Brachypodium as a Model for the Grasses: Today and the Future[1][W]

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THE NEED FOR A NEW MODEL GRASS

Model systems not only allow scientists to investigate complex processes that are difficult to study in nonmodel organisms but also serve to focus community efforts and resources, significantly advancing research. Arabidopsis (*Arabidopsis thaliana*) has served as a plant model system for almost 30 years and is widely considered the preeminent model plant. The success of Arabidopsis-related research has been driven not only by key features common to any model organism but also by the collaborative environment built by the Arabidopsis community. A decade after the Arabidopsis genome sequence was published, the development of model plants follows a different trajectory. In the past, the development of extensive resources and a large user community happened first and then sequencing the genome followed. Today, however, an organism is selected as a potential model and genome sequencing occurs prior to or concurrent with the development of experimental tools and a user community. Arabidopsis research has provided many scientific breakthroughs (Flavell, 2009). However, its utility as a model is limited to a certain extent when investigating monocot-specific processes.

Within the monocots, grasses provide the vast majority of human calories and are increasingly utilized as a sustainable source of energy. Traits including cell wall composition, plant architecture, grain properties,

intercalary meristems, and root architecture are best studied using grass model systems (Vogel, 2008; Watt et al., 2009). Rice (*Oryza sativa*) and maize (*Zea mays*) have numerous advantages as grass models, including sequenced genomes, large research communities, and substantial genetic resources and (http://www.gramene. org/ and http://www.maizegdb.org/). The major challenges associated with these species include the large size of the plants, long generation times, demanding growth requirements, and restricted access to germplasm due to quarantine restrictions and intellectual property concerns (Jung et al., 2008).

Brachypodium (*Brachypodium distachyon*) was first proposed as a model system in 2001 (Draper et al., 2001). A comparison with other plants (Table I) reveals that Brachypodium's attributes are well suited to a model system. Like Arabidopsis, Brachypodium has a small stature, short generation time, small genome, the ability to self-pollinate, and is easily grown under simple conditions (Draper et al., 2001). In addition, the phylogentic position of Brachypodium makes it a convenient model for grasses with significant genome expansions (e.g. wheat [*Triticum aestivum*], rye [*Secale cereale*], and cool season pasture grasses [http://www. umsl.edu/services/kellogg/gpwg/default.htm]). Re-

moving a significant limitation to genetic analysis, the Vogel and Garvin laboratories have recently optimized methods to efficiently cross Brachypodium (approximately 80% efficiency; for links to the methods, see Table II). The benefits of conducting experiments rapidly in a small space are apparent when Brachypodium is compared with biomass crops like switchgrass (*Panicum virgatum*) and *Miscanthus sinensis* (Table I). Thus, significant investments have been made in developing and using Brachypodium as a model for these emerging biofuel crops. In this context, it is noteworthy that Brachypodium is a "typical" grass at the genome level, as reflected by the overall similarity in gene content and gene families when compared with the rice and sorghum (*Sorghum bicolor*) genomes (International Brachypodium Initiative, 2010). Therefore, for the vast majority of traits (e.g. cell wall composition, yield, stress tolerance, cell wall biosynthesis, root growth, development, and plant-pathogen interactions), Brachypodium can serve as a useful functional model for the grasses, and initial studies on cell walls, grain development, and root growth support Brachypodium's utility as a model system (Watt et al., 2009; Larré et al., 2010; Guillon et al., 2011; Opanowicz et al., 2011; Wang et al., 2011).

Table I. *Comparison of select models and crops*

Parameter	Arabidopsis	Barley	Brachypodium	Foxtail Millet	M. sinensis	Maize	Rice	S. viridis	Sorghum	Switchgrass	Wheat
Height (cm)	15–20	50–120	15–20	120–200	150–300	120–300	100	10–250	50–250	200–300	50–100
Density[a] (plants m^{-2})	2,000	80–120	1,000	50	3–4	4	36	1,000	50	6	50
Growth requirements[b] (controlled conditions)	Simple	Intermediate	Simple	Intermediate	Demanding	Demanding	Demanding	Simple	Demanding	Demanding	Intermediate
Efficiently crossed?	Yes	Yes	Yes	No	Yes	Yes	Yes	No	Yes	Yes	Yes
Reproduction	Selfing	Selfing	Selfing	Selfing	Outcrossing	Outcrossing/ self-compatible	Selfing	Selfing	Selfing	Outcrossing	Selfing
Typical generation time (weeks)	8–12	10–20	8–12	11–13	12	8–15	12–24	6–8	13–18	26	10–20
Seeds per plant	>1,000	150–200	100–1,000	>10,000	>1,000	200–1,000	>1,000	>5,000	>1,000	>1,000	50–150
Transformation	Extremely easy	Efficient, but labor intensive	Highly efficient	Reported, but not efficient	Inefficient	Efficient, but labor intensive	Highly efficient	Efficient[c]	Inefficient	Efficient, but slow	Inefficient
Genome size (Mb)	119[d]	5,500	272[d]	515	5,000	2,300[d,f]	382[d]	515	700[d,e]	2,400	16,000
Assembled genome sequence	Finished genome sequence	Draft genome sequencing in progress	High-quality draft (finishing under way)	Draft genome	No	Draft genome[f]	Finished genome sequence	Draft genome sequencing in progress	Draft genome[d]	Sequencing in progress	Sequencing in progress
T-DNA resources	Extensive	None	10,000 lines available, 40,000 more planned	None	None	Transposon mutants are available	Extensive[g]	None	None	None	None
Cell wall type	Type 1	Type 2	Type 2	Type 2	Type 2	Type 2	Type 2	Type 2	Type 2	Type 2	Type 2
Photosynthesis	C_3	C_3	C_3	C_4	C_4	C_4	C_3	C_4	C_4	C_4	C_3

[a]High-density planting under laboratory conditions. [b]The difficulty of growing plants is dependent upon their size and the range of environmental conditions tolerated. Thus, small plants that tolerate varied conditions have simple growth requirements and large plants that need carefully controlled environmental conditions have demanding growth requirements. [c]Unpublished data (T. Brutnell). [d]Assembled genome size. [e]Approximately 20% of the genome was not assembled because of the repetitive nature. [f]Assembly consists of sequenced BACs many of which contain unordered genes because of the difficulty associated with assembling repetitive DNA. [g]While rice has extensive insertional mutant resources, the availability of the resources is constrained by quarantine restrictions and intellectual property concerns.

Table II. *Internet resources for Brachypodium research*

Resource	Institution	URL	Description
Arizona Genomics Institute	Arizona State University	http://www.genome.arizona.edu	BAC libraries
BrachyBase	Oregon State University	http://www.brachybase.org/	Genome sequence
BrachyBio	Boyce Thompson Institute for Plant Research	http://bti.cornell.edu/brachybio	TILLING population, resources for teachers
Brachypodium distachyon Information Resource	Oregon State University	http://www.brachypodium.org/	Central location for information
Brachypodium genome information	Munich Information Center for Protein Sequences	http://mips.helmholtz-muenchen.de/plant/brachypodium/	Genome sequence
Brachypodium resources	USDA-ARS, Western Regional Research Center	http://brachypodium.pw.usda.gov/	T-DNA lines, methods (crossing, mutagenesis, transformation), germplasm
BrachyTAG	John Innes Centre	http://www.brachytag.org/	T-DNA lines, protocols, transformation vectors, and service
CoGe	University of California, Berkeley	http://synteny.cnr.berkeley.edu/CoGe/	Comparative genomic tools
ELEMENT	Oregon State University	http://element.cgrb.oregonstate.edu/	Promoter searching tool
Garvin laboratory	USDA-ARS Plant Science Research Unit	http://www.ars.usda.gov/pandp/docs.htm?docid=18531	Germplasm, crossing method
GrainGenes	USDA-ARS, Western Regional Research Center	http://wheat.pw.usda.gov	Comparative genomics tools
Gramene	Cold Spring Harbor Laboratory	http://www.gramene.org	Comparative genomics tools
Iowa State University Plant Transformation Facility	Iowa State University	http://www.agron.iastate.edu/ptf/index.aspx	Transformation service
ModelCrop	John Innes Centre	http://www.modelcrop.org/	Genome sequence
NASC's International Affymetrix Service	University of Nottingham	http://affymetrix.arabidopsis.info/	Microarray service
Phytozome	JGI and Center for Integrative Genomics	http://www.phytozome.net/	Comparative genomic tools
PlantGDB	Iowa State University	http://www.plantgdb.org/	Comparative genomic tools
PlexDB	Iowa State University	http://www.plexdb.org/	Expression data (Brachypodium data are expected in mid 2011)
QuantPrime	University of Potsdam	http://www.quantprime.de/	Design oligonucleotides for quantitative PCR
TILLING database	Unité de Recherche en Génomique Végétale	http://urgv.evry.inra.fr/UTILLdb	TILLING collection
USDA NPGS	USDA	http://www.ars-grin.gov/npgs/	Germplasm
WMD3 Web MicroRNA Designer	Max Planck Institute for Developmental Biology	http://wmd3.weigelworld.org/	Tools for designing artificial microRNAs

Brachypodium, like Arabidopsis, is particularly useful for basic research that requires large numbers of individual plants, carefully controlled growth conditions, multiple generations, and genetic analyses. Conversely, research on crop plants themselves should be favored if the question under study is close to creating an improved variety (translational research), if the trait under study is unique to the crop, and/or if the goal is to immediately improve the crop. In this context, it is important to note that even with the explosion of sequence information available for crop plants, model systems will continue to play an important role in gaining fundamental knowledge about genetic pathways and gene functions simply due to the ease with which experiments can be conducted with model species.

Brachypodium, the small grains (e.g. wheat, oat [*Avena sativa*], and rye), and temperate forage grasses all belong to the Pooideae subfamily of the family Poaceae. This close phylogenetic relationship suggests that the Brachypodium genome may be useful for structural genomic studies in this group of grasses, even though the extensive rearrangements in the wheat-barley (*Hordeum vulgare*) lineage place limits on colinearity over large genomic regions (Opanowicz et al., 2008; International Brachypodium Initiative, 2010). Likewise, the sorghum and foxtail millet (*Setaria italica*) genomes will be useful as structural models for the emerging biomass crops switchgrass and *Miscanthus*. However, in all cases, comparison of multiple grass genomes will provide insights not available through simple pairwise comparisons.

Similar to rice, wheat, and barley, Brachypodium uses the C_3 photosynthetic pathway. However, maize, sorghum, and many of the emerging biomass crops use a C_4 photosynthetic pathway, which is more efficient under hot, dry conditions. Thus, Brachypodium alone is not suitable to study C_4 photosynthesis.

Setaria viridis, another small, annual grass being developed as a model, could be particularly useful in this regard (Doust et al., 2009; Brutnell et al., 2010). We envision that these two plants will serve as dual model systems and that comparative studies may accelerate functional genomic studies in the grasses (Brutnell et al., 2010). Indeed, Brachypodium is being used as a model system to explore the feasibility of installing a C_4 photosystem into C_3 target organisms such as rice and wheat (T. Brutnell, unpublished data). Candidate genes identified from C_4 grasses that are likely to be important for establishing major C_4 traits are being introduced into Brachypodium to see if it is possible to alter photosynthetic characteristics.

A GROWING CADRE OF BRACHYPODIUM RESOURCES

Genome Sequence

The high-quality draft Brachypodium sequence is available (International Brachypodium Initiative, 2010), and at 272 Mb, Brachypodium possesses one of the smallest grass genomes. The draft genome assembly is of unprecedented quality, and only 0.4% of the Brachypodium sequence reads were not contained in the final assembly. Furthermore, gaps in the Brachypodium genome assembly are predicted to amount to only 0.4% of the genome sequence. The high assembly quality is largely due to the comparatively low percentage of repetitive DNA in the genome (28%) and to the large number of bacterial artificial chromosome (BAC) end sequences used in the assembly (International Brachypodium Initiative, 2010). A recently initiated U.S. Department of Energy (DOE) Joint Genome Institute (JGI) project will close the remaining gaps and improve the sequence quality in currently ambiguous or unsequenced regions (J. Schmutz, personal communication). As a result of these efforts, Brachypodium is moving into the elite class of organisms with "finished" genome sequences.

Microarrays

Led by the Mockler laboratory at Oregon State University, the draft genome was used to create a Brachypodium Affymetrix microarray. This array contains approximately 2.55 million expression probes covering all gene models (including exons and intron sequences) and approximately 3.95 million probes tiling intergenic sequences (described at http://arrays.brachypodium.org/). Individual exons and introns are represented by multiple probes (average, 11; median, seven), and 95% of exons or introns are targeted by at least five probes. Currently, users can order individual arrays from the Nottingham Arabidopsis Stock Centre (http://affymetrix.arabidopsis.info/) or directly from Affymetrix (part no. Bradi-AR1b520742; www.affymetrix.com) if large numbers are required. This array is being used to create an expression atlas for Brachypodium by the Mockler and S. Persson laboratories, the latter in collaboration with the Institut National de la Recherche Agronomique (INRA)-Versailles (T. Mockler and R. Sibout, unpublished data). This last effort is resulting in the construction of a whole-genome Brachypodium coexpression network (BrachyNet) similar to what has already been done in Arabidopsis (http://aranet.mpimp-golm.mpg.de/; Mutwil et al., 2010). BrachyNet will be useful to infer candidate genes associated with particular biological processes (Persson et al., 2005; Gu and Somerville, 2010) and incorporates expression data from a large number of different organs/tissues. The Mockler laboratory has focused on profiling gene expression in abiotic stress conditions, different light environments, and diurnal and circadian time courses. Over 100 of these data sets are available as genome viewer tracks in BrachyBase (Table II).

Transformation and T-DNA Mutants

Highly efficient *Agrobacterium tumefaciens*-mediated transformation methods have been developed for Brachypodium (Păcurar et al., 2008; Vain et al., 2008; Vogel and Hill, 2008). Transformation efficiencies (defined as the percentage of callus pieces cocultivated with *Agrobacterium* that produce a transgenic plant) now approach 50% in a production setting where hundreds of T-DNA insertion lines are generated every week (for up-to-date protocols, visit http://brachypodium.pw.usda.gov/ and http://www.brachytag.org/). Most laboratories can readily accomplish Brachypodium transformation with a modest investment in tissue culture training. At least two Brachypodium transformation services are available to the public (http://www.brachytag.org/ and http://www.agron.iastate.edu/ptf/index.aspx).

With efficient Brachypodium transformation methods, it is now feasible to create large collections of sequence-indexed T-DNA mutants. Two groups have initiated large-scale projects to create T-DNA mutant collections. The BrachyTAG project at the John Innes Centre lists 5,000 T-DNA lines and has distributed mutants since 2008 (http://www.brachytag.org/; Thole et al., 2010). The U.S. Department of Agriculture (USDA) Brachypodium Genome Resources collection contains 8,700 lines and has funding to create another 30,000 lines (http://brachypodium.pw.usda.gov/TDNA/). In addition, the International Brachypodium Tagging Consortium was formed to facilitate the pooling of T-DNA mutants produced by multiple laboratories, with the ultimate goal of making enough T-DNA mutants such that there is a high probability of finding an insertion in any particular gene. Seven laboratories from five countries (United States, United Kingdom, China, Korea, and Canada) are currently creating T-DNA mutants, and these mutants will be integrated into genome browsers such as BrachyBase and ModelCrop (Table II), allowing easy identification and ordering. It is forecasted that, as a result of the

combined effort of the initiatives mentioned above, approximately 50,000 T-DNA lines will be made available to the community by 2013. A collection of this size has a 45% chance of containing an insertion in any particular gene. Transposons are another potential tool for creating insertional mutants. Unfortunately, initial experiments with Activator/Dissociator and Enhancer/Suppressor-Mutator transposon tagging systems suggest that these transposons are lethal to Brachypodium (J.P. Vogel, unpublished data). However, other transposon systems may yet prove valuable for mutagenizing Brachypodium.

The extensive use of sequence-indexed T-DNA mutants by Arabidopsis researchers provides an indication of the potential utility of Brachypodium T-DNA mutants. Ever since the first donation of 4,900 Arabidopsis T-DNA insertion lines in 1992 (Meinke and Scholl, 2003), T-DNA pools and individual lines have continued to represent the majority of the seed stocks distributed to the plant community. Their contribution to total seed distribution at the Arabidopsis Biological Resource Center (ABRC) has remained steady at about 80% for the past 10 years. The continuing distribution of sequence-indexed Arabidopsis lines underscores the value of this resource for the plant research community.

Another use for high-efficiency transformation is for the characterization of gene function through overexpression or gene silencing. Both approaches have been used with Brachypodium (Olsen et al., 2006; Demircan and Akkaya, 2009; Pacak et al., 2010), and recently, a T-DNA mutation in Brachypodium has been complemented with an Arabidopsis ortholog, bridging dicotyledonous and monocotyledonous models (Vain et al., 2011). In addition, biotechnological approaches for crop improvement can be tested by introducing and expressing heterologous genes (e.g. microbial cell wall-degrading enzymes) in Brachypodium.

Mutagenesis and TILLING

Chemical and radiation mutagenesis have been cornerstones in plant genetics research. The capacity of chemical mutagenesis to generate lines with large numbers of mutations in each plant translates into the need to screen smaller populations of plants to identify a mutation in any particular gene. The single-base changes caused by common mutagens can result in partial loss-of-function, conditional, or the occasional gain-of-function mutations that can be especially useful for the study of essential or redundant genes. Radiation-induced mutations typically generate deletions that can often be easily detected by tiling arrays or NextGen sequencing (Bolon et al., 2011).

Protocols for the mutagenesis of Brachypodium with ethyl methanesulfonate (EMS; http://brachypodium.pw.usda.gov/) and sodium azide (R. Sibout, unpublished data) have been developed. EMS and sodium azide mutants lend themselves to reverse genetic

screens through the use of Targeting Induced Local Lesions in Genomes (TILLING; McCallum et al., 2000). Two Brachypodium TILLING populations have been created. One is part of the BRACHYTIL project at INRA in Versailles and Evry in France. This collection currently contains 6,000 M2 families derived from sodium azide-treated M1 plants. Many of the families have been phenotyped, with data deposited in a searchable database (http://urgv.evry.inra.fr/UTILLdb). Preliminary results show a mutation rate of one per 550 kb, which is close to published Arabidopsis collections (Greene et al., 2003). A second population has been established at the Boyce Thompson Institute. This collection currently contains 3,000 M2 families derived from EMS-treated seeds. Pilot screens are now under way to use NextGen sequencing to identify mutations. This population is being phenotyped by a team of citizen scientists (largely high school students) working through the myPlant module at iPlant (http://bti.cornell.edu/brachybio).

Natural Diversity

Another key resource required for a model system is an extensive collection of natural accessions and inbred lines that vary in traits of interest. The first freely available collection of inbred lines was developed from the accessions available from the USDA National Plant Germplasm System (NPGS; Vogel et al., 2006; Vogel and Hill, 2008). Another collection consisting of accessions from various locations is maintained at the University of Aberystwyth and is governed by a material transfer agreement (Jenkins et al., 2003). The first large collections of inbred diploid lines were developed from material collected across Turkey (Filiz et al., 2009; Vogel et al., 2009). The initial phenotypic and genetic characterization of this freely available collection has revealed considerable diversity. A subsequent study found significant variation in drought tolerance (Luo et al., 2011). Two projects have been initiated to study this collection in more detail. One is using phenomics to characterize 100 accessions, and the other is resequencing 56 lines (J.P. Vogel, unpublished data). In addition to the Turkish collection, more than 2,000 accessions have recently been collected in Spain, Portugal, and France (L.A.J. Mur and A. Manzaneda, unpublished data) and other counttries (A. Caicedo, unpublished data).

A powerful method to gain insight into the genetic basis for natural diversity is to create recombinant lines that segregate diverse alleles. An F2 population from a cross between inbred lines Bd21 and Bd3-1 was used to generate the first genetic linkage maps for Brachypodium (Garvin et al., 2010; Huo et al., 2011). Recombinant inbred (RI) lines are particularly useful due to their "immortal" genetic composition. The development of the first RI lines, including those from crosses between Bd21 and both Bd3-1 and Bd2-3, have been completed. Other RI populations have been produced using five inbred lines from Turkey.

Each of these RI populations has more than 400 lines, and they are being phenotyped under open-field conditions to investigate drought-related traits (H. Budak, unpublished data). Genetic linkage maps will soon accompany each set of RI lines, which will facilitate the exploration of the genetic basis of natural trait variation.

To explore the potential utility of Brachypodium natural accessions and RI populations, we again look to Arabidopsis. In the 2000 to 2005 period, RI line requests accounted for approximately 13% of Arabidopsis seed distribution. This has dropped over the years, and in 2010, RI requests accounted for a little over 3% of seed distribution. Conversely, requests for the 3,600 natural accession stocks stored at the ABRC have increased steadily, from 3% of total distributions in 2005 to 11% in 2010. The resequencing of Arabidopsis natural accessions (http://www.1001genomes.org/) has greatly stimulated interest in this resource and will likely increase interest in RI populations as well. For Brachypodium, the natural accession collection is already extensive enough to ensure a high level of distribution, and easy accessibility would ensure broad community use.

DNA Libraries

DNA clone libraries (cDNA, BAC, and specialty plasmids) are another important resource for molecular studies in model systems. Several Brachypodium libraries have been made including BAC libraries for two different accessions. The largest BAC libraries, with a total of 56-fold genome coverage (184,320 clones), were made from line Bd21, used also for the reference genome (Huo et al., 2006, 2008; Febrer et al., 2010). The BACs in these libraries have been end sequenced and thus can be aligned along the genome (Huo et al., 2009; Febrer et al., 2010). Another BAC library, with a 10-fold genome coverage, was made from line Bd3-1 (M. Bevan, unpublished data). The clones in these libraries will be very useful for cloning genomic fragments too large to amplify by PCR and for cloning genes that may differ between Bd3-1 and the reference genome. The number of requests for these clones stays high, even with a sequenced genome. A similar pattern was observed for Arabidopsis, where 3 years after sequencing the genome BAC and genomic clones represented 25% of total DNA resource distribution by the ABRC. Continuing demand for these stocks from the ABRC indicates their lasting utility.

To ensure broad genome coverage, several standard cDNA libraries have been made from various tissues and treatments (Vogel et al., 2006; International Brachypodium Initiative, 2010). Together, these libraries contain approximately 127,000 clones. Full-length cDNAs created by selectively cloning only fully intact mRNAs are particularly useful for annotation and creating constructs designed to express particular genes. Recently, RIKEN has initiated a project to create and sequence 39,000 full-length Brachypodium cDNA clones (K. Mochida, unpublished data). Two Gateway-ready Brachypodium cDNA libraries, suitable for various downstream applications, were created from plants grown under different photoperiods or phytohormone treatments (Cao et al., 2011) Initial proof-of-concept screening demonstrated that these libraries can be readily transferred to a Gateway yeast two-hybrid vector and effectively used to identify both expected and novel protein interactions. Both libraries, in their entry and yeast two-hybrid vector forms, are available for free distribution from the Holt laboratory. With the exception of three of the five BAC libraries that are available through the Arizona Genomics Institute (http://www.genome.arizona.edu), DNA resources are only available through the laboratories that generated the clones.

Bioinformatic Resources

The bioinformatic infrastructure to support the Brachypodium community is reaching maturity, and a list of URLs for the Web sites mentioned below is found in Table II. Brachypodium.org is the central hub of the Brachypodium community, with links to many different resources and tools, including a Brachypodium BLAST portal, a gene annotation database, Brachypodium microarray analysis tools and resources, and BrachyBase, a Brachypodium-specific genome portal and viewer. BrachyBase contains standard genome information like gene models from the primary annotation, EST alignments, and deduced protein and cDNA sequences (Fig. 1). BrachyBase also contains Illumina RNA-Seq transcriptome data, which was used to develop empirical annotations. The locations of T-DNA mutant insertions in the genome are also provided in BrachyBase. Support for the analysis of predicted Brachypodium promoter sequences and the prediction of putative transcription factor binding sites can be found on the ELEMENT Web site. Support for designing artificial microRNAs for Brachypodium is available on the WMD3 Web MicroRNA Designer, and QuantPrime can be used to design oligonucleotides for quantitative PCR in Brachypodium. As they become available, resequenced genomes will also be available through BrachyBase. Another Brachypodium genome browser that allows easy downloading of gene, protein, and coding sequences is available at the Munich Information Center for Protein Sequences. Several smaller project-based Brachypodium Web sites also exist, including BrachyTAG and the Western Regional Research Center T-DNA collection pages. The ModelCrop Web site displays the BrachyTAG T-DNA insertions. In addition to these Brachypodium-specific Web sites, the Brachypodium genome has been incorporated into several more general databases geared toward comparative genomics, including Phytozome, Gramene, CoGe, PlantGDB, and GrainGenes (Table II). In comparison with what is available for Arabidopsis, the suite of bioinformatics resources for Brachypodium is more modest, but it covers the most critical tools and databases necessary to use Brachypodium as a model system.

Figure 1. Brachypodium resources contained in BrachyBase. Shown is a Gbrowse screenshot depicting the cold-responsive gene Bradi2g57800, which encodes a basic helix-loop-helix DNA-binding superfamily putative transcription factor. The T-DNA insertions track shows one T-DNA insertion in this gene. The JGI SNP tracks show single-nucleotide polymorphisms identified in resequenced accessions. The Bd21 transcriptome perfect alignments track represents Illumina RNA-Seq data. The Bd21 Array Data tracks show data from the Brachypodium expression atlas project indicating that the gene is induced by cold stress.

THE USE OF BRACHYPODIUM IS GROWING

To gauge the acceptance of Brachypodium as a model system, it is useful to appreciate the number of germplasm orders and the trajectory of publications involving this plant. Order numbers increased significantly after JGI announced that the genome would be sequenced in 2006 (Fig. 2A). The number of orders for Brachypodium in the years after is similar to the number of orders for Arabidopsis from the ABRC in 1992, the first year after the Center was established (Fig. 2B). In comparison, early distribution of Arabidopsis resources was fairly modest, since it was handled by individual researchers. For example, Albert Krantz distributed approximately 1,600 individual wild-type and mutant stocks between 1974 and 1987 (Kranz and Kirchheim, 1987; Somerville and Koornneef, 2002).

Publication rate is a vital (although lagging) indicator for the growth of a developing research community. The exponential increase in publications using

Brachypodium as a model system indicates that Brachypodium is on a very strong trajectory (Fig. 2C), similar to Arabidopsis in the early years (Fig. 2D). The adoption of Brachypodium as a model system can also be examined relative to total infrastructure and research investments. Approximately $11 million in competitive grants has been awarded for about 30 projects in the United States, mostly from the DOE. These projects include targeted infrastructure investments (T-DNA population and microarray development) as well as those with more traditional hypothesis-based research questions but that also may have significant infrastructure objectives (e.g. small RNA sequencing and analysis, phenomic characterization of T-DNA mutants and natural accessions, cell wall cross-linking, transcription factors, phosphate uptake, and mycorrhizal associations). In addition, JGI has invested a few million dollars to develop sequence resources. In Europe, some support for Brachypodium research has been provided by national agencies or by institutional

Brachypodium
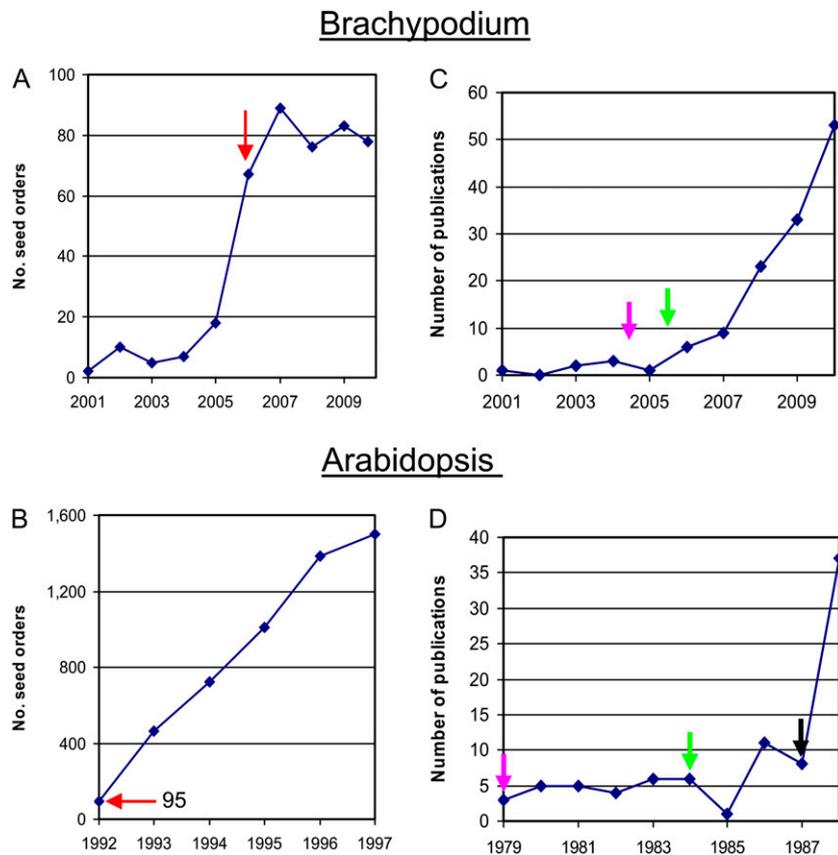
Arabidopsis

Figure 2. Seed distribution and publication comparison between Brachypodium and Arabidopsis. A, Combined Brachypodium seed distribution from three major U.S. sources of Brachypodium seeds (NPGS, David Garvin, and John Vogel). For 2010, the number reflects orders up to September. Since 2001, 435 orders have been placed. Since secondary distributions were encouraged, the actual number of informal "orders" would greatly increase the number of seed distributions. Seed distributions from laboratories and stock centers outside the United States are not included. The approval of the genome sequencing project in 2006 (red arrow) coincides with a large increase in demand for seed. B, Distribution of Arabidopsis seed resources from the ABRC. Data shown are number of orders per year starting 1 year after the ABRC was established. Note that the number of orders in 1992 is very similar to the number of Brachypodium orders in the last couple of years (red arrow). C, Number of publications using Brachypodium as a model system. The magenta arrow indicates the announcement that JGI will sequence Brachypodium. The green arrow represents the release of the 4× draft genome sequence to the community. D, Number of publications using Arabidopsis as a model system. The magenta arrow indicates the establishment of Arabidopsis as a model for embryo development. The green arrow indicates the recognition of the small size of the Arabidopsis genome. The black arrow indicates the proposal to use positional cloning to study biochemical, physiological, and developmental processes in Arabidopsis.

funding (Supplemental Fig. S1). However, there is an urgent need to mirror the U.S. effort on Brachypodium research at the European Union level to facilitate functional genomics and genetic resource distribution.

Several institutions have facilitated the adoption of this model system by developing Brachypodium research groups. These "Brachypodium working groups" decrease the investment necessary by individual laboratories to establish Brachypodium as model system and therefore foster its local adoption. At the USDA-Agricultural Research Service (ARS) Western Regional Research Center and the colocated Plant Gene Expression Center, eight laboratories are using Brachypodium for various projects, including cell wall biology, flower morphology, auxin signaling, grain properties, promoter mining, disease resistance, and comparative

genomic studies. At the University of Massachusetts, Amherst, 12 laboratories have established the UMass Brachypodium Consortium (www.bio.umass.edu/brachypodium/). Other working groups include seven laboratories at the Great Lakes Bioenergy Research Center, several laboratories at the John Innes Centre and the Institute of Biological, Environmental, and Rural Science (United Kingdom), and groups at INRA (France), Seoul National University (South Korea), and Oregon State University. In Japan, RIKEN is developing heavy ion beam mutants and full-length cDNAs as part of their new biomass engineering program (http://www.riken.go.jp/engn/r-world/research/lab/biomass/). In Australia, several projects are under way, including a phenomics project at the High Resolution Plant Phenomics Centre (http://www.plantphenomics.

org.au/HRPPC) and a project to fast-track gene discovery in wheat by using Brachypodium as a model for root architecture and disease traits at the Commonwealth Scientific and Industrial Research Organization in Canberra. This summary only represents a subset of overall interest; nevertheless, the large number of projects and groups tells a convincing story of the growing adoption of Brachypodium as a model system.

Examples of Brachypodium-Enabled Research

Brachypodium has proven particularly useful for comparative genomics because it is the first representative from the Pooideae subfamily of grasses to be sequenced. This allows comparisons between genomes from the three most economically important grass subfamilies. A key insight that came from this comparison was the role of whole chromosome insertions into centromeric regions as a mechanism for the reduction of chromosome number through evolution (International Brachypodium Initiative, 2010). While evidence for such insertions was previously noted (Kellogg, 2001; Srinivasachary et al., 2007; Luo et al., 2009), the comparison of the Brachypodium and rice genomes provided the most clear evidence of the role such insertions play in grass genome evolution. Along similar lines, a comparison of the synteny of genes in functional and ancient centromeric regions was used to trace the evolution of these regions in Brachypodium, rice, and wheat (Qi et al., 2010).

Other research areas are beginning to bear fruit as well, now that a large ensemble of resources is available to the plant biology research community. For instance, since flowering time pathway details differ between the grasses and Arabidopsis, flowering time questions can be addressed in Brachypodium, a long-day plant, that cannot be addressed in rice, a short-day plant, or Arabidopsis. A genome-wide comparison of known flowering time and vernalization genes in Brachypodium, rice, and Arabidopsis set the stage for determining how these genes control flowering in grasses with a long-day flowering strategy (Higgins et al., 2010). Using a transgenic approach, Brachypodium was used to show that a perennial rye gene, *LpTFL1*, and its Arabidopsis ortholog, TFL1, could delay flowering in a temperate grass (Olsen et al., 2006). Furthermore, Brachypodium is particularly useful for studying mature root systems because grass root systems differ substantially in structure and development from Arabidopsis. Unlike the root systems of rice, maize, and wheat, which are too large to study under controlled conditions, Brachypodium roots can be readily assessed in this manner (Watt et al., 2009). Lastly, a model for temperate grass diseases would be exceptionally useful, and Brachypodium has recently been shown to be susceptible to a major wheat disease, *Fusarium* head blight, with floral disease symptoms being the same as those in wheat (Peraldi et al., 2011). Recent research has also demonstrated that Brachypodium exhibits natural variation for resistance to *Puccinia graminis*, the causal

agent of stem rust, and mutant screens have been successful in identifying both resistant and susceptible lines (D.F. Garvin, unpublished data). These diverse examples provide a glimpse into the broad utility of Brachypodium for exploring novel frontiers in plant biology, and in coming years, novel discoveries emerging from Brachypodium research will grow.

Critical Areas for Future Investments

The remarkable strides in developing Brachypodium as a model system have led to the creation of substantial biological resources. New and continued investments in a few areas are necessary to sustain this momentum and ensure that the information and biological materials remain available for the long term. The ABRC played a crucial role in fostering the development of Arabidopsis as a model system. By efficiently storing and distributing restriction-free, reasonably priced seed and DNA resources, the ABRC continues to ensure that biological resources developed by the Arabidopsis community are maintained and are readily available. The importance of a freely available collection cannot be overstated and has been the cornerstone of the success of Arabidopsis as a model system. The Brachypodium community is rapidly approaching the point where it will no longer be feasible to rely on individual laboratories to distribute all of their biological materials (e.g. tens of thousands of T-DNA lines, TILLING populations, natural accessions, RI line populations, and many thousands of DNA clones). Fortunately, the USDA NPGS has committed to distribute seeds from natural accessions and T-DNA lines (V. Bradley, personal communication). However, they are unable to propagate transgenic material, and they are not able to distribute DNA stocks, so this is a stop-gap solution until a dedicated stock center (or expanded NPGS capability) is established. A comparable need for a stock center is also emerging in the European Union.

Another area for continued investment is the development of sequence-indexed T-DNA lines. Simply put, it will take over 125,000 lines to have a 90% chance of tagging any particular gene. Even higher numbers are necessary to identify a flanking sequence tag in any particular gene, because only a fraction of all insertions produce a usable flanking sequence tag. To generate this number of lines is beyond the capacity of individual laboratories, thus requiring coordinated international efforts. The DOE has taken the lead in this area by funding a project to create over 37,500 lines, and researchers from the International Brachypodium Tagging Consortium have plans to produce approximately 20,000 lines. However, additional funds will be required for these groups to reach the number of lines required to approach saturation.

CONCLUSION

One of the great successes of Arabidopsis has been the democratization of plant biology. This experimen-

tal system has enabled large and small laboratories around the world to identify functionally important genes using a common experimental toolkit. With the emergence of Brachypodium as a tractable model grass, we hope the future of basic studies focused on grass biology will follow a similar path. The development of Brachypodium as a model system has followed a new paradigm in which the development of genome sequence resources occurred simultaneously with the development of other key resources such as highly efficient transformation, the establishment of large collections of natural accessions, and the development of essential techniques like efficient crossing and chemical mutagenesis. The convergence of all these resources makes a compelling case for Brachypodium as an important plant model system.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Brachypodium grants.

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