The Plant Genome
Activities & Resources

Development of Genetic and Genomic Research Resources for *Brachypodium distachyon*, a New Model System for Grass Crop Research

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Abstract

Grass crop genomics research frequently is hindered by large genome sizes and polyploidy. While rice is an attractive system for grass genomics due to its small genome size and available genome sequence, it is not particularly well-suited as a robust model system for all grass crops. The wild grass species *Brachypodium distachyon* (L.) P. Beauv. (Brachypodium) has recently gained favor as a new model system for grass crop genomics research because it possesses a suite of biological traits desired in a model system. Further, it is more closely related to the large and diverse group of cool season grass crops than is either rice (*Oryza sativa* L.) or sorghum [*Sorghum bicolor* (L.) Moench.], the second grass crop species whose genome has been sequenced. Thus, by virtue both of its biological attributes and its evolutionary history, Brachypodium fills an important gap in grass crop genomics research. A surge in interest in Brachypodium has led to rapid and significant advances in the acquisition of knowledge and development of resources needed to exploit this species as a model system, including. the impending completion of a draft nuclear genome sequence of Brachypodium. Integration of diverse genetic and genomic resources developed or under development for Brachypodium with the genome sequence will encourage further adoption of this species as a bona fide model plant system.

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Before 2001, the unassuming grass genus *Brachypodium* was perhaps more known for its enigmatic features such as the diversity of chromosome numbers in the genus (Robertson, 1981) and the proper placement of the genus in the evolutionary tree of the grass family Poaceae than for anything else (Catalan et al., 1995; Kellogg, 2001). However, at the end of 2001 a seminal paper was published that touted a member of this genus, *Brachypodium distachyon* (L.) P. Beauv. (referred to as Brachypodium throughout the rest of this paper), as a new model system for grass genomics (Draper et al., 2001). A compelling case was made in this publication for Brachypodium to be given consideration as a new model system for genomics. In particular, Brachypodium possesses the suite of traits desired in a model

Abbreviations: BAC, bacterial artificial chromosome; BES, BAC end sequence; cDNA, complimentary DNA; DNA, deoxyribonucleic acid; DOE, Department of Energy; EST, expressed sequence tag; FISH, fluorescent in situ hybridization; GUS, β-glucoronidase; HR, hypersensitive response; JGI, Joint Genome Initiative; kb, kilobase pair; NPGS, National Plant Germplasm System; ORF, open reading frame; PCR, polymerase chain reaction; QR, qualitative resistance; QTL, quantitative trait locus; RNAi, ribonucleic acid interference; SAR, systemic acquired resistance; SNP, single nucleotide polymorphism; T-DNA, transfer-DNA.

plant including a small genome, availability of diploid ecotypes, self-fertility promoting inbreeding, transformability, small stature, and a moderately rapid life cycle. Indeed, these desirable biological features in Brachypodium are quite similar to those found in the dominant model plant *Arabidopsis thaliana* (L.) Heyhn (*Arabidopsis*). Readers are directed to Draper et al. (2001) and the following publications for additional reading that describes various aspects of Brachypodium that are complementary to the present review (Jenkins et al., 2003; Hasterok et al., 2004; Jenkins et al., 2005; Garvin, 2007).

This 2001 Brachypodium paper (Draper et al., 2001) prompted interest among a range of research groups, and since its publication major strides have been made in helping Brachypodium mature into a full-fledged model plant system. This has included the emergence of an international Brachypodium research community, leading to the formation of the International Brachypodium Initiative (IBI) in 2005 to foster communications among researchers. In January 2006, the first *Brachypodium distachyon* Genomics Workshop was held at the XIV Plant and Animal Genome Conference in San Diego, California, and the first meeting of the IBI was held at the same meeting. Further, a website (http://www.Brachypodium.org; verified 26 Nov. 2007) was established to provide basic information on the species for interested parties.

Similarly, research with Brachypodium has rapidly accelerated. A crowning achievement for Brachypodium that will soon emerge is a draft nuclear genome sequence. In 2006, the U.S. Department of Energy's Joint Genome Institute (DOE-JGI) accepted a proposal to undertake whole genome sequencing of the Brachypodium genome, to 8X genome coverage (see http://www.jgi.doe.gov/sequencing/cspseqplans2007.html; verified 26 Nov. 2007). This will be accompanied by a complementary integrated project to sequence almost 200,000 expressed sequence tags (ESTs) from diverse tissues to provide a view of the Brachypodium transcriptome. The completion of the Brachypodium genome sequence is expected in 2008, as is the transcriptome sequencing.

Since the genus *Brachypodium* is a sister group to the four major cool season grass tribes of greatest economic importance (Triticeae, Aveneae, Poeae, and Bromeae) (Kellogg, 2001), the Brachypodium genome is expected to exhibit far greater synteny to the genomes of major cool season cereal grains and forage and turf species than will the genomes of rice or sorghum, which have been sequenced. Thus the Brachypodium genome sequence will find great utility as a surrogate to assist gene discovery in the large genomes of its cool season crop relatives. Similarly, access to the genome sequence of Brachypodium will be a boon for wide ranging functional genomics research in grasses, such as investigating the impact of specific modifications to the plant cell wall on cellulosic ethanol conversion (U.S. DOE, 2006). Lastly, the Brachypodium genome sequence will be a representative of a third major grass lineage, with the rice and sorghum genome sequences representing two other such lineages (Kellogg, 2001). This will provide an unprecedented opportunity to examine genome evolution in this most economically important plant family.

The Brachypodium genome sequence and EST collection must be accompanied by additional genomics tools to be efficiently exploited by the research community. With the impending release of the Brachypodium genome sequence, it is timely to review the diverse array of Brachypodium genome resources and technologies that have already been developed or are being developed, and that will play a major role in optimizing the ability to use Brachypodium as a model system.

Genetic Resources

There are two main collections of Brachypodium available. The first source of Brachypodium germplasm is the USDA National Plant Germplasm System (NPGS). Approximately 30 Brachypodium accessions are available from the NPGS, and details of these accessions can be viewed by the appropriate querying of the NPGS Germplasm Resources Information Network website (http://www.ars-grin.gov/npgs; verified 26 Nov. 2007). In addition, complete passport data for these accessions from annual Plant Inventory reports can be found at the following website (http://www.brachypodium.org). These accessions have been collected and deposited into the NPGS over the last several decades. The USDA NPGS accessions are designated by the prefix "PI" (eg., PI 185133). The second set of ecotypes is held at Brachyomics in Aberystwyth, Wales. The following website http://www.aber.ac.uk/plantpathol/germplasm.htm (verified 26 Nov. 2007) lists this collection. The Brachyomics ecotypes are designated with the prefix designation "ABR" (e.g., ABR1). The Brachyomics website provides information on both chromosome numbers and origin of the ABR ecotypes. The Brachyomics collection includes 38 ecotypes, with the majority deriving from the USDA NPGS collection itself. For a table that lists much of this overlap as well as chromosome numbers, see Jenkins et al. (2003). But it is worth noting that this publication likely misses some of the redundnacy, based on comparing USDA NPGS passport data and the listed geographic locations of some of the ABR lines. Efforts are being made in the

community to completely resolve the synonymy of the NPGS and ABR lines.

For purposes of genomics research, it is imperative to have community genetic stocks that are genetically homogeneous and are also homozygous. Although Brachypodium is inbreeding, accessions acquired by sampling populations in the wild may each harbor varying degrees of genetic diversity, as has long been observed in other species (eg, Brown et al., 1978). So, there is no expectation that the original accessions are genetically homogeneous. Thus, a set of inbred Brachypodium lines from the NPGS collection was developed by single-seed descent (Garvin, 2007; Vogel et al., 2006a). Inbred lines developed from 28 different NPGS accessions have been designated with the prefix "Bd". In nearly all instances, the inbred lines each have the designation "Bd", followed by two numerals separated by a hyphen (e.g., Bd2–3). The first numeral is an identifier of the NPGS accession from which it was derived. For instance, the single seed descent inbred line Bd1–1 was developed from the NPGS accession PI 170218. The second numeral was included because in many instances two separate inbred lines were developed from a single accession.Thus, Bd3–1 and Bd3–2 are each single seed descent inbreds independently developed from PI 185134. (See Vogel et al. 2006a, for inbred line derivations.)

One significant case where this nomenclature is not followed is for Bd21. This inbred line is a single seed descent line derived from Bd 254867, but does not have a numerical suffix because just a single inbred line was initially developed. It is important to clarify this because inbred line Bd21 has been adopted as the canonical genotype for much of the genomics research being pursued, and is serving as the source of DNA and RNA for both the whole genome and EST sequencing projects by DOE-JGI.

Because Brachypodium is described as a polyploid series, flow cytometry was used to infer ploidy levels of the Bd inbred lines. Currently, a c-value of 0.36 for diploid Brachypodium (Bennett and Leitch, 2005) has been adopted because the far lower c-value reported by Draper and colleagues (2001) and in some subsequent publications has not been confirmed. Inbred lines derived from PI 170218 (Bd1–1), PI 185133 (Bd2–3), PI 185134 (Bd3–1, Bd3–2), PI 245730 (Bd18–1), and PI 254867 (Bd21) were hypothesized to be diploid based on c-values approximating 0.36 (Vogel et al., 2006a). Subsequent chromosome counts support their diploid (2n = 10) nature (R. Hasterok, personal communication, 2006). The other inbred lines appeared to be polyploid based on c-values approximately twice as large as those of the diploids. A core set of these inbred diploid lines has been widely disseminated to the research community; these are available on request (contact: D.F. Garvin, garvi007@umn.edu). Though the number of lines is small, they reveal phenotypic variation in many traits of agricultural relevance in crops (Garvin, 2007). To date, just one one morphological trait—pubescence on leaf sheaths—has been analyzed genetically and is controlled by a single dominant gene, termed *Spub* (D. Garvin, unpublished data, 2007).

Diploid genotypes are the most desirable for genomics research, and between the Brachyomics/Abersytwyth and NPGS collections it appears that there are perhaps just 14 unique diploid ecotypes/accessions. These have been collected across a wide geographic range in Europe to the middle east, but this nonetheless highlights the fact that more diploid inbred lines will be needed to capture additional genetic diversity in the species. One strategy for doing so would be to develop additional inbred lines from each original accession to try to capture additional variation that may be present. Indeed, besides Bd21, a second inbred line designated Bd21–3 has been developed from PI 254867 (Vogel and Hill, in press) that exhibits distinct patterns of molecular variation that differentiate it from Bd21 (Vain et al, in press, 2008). Similarly, Christiansen et al. (2005) allude to the presence of molecular variation in plants from the same accession. Additionally, further collection of diploid germplasm is warranted. The broad range of diploid Brachypodium (see the following section on geographic distribution) suggests that this should be possible.

A cornerstone resource for any model system is segregating populations. The only report of a segregating population was published in 2004 (Routledge et al., 2004), which examined disease resistance segregation in a small population. But, routine crossing of Brachypodium has proven to be a challenge due to the small size of the floral structures and the fact that the anthers reside between folds in the palea. Thus, development of a protocol that will permit routine crossing is needed. Nonetheless, segregating populations between all combinations of the diploids Bd1–1, Bd2–3, Bd3–1, and Bd21 have been created (Garvin, 2007). Ultimately, development of immortalized recombinant inbred populations for the various combinations among the Bd diploids will be completed. The final intended inbred populations will derive from either F_7 or F_8 individuals. It is expected that the first RIL populations will be completed in 2008.

A molecular map of Brachypodium is essential for many purposes, and currently a community effort is underway to to develop a primary molecular

map of Brachypodium for comparing its genome organization to that of rice and other grasses. This project involves an F_2 population from the cross Bd3–1 × Bd21, with markers primarily derived from simple sequence repeat length polymorphisms identified in EST sequences. Subsequently, a larger population of this same cross will be used to expand map resolution by populating it with a large number of single nucleotide polymorphism (SNP) markers.

Geographical Distribution

Since collection of additional germplasm is a priority for Brachypodium research, it is useful to review the extent of the geographic range of both native and introduced Brachypodium. According to herbarium records, field observations, and a small characterized germplasm collection, the geographic distribution of both native and introduced Brachypodium appears to be widespread (Fig. 1). The native range is likely to be very broad, extending throughout the Mediterranean basin and into the Indian subcontinent. All but three of 35 apparently unique accessions characterized for genome size originate from this area (Draper et al., 2001; Hasterok et al., 2004; Hasterok et al., 2006; Vogel et al., 2006a). A majority of the diploid accessions with a haploid genome of five chromosomes were collected in northern Spain while others hale from France, Italy, Slovenia, Iraq, and Turkey. The island of Formentera in the Mediterranean Sea is home to the only accession, ABR114, known to have a haploid genome of ten chromosomes (Draper et al., 2001; Hasterok et al., 2006; Jenkins et al., 2003). The known range of polyploids with a haploid genome of fifteen chromosomes extends outside the aforementioned range as far north as Belgium and as far east as Pakistan. In addition to a broad native latitudinal distribution, there is a broad range of altitudes where populations are common in both coastal sea level territory as well as mountainous areas in Turkey (Kaman Kirschir, 1100 masl, PI 245730), Iraq (Arbet, 675 masl, PI 254868), Iran (Kashmar, 1220 masl, PI 239714), and Afghanistan (Jabal-Us-Siraj, 1600 masl, PI 220567).

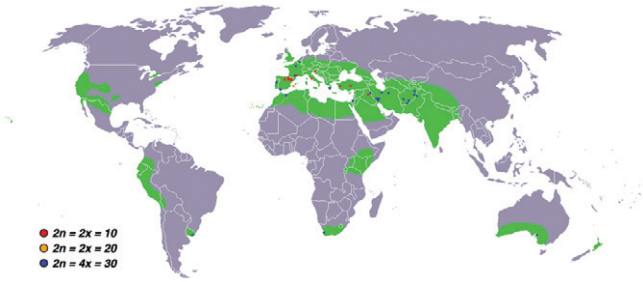

Figure 1. Geographic distribution of Brachypodium. Green shaded areas correspond to the species distribution inferred from herbarium records, field observations, and germplasm collection passport data. The 2n = 4x = 30, 2***n*** = 2x = 10, and 2***n*** = 2x = 20 accessions characterized for DNA content or chromosome number are plotted as blue, red, and orange dots, respectively.

With the available data, some speculation is required to define the native habitat. Outside this area, where the tribe Brachypodieae is not believed to be native, Brachypodium is prevalent and often considered invasive. Three characterized accessions are from nonnative areas (Uruguay, South Africa, and Australia) and are described as having a haploid genome of fifteen chromosomes (Jenkins et al., 2003). Records also denote Brachypodium populations in east Africa and northwest South America at elevations as high as 3600 masl (Cajamarca, Peru). Among other institutions, the Missouri Botanical Garden and the Jepsen Herbarium maintain records of the location of many Brachypodium collections made in North America, specifically California and neighboring areas in the Southwest United States, where it is considered an exotic pest.

As is the case in other model system species (e.g., Anholt and Mackay, 2004; Koornneef et al., 2004), natural genetic variation will be a valuable resource to exploit to define gene function in Brachypodium. The environments in which Brachypodium is most commonly found often experience hot, dry summers and mild, wet winters, such as the climate found around the Mediterranean and Californian chaparral communities. Additionally, other conditions include the steady equatorial climate of the Peruvian highlands, cold and snowy Central Anatolian winters, and mild temperate England. These numerous environmental milieus represent a wide range of biotic and abiotic conditions that may be indicative of the presence of an ample amount of adaptive natural genetic variation within Brachypodium that will facilitate functional genomic analysis.

Molecular Cytogenetic Characterization of Brachypodium

FISHing for Karyotypes

An important part of the development of Brachypodium as a functional genomics bridge to the temperate cereals and grasses is an understanding of its cytogenetics and that of its relatives within the genus. Accurate descriptions of its chromosomes with the aid of fluorescence in situ hybridization (FISH) enable facile identification of its chromosomes in squash preparations of root meristems, and facilitate the integration of genetic and physical maps. Quality karyology is especially important in

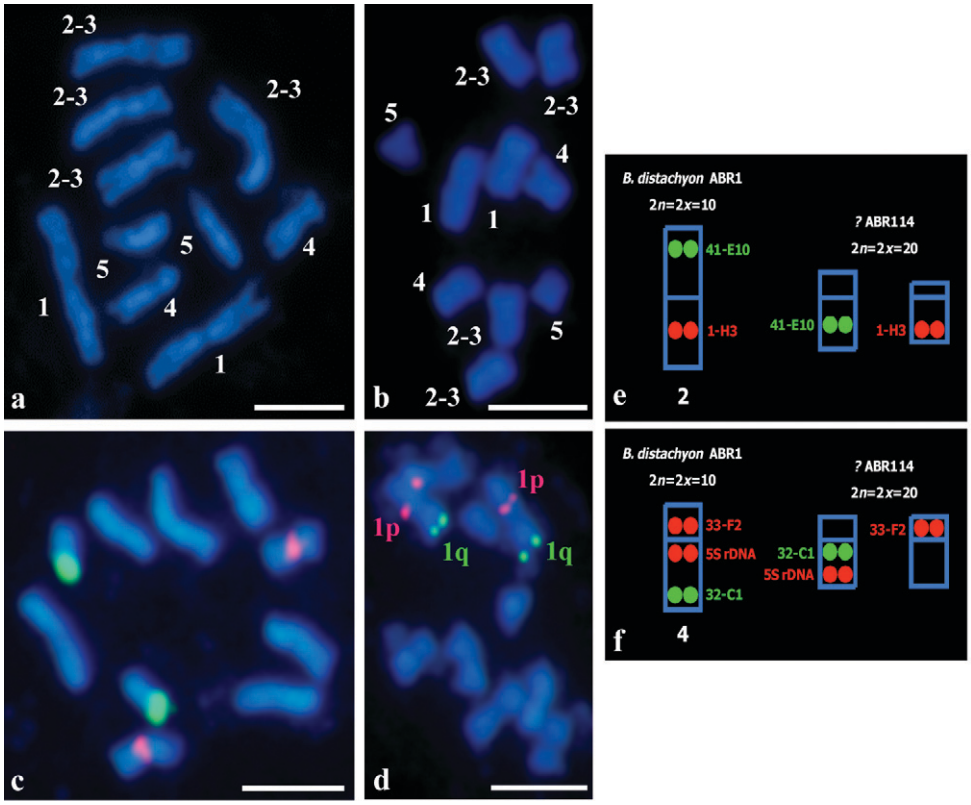

Figure 2. (a-b) Somatic chromosomes of Brachypodium stained fluorescently with DAPI. (a) Early metaphase chromosomes of line ABR1 displaying very good morphology. (b) Highly condensed late metaphase chromosomes of line Bd21 with lower resolution of morphometrical features. White numerals in (a) and (b) indicate chromosome identities. (c) Dual color FISH of 5S rDNA (red fluorescence) and 25S rDNA (green fluorescence) probes to somatic metaphase chromosomes of Bd21. (d) Dual color FISH with BAC clones ABR1–26-H1 (red fluorescence) and ABR1–63-E11 (green fluorescence). Colored annotation indicates localization of BACs to particular chromosome arms. (e-f) Idiograms showing comparative "landing" of selected Brachypodium BAC clones onto chromosomes of ABR1 (2*n* = 2x = 10) and ABR114 (2*n* = 2x = 20). The ideograms indicate that the two lines appear to be related to each other by complex centric fission or fusion. Photographs (b-d) courtesy of Elzbieta Wolny, Department of Plant Anatomy and Cytology, University of Silesia Katowice. Bar, 5 μm.

this genus, given the unusual variation in basic chromosome number (Robertson, 1981) and confusing phylogenetic relationships within this genus.

Much of our understanding of the cytogenetics of Brachypodium has come from scrutiny of two diploid reference lines (ABR1 and ABR5) from the collection managed by Brachyomics in Aberystwyth (Draper et al., 2001; Jenkins et al., 2003; Hasterok et al., 2004; Jenkins et al., 2005; Hasterok et al., 2006). These studies have shown that these two reference lines have indistinguishable karyotypes, comprising five pairs of very small chromosomes, reflecting the inordinately small genome size in this species (Bennett and Leitch, 2005). The karyotypes are distinctly asymmetrical, with the longer chromosomes exceeding twice the length of the shorter ones. At early somatic metaphase (Fig. 2a), the pair of metacentric chromosomes 1 is easy to discriminate, as it is the longest of the complement (>7 μm) and substantially longer than the other pairs. Metacentric chromosomes 2 and 3 are significantly smaller than 1 (5–6 μm) and are difficult to distinguish from one another in routine squash preparations. The two smallest (3.5–4.0 μm) pairs of chromosomes are acrocentric and can be identified unequivocally, due to the presence of a nucleolar organizing region on the short arm of chromosome 5 only. Thus, three out of the five pairs of chromosomes can be identified in prometaphase squash preparations using morphometrical features revealed by phase contrast microscopy or basic fluorescence staining of chromatin. Identification of all of the chromosomes of the complement requires FISH with probes specific for chromosomes 2 and 3.

In addition to ABR1 and ABR5, the "Bd" inbred reference lines are now widely used (Garvin, 2007; Vogel et al., 2006a). Since one of these lines, Bd21, is now the reference genotype for much of the current

genomics resource development, it is imperative that its karyotype be carefully examined and contrasted with information previously obtained for ABR1 and ABR5. We present here the first molecular cytogenetic analysis of Bd21. Not surprisingly, this inbred line has the same karyotype as ABR1 (Fig. 2b). The 5S ribosomal DNA locus is located on a proximal region of the long arm of chromosome 4, and the 25S ribosomal DNA locus occupies a distal part of the short arm of chromosome 5 (Fig. 2c). This is concordant with results reported in Draper et al. (2001). Furthermore, two fluorescently-labeled clones from a BAC library of ABR1 "land" onto the same short arm (p) and long arm (q) chromosomal sites of Bd21 (Fig. 2d; Hasterok et al., 2006). Therefore, we are confident that all of the cytogenetic resources available for the ABR lines will be transferable to the Bd line collection.

FISHing for Synteny

One of the aims of establishing Brachypodium as a new model is to gain access to genetic regions of interest in its less tractable crop relatives by alignment to syntenic regions in Brachypodium. The closer phylogenetic position of the temperate cereals and grasses to Brachypodium relative to rice would imply that the Brachypodium genome will be substantially more colinear and will harbor a more similar repertoire of genes. To assay the synteny between Brachypodium and its temperate relatives, and to test its utility as a "bridge", we screened by PCR BAC libraries of ABR1 and ABR5 using primers based on mapped genomic sequences of several members of the Poaceae (Hasterok et al., 2006). Orthologous sequences were detected in the libraries, showing that there is homology between Brachypodium and these species.

However, to determine if contiguous sequences from rice and ryegrass mapped physically to the same locus, we landed marker-selected BACs onto chromosomes of Brachypodium using FISH. For example, BACs containing sequences orthologous to the QTL *Hd3* and the *Hd3a* gene that control heading date in rice (Monna et al., 2002) were hybridized in situ to somatic metaphase chromosomes. Many of these "landed" on a single locus in an interstitial part of chromosome 1q (Hasterok et al., 2006). Similarly, two BACs corresponding to two close markers from chromosome 6 of *Lolium perenne* and chromosome 2 of rice (Armstead et al., 2004) map to the subterminal part of chromosome 3q in Brachypodium (Hasterok et al., 2006). Clearly, these preliminary results suggest that the genome structure of Brachypodium is sufficiently well conserved compared to other grasses to enable comparative genomic studies. Additional planned studies will continue "landing" other marker-selected, single-locus BACs to achieve dense coverage of the entire genome of Brachypodium. Such a physical map would provide a useful resource for reconstructing the archetypal grass genome, and for determining the patterns of genome evolution in the Poaceae.

FISHing for Phylogeny

The genus *Brachypodium* has relatively few species with a wide distribution. The species have different basic chromosome numbers, and some have a polyploid series as well. These two features together have complicated the taxonomy and have precipitated reclassifications and reappraisals of phylogenetic relationships (Robertson, 1981). On the basis of the identification of 10, 20, and 30 chromosome cytotypes, it was accepted for many years that Brachypodium had evolved an autopolyploid series based on multiples of 10 chromosomes. However, it has been shown recently (Hasterok et al., 2004) by molecular cytogenetics that the 20 chromosome type not only appears to be a different diploid of unknown origin, but also a possible progenitor of the 30 chromosome cytotype. This has necessitated a change in status of the latter from autohexaploid to allotetraploid. Furthermore, comparative BAC "landing" in the 10 and 20 chromosome cytotypes had shown that they may be related by multiple Robertsonian translocation events (Fig. 2e-f). However, the story appears even more complex than this. A BAC clone mapping to the subterminal part of chromosome 4p, and one to the distal part of chromosome 4q of Brachypodium, have a similar distribution in related species such as *Brachypodium sylvaticum* (L.) Beauv. ($2n = 18$), *B. sylvaticum* subsp. *glaucovirens* ($2n = 16$) and *Brachypodium pinnatum* (L.) Beauv. ($2n = 28$), despite differences in chromosome number. However, in the 20 chromosome cytotype the clones map to two subterminal and pericentric regions of two different chromosomes. The implication is that the genome of the 20 chromosome cytotype differs substantially from the others and may be a taxonomic outlier.

Brachypodium Growth and Development

One early concern faced by Brachypodium researchers was the belief that vernalization was needed to help induce flowering (Draper et al., 2001). This was a potential logistical obstacle since it would require extensive cold room space for high-throughput research that was envisioned, and would add weeks to generation times. Initial experiments with the NPGS Brachypodium accessions in 2002 revealed that a majority of the accessions flowered readily in a greenhouse without any vernalization, while others did not. The accessions subsequently determined to

be diploid (Vogel et al., 2006a) uniformly appeared to require vernalization, although pilot studies determined that the vernalization requirement varied considerably among lines (D. Garvin, unpublished data, 2003). Thus, efforts to develop single seed descent inbred lines from the NPGS collection proceeded at different rates, depending on whether or not the lines needed vernalization.

Subsequent research in growth chambers, using a generic setting of 20°C constant temperature and a mix of fluorescent and incandescent lights, led to the discovery that long daylengths induced flowering in the majority of the inbred diploid Bd lines, thus bypassing the need for a vernalization period. Long daylengths successfully induce flowering in Bd2–3, Bd3–1, and Bd21 (Vogel et al., 2006a). While the long day response has not yet been quantified in detail, these three lines will flower readily with 20h daylength, but will not when daylength is 14 h. Flowering can be accelerated with more extreme light regimes; in 24 h conditions inflorescences of Bd21 plants have been observed to begin emerging in as few as 17 d (D. Garvin, unpublished data, 2006). Thus, modulation of daylength is an effective method of accelerating generation time in these three inbred lines (Fig. 3a,b), with Bd21 flowering most rapidly, followed by Bd3–1, and then Bd2–3 (Fig. 3c). In contrast, Bd1–1 and Bd18–1 do not rapidly flower in long daylengths (Fig. 3c), although Bd1–1 will eventually begin to flower sporadically after three or more months in long days. Thus, vernalization of approximately eight weeks at 5°C can be used to induce flowering in these two lines.

By selecting the appropriate daylength regimes, it is possible to obtain plants of a particular size depending on what the end use is. For instance, if one desires a large amount of vegetative biomass from Bd21, this could be obtained by growing plants under 12-hour daylengths so as to suppress the transition to flowering. Alternatively, if one desires to advance a generation as rapidly as possible, long days will result in small plants (Garvin, 2007; Vogel et al., 2006a; Fig. 3d) from which the first seeds will be ready to harvest from Bd21 in as little as two months. Owing to the presence of a relatively significant endosperm, the seeds themselves are approximately 17% of the size of a wheat seed, and 35 to 40% the size of a polished rice grain, with the hull adhering to the seed (Fig. 3e).

Brachypodium Transformation

An efficient transformation system is essential for a model plant system, and has been one of the pillars supporting the tremendous success of the dicot model *Arabidopsis*. Unlike the *Arabidopsis* floral dip method of transformation, methods used for grass transformation are laborious, involve extensive tissue culture, and are often inefficient. Rice has the best developed transformation system of any grass, and can be transformed by both *Agrobacterium tumefaciens* (*Agrobacterium*) and biolistic methods at high efficiencies. A tremendous amount of effort has been directed at optimizing rice transformation and this has resulted in transformation efficiencies increasing from less than 1% in the first reports to greater than 40% today (reviewed in Tyagi and Mohanty, 2000). For the major grass crops most closely related to Brachypodium such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.), transformation remains inefficient. In contrast, research to date indicates that Brachypodium transformation efficiency can approach that of rice.

The stage for Brachypodium transformation was set with the identification of tissue culture conditions for the induction of embryogenic callus and regeneration of fertile plants (Bablak et al., 1995). This was a key development because embryogenic callus is a preferred target for transformation due to its highly regenerable nature. In this study, three diploid accessions (B200, B373, B377) produced several types of callus, including embryogenic callus, when mature seeds were incubated on callus-inducing media. The optimal callus-inducing medium contained LS salts, 3% sucrose, and 2.5 mg l^{-1} 2,4-D. Efficient regeneration of fertile plants was observed on several different media. These conditions are similar to those used for other grasses, indicating that Brachypodium is not unusual in this regard.

Both particle bombardment and *Agrobacterium* have been used to transform several grass species, and each offers unique advantages and disadvantages. Particle bombardment has the advantages of not being dependent on the biological limitations of *Agrobacterium*, and being less dependent on the plant genotype used. The main requirement for transformation by particle bombardment is that plants can be regenerated from the bombarded explant. Since conditions for inducing embryogenic callus were already established, it is no surprise that the first report of Brachypodium transformation used particle bombardment of embryogenic callus (Draper et al., 2001). In this study the authors transformed a polyploid accession (ABR100) via particle bombardment, with an average efficiency of five transformations per gram of starting embryogenic callus.

This demonstrated that Brachypodium could be transformed; however, it begged the question of whether a diploid Brachypodium line could be transformed. The answer came when a more detailed account of biolistic transformation was published

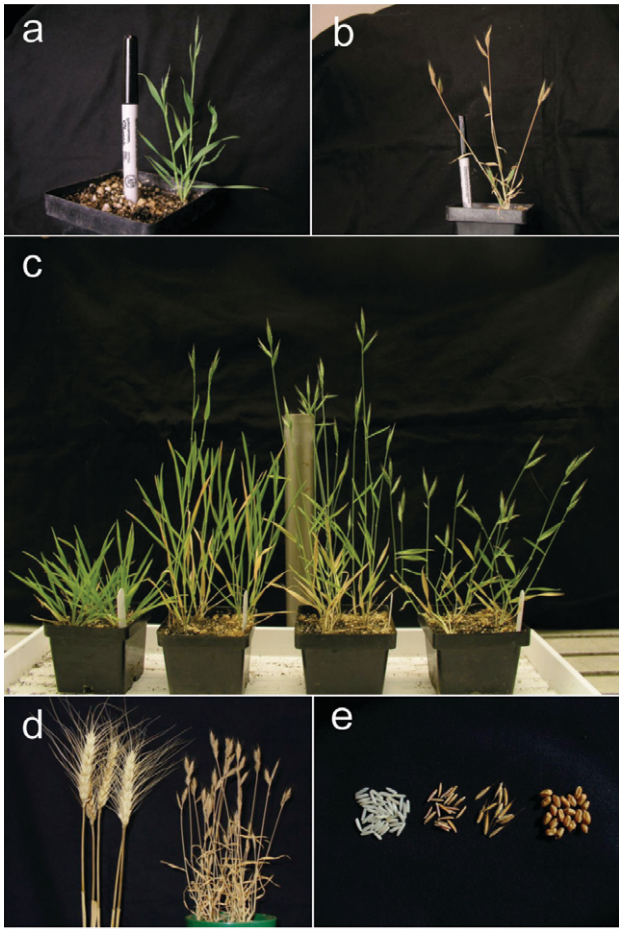

Figure 3. Descriptive images of Brachypodium. (a-b) Bd21 grown under 24 h light. (a) Three week-old plant. (b) Same plant at 6 wk, with seeds maturing and plant senescing. (c) Differences in developmental rate among four inbred diploid Brachypodium lines grown in 20 h days. Left to right: Bd1–1, Bd2–3, Bd3–1, and Bd21. Each pot has three plants. (d) Comparison of plant size between wheat spikes and a pot of six mature Bd21 plants. (e) Comparison of seed size between rice (left), wheat (right) and intact and dehulled Bd21 seeds (center).

(Christiansen et al., 2005). In this paper the authors generated embryogenic callus from 10 accessions (two diploid and eight polyploid) and attempted to transform four accessions (two diploid and two polyploid). One diploid accession (BDR018) was successfully transformed with an average efficiency of 5.3% of bombarded calli producing transgenic plants. The other diploid accession (BDR001) proved extremely recalcitrant to transformation. The two polyploid accessions tested (BDR017 and BDR030) were transformed at frequencies of 3.8 and 4.1%, respectively. These results compare favorably with the 3.8% efficiency first reported for biolistic rice transformation (Christou et al.,1991). Thus, Brachypodium is similar to other species in that genotype has a profound effect on transformation efficiency and underscores the need to examine multiple genotypes when developing transformation systems.

While the relative simplicity of biolistic transformation made it a logical choice for initial attempts to transform Brachypodium, the method has disadvantages. Biolistic transformation requires specialized equipment that may not be accessible to researchers in smaller institutions. A more serious disadvantage is the complexity of the transgene loci which typically contain many copies of the inserted DNA, including truncated pieces, interspersed with genomic DNA that may span megabases of the host genome (Svitashev and Somers, 2002; Kohli et al., 2003). These complex insertions can interfere with downstream applications that require simple insertions, and may lead to silencing of transgenes in later generations. Attempts to minimize the complexity of biolistic loci by using linear DNA instead of circular plasmid DNA have produced mixed results. In one report transformation of rice with linear DNA resulted in simpler insertions than plants transformed with circular DNA (Fu et al., 2000) while another study reported that linear and plasmid DNA had similarly complex insertion patterns (Loc et al., 2002).

In contrast to biolistic transformation, *Agrobacterium*-mediated transformation typically results in simple insertion patterns (for a direct comparison of methods see Dai et al., 2001; Travella et al., 2005). *Agrobacterium*-mediated transformation of both rice and *Arabidopsis* produces an average of approximately 1.5 transgenes per line (Feldmann, 1991; Jeon et al., 2000). Thus, many researchers favor *Agrobacterium*-mediated transformation. However, the development of an efficient *Agrobacterium*-mediated transformation system for a species can be difficult and time consuming. It is fortunate then that Brachypodium has proven to be amenable to *Agrobacterium*-mediated transformation. The first report of *Agrobacterium*-mediated transformation of Brachypodium was published in 2006 (Vogel et al., 2006a). In this study, 19 accessions and inbred lines (16 polyploid and three diploid) were evaluated for transformability. The highest transformation efficiency was obtained in the polyploid inbred line Bd17–2, for which 14% of the callus pieces co-cultivated with *Agrobacterium* produced transgenic plants. The diploid NPGS accession PI 254867 was also transformed. However, the transformation frequency was much lower (2.5%). Since only three diploid accessions/inbred lines were tested in this experiment, it is likely that diploid lines with higher transformation efficiency will be identified as more are tested. In this context, it is noteworthy that only one of the 16 polyploid

accessions tested had an average transformation efficiency exceeding 10%. In fact, most had transformation efficiencies less than 1%. Southern blot analysis and segregation of the transgene in the T1 generation indicated that most transgenic plants contained simple low-copy insertions typical of *Agrobacterium*-mediated transformation.

Since the initial efficiency of transforming diploids was not adequate for high throughput applications, research toward improving the transformation method has continued. By altering the conditions of the co-cultivation step, the efficiency of transformation of the diploid inbred line Bd21–3 that was derived from the same accession as Bd21 was increased 10-fold to approximately 30% (Vogel and Hill, in press). Similarly, by altering various conditions, transformation frequencies of Bd21 itself have exceeded 30% (Vain et al., in press). These transformation efficiencies are approximately the same as rice, and make experiments such as T-DNA tagging feasible. Indeed, the first T-DNA flanking sequence tag from Brachypodium has already been deposited in Genbank (accession number EF165098).

The utility of Brachypodium transformation for functional genomics of temperate grasses was clearly demonstrated in a recent study of flowering genes. In this report (Olsen et al., 2006) the authors used biolistic transformation to introduce two orthologs of Terminal Flower 1, LpTFL1 from perennial ryegrass (*Lolium perenne* L.) and TFL1 from *Arabidopsis* into Brachypodium. By studying the resulting transgenic plants the authors were able to conclude that both orthologs acted as floral repressors in a temperate grass, a conclusion that could not have been reached using *Arabidopsis* or rice.

Brachypodium transformation is still in its infancy and continued improvements are expected. The rapid progress in Brachypodium transformation made by a few groups with limited resources underscores the promise of Brachypodium for high-throughput transformation. Indeed, early reports of Brachypodium transformation efficiency compare favorably to the initial reports of rice transformation, which was assisted by a large grant program aimed at developing rice transformation and biotechnology. Thus, the requirement for an efficient transformation system has been satisfied for Brachypodium as a model system.

Brachypodium BAC Library Resources

Large insert DNA libraries have many biological applications. These include isolation of genes or gene clusters from regions of interest (Kuang et al., 2005), whole-genome physical mapping (Chen et al., 2002), genome structure analysis (SanMiguel et al., 2002; Wicker et al., 2005), and comparing the genome organization of related genomes (Gu et al., 2004; Chantret et al., 2005; Isidore et al., 2005). Bacterial artificial chromosome (BAC) libraries have emerged as the preferred method for constructing large-insert clone libraries. Relative to alternative large insert libraries, BACs are easier to manipulate, have fewer chimeric clones, and can be stably propagated in *E. coli* (Shizuya et al., 1992; Woo et al., 1994).

BAC library resources have been developed both for different *Brachypodium* species and for different inbred lines of Brachypodium. The first genus *Brachypodium* BAC library was constructed for *B. sylvaticum*, a perennial member of the genus with an estimated genome size of 470 Mb (Foote et al., 2004). This library contains 320,228 clones with an average insert size of 102 kb, representing 6.6X genome equivalents. To compare genome organization between this species and rice and wheat, the authors screened BAC library filters with a set of single/low-copy DNA probes, some of which were derived from rice, barley, or wheat sequences (Foote et al., 2004). Seven BAC contig blocks were identified, and within these blocks 22 of the 25 (88%) markers anchored to *B. sylvaticum* BACs were colinear with rice and/or Triticeae markers, indicating the maintenance of colinearity between genus *Brachypodium* genomes and major cereal crops (Foote et al., 2004). The isolation of the major chromosome pairing locus *Ph1* in polyploid wheat demonstrated the usefulness of the *B. sylvaticum* BAC clones for comparative genome analysis and marker development in targeted wheat regions (Griffiths et al., 2006).

The first Brachypodium BAC library was constructed from two diploid genotypes (ABR1 and ABR5) (Hasterok et al., 2006). The library consists of 9100 clones with an average insert size of 88 kb, representing 2.22X genome equivalents. PCR primers designed from 15 markers targeted to a contiguous region of approximately 884 kb on rice chromosome 6 were used to screen this BAC library; all but two markers were physically associated with at least one other marker, indicating a significant degree of colinearity between the targeted rice genome region and the Brachypodium BACs in the syntenic region of Brachypodium. In addition, fluorescent in situ hybridization of the marker-selected BACs to Brachypodium chromosomes revealed that contiguous BACs co-localized on a single chromosome, further confirming the conservation of genome synteny at the chromosomal level (Hasterok et al., 2006).

In an effort to develop physical maps of the Brachypodium genome, two other BAC libraries have been developed from the inbred diploid Brachypodium line Bd21 (Huo et al., 2006). The

libraries were constructed with two different restriction enzymes (*Hin*dIII and *Bam*HI) to avoid bias and cloning artifacts associated with BAC libraries constructed with just one restriction enzyme (Zhang and Wu, 2001). A total of 36,842 clones were picked for each BAC library. The average insert sizes for the *Hin*dIII and *Bam*HI libraries are 105 kb and 100 kb, respectively, with insert sizes ranging from 30 kb to 200 kb. Based on a genome size of 355 Mb (Bennett and Leitch 2005), individually the two libraries theoretically represent 9.9 and 9.4 equivalents of the Brachypodium genome, respectively. Screening the BAC libraries with starch biosynthesis genes verified the genome equivalents represented by each library. These BAC libraries and high-density colony filters are available to the public (to order, visit the website http://wheat.pw.usda.gov/wgc/resources.html; verified 26 Nov. 2007). An additional BAC library for Brachypodium inbred diploid line Bd3–1 was also recently constructed; this library has an average insert size of 130 kb, and is estimated to represent 10X genome equivalents. This library is also available (see the website http://www.genome.arizona.edu; verified 26 Nov. 2007).

To obtain a broader view of the sequence composition of the Brachypodium genome, a pilot BAC end sequencing of the Bd21 BAC library clones was performed (Huo et al., 2006). A total of 2185 high-quality BAC end sequences (BES) were generated, with an average length of 563 bases per BES. These BES represent a random sample of genomic sequences from Brachypodium. The G+C content of the BES is 45.2%, which is very similar to those obtained previously for other monocot genomes (SanMiguel et al., 2002; Yu et al., 2002). Sequence alignments revealed that 40% of BES had matches in the dbEST database with *E* values less than 10^{-10}. Even at an *E* value of less than 10^{-50}, 16.5% of BES still found a match. This suggests that a considerable portion of the Brachypodium genome is similar to known transcribed sequences and possesses a much lower content of repetitive DNA than in other cereal genomes (Mao et al., 2000; Peterson et al., 2002; Akhunov et al., 2005; Haberer et al., 2005).

When BES were compared to ESTs from cereal crops, 23% have a significant match to wheat ESTs at *E* values < 10^{-10}, compared to 14.8% and 18% to maize and rice, respectively. Moreover, BES could readily be anchored to both the rice genome and wheat EST-deletion bin maps (Qi et al., 2004). Thus these BES are not only useful for developing a BAC-based physical map for the Brachypodium genome, but also facilitate a direct comparison of genome structure and organization between Brachypodium and other cereal crops (Huo et al., 2006).

One of the important applications of BAC libraries in genomics research is to develop BAC-based physical maps for the entire genome of targeted species. In this approach, random BAC clones are digested with one or more restriction enzymes and the profiles of the resulting restriction fragments act as fingerprints of the BAC clones. Based on shared fingerprint patterns, clones derived from the same regions of a genome can be assembled into contigs (Soderlund et al., 2000). High-throughput fingerprinting of BAC clones and automated sizing of DNA fragments by capillary electrophoresis were developed to facilitate physical mapping of large and complex genomes (Luo et al., 2003). Integration of a whole-genome BAC-based physical map with linkage maps permits ready identification of specific physical genome regions to explore for genes of interest in a given region. BAC-based physical maps can be readily generated by fingerprinting deep-coverage BAC libraries to permit assembly of BAC contigs spanning large chromosome regions (Zhang and Wu, 2001). The resulting integrated physical and genetic map, together with associated BES information, will also be invaluable for aligning and ordering sequence contigs obtained from the DOE-JGI Brachypodium whole-genome shotgun sequencing project, and for sequence completion by filling identified gaps (Vollrath and Jaramillo-Babb, 1999).

EST Resources and Transcriptome Sequencing

A powerful tool to gain insight into an undescribed genome is to partially sequence random cDNA clones to generate a collection of expressed sequence tags (ESTs) (Adams et al., 1991). ESTs serve many purposes such as being employed for microarray design, for analysis of gene expression, for generation of molecular markers, for physical mapping, and for annotation of genes in genomic sequence. Since ESTs provide a wealth of sequence information relatively quickly and inexpensively, they have become a standard genomic resource for many plants. As of September 2006, six grasses: wheat, maize (*Zea mays* L.), rice, barley, sorghum, and sugarcane (*Saccharum officinarum* L.) have greater than 200,000 ESTs. Three other grasses: tall fescue (*Lolium arundinacea* Schreb.), Brachypodium, and switchgrass (*Panicum virgatum* L.) have greater than 10,000 ESTs each deposited in GenBank.

In 2005, 20,440 Brachypodium ESTs representing approximately 6000 genes were deposited in GenBank bringing the total number of publicly available Brachypodium ESTs to 20,449 (Vogel et al., 2006b). This project provided the first significant

genomic resource for this model species. The ESTs were derived from five cDNA libraries constructed from RNA of different tissues (stem plus leaf sheath, leaf, callus, developing seed heads, roots) of the inbred diploid line Bd21. Using ESTs for 20 genes, the authors constructed a phylogenic tree that supported the close relationship of Brachypodium to wheat and barley that has been previously reported based on much smaller datasets (Kellogg, 2001). This EST collection was shown to contain homologs for all the genes involved in the biosynthesis of lignin monomers, which is of particular interest to researchers using Brachypodium as a model for energy crops. These EST sequences have also been used to identify microsatellites and to generate SSLP markers being used to construct the first-generation genetic map of Brachypodium discussed earlier in this paper.

While these EST sequences have provided a first step toward defining the Brachypodium transcriptome, Brachypodium lacks the abundant expressed-transcript data necessary to develop it fully as a research model for grasses. Thus, deeper sequencing of the Brachypodium transcriptome is extremely important and will complement the rich genomic information that will emerge from the DOE-JGI Brachypodium whole-genome shotgun sequencing project. In addition to sequencing the Brachypodium genome, the DOE-JGI will generate at least 180,000 EST sequences from normalized Bd21 cDNA libraries for the complementary Brachypodium EST sequencing project. These ESTs will be derived from independent normalized cDNA libraries enriched for full-length transcripts. The libraries will represent a diverse collection of tissues, developmental stages, diurnal sampling, and various treatments selected to maximize transcript diversity.

This high-throughput EST sequencing effort will achieve significant transcriptome coverage but not all genes will be sampled. Nonetheless, the sequence data generated will have numerous uses. For instance, the EST sequences will provide experimental evidence for many gene models in the Brachypodium genome and thus will greatly improve the initial Brachypodium genome annotation efforts. Moreover, the resulting EST sequences will be useful for optimizing *ab initio* gene-finding algorithms and will thus lead to improved annotation methods. The Brachypodium EST sequences will also enable diverse functional genomic studies and tool development. This includes overexpression of candidate genes in transgenic plants, directed knock-down or targeted mutation of candidate genes by TILLING (McCallum et al., 2000), delete-a-gene (Li and Zhang, 2002), RNAi or artificial microRNAs (amiRNAs) (Schwab et al., 2006), development of a full-length cDNA collection for recombination-based manipulation of ORFs into epitope tags, yeast two-hybrid baits, and overexpression constructs for systematic studies of gene function, and development of microarrays that will find use in a diverse range of studies (Mockler et al., 2005).

Bioinformatics Resource Development

With Brachypodium genome sequence acquisition poised to grow at an extremely rapid rate, bioinformatics becomes centrally important for pursuing biology associated with this sequence information. Annotation of eukaryotic genomes such as that of Brachypodium is a challenging problem that involves integrating multiple lines of evidence to infer gene models and to make functional predictions. While computational annotations are adequate for many applications such as comparing general characteristics of several genomes, in the development of a model system such as Brachypodium a scientifically rigorous investigation of the genome should rely on manual curation that provides a "consensus" annotation of gene models and functional classifications.

A curated annotation is the long-term goal of the Brachypodium community; however, a short-term goal will be to establish a public genome database featuring automated annotations. Without an easily accessible annotation, it will be very difficult for biologists to exploit the information content of the Brachypodium genome sequence. The DOE-JGI large-scale Brachypodium EST sequencing project will provide experimental evidence to improve many gene structure models and annotations. But, because there will be relatively little empirical transcript evidence, bioinformatics approaches will be required to define gene structures and annotate Brachypodium genes. Thus, *ab initio* gene-finding algorithms (Lukashin and Borodovsky, 1998; Salamov and Solovyev, 2000; Korf et al., 2001; Majoros et al., 2004) will be used to predict gene models. Sequence database searches will identify homologous sequences, and predicted proteins will be compared against domain/profile databases (Zdobnov and Apweiler, 2001) to provide information about predicted protein structure and compute gene ontologies. Transcript evidence and homology estimates will enable researchers to evaluate gene predictions, and the evidence (EST, cDNA, or homology) that supports a predicted gene model will provide confidence in the computational annotations.

To facilitate exploitation of the forthcoming Brachypodium genomic sequence from both the

DOE-JGI whole genome and EST sequencing projects, a new database is needed to provide public access to sequence data from the Brachypodium genome and EST sequencing projects. Such a database, BrachyBase (http://www.brachybase.org; verified 26 Nov. 2007) is being developed at Oregon State University. BrachyBase is based on the GMOD genome browser (gbrowse; Stein et al., 2002), and the current prototype version uses the TIGR japonica rice genome assemblies (International Rice Genome Sequencing Project, 2005) as a surrogate genome scaffold for Brachypodium sequence alignments (Fig. 4). BrachyBase will incorporate all available Brachypodium genome and transcriptome data from the DOE-JGI sequencing projects and other public data sources to make these sequence resources widely available to the research community through a user-friendly web-based interface. The design of BrachyBase anticipates the adoption of high-throughput genomics technologies such as emerging high-throughput short-read sequencing technologies.

Brachypodium: A Model for Plant-Pathogen Interactions

To be a useful model for plant–pathogen interactions, Brachypodium must serve as a host for a range of commercially relevant cereal and grass pathogens. Considerable efforts have already been made to screen the responses of Brachypodium accession to pathogens. The most well characterized interaction involves the rice blast pathogen, *Magnaporthe grisea* (Hebert) Barr. This is a devastating cereal disease with a wide host range that includes most temperate cereals and forage grasses as well as rice (Valent and Chumley, 1991; Talbot, 2003). Screening different accessions of Brachypodium with *M. grisea* has revealed a range of responses from a hypersensitive response (HR)-mediated resistance to full susceptibility. In one genotype, ABR5, segregation analysis suggested the resistance to *M. grisea* strain Guy-11 was dependent on a single R gene. Crucially, the cytology of both susceptible and resistant interactions in Brachypodium appeared to be very similar to those reported in rice, suggesting that observations made in either species could be directly comparable (Routledge et al., 2004).

> These preliminary pathogen screens demonstrate that Brachypodium offers a wealth of targets for further analysis: multiple pathogenic interactions, QR, nonhost resistance, and SAR.

Brachypodium accessions have also been extensively screened with strains of stripe rust (*Puccinia striiformis* Westend.), and wheat leaf rust (*Puccinia triticina* Eriks.), and barley leaf rust (*Puccinia hordei* G. Otth.), as well as crown rust (*Puccinia coronata* Corda) (Draper et al., 2001) adapted to the genus *Lolium*. Only in one instance—the interaction of *P. coronata* strain 7–02 with ABR100- were symptoms of full susceptibly observed. In all other cases, a range of phenotypes were noted, ranging from immunity (rarely), to highly localized HR-type cell death, and to the formation of disease-associated pustules (uredinia) in necrotic regions (Draper et al., 2001). This range of responses was suggestive of qualitative resistance (QR) with the possibility of some single gene resistance within HR interactions. Since surprisingly little is known of the interaction of rusts with cereals, the development of a rust–Brachypodium pathosystem could represent a major opportunity to develop our understanding of this complex disease. In contrast, the interactions of powdery mildew fungi (*Blumeria graminis* DC) and cereals have been exceptionally well investigated (Huckelhoven, 2005). Screening the ABR Brachypodium collection with barley and oat strains of *B. graminis* failed to identify any interaction leading to disease symptoms, suggesting the presence of nonhost resistance. Such resistance is in itself an important target for further study and should provide genes that could be exploited in agriculture (Ellis, 2006).

Wheat head blight caused by *Fusarium graminearum* Schwabe is an increasing problem, with losses associated not only with disease but crop contamination with mycotoxins (Parry et al., 1995; McMullen et al., 1997). Trials of *F. graminearum* strains on Brachypodium have been limited, but on each occasion disease symptoms were observed. Investigations of any future model *F. graminearum*–Brachypodium interaction would undoubtedly be eased by the relatively large seed heads that are a feature of Brachypodium (Draper et al., 2001). Equally, a model interaction with the important necrotrophic pathogen *Stagnospora nodorum* [(Berk.) Castellani & Germano] should be developed. Both of the two tested accessions exhibited the formation of necrotic flecks but virulence was considerably reduced compared to wheat controls. However, these results are likely not to reflect universal responses of Brachypodium to these two pathogens, because it is likely that wider screens of both plant and pathogen germplasm would identify interactions leading to more pronounced disease symptoms.

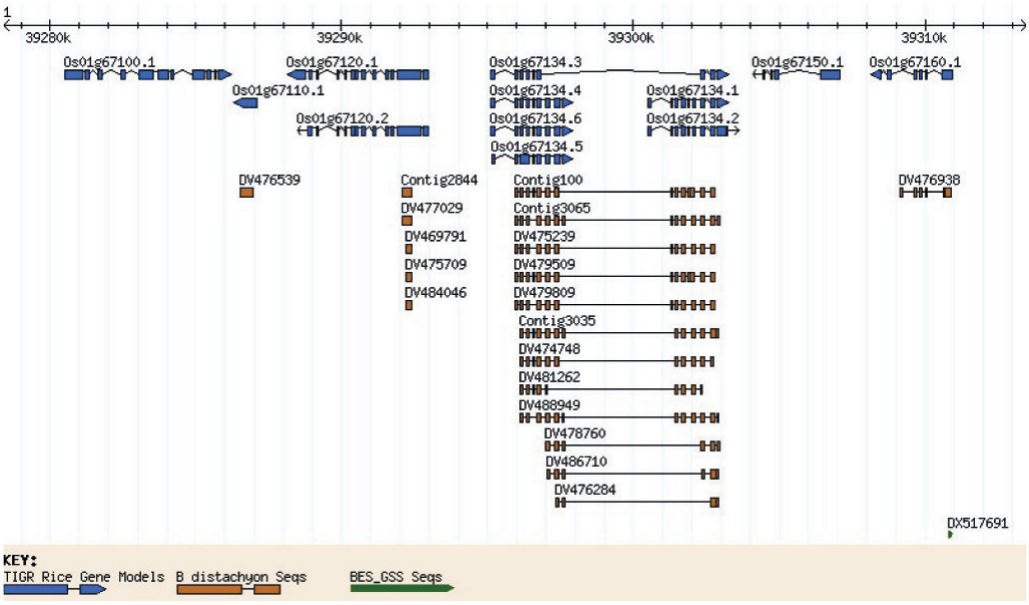

Figure 4. BrachyBase Genome Viewer. A screenshot from the BrachyBase genome viewer showing Brachypodium ESTs and EST assemblies mapped onto rice chromosome 1 and in comparison to rice gene models.

The response of Brachypodium to the dicot bacterial pathogen *Pseudomonas syringae* pv. *phaseolicola* elicited a HR that proved to be a reasonably efficient inducer of systemic acquired resistance (SAR). This has been extensively characterized in model dicotyledonous species but appears to be qualitatively different in cereals (Gorlach et al., 1996; Molina et al., 1999). Accordingly, SAR in Brachypodium did not appear to be associated with the synthesis of salicylic acid or the expression of genes encoding pathogenesis-related protein (L. Mur, unpublished data, 2007), both markers for SAR in dicot species (Ryals et al., 1996). Interestingly, this was in contrast with the wound-induced systemic expression of a proteinase inhibitor, which, as in dicots, appeared to be regulated by jasmonic acid (Mur et al., 2004). Given the potential of SAR as a source of field resistance, Brachypodium could serve as a model to characterize this phenomenon in cereals.

These preliminary pathogen screens demonstrate that Brachypodium offers a wealth of targets for further analysis: multiple pathogenic interactions, QR, nonhost resistance, and SAR. The next challenge is to exploit these opportunities. In many cereal crop genomes, the amount of repetitive DNA and polyploidy pose a particular problem for genomic analysis. As pointed out elsewhere, the Brachypodium genome could act as a genomic bridge to cereal species and thereby considerably ease the task of mapping orthologous genes, including *R* genes and QTLs associated with QR. This strategy requires the establishment of the syntenic relationships between Brachypodium and other species; this is being implemented (Hasterok et al., 2006). This implementation will be accelerated further as the community molecular mapping project and ultimately the DOE-JGI genome sequencing project are completed.

Pathologists seeking to isolate cereal defense genes orthologous to those characterized in *Arabidopsis* are likely to choose to focus on rice, given its already completed sequence. However, Brachypodium may well emerge as the model of choice in mutagenesis programs and functional genomic studies. The advantages of Brachypodium for mutagenesis have been exhaustively considered (Draper et al., 2001; Jenkins et al., 2005). Further, a protocol for high throughput *Agrobacterium*-mediated transformation of Brachypodium (Vogel et al., 2006a) makes the development of T-DNA or transposon-tagged populations possible. The potential value of such resources to pathologists is illustrated by the use of *Arabidopsis* T-DNA populations to isolate many genes involved in pathogen resistance (eg., Torres et al., 2002; Maldonado et al., 2002; Dellagi et al., 2005; Zeidler et al., 2004; Liu et al., 2005; Ramonell et al., 2005).

The value of Brachypodium functional genomic analyses is already being realized. The Mur lab in Wales has adopted a targeted approach to construct a subtractive library representing approximately 4000 genes that are differential expressed during disease development in genotype ABR1 following challenge with *M. grisea*. Using these sequences, cDNA microarrays have been constructed and screened. While analyses are ongoing, genes involved in the

indole biosynthesis and amino acid metabolism have been shown to be up-regulated (L. Mur, unpublished data, 2007) as found in defense reactions in *Arabidopsis* (Truman et al., 2006). Analyses using metabolomic approaches are more advanced and have also focused on the interaction of *M. grisea* with ABR1 (susceptible) and ABR5 (resistant). Brachypodium proved to be a highly suitable subject for metabolomic studies, where reproducible metabolite profiles indicated that differential phospholipid processing was a feature of the different interactions (Allwood et al., 2006). This accorded with an earlier study which indicated the phospholipid derivative, jasmonic acid, was a feature of the response of Brachypodium to *M. grisea* (Mur et al., 2004). Such studies may be very much in their infancy but they demonstrate that Brachypodium is a highly suitable model for functional genomic studies into plant-pathogen interactions.

Most of the Brachypodium resources described in this paper have been or are being developed without significant external grant support. An early willingness by many scientists to commit to such resource development without large dedicated grant support reflects a desire both to have a model system more amenable for cool season grasses than is rice, and to have a model grass system with the attributes to permit high throughput functional genomics research similar to what is possible with *Arabidopsis*. As detailed here, in just a few years a large ensemble of genetic and genomic resources has been developed for Brachypodium, and during the next few years we can expect to see virtually all of the resources desired in a model plant system ushered in for Brachypodium.

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