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Update on the genomics and basic biology of *Brachypodium*

International *Brachypodium* Initiative (IBI)

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The scientific presentations at the First International *Brachypodium* Conference (abstracts available at http://www.brachy2013.unimore.it) are evidence of the widespread adoption of *Brachypodium distachyon* as a model system. Furthermore, the wide range of topics presented (genome evolution, roots, abiotic and biotic stress, comparative genomics, natural diversity, and cell walls) demonstrates that the *Brachypodium* research community has achieved a critical mass of tools and has transitioned from resource development to addressing biological questions, particularly those unique to grasses.

A model for grass genome organization

This report highlights recent advances made in *Brachypodium* research, focusing on the use of *B. distachyon* and related species to understand biological processes. Its experimental and genomic tractability allow *B. distachyon* to act as a functional genomic test-bed to accelerate the improvement of grain, forage, and biomass crops. Its strengths as a model plant (e.g., short generation time, efficient *Agrobacterium*-mediated transformation, and availability of mutant collections) are described in [1] and other reviews published in the past 5 years. The main web portal for *Brachypodium* (http://www.brachypodium.org) contains a genome browser and links to community resources. In addition, two project-specific websites (http://brachypodium.pw.usda.gov/ and http://www-urgv.versailles.inra.fr/tilling/brachypodium.htm) provide access to T-DNA and Tilling resources, respectively.

The compact nature of the fully sequenced [2] *Brachypodium distachyon* genome is a major reason for the success of *B. distachyon* as a model system, and provides unique opportunities to study various aspects of grass genome organization and evolution. Moreover, as a monocot reference, it permits comparisons of genomic landscape dynamics with the dicot model *Arabidopsis thaliana*. Thus, *B. distachyon* has become an appealing target for plant molecular cytogenetics.

One of the most informative cytomolecular tools is chromosome painting (CP), which enables unique and unambiguous visualization of individual chromosomes or large segments, both during cell division and even at interphase, using fluorescence *in situ* hybridization with specific DNA probes. CP was initially applied to vertebrate systems. Whole-genome sequencing (WGS) and large-insert genomic DNA libraries allow its application to small-genome plants such as *A. thaliana*. The sequencing of the *B. distachyon* genome [2] combined with its low (5) chromosome number and a well-developed cytogenetic infrastructure has allowed the CP of several *Brachypodium* species [3], a pioneering application of CP in monocots.

The chromosomes of *B. distachyon* can be selectively painted to address important questions about grass genome

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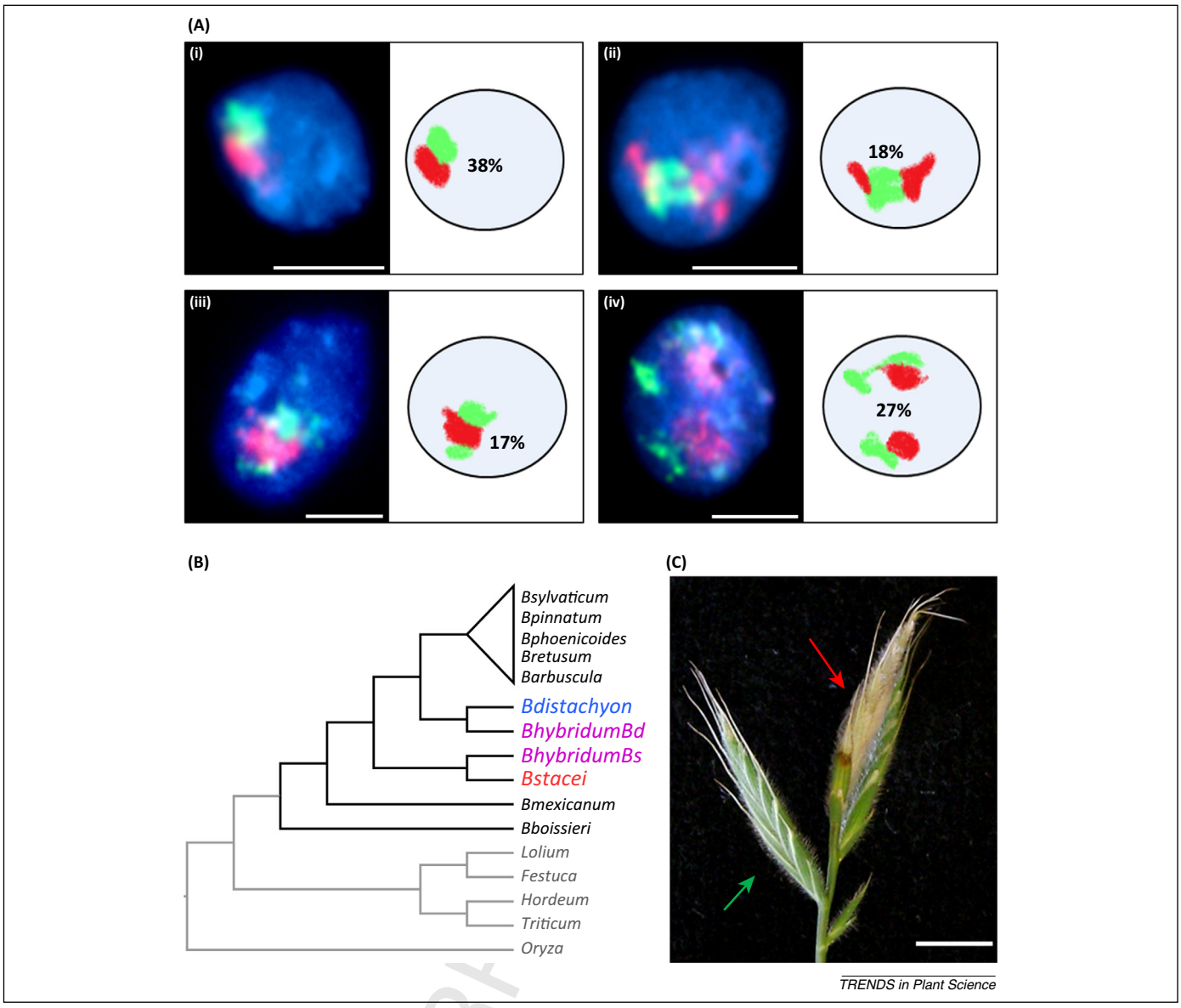

Figure 1. **(A)** Different arrangements of the Bd2 homologous chromosome territories (CTs) and the observed frequencies (%) of their occurrence in interphase nuclei of roots of *B. distachyon*: (i) complete association; (ii) top (green fluorescence) and (iii) bottom (red fluorescence) arm-only association; (iv) complete separation. Chromatin stained with DAPI (4′,6-diamidino-2-phenylindole; blue fluorescence). Scale bars: 5 μm. For better visualization the respective arrangements of CTs have been schematically shown on the diagrams next to photomicrographs. Documentation courtesy of Ewa Breda (University of Silesia in Katowice, Poland). **(B)** Evolution of the three *B. distachyon*-complex species. Summarized low-copy nuclear GIGANTEA (GI) gene tree showing the phylogenetic reconstruction of *B. stacei*, *B. distachyon*, and *B. hybridum* [*B. hybridum* shows GI copies from both stacei-type (BsBs genome) and distachyon-type (BdBd genome) parents, coinherited from bidirectional crosses]. **(C)** *Fusarium* head blight symptoms on Bd21 spikes following point inoculation (red arrow) with *Fusarium graminearum* strain FgUK1 at 6 days after inoculation. An asymptomatic spike (green arrow) is shown for comparison. Scale bar, 1 cm. Photograph courtesy of Paul Nicholson and Antoine Peraldi (John Innes Centre, UK).

structure and evolution (in Figure 1 we demonstrate how they are arranged at interphase). CP of *B. distachyon* chromosome 2 (Bd2) in the nuclei of root cells revealed that Bd2 homologous chromosome territories can assume four different configurations that are observed at different frequencies (Figure 1A). This is one example where research in *Brachypodium* could lead the way in determining whether and how nuclear structure is linked to cell differentiation and tissue-specific gene expression.

A tractable model for inter- and intraspecific diversity

The genus *Brachypodium* contains 15–18 species with unusually variable chromosome numbers and ploidy levels. This diversity was a subject of interest long before *B. distachyon* became a model grass. WGS of *B. distachyon* [2], together with the advent of inexpensive next-generation sequencing (NGS) technologies, set the stage for high-resolution investigation of the genomic diversity and evolutionary relationships in the genus.

It was recently demonstrated that '*B. distachyon*' is a complex of three separate species: two diploids (*B. distachyon*, *B. stacei*) and their derived allotetraploid (*B. hybridum*) ([4] and Figure 1B). The genomes of *B. stacei* and *B. hybridum* are being sequenced and gene expression is being compared between all three species, to serve as a model for speciation through adaptation and polyploidization. To develop further this trio of species as a model for plant polyploidy, allopolyploids are being developed through interspecific hybridization between *B. distachyon* and *B. stacei* with the aim of reproducing *B. hybridum*

(Vinh Ha Dinh Thi and B.C., unpublished). This system offers experimental advantages (compact genomes with little repetitive DNA, small and easily grown plants that can be transformed efficiently) that will facilitate investigating the role of polyploidy in speciation and adaptation, a topic of great interest to cereal breeders.

The analysis of intraspecific diversity in *B. distachyon* is also underway through NGS resequencing of 54 diverse natural accessions. High polymorphism rates of up to one single-nucleotide polymorphism (SNP) per 200 bp have been detected (J.P.V., unpublished). Because the *B. distachyon* accessions display considerable phenotypic variation in a plethora of economically important traits (e.g., seed size, biomass, cell wall composition, flowering time), knowledge of genome sequence variation will facilitate genome-wide association mapping and positional cloning of economically important genes. In addition, several mutant populations have been established [1] that will greatly increase the utility of these new sequence resources.

A model for abiotic stress

B. distachyon, B. stacei, and *B. hybridum* all grow in a wide range of habitats under marked environmental gradients. Distinct genotypes are thus subject to different abiotic stresses which might have exerted, from speciation until present, different selective pressures on stress tolerance-related traits.

Detection of adaptive variation of stress-tolerance traits in response to abiotic conditions requires the following: (i) significant genetic variation in the trait of interest, (ii) a match between adaptive genetic variation and environmental variation (e.g., local adaptation across the gradient), and (iii) positive selection for these traits in genotypes growing under abiotic stress. Progress has been made in screening for natural variation in stress tolerance among *B. distachyon* accessions (reviewed in [1]), the first step towards determining the heritability of adaptive traits. However, full understanding of the adaptive significance of tolerance trait variation awaits experimental evaluation of the effects of such variation on fitness in natural populations.

Progress toward understanding abiotic stress adaptation at a molecular level is being made. Promising results come from the recent characterization of a microRNA (miRNA) network controlling cell division during stress, part of a search for epigenomic regulatory mechanisms underlying drought stress [5]. Further, evidence of ancient adaptive evolution of temperate Pooideae species was inferred from nucleotide substitution rates and signatures of positive selection in genes induced by low temperature [6]. Finally, adapted genotypes of *B. distachyon* and *B. hybridum* may reveal how genomic changes such as whole-genome duplication influence ecological tolerances to abiotic stress. In fact, differential tolerance to water stress between *B. distachyon* and *B. hybridum* seems to drive the ecogeographical differentiation of these species [7].

A model pathosystem for multiple cereal diseases

Pests and pathogens are major contributors to global food insecurity. Shifting climate patterns are altering disease ranges and facilitating the emergence of virulent strains. Thus, we need a better understanding of plant–pathogen interactions to develop rapidly new and preferably durable sources of disease resistance. *B. distachyon* has emerged as a powerful tool to elucidate defense responses in the Poaceae. A major advance has been the demonstration that *B. distachyon* serves as a host for many pathogens that cause diseases such as rice blast, *Fusarium* head blight (FHB; Figure 1C), and barley stripe mosaic virus (BSMV) (reviewed by [4]). Studies are now exploiting the genetic and functional genomic resources available for *B. distachyon* to elucidate host responses to pathogens. An elegant example is the characterization of *B. distachyon* UDP-glycosyltransferases that can detoxify the mycotoxin deoxynivalenol produced by *Fusarium graminearum*, the casual pathogen of FHB [8]. As such studies progress they may identify commonalties in host responses to pathogens which could represent key defense nodes that are potential sources of durable resistance to many pathogens. Translation of this knowledge into improved crop varieties will involve identifying orthologous genes or linked molecular markers in crop germplasm. Such a strategy contributed to the targeting of *Pch1* eyespot resistance in wheat (*Triticum aestivum*) [9]. However, the absence of an ortholog of the wheat *Lr34* leaf rust resistance gene in *B. distachyon* [10] indicates that successful transfer of information between *B. distachyon* and grass crops is not guaranteed. Another powerful means of increasing crop resistance is the direct transfer of genes from *B. distachyon* into elite cereal germplasm through transformation. The success of this approach is enhanced by the close relationship between *B. distachyon* and the cereals.

A model for the grass cell wall and biomass accumulation

Similarly to other grasses, *B. distachyon* has a type II wall that differs markedly from the type I walls found in dicots. Until now, few studies have focused on characterizing *B. distachyon* cell wall polysaccharides and their biosynthetic enzymes. Detailed biochemical characterization of these polysaccharides, their distribution in different tissues and organs, and their roles during development need to be investigated. Characterization of mixed linkage glucans (a polymer unique to grasses) in *B. distachyon* seeds showed surprising enrichment in (1→3) linkages and that arabinoxylans were more substituted compared to wheat, barley (*Hordeum vulgare*), or oat (*Avena sativa*).

In comparison with polysaccharides, *B. distachyon* lignin has been studied in greater detail by genetic and biochemical characterization of some of the enzymes required for lignin biosynthesis. The enzymes are encoded by gene families, but each gene has a distinct function, and are thus good targets for mutagenesis or introgression [11]. As an elegant example of the potential of the model grass, mutation of the cinnamyl alcohol dehydrogenase (CAD1) gene has been shown to lead to a 25% decrease in lignin content, resulting in improved saccharification [12]. These results, taken together with the possibility to increase biomass by modifying polysaccharide-related metabolism, demonstrate that *B. distachyon* is an excellent model for identifying genes important for developing biomass crops with improved conversion into bioenergy or new materials (Box 1).

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Box 1. *Brachypodium distachyon* root and rhizosphere for underground discoveries

Plant roots provide a multitude of essential functions like mechanical support, water and nutrient uptake, defense against soil pathogens and toxins. Compared to shoots, roots have been understudied, and offer important opportunities to increase global food production, and save water, land, and fossil fuels. Most molecular root research has been conducted on *Arabidopsis*, but its dicotyledonous root system has a different morphology, architecture, anatomy, and biochemistry from cereals such as wheat or barley. Thus, *B. distachyon* now has many of the tools available for *A. thaliana* but a root system similar to that of temperate cereals (Figure I).

B. distachyon root system is composed of three root types (Figure I). A single primary seminal root (PSR) emerges at germination; this is followed approximately 2 weeks later by one or two coleoptile nodal roots (CNR) from the coleoptile node located on the mesocotyl, about half way between the seed and the leaf nodes, and, finally, 3–4 weeks after germination, leaf nodal roots (LNR) start to emerge from the leaf nodes [13]. In *B. distachyon* the number of nodal roots, but not the seminal primary root, varies genetically and in response to water, opening the possibility of selecting root systems for specific soil conditions (V.C., unpublished). *B. distachyon* root variation can be studied in much smaller volumes of soil than maize or rice, permitting the characterization of the role of mature root systems during flowering and seed development [13]. Flowering and seed development are highly susceptible to drought, and knowledge of root genes at these stages can be applied to crop improvement through marker-assisted breeding.

Since the emergence of *B. distachyon* as a molecular model it has been applied in several fields relevant to roots, including root system architecture of cereals, response to biotic (pathogens such as *Fusarium*, *Rhizoctonia*) and abiotic (nutrient levels, drought) stresses, auxin homeostasis [14], and symbiotic root–microbe interactions such as arbuscular mycorrhizal fungi [15]. *B. distachyon* is an exciting new model for root research, opening the way to understanding monocotyledon root biology, and eventually leading to the improvement of major temperate crops.

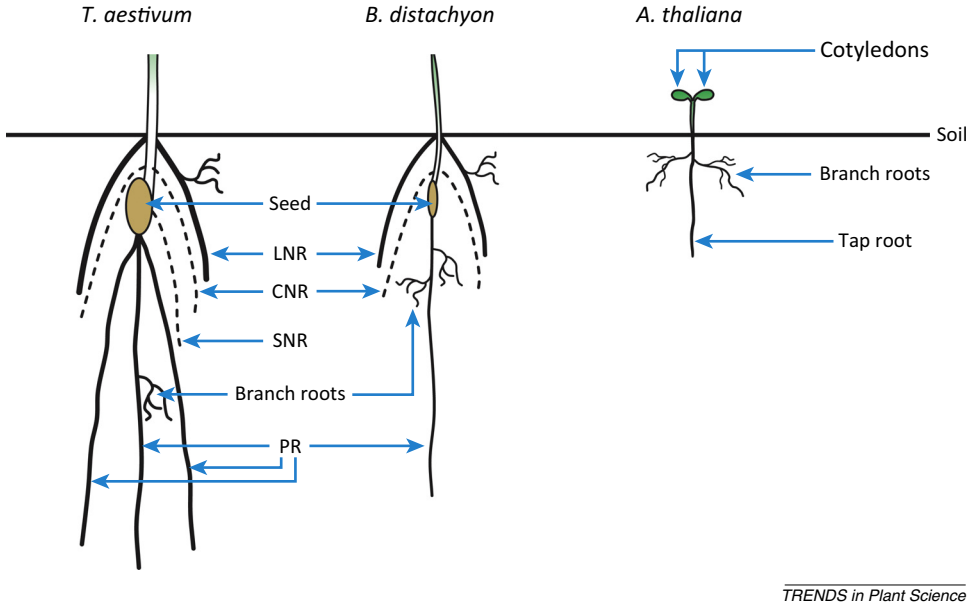

Figure I. Comparison of wheat (*T. aestivum*), *Brachypodium distachyon*, and *A. thaliana* root systems. Horizontal line, soil level. Abbreviations: CNR, coleoptile node axile root; LNR, leaf node axile root; PR, primary axile root; SNR, scutellar node axile root.

Taken together, these advances demonstrate the wide applicability of *Brachypodium* as a model system and underscore the maturity of the system.

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