-specific and expression analysis is consistent with the phenotype as expression is mainly localized to the root tip. We identified a second *Bdbuzz* allele in with a roothairless phenotype suggesting the original EMS allele is a functional null. We then identified the putative BUZZ ortholog in *A. thaliana.* We describe the function of the *A. thaliana* BUZZ kinase by characterization of two independent T-DNA lines. Together these data show that the function of the BUZZ kinase has diverged in grasses as compared to the model dicot *A. thaliana.*

Keywords : root development, kinase

Session 2: Development,epigenetics and growth

Gene silencing by endogenous and exogenous miRNAs in flowering-time control in *Brachypodium distachyon*

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Abstract

The switch from vegetative to reproductive growth is a critical developmental event in plants. Florigen, a small globular protein encoded by *FLOWERING LOCUS T* (*FT*), associates with FD, abZIP transcription factor, and 14-3-3 family proteins to form flowering initiation complex, functioning at the core node in multiple flowering pathways in plants. microRNAs (miRNA), a class of small RNAs with a stem-loop precursor structure, play versatile parts in plant development and adaptation to environments. In *Brachypodium distachyon*, on the one hand, we identified a*Pooideae*-specific miRNA, miR5200, that targets two *FT*orthologs, *FT1* and *FT2*, for mRNA cleavage. miR5200 is highly induced under short-day (SD) conditions, but dramatically repressed in long-day (LD) environments. Our over-producing miR5200 transgenic *B.distachyon* exhibits a significant delay of flowering, whereas interfering its activity by a target mimicry strategy accelerates flowering under SDs, indicating an important role of this endogenous miRNA in photoperiod-mediated flowering-timeregulation. On the other hand, we identified two alternative splicing variants*of FT2,* namely*FT2α* and *FT2β.* Through introducing artificial miRNAs, we obtained transgenic*B.distachyon* specific silencing *FT2α* and *FT2β*, respectively. We found an early flowering transition in *FT2β* silencing plants, in contrast with a severe delay of flowering onset in *FT2α* repressing plants, suggesting a negative role of FT2β while a positive role of FT2αin flowering control. Since gene editing at the genome level cannot get a loss-of-function mutant of specific splicing variant, our approach by introducing an exogenous miRNA provides a useful tool to inhibit a certain splicing variant activity in transgenic plants to explore its biological relevance. Taken together, we characterize an endogenous miRNA that modulates two *FT* gene expressions, and take advantage of an artificial miRNA to degrade a specific *FT* splicing variant transcript in flowering-time control in *B.distachyon.*

Keywords : endogenous miRNA, artificial miRNA

Session 2: Development,epigenetics and growth

Brachypodium tissue culture as a model system to reveal the functions of the components of the cell wall

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Abstract:

*Brachypodium Beauv.*is believed to be one of the oldest genera within the Poaceae. This genus is predominantly distributed in Europe and Asia with disjunctions occurring in Central America and Southern Africa. *Brachypodium distachyon* (*Brachypodium*) is a model system for functional genomics in grasses. Although there are some studies of invitro Brachypodium cultures including somatic embryogenesis, detailed knowledge of the composition of the components of the maincell wall in the embryogenic callus in this species is lacking. Therefore, we used histological, scanning electron microscopy as well as the immunocyto chemical approach against the arabinogalactan proteins (AGP), extensins and hemicelluloses to understand the localisation and possible functions of these cell wall components during the embryogenic mass that appears in Brachypodium callus. We found that the distribution of pectins, AGPs and hemicelluloses can be used as molecular markers of the embryogenic cells. Furthermore, we showed that AGPs and pectins are components of the extracellular matrix. The presented data extends our knowledge about the chemical composition of the embryogenic cells in the Brachypodium callus. This work was supported by the National Science Centre, Poland [grant no. DEC-2014/14/M/NZ2/00519].

Keywords:*Brachypodium distachyon*, cell wall, embryogenic callus, extracellular matrix.

Session 2: Development, epigenetics and growth

A FLOWERING LOCUS C homolog is a vernalization regulated repressor in Brachypodium and is cold-regulated in wheat

Neha Sharma [1], Philip Ruelens [1] Mariëlla D'hauw [2], Thomas Maggen [2], Niklas Dochy [1], Sanne Torfs [1], Kerstin Kaufmann [3], Antje Rohde [2], and Koen Geuten [1]*

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Koen Geuten

Abstract:

Winter cereals require prolonged cold to transition from vegetative to reproductive development. This process, referred to as vernalization, has been extensively studied in Arabidopsis (*Arabidopsis thaliana*). In Arabidopsis, a key flowering repressor called *FLOWERING LOCUS C* (*FLC*) quantitatively controls the vernalization requirement. By contrast, in cereals, the vernalization response is mainly regulated by the *VERNALIZATION* genes, *VRN1* and *VRN2*. Here, we characterize *ODDSOC2*, a recently identified *FLC* ortholog in monocots, knowing that it belongs to the *FLC* lineage. By studying its expression in a diverse set of Brachypodium accessions, we find that it is a good predictor of the vernalization requirement. Analyses of transgenics demonstrated that *BdODDSOC2* functions as a vernalization-regulated flowering repressor. In most Brachypodium accessions *BdODDSOC2* is down-regulated by cold, and in one of the winter accessions in which this down-regulation was evident, *BdODDSOC2* responded to cold before *BdVRN1*. When stably down-regulated, the mechanism is associated with spreading H3K27me3 modifications at the *BdODDSOC2* chromatin. Finally, homoeolog-specific gene expression analyses identify *TaAGL33* and its splice variant *TaAGL22* as the *FLC* orthologs in wheat (*Triticum aestivum*) behaving most similar to *Brachypodium ODDSOC2*. Overall, our study suggests that *ODDSOC2* is not only phylogenetically related to *FLC* in eudicots but also functions as a flowering repressor in the vernalization pathway of *Brachypodium* and likely other temperate grasses. These

insights could prove useful in breeding efforts to refine the vernalization requirement of temperate cereals and adapt varieties to changing climates.

Keywords: *FLC*, *MADS*,cereals, flowering

Session 2: Development,epigenetics and growth

The preferential silencing of *B. stacei*-inherited rRNA genes in *Brachypodium hybridum* - an epigenetic point of view

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Abstract:

Nucleolar dominance (ND) was among the first epigenetic phenomena that had been described. It was found in plant and animal allopolyploids and/or hybrids and consisted of reversible silencing of 35/45S rRNA gene loci inherited from one of the two or more ancestral species. Recent studies revealed that the large-scale silencing of rRNA genes via ND is of an epigenetic origin. However, the mechanisms according to which one parental rDNA set is chosen to be silenced still remain unclear.

Brachypodium hybridum is a natural allotetraploid (2n=30) with putative parental genomes originating from two diploid species: *B. distachyon*(2n=10) and *B. stacei* (2n=20). Selective silencing of *B. stacei*-like rDNA loci was observed in this allopolyploid. This presentation outlines the studies on ND mechanisms in several *B. hybridum* genotypes originated from distinct geographic locations. The distribution of 35S rDNA loci inherited from ancestral species was determined in both mitotic and meiotic cells. Moreover, we aimed to investigate the epigenetic status of 35 rRNA gene lociin *B. hybridum* and its putative ancestral species by the determination of DNA methylation and selected histone modification (e.g. H4K5ac, H4K16ac, H3K9ac, H3K9me2) immune patterns. We also show the results of molecular characterisation of intergenic spacers (IGS) between 25S rDNA and 18S rDNA in *B. hybridum* and its progenitors as well as their physical localisation in metaphase chromosomes and interphase nuclei. In all IGS sequences we identified putative transcription initiation sites and spacer promoters followed by subrepeats.

This work was supported by the Polish National Science Centre (grants no.DEC-2012/04/A/NZ3/00572 and DEC-2011/01/B/NZ3/00177).

Keywords: DNA methylation, histone modifications, nucleolar dominance, rRNA genes

Session 3: Natural variation and evolution

Population structure of the Brachypodium species complex and genome wide dissection of agronomic traits in response to climate

Pip Wilson, Jared Streich, Steve Eichten, Riyan Cheng, Kevin Murray, Niccy Aitkin, Kurt Spokas, Norman Warthmann, Accession Contributors*, Justin Borevitz

Abstract

The development of model systems requires a detailed assessment of standing genetic variation across natural populations. The Brachypodium species complex has been promoted as a new plant model for grass genomics with translational to small grain and energy crops. To capture the global genetic diversity within this species complex, thousands of Brachypodium accessions from around the globe were collected and sequenced using genotyping by sequencing (GBS). Samples were initially separated into two diploid or allopolyploid species defining overlapping and invasive ranges and climate niches. A core set of high diversity *B. distachyon* diploid lines were selected for whole genome sequencing and high resolution phenotyping. Genome-wide association studies was used to identify candidate genes and pathways tied to key fitness and agronomic related traits. A total of 9, 22 and 47 QTLs were identified for flowering time, early vigour and energy traits, respectively. Overall, the results highlight the genomic structure of the species complex and allow powerful complex trait dissection within an emerging model species.

Keywords: GWAS, hapmap, cryptic species

Accession Contributors

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Session 3: Natural variation and evolution

Insertion/deletion markers for assessing the genetic variation and the spatial genetic structure of Tunisian *Brachypodium hybridum* populations

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Abstract

The wild annual grass *Brachypodium distachyon* has been widely investigated across the word as a model plant for the temperate cereals and biofuel grasses. This annual plant shows three cytotypes that have been recently recognized as three independent species, the diploid species *Brachypodium distachyon*(2*n* = 10) and *Brachypodium stacei*(2*n* = 20) and their derived allotetraploid *B. hybridum* (2n = 30). The last species appear to be the most relevant in Tunisia. In order to analyze the genetic structure and the ecogeographical adaptation of this species, it is necessary to increase the number of polymorphic markers currently available for the species. In this work, the possibility of using syntenic Brachypodium indels as a new source of markers for this purpose has been explored. From 24 *B. distachyon* indels tested for transferability and polymorphism in the *B. hybridum*, 11 primer pairs (45%) gave cross-species transferability and 8 primer pairs (33%) showed polymorphism. The latters were used to examine the spatial distribution of genetic variation of *B. hybridum* across its entire range in Tunisia and to test underlying factors that shaped its genetic variation. Population genetic analyses were conducted on 145 individuals from 9 populations. Indels markers showed a total of 20 alleles overall all loci and a high level of genetic diversity overall populations (average of polymorphism rate

84,72%, allelic richness 1.95, Nei's gene diversity 0.35 and observed heterozygosity 0.31). Analysis of molecular variance (AMOVA) showed that 20% of the total genetic variability was partitioned among populations (ΦPT = 0.20), a very little part of this variability (5%) was due to the eco-regional effect and no altitudinal effect. These results suggest that there is probably an important gene flow among populations (Nm = 2.75). UPGMA cluster analyses, PCoA and Bayesian clustering divided the analysed populations into 3 groups without any geographical or altitudinal differences (r = 0.023, p = 0.39; r = -0.017, p = 0.48). Overall, the obtained results suggest that the genetic structure of the Tunisian *B. hybridum* populations was mainly governed by a high level of long distance seed dispersal between populations.

Keywords: *Brachypodium hybridum*, syntenic, genetic diversity, insertion/deletion, long-distance seed dispersal, spatial genetic structure

Session 3: Natural variation and evolution

Diversity of Brachypodium samples in Israel revealed by molecular and cytogenetic methods

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Abstract

As a temperate wild grass species, *Brachypodium distachyon*(2n=10) is becoming a promising model organism for studies of grass genomics and grain crop improvement. The annual species of genus Braypodium with 2n=20 and 2n=30 were described as species of *B. stacei* and *B. hybridum*, respectively. The characterization of diversity of natural populations of Brachypodiumspecies is important for genetic and evolutionary researches. In the present study, we investigated the phenotypical and cytological variation of nine extensively collected natural populations of Braypodium species with different macrogeographic scales originating from Israel. Total 174 genotypes of Braypodium samples were developed. Extensive phenotypical variation including plant height and tiller variation among genotypes were observed. The observation of chromosome number indicated 66 and 108 samples were *B. stacei* and *B. hybridum*, respectively, while no *B.distachyon* species was identified. The genetic diversity of *B. stacei* and *B. hybridum* samples was larger among population (62.1%) than within population (27.9%). Based on the values of Nei's genetic diversity (He) and Shannon's information index (I) correlated with the ecological factors, we found that the distribution of two species was significantly correlated to the environmental ecological factors. The results indicated that the*B. Hybridum* is largely positively correlated with higher environmental climatic stresses of temperature and drought. Identifying chromosomal mechanisms of *Brachypodium* species associated with population genetics and adaptation to climatic variation are needed to advance in

future studies. The high productivity of *Brachypodium*sampleswill be useful for further genetic and biofuel economic studies.

To evaluate the molecular ecology and evolution of *Brachypodium* with respect to population distribution, we investigated the genetic variation within and among populations using sequences at drought responsive genes dehydrin 1 (*BdDhn1*) locus of 118 Brachypodium samples from nine climatically divergent sites across Israel. We identified 37 single nucleotide polymorphisms (SNPs) in the *BdDhn1* locus including the sequence of 340bp promoter regions and 720-726bp gene coding regions. Total 20 haplotypes were defined by sequence analysis. Fifteen SNPs present at promoter regions, five in intron, seventeen in coding regions while eight of which caused the amino acid substitution from different Brachypodium samples. The association between *BdDhn1* gene diversity and 16 ecogeographical factors of the samples collection was estimated. The genetic diversity of SNPs at the *BdDhn1* locus was negatively correlated with key climatic factors of temperature and water variables. It was suggested that the SNPs in the BdDhn1 have been subjected to natural selection, and ecological factors had an important evolutionary influence on gene differentiation at specific loci. The results provide a strong support for the hypothesis that divergent natural selection is currently maintaining adaptive differentiation and promoting ecological population in Brachypodium species.

Keywords: Brachypodium, chromosome number, diversity *, BdDhn1*, Adaptive evolution.

Session 3: Natural variation and evolution

Environmental isolation explains Iberian genetic diversity in the highly homozygous model grass *Brachypodium distachyon*

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Abstract

Brachypodium distachyon, an annual Mediterranean Aluminum (Al)-sensitive grass, has been increasingly investigated as a model plant for temperate cereals, forage grasses and biofuel grass crops. However, despite being a model plant, we still know very little about its genetic diversity. We used nuclear Simple Sequence Repeats (nSSR) to study the patterns of genetic diversity and population structure of *B. distachyon* in 14 populations collected across the Iberian Peninsula. We detected very low levels of genetic diversity, allelic number and heterozygosity in *B. distachyon*, congruent with a highly selfing system. Our results indicate the existence of at least three genetic clusters; populations growing on basic soils (NE and S Spain) were significantly more diverse than those growing in acidic soils (NW Spain). A partial Mantel test confirmed a statistically significant Isolation-By-Distance (IBD) among all studied populations, as well as a statistically significant Isolation-By-Environment (IBE), revealing the presence of environmental-driven isolation as one explanation for the genetic patterns found in the Iberian Peninsula. Despite the low values of allelic and genetic diversity and the low levels of heterozygosity detected in *B.*

distachyon, the finding of higher genetic diversity in eastern Iberian populations occurring in basic soils suggests that these populations can be better adapted than those occurring in western areas of the Iberian Peninsula where the soils are more acidic and accumulate toxic Al ions. This suggests that the western Iberian acidic soils might prevent the establishment of Al-sensitive *B. distachyon* populations, potentially causing the existence of more genetically depauperated individuals.

Keywords: Al-sensitiveness,*Brachypodium distachyon*, isolation-by-distance, isolation-by-environment, SSR genetic diversity.

Session 4: Plant-biotic and abiotic interactions

Generation of *Brachypodium distachyon* T-DNA mutant population for studying nonhost resistance to wheat stripe rust

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Abstract:

Brachypodium distachyon has become a model system for temperate grasses' functional genomics research. Establishing a large insertion mutant population was very important for functional genomics. Here we reported the generation of about 7,000 T-DNA insertion lines based on a highly efficient *Agrobacterium*-mediated transformation system. Then a very powerful method for isolating flanking sequences of T-DNA insertion site was developed from the previous inverse PCR. Meanwhile, a serial of Perl scripts were utilized to rapidly process sequence data and identify insertion sites combining with network resources. A total of 794 flanking sequences was isolated and analyzed in detail using this method.

The resource of the T-DNA mutants provided us the convenience to study the molecular resistance mechanism against wheat stripe rust, caused by *Puccinia striiform*is f. sp. *tritici*(PST). The phenotypic evaluation showed that the response of *Brachypodium distachyon* to PST was nonhost resistance (NHR), providingagood plant-pathogen system for study of the immune responses and the molecular mechanism underlying wheat-PST interactions. More than 200 mutant lines that were more susceptible or resistant to the wheat stripe rust were identified from our T-DNA insertion population. The infection type was assessed according to the pathological phenotype. One highly susceptible mutant line, T1415, was isolated and studied thoroughly.

Keywords: *Brachypodium distachyon*, transformation, T-DNA insertion, flanking sequence, wheat stripe rust.

Session 4: Plant-biotic and abiotic interactions

Brachypodium: A useful model host for cereal-fungal pathogen interactions

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Abstract:

Fusarium pathogens cause serious yield and quality losses on cereal crops such as wheat and barley. There is no complete resistance against these pathogens and available resistance acts quantitatively, implying that many genes in the host, each with small effects, are involved in disease resistance. We have recently adopted Brachypodium as a model host to dissect cereal-*Fusarium* pathogen interactions. We have first developed a *Fusarium* infection assay for Brachypodium. We have then comparatively analysed the molecular responses of wheat and Brachypodium to *Fusarium* infection by RNA-seq and metabolite analyses. These analyses have revealed significant overlapping responses between Brachypodium and wheat. In addition, to understand the importance of salicylic acid (SA) in defence against *Fusarium* pathogens, we have generated a Brachypodium resource by over-expressing the bacterial *nahG* gene encoding a SA degrading enzyme. *nahG* over-expressing Brachypodium plants with very low basal SA levels were then exposed to pathogen infection and differential gene expression patterns of mock- and pathogen-inoculated plants were analysed by RNA-seq. This enabled us to identify Brachypodium genes that require SA for their induction by *Fusarium* pathogens. Together, our results suggest that Brachypodium is an excellent model host to dissect cereal-fungal pathogen interactions.

Keywords: Fungal defense, Fusarium, Biotic stress, Transcriptomics, nahG

Session 4: Plant-biotic and abiotic interactions

Linking phenotype to genotype: A metabolomics approach to build trait association network models for Brachypodium

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Abstract:

Prognostic understanding of the terrestrial carbon cycle in the face of global change is necessary to secure sustainable energy, water, and food for our nation and the world. To meet this challenge, we urgently need robust predictive models of net carbon flux in terrestrial ecosystems. A step in this direction is to explore the power of multi-scale plant modeling, where the phenotypic expression at one level is prognostic of emergent properties at the next integrative levels. Plant phenotype is shaped as a function of its genotype and interactions with the environment. Thus, for example, how does the genotype of a plant inform phenotypic expression at the level of metabolite and/or protein profiles, and how do these molecular-level phenotypes inform phenotypic expression at the cellular and organismal levels? Plants produce a wide variety of metabolites/ small molecules that are crucial for its growth and development. Metabolomics, the study of these molecules is becoming an increasingly important and powerful tool in predicting the effects of various aspects of plant physiology and biology, enabling our understanding and knowledge of plant growth, development and stress responses. In this study we have used above ground and below ground dry biomass as phenotypic markers to study carbon cycling under well watered and drought conditions in thirty different *Brachypodium distachyon* accessions coupled with metabolomics to build predictive metabotype-trait models. Here we demonstrate the power of metabolomics in defining the plant genotype and predicting the metabolite drivers of drought tolerance.

Keywords: Metabolomics, Biomass, Drought, Carbon Cycling, Statistical Modeling.

Session 4: Plant-biotic and abiotic interactions

Expression profiling of marker genes for defense-associated phytohormones in *Brachypodium distachyon* highlights its similar immune systems to rice

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Abstract:

Brachypodium distachyon is a model plant closely related to economically important cereals and biomass crops. It has been started to be used as a platform to study plant disease resistance and can be a counterpart of *Arabidopsis* and rice to illustrate the specificity and commonality of immune systems among plant species. To obtain marker genes to evaluate responses of *B. distachyon* to defense-related phyrohormones, 34 candidate marker genes for salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) were selected based on the similarities of protein sequences to the known marker genes of *Arabidopsis* and rice, and analyzed their responsiveness to each phytohormone at 24 and 48 h after treatments. Two genes for SA, 7 for JA, and 2 for ET were significantly induced at either or both time points. Next, we compared phylogenetic relationships and expression profiles of *PR1* family genes among *Arabidopsis*, rice and *B. distachyon*. The constitution and phytohormone responsiveness of *BdPR1* genes were shown to be similar to rice but not *Arabidopsis*, suggesting that monocots share a characteristic immune system, defined as the common defense system in contrast to dicots. Since we recently established a model pathosystem for sheath blight disease using *B. distachyton*, the infection process of *Rhizoctoniasolani* on *B. distachyon* was evaluated by using these

identified marker genes. The JA marker *BdAOS* was strongly expressed accompanied by JA accumulation at 24 h after inoculation, although SA and ET markers were not induced. The result clearly demonstrated the necrotrophic nature of this pathogen.

Keywords: Phytohormone, Marker gene, Plant immunity system

Session 4: Plant-biotic and abiotic interactions

Interaction of Bsr1 and TGB1 confers Barley Stripe Mosaic Virus resistance in Brachypodium, barley and wheat

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Abstract:

Barley stripe mosaic virus (BSMV) is a model for studies of viral pathogenesis and movement. *Brachypodium distachyon* is a member of the Poaceae subfamily Pooideae and has emerged as a model species for the study of cool season cereal crops. By applying map-based cloning approach, the first BSMV resistance gene *Bsr1*, encoding a typical CC-NBS-LRR protein, was isolated from *Brachypodium* inbred line Bd3-1. Domain switch of the Bsr1 and bsr1 alleles indicated that the LRR domain and the C terminal are critical region for the BSMV resistance of Bsr1. Transgenic experiments showed that *Bsr1* overexpression transgenic Bd21-3 plants performed BSMV resistance against ND18 strain, indicating that *Bsr1* is the causal gene that confers resistance to BSMV ND18 in Bd3-1.Furthermore, we transformed the complimentary construction pCBsr1 into barley cultivar "Golden promise" and wheat cultivar "Kenong199" (KN199), and the results imply that the foreign gene *Bsr1* has function in barley and wheat and the resistance genes from *Brachypodium* could be an assistant to crop breeding.The allelic variations of *Bsr1* in *Brachypodium distachyon* accessions collected mainly from Turkey-Iraq and *Bsr1* in *B. stacei* and *B. hybridum* accessions collected mainly from Israel were explored. The results conformedthat *B. distachyon* and *B. stacei*were the genome donors of *B. hybridum*. Further studies demonstrate that the $TGB1_{ND}$interacts Bsr1 to stimulate *Bsr1*

mediated resistant in *N. benthamiana*, $TGB1_{ND}$ R390 and T392 amino acid residues play the key role to Bsr1-$TGB1_{ND}$ interaction. Bimolecular fluorescence complementation (BiFC) assays provide evidence that $TGB1_{ND}$ and Bsr1 proteins interact directly in vivo in *N.benthamiana*. Additionally, subcellular localization data shows that the Bsr1 protein localizes to both the cytoplasm and nucleus, even to nucleolus. In the presence of $TGB1_{ND}$, localization of Bsr1 was not changed, and localized to the cytoplasm along with $TGB1_{ND}$, which may have an important role in *Bsr1*-mediated resistance responses.

Keywords: *Brachypodium distachyon*, barley stripe mosaic virus (BSMV), *Bsr1*, triple gene block 1 (TGB1)

Session 4: Plant-biotic and abiotic interactions

Using the JGI Brachypodium T-DNA collection to reveal novel transcription factor roles in abiotic stress responses

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Abstract:

Abiotic stresses such as drought and heat negatively impact the growth, development, yield, and seed quality of crops and other plants. To understand the molecular basis of plant responses to these adverse stresses, we utilized the JGI Brachypodium T-DNA collection to study the specific functions of transcription factors (TFs) under different environmental conditions. The JGI collection contains 23,649 tagged T-DNA lines in *Brachypodium distachyon* Bd21-3; the T-DNA insertion sites in each mutant line have been mapped and are publically accessible. We identified and obtained 348 lines that contain the activation tag T-DNA construct pJJ2LBA in or near transcription factors. We first screened these lines over two generations to obtain homozygous mutant lines, including target gene expression using Q-PCR. The expression of these target genes was also examined in wild type plants using publically available Brachypodium RNA-seq data generated under different experimental conditions such as cold, drought, salt, and heat. At present, we have a set of 43 lines with different gene expression patterns: 5 are knockouts, 11 are downregulated, and 27 are upregulated compared to Bd21-3. These genes are from several different TF families; most are transcription factors that are poorly understood in temperate grasses. We are intensely screening these lines for phenotypes during heat, drought, and salt stresses. Several promising lines involved in heat sensitivity and salt resistance have been identified, along with various morphological phenotypes.This screening method will allow us to uncover novel roles for genes that may be difficult to detect in classic genetic screens.

Keywords: Brachypodium, T-DNA mutant, Abiotic stresses, Gene expression.

Session 4: Plant-biotic and abiotic interactions

Cool season turf grass heat tolerance study through genomic and genetic analyses with *Brachypodium distachyon*

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Session 4: Plant-biotic and abiotic interactions

Homoeolog-specific activation for heat acclimation in the allopolyploid grass *Brachypodium hybridum*

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Abstract:

Allopolyploid plants often show wider environmental tolerances than their ancestors; this difference would be expected due to the merger of multiple distinct genomes with a fixed heterozygosity. The allopolyploid grass *Brachypodium hybridum* and its ancestor *Brachypodium stacei* show long-term heat stress tolerance, unlike its another ancestor *Brachypodium distachyon*. To understand physiological differences between these species, we compared the transcriptome of the allopolyploids and its ancestors grown under normal and heat stress conditions. We found that *B. distachyon* was transcriptionally insensitive, whereas *B. hybridum* and *B. stacei* were sensitive to heat at 3 days after stress exposure, and at 15 days after heat exposure, *B. hybridum* and *B. stacei* maintained transcriptional states similar to those under normal conditions. These results suggested an earlier response to heat that was specific to homoeologs originating from *B. stacei* and that contributed to cellular homeostasis under long-term heat stress. Our results provide insights into different regulatory events of the homoeo-transcriptome that are associated with stress acclimation in allopolyploid plants that evolved through all opolyploidization.

Keywords: all opolyploidization, *Brachypodium hybridum*, abiotic stress, transcriptome

Session 4: Plant-biotic and abiotic interactions

Influence of Supplemental Lighting with Different Light Quality on the Turf Growth of *Festuca Arundinacea*

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Abstract:

Plant factorywas widely used in developed countries, which provided a new model for factory production of soil-free turf.As light was an indispensable factor for plant growth, artificial supplemental lighting was the most effective way to improve the light deficiency in plant factory. In order to study the effects of supplemental lighting with different light quality on turf grass growth, four kinds of light with red and blue 3: 1 (3R/B), red and blue 4: 1 (4R/B), red and blue 5: 1 (5R/B) and full spectrum（control）were used in the experiment as supplemental lights for 10 hours each day.The soil-free substrate was mixed by the same volume of coirdustand straw ash. The seeds of *Festuca Arundinacea* were sown by 30 grams per square meteron December 9, 2016. The laboratory temperature was 22□ up to 25□, the relative humidity 70% up to 80%. The LED lamps were about 50cm above the canopy of the turf. The experiment was ended on January 16, 2017 and the average stem diameter,leaf width,underground biomass, coverage and turf color index was tested.

Results showed that the average stem diameter decreased gradually **w**ith the increase of red light proportion. The stem diameter of the controlplant was the thickest (2.04mm), and that treated with 5R/B was the weakest. The average leaf-breadth of the plant treated with 3R/B was 3.47mm, about the same of the control. Leaf width of 3R/B treatment was significantly higher than those of 4R/B and 5R/B treatments.

Results also showed that underground biomass reduced with the increase of red light proportion. The underground biomass of the control (687.00mg) was significantly higher than other treatments, 498.87mg more than 5R/B treatment (188.13mg).And the turf color index decreased with the increase of red light proportion. The turf color index of 3R/B

treatment was the biggest (4.91), and that of 5R/B treatment was the lowest (4.28). The coverage of 3R/B treatment was the biggest (50.00%), while that of the controlwas only 41.20%.

As a conclusion, the average stem diameter, underground biomass and turf color index all decrease drespectively with the increase of red light proportion. The turf color index and coverage were the key indicators for turf. So supplemental lighting with 3R/B could improve the turf grass growth and shorten the production time.The results would behelpful for the research of influence of supplemental lighting intensity and time on turfgrass growth.

Tab 1 Influence of Different Light Quality on the Growth of *Festuca Arundinacea*

Treat-ments	Stem Diameter/mm	Leaf Width/mm	Underground Biomass/mm	TurfColor Index	Coverage/ %
3R/B	2.00aA	3.47aA	406.13bAB	4.91aA	50.00aA
4R/B	1.84aA	2.88bA	215.63bB	4.34aA	45.30aA
5R/B	1.76aA	3.00bA	188.13bB	4.28aA	48.60aA
CK	2.04aA	3.46aA	687.00aA	4.74aA	41.20aA

Keywords: supplemental lighting; light quality; turf color index; coverage; turfgrass growth

Session 4: Plant-biotic and abiotic interactions

Establishing *Brachypodium distachyon* as a model in analyses of plant genome stability after mutagenic treatment

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Abstract:

Due to their high sensitivity higher plants are widely used for screening and monitoring environmental genotoxicity. *Brachypodium distachyon*, an internationally accepted model grass species, would be a convenient system in mutagenesis to analyse "hot spots" of DNA damage in nuclear genome and consequently could find practical application in the environmental monitoring. The chromosome rearrangements are commonly identified using classical cytogenetic techniques. Physical mapping technology together with the availability of BAC libraries of *B. distachyon* nuclear DNA, allow comprehensive analyses of mutagenic effects at the chromosomal level and extend our understanding of the mechanisms of chromosomal aberrations. The visualisation of mutagen-induced genome changes, including micronuclei formation and alterations of chromosome territories in interphase nuclei using fluorescence *in situ* hybridisation (FISH) with selected chromosome-specific BAC clones, as well as ribosomal DNA and chromosome region-specific, i.e. centromeric and telomericprobes are presented. This work was supported by the Polish National Science Centre (grant no. 2012/04/A/NZ3/00572).

Keywords:*Brachypodiumdistachyon*, FISH, micronuclei, mutagenesis, model grass

Phenotypic and metabolomic variation in the model annual grasses *Brachypodium distachyon*, *B. stacei*, and *B. hybridum*

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Abstract:

Morphological traits and metabolite fingerprinting were used to investigate inter- and intra species variation within the model annual grasses *B. distachyon*, *B. stacei* and *B. hybridum*. Phenotypic variation of 15 quantitative and 5 qualitative morphological charactersand 2219 nominal mass (*m/z)* signals generated using Flow Infusion Electrospray ionisation - Mass Spectrometry were analysed in individuals from 174 natural populations and 6 inbred lines, and 12 Iberian lines of the three species, respectively. Basic statistics and multivariate Principal Component Analysis (PCA) and Discriminant Analysis (DA) were used to differentiate inter- and intraspecific variability of phenotypic and metabolomic variables, and their association was assayed with *rcorr*. Eight quantitative [(stomata) leaf guard cell length, pollen grain length, (plant) height, second leaf width, inflorescence length, number of spikelets per inflorescence, lemma length, awn length] and five qualitative (leaf color, softness, shape, and hairiness, and presence of short rhizomes) phenotypic characters, and 434 tentatively annotated metabolite signals significantly discriminated the three species. The three species showed different metabolomic profiles. DA significantly discriminated the three taxa with both, morphometric and metabolome traits and the intraspecific phenotypic diversity within *B. distachyon* and *B. stacei*. The populations of *B. hybridum* were considerably less differentiated. Highly explanatory metabolite signals together with morphological characters revealed concordant patterns of differentiation of the three taxa. Significant association was found for pollen grain

length and lemma length and 10 and 6 metabolomic signals, respectively. These results would guide the selection of new germplasm lines of the three model grasses in on-going GWAS experiments.

Keywords: association studies, annual *Brachypodium* model grasses, metabolite fingerprinting, phenotypic traits, statistic analyses.

How does *CUC2* regulate leaf serration development in Arabidopsis?

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Abstract:

A key question in developmental biology is how developmental patterning generates the final form of an organ. *Arabidopsis thaliana* produces simple leaves bearing repeated marginal outgrowths termed serrations, providing a good opportunity to study this question. The NAC domain transcription factor *CUC2* regulates serration formation by promoting the formation of PIN1 convergence points and auxin activity maxima along the leaf margin during leaf development (Bilsborough et al., 2011). However, how *CUC2* regulates repeated formation of auxin maxima and serrations remains enigmatic. To address this question, we performed an EMS-mutagenesis screen in a *cuc2-3* mutant background to identify novel components in regulating serration formation, specifically looking for suppressors that restore serration development. Among the suppressors identified, we primarily investigate #51 because it displays the strongest suppression effect and no other developmental defects. We found that #51 could restore auxin maxima and PIN1 convergence points along the *cuc2* leaf margin. Currently we are trying to identify the molecular basis for the #51 mutation and understand how the gene defined by this mutation regulates PIN1 convergence point and auxin maxima formation.

Keywords: Leaf serration, CUC2

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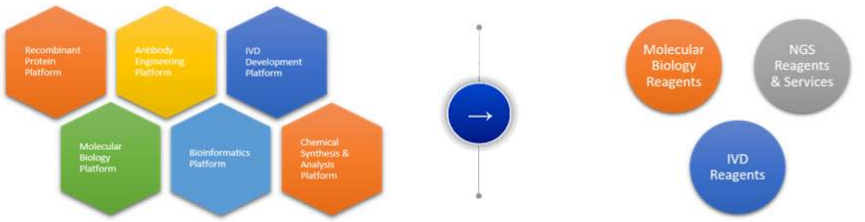

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