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REVIEW

Brachypodium: A Monocot Grass Model Genus for Plant Biology[OPEN]

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The genus *Brachypodium* represents a model system that is advancing our knowledge of the biology of grasses, including small grains, in the postgenomics era. The most widely used species, *Brachypodium distachyon*, is a C_3 plant that is distributed worldwide. *B. distachyon* has a small genome, short life cycle, and small stature and is amenable to genetic transformation. Due to the intensive and thoughtful development of this grass as a model organism, it is well-suited for laboratory and field experimentation. The intent of this review is to introduce this model system genus and describe some key outcomes of nearly a decade of research since the first draft genome sequence of the flagship species, *B. distachyon*, was completed. We discuss characteristics and features of *B. distachyon* and its congeners that make the genus a valuable model system for studies in ecology, evolution, genetics, and genomics in the grasses, review current hot topics in *Brachypodium* research, and highlight the potential for future analysis using this system in the coming years.

INTRODUCTION: DEVELOPING *BRACHYPODIUM* AS A MODEL GENUS

Advances in biology have both been based on and benefited from research on model organisms. *Arabidopsis thaliana*, of course, has been transformative as a model system for plant biology research, with its short life cycle, ease of genetic manipulation, small diploid genome, and simple cultivation in the laboratory. In the past two decades, several experimental plants, including rice (*Oryza sativa*); the N_2-fixing legumes *Lotus japonicus* and *Medicago truncatula*; *Populus* species; *Nicotiana benthamiana*; and *Mimulus guttatus* (also known as *Erythranthe guttata*) and *Nicotiana attenuata* (for population biology and evolutionary studies), have spearheaded current advances in plant biology. However, as cogently pointed out by Leonelli and Ankeny (2013), experimental organisms and model organisms differ, although both are essential for advances in biology. Specifically, model organisms are vital resources for studies in large-scale biology, ecology, evolution, genetics, and cell biology, with a plethora of diverse lines (wild, inbreds, and mutants) and infrastructure (databases, seeds, and so on) readily available. In addition, there is a culture of sharing such resources, which exhibit a short life cycle, are easily and inexpensively cultivated and are readily manipulated in the laboratory using standard molecular biology techniques (Table 1). By contrast, experimental organisms are used to solve a specific question, excel as a tool, or are interesting organisms or objects of scientific curiosity (Leonelli and Ankeny, 2013). Based on these criteria, Arabidopsis (for dicots) and *Brachypodium* (for monocots) can be defined as model organisms for plant biology.

The genus *Brachypodium* has come to the forefront for research on grasses (Poaceae). The development of its temperate flagship species, *Brachypodium distachyon*, as a model organism has been described previously (Brutnell et al., 2015; Kellogg, 2015b; Lyons and Scholthof, 2015, 2016; Vogel, 2016) and is briefly summarized below. *B. distachyon* was accepted at a remarkably rapid rate as a dynamic research tool that offers a plethora of opportunities for comparative work—with Arabidopsis as the model dicot—and fundamental advances in grass biology. Similar to Arabidopsis, building *B. distachyon* as a model grass species (Lyons and Scholthof, 2015; Vogel, 2016) has required the development of cultivation and transformation methods, plus the genetic and genomic tools deemed essential for 21st century plant biology studies. In addition, *Brachypodium* research has been greatly accelerated by next-generation sequencing (NGS) technologies and the remarkable efforts of community-based projects supported by the U.S. Department of Energy Joint Genome Institute (DOE-JGI) and the USDA.

Model organisms for laboratory research have primarily been used to dissect specific aspects of plant biology following a reductionist approach. With the rise of *B. distachyon*, scientists have found many opportunities to work across the spectrum of basic-translational-applied research, as elaborated below. For instance, *B. distachyon* has been rapidly subsumed into fundamental research on plant development, plant-microbe interactions, abiotic stress, evolutionary biology, systems biology,

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Table 1. Key Features of Purple False Brome *B. distachyon* (*Brachypodium*, Brachypodieae, Pooideae, Poaceae, and Poales) as a Model Organism for Plant Biology

Key Features
Diploid[a]
Annual[b]
Self-fertile
Short seed-to-seed life cycle (~8 weeks)
Wild species[c]
Ease of outcrossing for genetics
Ease of growth under laboratory conditions
Broadly susceptible to plant microbes[d]
Evolutionary taxonomy of Brachypodium species[e]
Small genome size[f]
Synteny with major small grains (wheat, maize, millet, rice, barley)
Completed genome, transcriptome[g]
Inbred lines[g]
Mutants (indels, SNPs, T-DNA insertions, CRISPR amenable)[h]
Community resources (seed, tools, genomic databases)[h]

[a]Key features of other Brachypodium species, both diploid and allopolyploid, are discussed in the text. See Table 2 for additional features of specific annual *Brachypodium* species.

[b]Three annual species, *B. distachyon*, *B. stacei*, and *B. hybridum*, have been sequenced. *B. sylvaticum*, a perennial diploid species, has been sequenced, inbred lines have been developed, and it is amenable to Agrobacterium-mediated transformation (Steinwand et al., 2013). Other perennials are discussed in the text.

[c]*B. distachyon* is found in its native Mediterranean region (Catalán et al., 2016a), and its close congener *B. hybridum* is found worldwide.

[d]See Table 4 for a list of microbes and citations.

[e]For more information, refer to updated taxonomic reports of annual (Catalán et al., 2016a) and of annual and perennial (Catalán et al., 2016b) *Brachypodium* species.

[f]The 272-Mb genome of *B. distachyon* comprises five chromosomes. Genomes of other members of the genus *Brachypodium* are discussed in the text.

[g]To date, 54 inbred lines of *B. distachyon* have been sequenced (Gordon et al., 2017), and several other have been characterized genomically.

[h]See Table 3 for resources with pertinent links and citations.

ecology research, and for the development of new tools and concepts toward improving other temperate C_3 grasses—such as wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*)—that are crucial small grain crops used worldwide for the production of food, forage, and feed. Today, *B. distachyon* provides an ideal and robust platform for discovery-based research in each of these aspects of plant biology (Table 1). Additionally, *Brachypodium* species have maintained their wildness, providing a treasure-trove of resources for plant ecology researchers to study the plants in situ.

Here, we describe key features of *B. distachyon* and other congeners, including descriptions of their origin, morphology, distribution, ecology, and genetic characteristics and provide examples of how they have been and can be used as genetic tools to understand and solve problems in plant biology.

BRACHYPODIUM SYSTEMATICS, EVOLUTION, SPECIATION, AND MORPHOLOGY

For more than a century, *B. distachyon* (Palisot de Beauvois, 1812) was considered the single annual representative species of the genus *Brachypodium* (Schippmann, 1991), and for more than three decades, three cytotypes of 2n=10, 20, and 30 chromosomes were recognized within the species, although they were thought to be diploid, tetraploid, and hexaploid individuals of an ascendant autopolyploid series with x=5 (Talavera, 1978). It was not until recently, however, that the accrued phenotypic, cytogenetic, and molecular phylogenetic evidence demonstrated that the three cytotypes corresponded to three independent species: two diploids, *B. distachyon* (2n=2x=10, x=5) and *B. stacei* (2n=2x=20, x=10), and their derived allotetraploid *B. hybridum* (2n=4x=30, x=10+5) (Catalán et al., 2012; Table 2). Despite having twice the number of chromosomes, the genome size of *B. stacei* (0.564 pg/2C; reference *Brassica rapa* Goldball 0.97pg/2C) is roughly similar to that of *B. distachyon* (0.631 pg/2C; reference *Brassica rapa* Goldball 0.97 pg/2C), whereas the genome size of *B. hybridum* corresponds to the sum of the two progenitor genomes (1.265 pg/2C; reference *Lycopersicum esculentum* Stupicke 1.96pg/2C) (Catalán et al., 2012). Molecular evolutionary data indicate that *B. stacei* is the oldest diploid lineage within the genus *Brachypodium*, splitting from the common ancestor ~10 million years ago (Mya; 10^6 years). This was followed by the divergence of the *B. distachyon* lineage (~7 Mya), which preceded the split of a clade of recent perennial lineages (core perennial clade; ~3 Mya). The allotetraploid *B. hybridum* species originated ~1 Mya (Catalán et al., 2012). The divergence estimations have been confirmed through nested-dating analysis within a (grass) family-wide phylogenomic analysis, based on plastome data that also dated the origin of the parental *B. distachyon* species to ~1 Mya, concurrent with that of its descendant hybrid (Sancho et al., 2018).

Analysis of maternally inherited plastid genes support the recurrent origin of allotetraploid *B. hybridum* from bidirectional crosses of its parents, followed by whole-genome duplication of the unfertile interspecific hybrid (López-Alvarez et al., 2012). These authors also showed that most of the studied circum-Mediterranean *B. hybridum* populations were derived from a maternal *B. stacei* parent, whereas only relatively few western-Mediterranean populations were derived from a maternal *B. distachyon* parent.

Phylogenomic studies of *B. distachyon* based on 54 resequenced ecotypes showed a main split of two intraspecific lineages characterized by their flowering time. These included the extremely delayed flowering (EDF+) versus non-extremely delayed flowering (non-EDF+) lineages and their respective coevolving molecular variants of genes known to regulate vernalization (e.g., *VRN1* and *VRN2*) and flowering (e.g., *CO2*, *FTL9*, *FTL13*, *PHYC*, and *PPD1*). However, none of those traits coevolved with latitude (Gordon et al., 2017). Both lineages branched off approximately half a million years ago (Sancho et al., 2018): The first clade contained lines distributed across the Mediterranean region, and the second clade showed the divergence of two geographically constrained eastern Mediterranean (Turkey and other countries, T+) and western Mediterranean (Spain and other countries, S+) groups. Comparative genomics of nuclear and plastome data further identified four introgressions (two of them also plastomic) and nine chloroplast capture events between the three groups, suggesting that flowering time variation is the main factor driving rapid intraspecific divergence

Table 2. Key Features of *B. distachyon*, *B. stacei*, and *B. hybridum*

Trait	*B. distachyon*	*B. stacei*	*B. hyridum*
Ploidy	Diploid	Diploid	Allotetraploid
Annual	Yes[a]	Yes	Yes
Origin[b]	~7 Mya (lineage) ~1 Mya (species)	~10 Mya (lineage)[c]	~1 Mya (species)[c]
Chromosome	2n=2x=10, x=5	2n=2x=20, x=10	2n=4x=30, x=10+5
Genome size	0.631 pg/2C 272 Mbp	0.564 pg/2C 234 Mbp	1.265 pg/2C 509 Mb
Occasional short rhizomes	No	Yes	Yes
Leaf blade color	Bright green	Pale green	Dark green
Leaf blade shape	Straight	Curled	Straight
Leaf softness	Brittle	Soft	Brittle
Leaf hairiness	Scarcely hairy or glabrous	Densely hairy	Scarcely hairy or glabrous
Vernalization[d]	Yes	No	No

[a]Annual, although there are other *Brachypodium* species that are perennials.
[b]Origin indicates million years ago (Mya; 10^6 years) since the split from a common ancestor. Lineage indicates split from the stem ancestor, and species indicates split from the crown ancestor.
[c]*B. hybridum* is the derived allotetraploid of *B. distachyon* × *B. stacei*.
[d]Vernalization is required to induce flowering. Most, but not all, *B. distachyon* lines require vernalization (Vogel et al., 2009).

in *B. distachyon*, although it is counterbalanced by repeated introgression between previously isolated lineages (Gordon et al., 2017; Sancho et al., 2018), thus maintaining the biological reproductive concept of the species.

PERENNIAL *BRACHYPODIUM* SPECIES

Besides the three most intensively investigated annual species, the genus *Brachypodium* also contains ~17 perennial species distributed worldwide (Schippmann, 1991; Catalán et al., 2016b) (Figure 1). The 20 recognized *Brachypodium* taxa are characterized by their typical subsessile spikelet and exclusive forms of embryo development, seed storage proteins, polysaccharides and globulins, stem and leaf fructosans, small genome sizes, and large disploidy (i. e., species showing different chromosome base numbers) (Catalán et al., 2016b). The *Brachypodium* taxa belong to the monotypic tribe Brachypodieae and are evolutionarily placed in an intermediate position between the ancestral basal pooids and the recently evolved clade of core pooid lineages, including the economically important Triticeae + Bromeae and Poaceae (Sancho et al., 2018).

Perennial *Brachypodium* species vary widely both in phenotype and origin. They range from the short-rhizomatose, self-fertile American allotetraploid *B. mexicanum*, a species closely related to the oldest *B. stacei* lineage and biologically and genomically similar to the annual species, to the strong-rhizomatose, outcrossing, and recently evolved Eurasian and African diploid and allopolyploid species of the core-perennial clade. This clade includes some of the widely distributed palaearctic species, such as the diploids *B. pinnatum* and *B. sylvaticum*, together with other more restricted endemic species (Catalán et al., 2016b). Two Mediterranean high ploidy-level allopolyploids, *B. retusum* and *B. boissieri*, characterized by their branched woody stems and short inrolled leaves, have inherited ancestral, intermediately evolved and recent genomes, whereas core perennial allotetraploids *B. phoenicoides*, *B. pinnatum* 4x and *B. rupestre* 4x, characterized by their nonbranched stems and long flat leaves, have only inherited recently evolved genomes (Catalán et al., 2016b; Díaz-Pérez et al., 2018).

The diploid *B. sylvaticum*, the best-known perennial species of the genus, was recently selected as a model plant for perenniality (Gordon et al., 2016). Genomic and transcriptomic resources are available for *B. sylvaticum*, including its reference genome (*B. sylvaticum* Ain1) and a second resequenced line (*B. sylvaticum* Sin1) (see Phytozome). This plant is predominantly self-fertile (94.6%), with a small, compact genome (340 Mb) distributed on nine chromosomes that can be easily transformed (Steinwand et al., 2013). Though it belongs to the core perennial clade of predominantly robust strong-rhizomatose outbreeding species, *B. sylvaticum* shows a slender habit and rhizomes and a selfing reproductive system; however, the species, like the other perennials, is an overwintering plant, characterized by its hairy indumentum, soft leaves, nodding panicle, and long awned lemma (Catalán et al., 2016b). Its distribution covers the largest native Old World geographical range of any *Brachypodium* species, ranging from the Canary Islands (West) to Japan and New Guinea (East) and from Scandinavia and Siberia (North) to northern Africa and Malaysia (South), although some of the East Asian and Malaysian populations may correspond to different microtaxa (Catalán et al., 2016b).

Disploidy is a major feature of *Brachypodium*, a genus that contains diploid species with x=10, 9, 8, and 5 chromosomes and allopolyploid species with different combinations of chromosome base numbers (Catalán et al., 2016b). Phylogenetic and comparative chromosome painting data have been used to propose evolutionary hypotheses on descendant versus descendant-ascendant disploidy series along the *Brachypodium* tree (Betekhtin et al., 2014) and secondary origins for the allopolyploids (Catalán et al., 2016b). The advent of the sequenced reference genomes facilitated the reconstruction of the path of nested chromosome fusions that support the descendant disploidy hypothesis. Interspecific breeding barriers between *Brachypodium* species (Khan and Stace, 1999) are fully congruent with the *Brachypodium* phylogeny and explain the reproductive isolation of the early diverging *B. stacei* type (*B. hybridum*) and *B. mexicanum*. These barriers also explain the crossability of the intermediately evolved *B. distachyon* with the core perennial species and the highly fertile descendants of all attempted

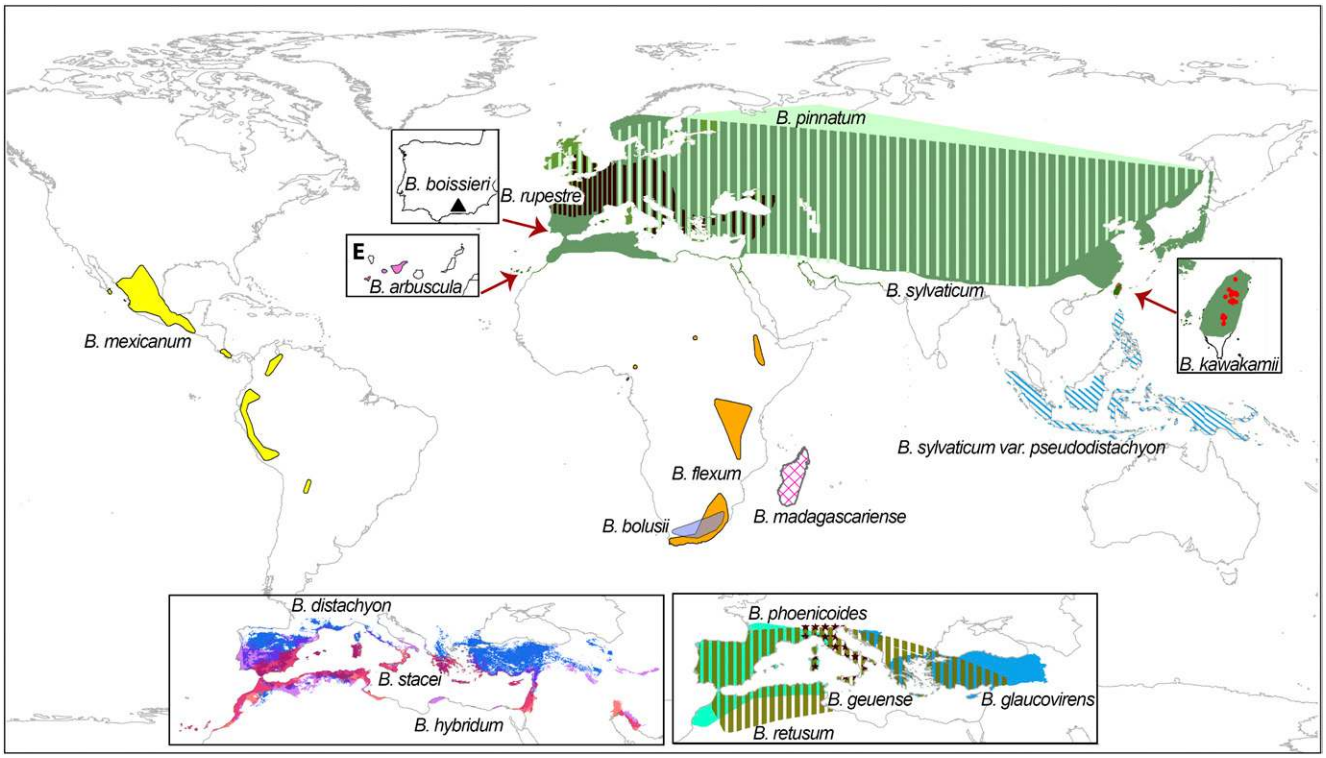

Figure 1. Worldwide Geographic Distribution of 18 *Brachypodium* Taxa.

The 18 worldwide species of *Brachypodium* taxa (*B. arbuscula*, pink; *B. boissieri*, black triangle; *B. bolusii*, violet; *B. distachyon*, dark blue; *B. flexum*, orange; *B. genuense*, dark-brown star; *B. glaucovirens*, light blue; *B. hybridum*, purple; *B. kawakamii*, magenta dot; *B. madagascariense*, open pink square; *B. mexicanum*, yellow; *B. phoenicoides*, aquamarine; *B. pinnatum*, light green; *B. retusum*, pale brown; *B. rupestre*, dark brown; *B. stacei*, red; *B. sylvaticum*, sage green; *B. sylvaticum* var *pseudodistachyon*, light-blue diagonal line). (*Image adapted from Catalán et al. [2016b], Figure 2, page 15, copyright 2016, Springer.*)

interspecific crosses between recently evolved core perennial taxa (Khan and Stace, 1999; Catalán et al., 2016b).

ECOLOGY OF *BRACHYPODIUM*

The ecology of the ~20 *Brachypodium* species varies drastically depending on their geographical distributions and adaptation to different climates and habitats. Among these, the three annual species and the perennials *B. retusum* and *B. boissieri* have adapted to xeric Mediterranean conditions. The Canarian endemic species *B. arbuscula* grows in more humid places, while the endemic South African species *B. bolusii* and Taiwanese *B. kawakamii* thrive in alpine vegetation belts. The tropical African *B. flexum* and Malagasy *B. madagascariense* grow in the Afromontane forests, and the American *B. mexicanum* is found in xeric to humid neotropical habitats. The western Mediterranean *B. phoenicoides* is adapted to mesic to dry places with humid soils, and the predominantly Eurasian *B. pinnatum*, *B. rupestre*, and *B. sylvaticum* species grow in mesic to humid open grasslands and forests (Catalán et al., 2016b). Two *Brachypodium* species have been confirmed as invasive species. *B. hybridum* has successfully and predominantly colonized other Mediterranean-type ecoregions (California, South Africa, South America, and southern Australia), while *B. sylvaticum* is spreading in humid, forested regions of western North America and Australia (Catalán et al., 2016b).

Detailed ecological studies have been conducted using the three annual circum-Mediterranean species of the *B. distachyon* complex. Environmental niche modeling analysis indicated that, overall, *B. distachyon* grows in higher, cooler, and wetter places north of 33°; *B. stacei* in lower, warmer, and drier places south of 40° 30'; and *B. hybridum* in places with intermediate ecological features and across latitudinal boundaries but also overlapping with those of its parents, more often with those of *B. stacei* (López-Alvarez et al., 2015; Catalán et al., 2016b). This concurs with the finding that most *B. distachyon* lines require vernalization treatment in order to flower, whereas *B. stacei* and *B. hybridum* lines do not (Vogel et al., 2009). Additionally, *B. stacei* grows in shady habitats, whereas *B. distachyon* and *B. hybridum* occur in open habitats (López-Alvarez et al., 2015; Catalán et al., 2016a). Paleoenvironmental modeling data support the notion that the Mediterranean basin and adjacent areas serve as long-term refugia for *B. stacei* and *B. distachyon*, and some of them serve as potential hybrid zones, which could have favored the recurrent origins of *B. hybridum* since the late Pleistocene.

Niche similarity tests showed evidence of niche conservatism for *B. hybridum* and each of its parents; the allotetraploid shares

niche occupancy with its progenitors but is reproductively isolated from both. Also, *B. hybridum* has the largest niche overlap with its parental niches but a similar distribution range and niche breadth, indicating that the hybrid does not outcompete its parents in their native ranges (López-Alvarez et al., 2015; Catalán et al., 2016b). Conversely, *B. hybridum* is the only species of the complex that has successfully colonized other non-native world regions. This suggests that the allotetraploid has greater ecological tolerance compared with the diploids, which could be associated with the boost in diversifying selection due to increasing genomic and epigenomic expression, as well as rapid shifts in physiological and adaptive traits such as photoperiod and weediness (Bakker et al., 2009; Catalán et al., 2016b).

Field analyses demonstrated that environmental aridity gradients in Spain affect the predominant northern and southern Mediterranean distributions of the less efficient water user *B. distachyon* and the more efficient water users *B. hybridum* and *B. stacei* (water use efficiency), respectively, under water-restricted growing conditions (Manzaneda et al., 2012; Martínez et al., 2018). Under drought conditions, *B. hybridum* individuals behave as drought escapists, maintaining higher photosynthesis and stomatal conductance and showing earlier flowering times to cope with water stress than the less adapted *B. distachyon* individuals (Manzaneda et al., 2015). Translocation experiments in mixed southern Spanish *B. distachyon–B. hybridum* populations have demonstrated the superior capability of the allotetraploid in colonizing densely occupied competitive habitats and the balance of intra/interspecies competition favoring the establishment of *B. hybridum* over *B. distachyon* populations under natural field conditions at the rear-edge distribution of the diploid *B. distachyon* parent (Rey et al., 2017). By contrast, field analyses in southern Mediterranean Israel microsites have revealed the predominant presence of allotetraploid *B. hybridum* over its diploid parents, especially the more frequent *B. stacei*, along a large-scale latitudinal range. However, the distribution of *B. hybridum* was not correlated with an aridity cline, although clustered patterns suggested that the distributions of *B. stacei* and *B. hybridum* were not random (Bareither et al., 2017). Ongoing ecogenomic studies of *B. distachyon–B. hybridum* populations and *B. stacei–B. hybridum* populations in Spain and Israel will further help to decipher the potential drivers of the ecological success of parental diploid and allotetraploid populations in different microenvironments.

GENETICS AND GENOMICS

B. distachyon has some obvious advantages over rice, its closest genetic competitor, as a grass model system, including its smaller habit, ease of cultivation in the laboratory, and shorter seed-to-seed life cycle (Figure 2). *B. distachyon* also has a smaller genome (~272 Mb) (International Brachypodium Initiative, 2010) than rice (~430 Mb) (Sasaki and Antonio, 2004), although genome size may become less important with the rapid advances in NGS technologies. Importantly, the genus *Brachypodium* is evolutionarily closer to several major cereal crops than rice, such as wheat, rye (*Secale cereale*), and barley, which makes it a better suited model system to study cereal biology (Draper et al., 2001; Brkljacic et al., 2011; Catalán et al., 2016b).

B. distachyon and rice, both C_3 plants, have less complex genomes compared with other grasses with larger genomes. For instance, comparative genomics analyses of *B. distachyon*, rice, sorghum (*Sorghum bicolor*; 730 Mb), and goat grass (*Aegilops tauschii*; 4020 Mb) revealed that sorghum and goat grass genes are distributed in clusters (gene insulae) in the genome. The gene insulae contain an average of 3.2 genes/cluster, with non-coding regions separating the clusters. The genes of rice and *B. distachyon*, on the other hand, are more uniformly distributed, with short intergenic distances. These differences highlight the complex effects of genome expansion on gene distribution and spacing in grasses (Gottlieb et al., 2013), and they underscore the simplicity of the *B. distachyon* genome.

The fully annotated reference genome sequences of two commonly used *B. distachyon* accessions (Bd21 and Bd21-3) are publicly available through Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html). The accession Bd21 assembly was completed in 2010, and versions (v) 2.1 and 3.1 are currently available through Phytozome. The Bd21 assembly was found to comprise ~272 Mb with an N50 of 6.4 Mb during the 2.1 version update and encodes 31,694 protein-coding loci. The recently completed Bd21 v3.1 update focused on inspecting smaller sized regions, which were verified by clone-based shotgun sequencing using Sanger and Illumina sequencing. This increased the assembly size by 1.43 Mb and filled several gaps that existed in the previous versions. The chromosomal genome assembly of accession Bd21-3 was completed using MECAT and refined using ARROW; this assembly is also ~272 Mb in size. Users can also browse a Phytozome database of more than 800,000 single-nucleotide polymorphism (SNP) mutations in this newly released Bd21-3 assembly.

In addition to accessions Bd21 and Bd21-3, de novo assemblies of 54 diverse *B. distachyon* inbred accessions were completed to enable identification of the full gamut of genes (or pan-genome) of *B. distachyon* (Gordon et al., 2017). The availability of the *B. distachyon* pan-genome (https://brachypan.jgi.doe.gov)—with its annotated genomes, transcriptomes and transposons—opens new avenues to uncover key physiological and adaptive processes in grasses. The *B. distachyon* pan-genome is based on 54 fully sequenced genome assemblies of geographically diverse ecotypes, 36 of which were also analyzed at the transcriptome level (Gordon et al., 2017). From this, 61,155 pan-genome clusters were classified as core (present in all lines), softcore (95–98%), shell (5–94%), and cloud (2–5%) genes, comprising nearly twice the number of genes present in any individual genome. The study showed that the core genes were enriched for essential biological functions (e.g., glycolysis) and were constrained by purifying selection, whereas shell genes were enriched for potentially beneficial functions (e.g., defense, development, and gene regulation), displayed higher evolutionary rates, were located closer to and were more functionally affected by transposable elements, and were less syntenic with orthologous genes in other grasses (Gordon et al., 2017). Shell genes contribute substantially to phenotypic variation and influence population evolutionary history within *B. distachyon*, as demonstrated for the three phylogenetic groups detected in the study (EDF+, T+, S+), which are characterized by different flowering time traits and their molecular regulators

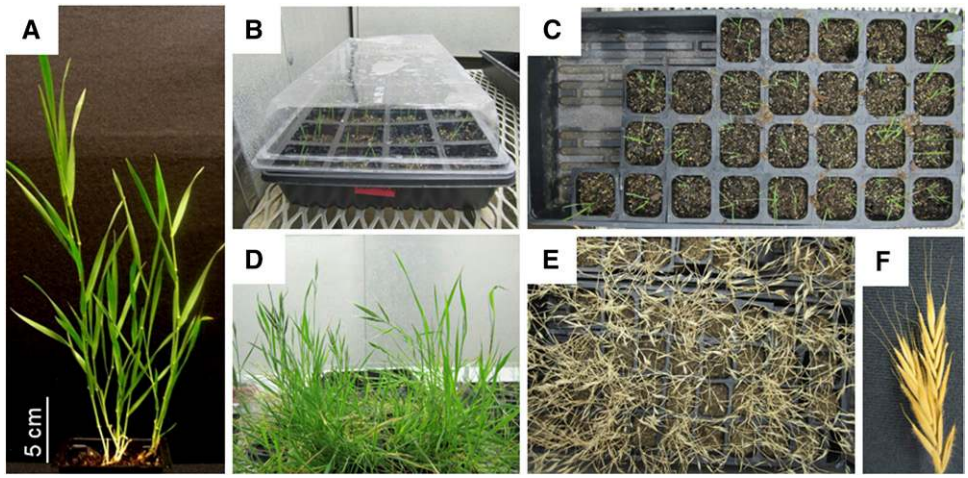

Figure 2. *Brachypodium* Life Cycle and Propagation.

(A) Mature *B. distachyon* (Bd21-3) at ~6 weeks old. Bar is an approximate estimate.
(B) Seven-day cold-treated (vernalized) Bd21-3 seeds are germinated in small 36-cell sheets in trays to accommodate several plants in a limited space. Typically, trays are covered until seeds germinate and seedlings are established approximately 1 week after planting.
(C) Uncovered trays containing two to three plants per pot in the early vegetative phase (~2 weeks after planting).
(D) Mature Bd21-3 plants with inflorescence (~8 weeks after planting).
(E) Senesced plants, ready for harvest ~12 weeks after planting.
(F) Close-up of dried inflorescence (panicle). The seeds can be separated from the panicle, desiccated, and stored at 4°C.

associated with different types of core and shell ingroup genes (Gordon et al., 2017).

Reference genomes of additional *Brachypodium* species, *B. stacei*, *B. sylvaticum*, and *B. hybridum*, were also recently completed and are publicly available at Phytozome. Although sequencing and annotation of the mitochondrial genome is yet to be completed for most species, the plastid genomes of *B. distachyon* (54 lines), *B. stacei* (1 line), and *B. hybridum* (2 lines) have been sequenced (Sancho et al., 2018). Plastome lengths varied from 134,991 to 135,214 bp in *B. distachyon* and between 136,326 and 136,330 bp in *B. stacei* and *B. hybridum*, as the two allotetraploid lines were derived from maternal *B. stacei*-type parents. The *B. distachyon* plastomes contained 133 genes, 76 of which were protein-coding genes (including seven duplicated genes), 20 nonredundant tRNAs (out of a total 38), four rRNAs in both inverted repeats, four pseudogenes (*trn*I, *rps*12a, *trn*T, and *trn*l), and two hypothetical open reading frames (ycf). The *B. stacei* and *B. hybridum* plastomes showed the same general features as the *B. distachyon* plastomes, except for a 1161-bp insertion between *psa*I and *rbc*L in the large single-copy region that corresponds to a coding sequence fragment annotated as pseudogene *rpl*23, and a deletion of an *rps*19 copy between *psb*A and *trn*H in the IRb repeat (Sancho et al., 2018). Together, these *Brachypodium* spp genomes are invaluable resources for grass evolutionary biology, polyploidy, and speciation studies.

AVAILABLE TOOLS, RESOURCES, AND DATABASES

A growing suite of tools, resources, and databases exist for the scientific community interested in embracing *Brachypodium* spp for their research. The DOE-JGI has spearheaded sequencing of additional *Brachypodium* species and accessions, as described above, to complement the Bd21 genome. Through the efforts of the International Brachypodium Initiative, the community aims to allow free access to genetics and genomics resources among *Brachypodium* research groups. To this end, a *B. distachyon* gene expression atlas was recently completed, which maps gene expression in major organs at different developmental stages (Sibout et al., 2017). The gene atlas is a new, invaluable tool to study gene function and to identify metabolic pathways. Another gene atlas project is underway, funded by the JGI Community Science Program (CSP), which will allow genome-wide identification of conserved and unique gene expression responses that occur during diverse *B. distachyon*-microbe interactions, including pathogenic and beneficial interactions (CSP503406; https://jgi.doe.gov/csp-2018-mandadi-gene-atlases-grass-microbe-interactions/).

Several functional genetics tools and resources are currently available for *Brachypodium* research at the molecular, cytogenetic, and biochemical levels (Table 3). These include a diverse collection of *B. distachyon* accessions; T-DNA and EMS mutants; and BAC, EST, and yeast two-hybrid (Y2H) libraries. Many of these are publicly available for scientific research.

Well-established *Agrobacterium tumefaciens*-based and biolistic-based transformation methodologies exist to transform *B. distachyon* using mature (Bablak et al., 1995; Vogel et al., 2006; Sogutmaz Ozdemir and Budak, 2018) and immature (Bablak et al., 1995; Draper et al., 2001; Christiansen et al., 2005; Păcurar et al., 2008; Vain et al., 2008; Vogel and Hill, 2008) embryos. Agrobacterium-based transformation of *B. distachyon* Bd21-3 can be successfully completed in ~22 to 31 weeks (Figure 3). Typically, immature embryos are dissected from developing

Table 3. Genetic and Genomic Resources for *Brachypodium*

Biologicals	Resource
Accessions	US: USDA Plant Germplasm, https://www.ars-grin.gov/npgs/ Spain: CRF-INIA, http://wwwx.inia.es/coleccionescrf/PeticionesCRFeng.asp France: INRA, https://www6.inra.fr/observatoire-vegetal_eng/Scientific-platforms/Center-for-Brachypodium-distachyon Japan: RIKEN Bioresource Center, http://epd.brc.riken.jp/en/brachypodium/bd21
T-DNA collection	https://jgi.doe.gov/our-science/science-programs/plant-genomics/brachypodium/brachypodium-t-dna-collection/
T-DNA insertion sites	https://phytozome.jgi.doe.gov/jbrowse/index.html?data=genomes%2FBdistachyon%2F&loc=Bd2%3A58875989..58904668&tracks=Transcripts%2CT-DNAInsertionSites&highlight
SNP mutant catalog[a]	https://phytozome.jgi.doe.gov/jbrowse/index.html?data=genomes%2FBdistachyonBd21_3_er%2F&loc=Bd2%3A51544007..51556076&tracks=Transcripts%2CMutant_Sites&highlight=
BAC, EST, and Y2H materials	Cao et al. (2011)
Tilling lines[b]	http://www-urgv.versailles.inra.fr/tilling/brachypodium.htm
Tilling database	http://urgv.evry.inra.fr/UTILLdb
Brachy mutant sequencing project	https://docs.google.com/spreadsheets/d/16p85FUkyHFGYygEkzBtcz2Se-scieclbIJsUd31xPCY/edit#gid=0
Key genomic websites	
Phytozome 12[c]	https://phytozome.jgi.doe.gov/pz/portal.html
Gramene	http://ensembl.gramene.org/Brachypodium_distachyon/Info/Index
DOE-JGI	https://jgi.doe.gov/our-science/science-programs/plant-genomics/brachypodium/
INRA Versailles-Grignon	Genomic Resources Center, https://www-ijpb.versailles.inra.fr/en/pave/equipes/paroi-secondaire/index.htm
Taxonomy	http://www.tropicos.org/
Pan-Genome (Brachypan)[d]	https://brachypan.jgi.doe.gov
Pan-Genome Software	GET_HOMOLOGS-EST pipeline, http://eead-csic-compbio.github.io/get_homologues/manual/
PlaNET	http://aranet.mpimp-golm.mpg.de/index.html
Gene Atlases of Grass-Microbe Interactions	https://jgi.doe.gov/csp-2018-mandadi-gene-atlases-grass-microbe-interactions/
Techniques	
Agrobacterium-mediated transformation	Vogel et al. (2006); Păcurar et al. (2008); Vain et al. (2008); Vogel and Hill (2008); Collier et al. (2016)
Hybridization/cross-pollination	https://www.ars.usda.gov/ARSUserFiles/1931/BrachypodiumCrossing.pdf http://1ofdmq2n8tc36m6i46scovo2e-wpengine.netdna-ssl.com/wp-content/uploads/2015/05/Vogel-lab-Crossing-Brachypodium-2-3-2010.pdf
Cytogenetics (FISH, GISH, CCP)	Hasterok et al. (2006); Wolny and Hasterok (2009); Idziak et al. (2011); Catalán et al. (2012)
Conferences	
4th International Brachypodium Conference	Spain, 2019; https://sites.google.com/view/brachypodium/home

[a]More than 800,000 SNP mutants are available for *B. distachyon* Bd21-3.
[b]Sodium azide mutagenesis of Bd21-3 (Dalmais et al., 2013; de Bang et al., 2018).
[c]Phytozome has resources for *B. distachyon* lines Bd21 and Bd21-3, plus three additional species of *Brachypodium* (*B. hybridum*, *B. stacei*, and *B. sylvaticum*).
[d]Brachypan represents the sequence, assembly, and annotation of the genomes of 54 *B. distachyon* accessions (Gordon et al., 2017).

seeds of vernalized Bd21-3 plants grown to maturity. To produce sufficient embryogenic callus for transformation, subculturing is performed twice on callus initiation medium at 28°C in the dark, with transfer to fresh medium every 2 weeks. Optimal calli that are well structured and yellow can be generated within ~6 to 8 weeks. These calli are then cocultured with Agrobacterium (OD_{600} = 0.6) containing the desired construct at 22°C in the dark for 3 d. The calli are then transferred to selection medium containing the bactericide Timentin (300 mg/L) and an appropriate antibiotic for the selectable marker (e.g., hygromycin B). After 2 weeks on selection medium at 28°C in the dark, healthy transformed calli are transferred to regeneration medium with the appropriate selection agents. Shoots typically emerge from the calli in ~4 weeks when incubated in a growth chamber with a 16/8-h light/dark cycle (28°C, ~65 μEm m^{-2} s^{-1}). For rooting, the shoots are transferred to MS medium with sucrose. Within ~2 to 3 weeks, the plants are ready for vernalization (to promote flowering) by cold treatment at 4°C for 2 weeks under continuous low light (~4 μEm m^{-2} s^{-1}), or the plants can be transferred to soil and propagated under long-day diurnal conditions (20/4 h, 24/18°C light/dark, ~150 μEm m^{-2} s^{-1}). T1 seeds can be harvested from vernalized plants in ~6 to 12 weeks. Bragg et al. (2015) have developed a comprehensive transformation protocol with detailed information about nutrient media compositions.

The transformation efficiency (TE), defined as the percentage of cocultivated calli that successfully produce transgenic plants, is highly dependent on several factors including the *B. distachyon* accession (e.g., Bd21 and Bd21-3), embryo stage, callus morphology, Agrobacterium strain, as well as the promoters and selectable markers used in the binary vector (Bragg et al.,

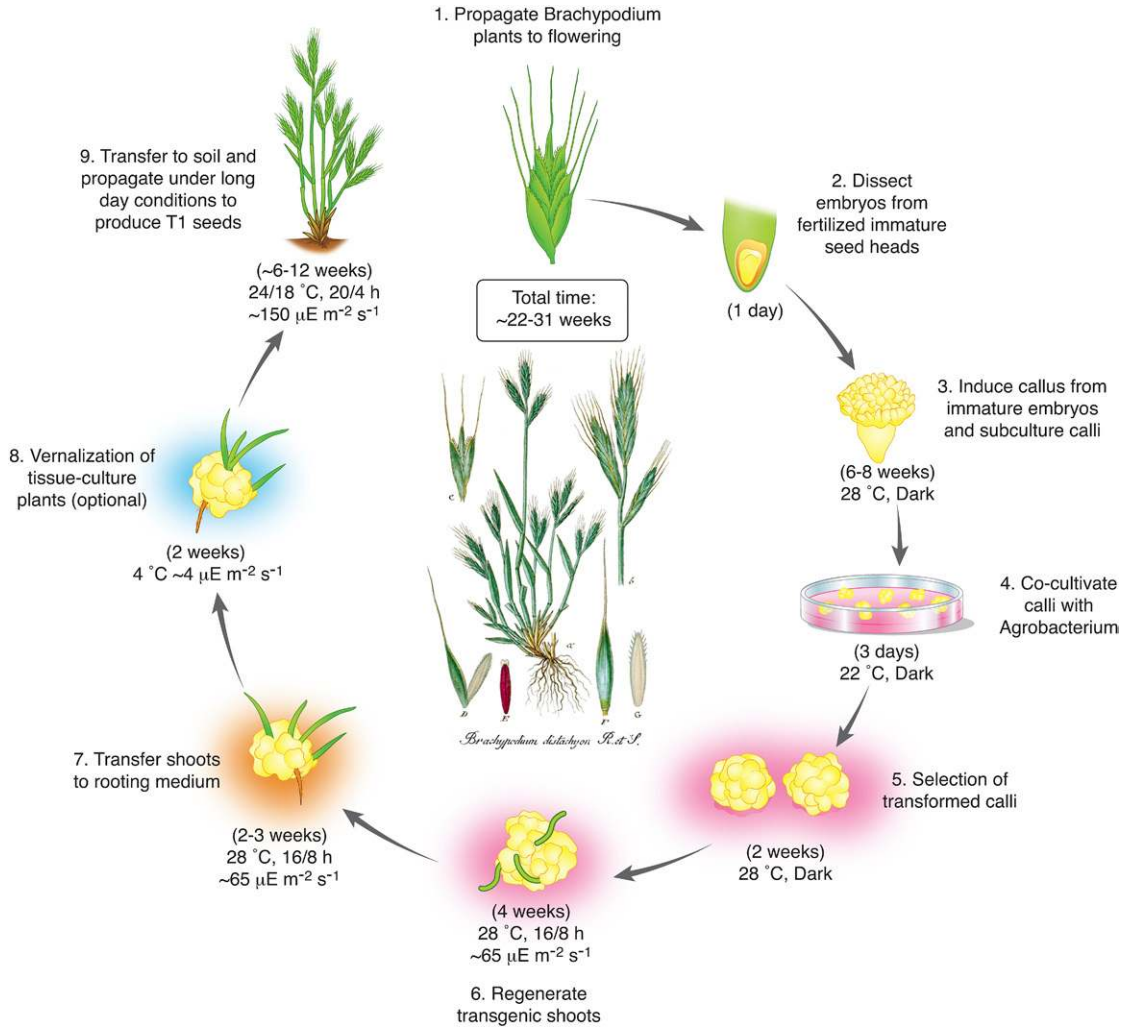

Figure 3. Overview of the *Brachypodium* Agrobacterium-Based Transformation Pipeline.

For a detailed protocol with nutrient medium composition, please refer to Bragg et al. (2015). Inset: public domain botanical illustration from Sturm (1843).

2015). When using immature embryos as the starting material, TEs of 50 to 70% can be achieved (Bragg et al., 2015). Typically, immature embryos <0.3 mm in size often produce the best embryogenic callus. It is also very important to only select and subculture compact, structured, yellow callus. Both Bd21 and Bd21-3 are amenable to transformation. However, Bd21-3 produces more structured and more yellow callus than Bd21, which is easily distinguished from inferior callus, resulting in higher TEs (Bragg et al., 2015). Therefore, Bd21-3 was used as the preferred background to develop the T-DNA knockout collection (Bragg et al., 2012; Hsia et al., 2017). Although multiple Agrobacterium strains and selection agents have been employed for *Brachypodium* transformation, Agrobacterium strain AGL1 and hygromycin selection are the materials of choice (Bragg et al., 2015). Commonly used monocot promoters, such as the maize (*Zea mays*) ubiquitin promoter, also work well in *B. distachyon*.

Rhizobium rhizogenes (formerly *Agrobacterium rhizogenes*, strain 18r12v) was recently evaluated for use with *B. distachyon* and *B. sylvaticum*, using paromomycin as a selectable marker (Collier et al., 2016). For *B. distachyon*, the TE using *R. rhizogenes* was comparable to or slightly greater (up to 60%) than that of Agrobacterium. However, the TE of *B. sylvaticum* was lower (~4–6%) irrespective of the strain or selection marker used, indicating species-specific recalcitrance within *Brachypodium* spp. A new transformation strategy using selective overexpression of two maize morphogenic regulators, *Baby boom* (*Bbm*) and maize *Wuschel2* (*Wus2*), dramatically enhanced the TE of several highly recalcitrant maize, sorghum, sugarcane (*Saccharum officinarum*), and rice lines, using either immature embryos or mature leaf (somatic) tissues as explants (Lowe et al., 2016). In this context, it may be possible to further enhance *Brachypodium* spp TE by combining the current transformation procedures with the *Bbm* and *Wus2* system for functional genetic studies.

Using Agrobacterium-based transformation, multiple libraries of insertional T-DNA mutants have been generated. BrachyTAG (John Innes Center, Norwich, UK) contains ~4117 T-DNA lines in the Bd21 background (Thole et al., 2010), but the collection is currently not accessible. The second T-DNA collection, which is accessible to users, was generated by the USDA and the DOE-JGI (https://jgi.doe.gov/our-science/science-programs/plant-genomics/Brachypodium/Brachypodium-t-dna-collection/) and consists of 23,649 T-DNA lines derived from the Bd21-3 accession, ~7145 of which were described previously (Bragg et al., 2012). Recently, using an Illumina-based NGS approach, ~21,165 T-DNA lines from this collection were sequenced and indexed (Hsia et al., 2017). This analysis identified ~31% of the *B. distachyon* genes that were tagged by the T-DNAs, either in the gene body or within 500 bp of the gene locus.

In addition to the T-DNA mutant collections, *Tnt1* retrotransposon lines (Gill et al., 2018) and chemical mutagen lines (Dalmais et al., 2013) are available for functional genetic studies. For instance, ~6000 sodium azide (NaN_3) and EMS-mutagenized M2 families in the Bd21-3 background are available as part of a BRACHYTIL collection (http://urgv.evry.inra.fr/UTILLdb), with a mutation rate of ~1 mutation per ~354 kb (Dalmais et al., 2013). These mutants are generated and maintained by the Genomic Resources Center for *B. distachyon* at INRA (Versailles-Grignon; https://www-ijpb.versailles.inra.fr/en/pave/equipes/paroi-secondaire/index.htm). Together, the *B. distachyon* mutant collections are a valuable resource for the research community to investigate various topics in monocot biology. Researchers can find a range of phenotypes in these collections. For instance, several mutants in BRACHYTIL and the T-DNA collection display morphological, anatomical, and physiological defects reflecting perturbations in plant growth, development, flowering time pathways, cell wall, wax, and reproductive pathways (Figure 4).

In addition to making these mutant lines available to the *Brachypodium* community, researchers at DOE-JGI, USDA, and INRA are spearheading an international collaborative project, with the goal of sequencing ~2000 interesting mutant lines. Several participants have initiated screening of the mutant collections for various traits of interest (https://docs.google.com/spreadsheets/d/16p85FUkyHFGYygEkzBtcz2Se-scieclbIJsUd31xPCY/edit#gid=0). Currently, ~859 user-selected mutant lines have been sequenced, and ~804,508 putative SNP mutations and ~5540 putative small deletions were identified (J. Vogel, personal communication). The mutants are indexed on the Bd21-3 (v1.1) reference genome sequence available through Phytozome.

In addition to the genetic resources, two Y2H libraries are available for *Brachypodium* researchers to conduct protein-protein interaction studies (Cao et al., 2011). These libraries represent genes/proteins expressed in short- and long-day grown shoots collected over 24 h, as well as from shoots and roots treated with eight hormones (e.g., salicylic acid, methyl jasmonate, and so on) collected 24 h after treatment. Both Y2H libraries have very good coverage of the *B. distachyon* genome, as represented by ~5.5×10^7 clones of ~1500 bp. As a proof-of-concept, an ortholog of one of the Arabidopsis nuclear factor-Y proteins (NF-YC) in *B. distachyon* (Bradi3g05270) was used as a bait to identify BdNF-YB and its chaperonin containing the T-complex polypeptide-1 (CCT) protein interactors from both Y2H libraries, along with several novel interactors, demonstrating the usefulness of the Y2H libraries (Cao et al., 2011). The compact genome, small chromosome base number (x=5), and availability of a genomic BAC library of *B. distachyon* make it amenable for cytogenetic studies across the genus. Recently, cytogenetic maps of *Brachypodium* spp were developed using fluorescence in situ hybridization (FISH), chromosome painting, and chromosome barcoding (Hasterok et al., 2015; Idziak-Helmcke and Betekhtin, 2018; and references therein). Together, these cytogenetic and biochemical tools, databases, and resources are of tremendous value for *Brachypodium* gene discovery and genetics pertaining to diverse biological processes.

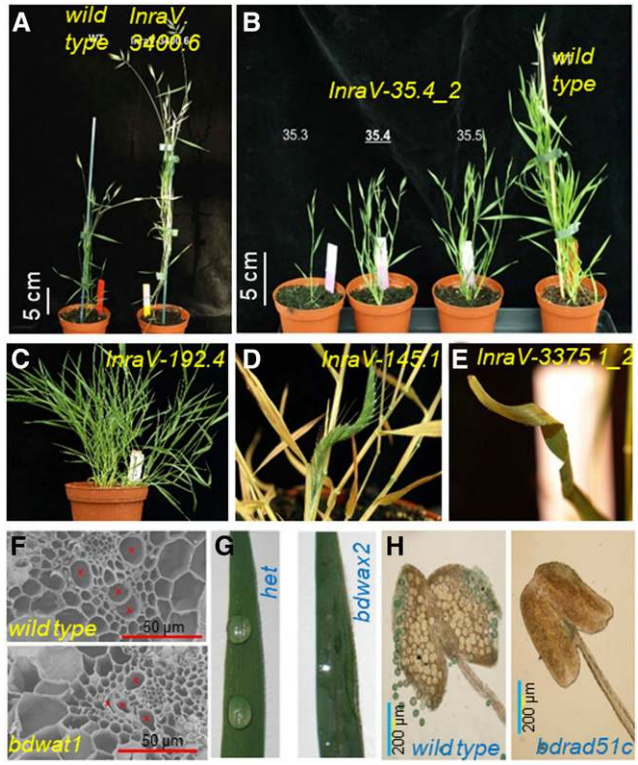

Figure 4. *B. distachyon* Mutant Resources and Selected Phenotypes.

Mutants from the BRACHYTIL sodium azide collection displaying abnormalities such as tall **(A)**, dwarf **(B)**, high tillering **(C)**, and curved panicle and leaf phenotypes (**[D]** and **[E]**). (*Images: R. Sibout, INRA, adapted from Dalmais et al. [2013], Figure 2, page 3, copyright 2013, PLoS ONE.*) Mutants from Brachy T-DNA collection showing anatomical and physiological defects in cell wall **(F)**, wax **(G)**, and reproductive tissues **(H)**. (*Images: J. Vogel, DOE-JGI, adapted from Hsia et al. [2017], Figures 3D, 4C, and 5C, pages 365–366, copyright 2017, The Plant Journal.*) Bars are approximate estimates.

HOT TOPICS IN *BRACHYPODIUM* RESEARCH

Given the availability of myriad community resources and the amenability of *Brachypodium* species for grass functional genetics, the genus has rapidly become a model for understanding diverse grass traits including, but not limited to, cell

wall biology, vernalization, evolutionary biology, root biology, host-microbe/microbiota interactions, and responses to abiotic stresses. Here, we detail some of the hot topics in *Brachypodium* research today and potentially the coming years.

Cell Wall and Saccharification

The composition and control of cell wall formation have primarily been studied in the eudicot model Arabidopsis. However, grasses are an evolutionarily separate group, and an appropriate model is needed to better study their cell walls (Coomey and Hazen, 2016). Grass cell wall carbohydrates predominantly include cellulose, β-glucans [(1,3;1,4)-β-D-glucans], and arabinoxylans. At the metabolic level, *B. distachyon* cell wall contents resemble those of field grasses (Bouvier d'Yvoire et al., 2013), making it an ideal model for studying cell wall biosynthesis. Furthermore, because of the similarity of *B. distachyon* vascular bundle anatomy to that of larger C_3 grasses, it is a good model to study vascular system composition and development—with implications toward increasing biomass and biofuel production in feedstock grasses (Matos et al., 2013). Cell wall composition depends on tissue type and developmental stage. In *B. distachyon*, variations in cell wall components (cellulose, hemicellulose, and lignin) have been studied in different organs at the seedling, elongating stem and mature stages. For instance, lignin levels are lowest in the leaf and sheath and higher in the stem, with levels increasing as the plant ages (Rancour et al., 2012; Matos et al., 2013).

Cellulose, the main cell wall component in grasses, is the major target of saccharification in biofuel production. To enhance this process, several groups have focused on understanding and modifying lignin contents and quality (Bouvier d'Yvoire et al., 2013; Dalmais et al., 2013; Trabucco et al., 2013). Marriott et al. (2014) screened a *B. distachyon* mutant population and identified 12 lines with reduced recalcitrance to lignin hydrolyzing enzymes, named *saccharification 1-12* (*sac1-12*). These lines show improved saccharification, with no major negative effects on plant size or mechanical strength. In another genetic screen, the *spaghetti1* (*spa1*) mutant was identified, which exhibited pleiotrophic phenotypes such as brittle and floppy stems. At the metabolic level, the *spa1* mutant contains reduced levels of crystalline cellulose but increased levels of xylan and lignins. Surprisingly, the mutants showed a 2-fold increase in saccharification compared with the wild type, despite the higher lignin content in the cell wall (Timpano et al., 2015). The authors suggest that perhaps the higher friability of the *spa1* biomass and the lower cellulose crystallinity could have enhanced its enzymatic digestibility. Another study used RNAi tools to knock down *B. distachyon* UDP-arabinopyranose mutase activity encoded by *BdRGP1*, *BdRGP2*, and *BdRGP3*, which resulted in altered cell wall composition, such as reduced levels of arabinose sugars, ferulic acid, and *p*-coumarates (Rancour et al., 2015). The *BdRGP1* RNAi lines also showed approximately 2-fold increases in released carbohydrate levels after enzymatic digestion (Rancour et al., 2015).

The mechanics of cellulose biosynthesis have been challenging to study, in part because the enzymes responsible become inactive when purified from the plant tissue. Recently, an indirect approach, based on studies with Arabidopsis, was used to help address this issue in *B. distachyon* (Liu et al., 2017). A fluorescent protein tag on the N-end of a cellulose synthase catalytic subunit (CESA) was used to monitor cellulose synthesis motility in *B. distachyon*. This fluorescence microscopy strategy also demonstrated that *B. distachyon* has a CESA motility practically indistinguishable from that of Arabidopsis (Liu et al., 2017).

At the transcriptional level, the regulation of cell wall formation has also been primarily described in Arabidopsis, while in grasses, these pathways have been less defined. However, with the use of *B. distachyon* as a model, an equivalent outline of cell wall transcriptional regulation in grasses is being established (Handakumbura and Hazen, 2012). As in Arabidopsis, there are several nascent polypeptide-associated complex (NAC) proteins in *B. distachyon* that regulate secondary wall biosynthesis. Of these, eight SECONDARY WALL NACs (SWNs) have been identified by phylogenetic analysis (Valdivia et al., 2013). The first six members of this family (BdSWN1-6) are orthologous to VASCULAR NAC DOMAIN transcription factors (Ohashi-Ito et al., 2010; Yamaguchi et al., 2011), while BdSWN7 and 8 are orthologous to the SND, NST1, and NST2 transcription factors in Arabidopsis (Mitsuda et al., 2005).

The mechanisms of cell wall assembly and remodeling during grain formation and the functions of cell wall proteins (CWPs) during this important process are poorly understood in grasses (Francin-Allami et al., 2016). Studies in *B. distachyon*, taking advantage of its similarities in grain development to that of other grasses, are helping to elucidate these pathways. A liquid chromatography-tandem mass spectrometry analysis of purified CWPs during three developmental stages in *B. distachyon* grain formation has helped increase our knowledge of the cell wall proteome in grasses. For instance, this study led to the identification of 111 new proteins. Many of these CWPs are likely involved in cell wall remodeling during grain formation (Francin-Allami et al., 2016). Of crucial importance for agriculture and crop research, the first comprehensive study of starch granule development in *B. distachyon* (Bd21) was performed and compared with Chinese Spring wheat and *Aegylops peregrina*. The results showed that starch granule size and development are highly similar between Bd21 and wheat, with most of the starch synthesis genes in *B. distachyon* being more similar to those in wheat than in rice (Chen et al., 2014). In summary, *B. distachyon* has proven to be an excellent model for studying grass cell wall biology, providing information that could be used to enhance food, biomass, and bioenergy-related traits in field grasses.

Flowering, Vernalization, and Cold Acclimation

An aspect of cold adaptation among temperate grasses and cereals is the requirement to undergo vernalization—a biological process that ensures that the transition to flowering will not occur before winter. Prolonged cold exposure in winter, followed by a gradual increase in daylength during spring and summer, is typically necessary for vernalization. Much of our knowledge of the underlying mechanisms of this phenomenon in monocots is based on genetic studies of "spring" and "winter" genotypes of barley and wheat, and even extrapolations from Arabidopsis. Briefly, three key genes regulate this process: *VERNALIZATION1*

(*VRN1*), *VRN2*, and *FLOWERING LOCUS T* (*FT*) (Ream et al., 2014; Woods et al., 2017). *VRN1* is upregulated by exposure to gradual cold temperatures, thus initiating a positive feedback loop involving the activation of *FT* that ensures that the flowering signal is maintained in the spring, while *VRN2* is the flowering repressor. Orthologs of the three vernalization genes were recently identified and characterized in *B. distachyon*. The vernalization mechanisms in *B. distachyon* appear to be conserved compared with wheat and barley. For instance, in a manner similar to wheat and barley, *VRN1* and *FT* are positive regulators of flowering in *B. distachyon*. Overexpression of *VRN1* and *FT* results in early flowering in *B. distachyon*, while loss of function in *VRN1* and *FT* delays this process (Lv et al., 2014; Ream et al., 2014; Woods et al., 2016).

For proper flowering induction, *VRN1* must also be repressed before cold exposure, but the mechanisms of this repression have not been studied. Recently, forward-genetic screening of *B. distachyon* mutants identified another protein, REPRESSOR OF VERNALIZATION1 (RVR1), that is required to downregulate *VRN1* before the onset of cold (Woods et al., 2017). *RVR1* encodes a protein with bromo-adjacent homology and transcriptional elongation factor S-II domain. RVR1 also appears to be conserved among other grasses and is likely required for the vernalization requirement in other pooid grasses (Woods et al., 2017). The function of VRN2 in *B. distachyon* as a flowering repressor was also recently investigated. Unlike in wheat and barley, the expression of *VRN2* is unaltered by cold and does not correlate with the levels of *VRN1* and *FT*. However, genetic studies utilizing an artificial microRNA to downregulate *VRN2* (amiVRN2) revealed early flowering phenotypes and enhanced *VRN1* and *FT* expression in amiVRN2 lines (Woods et al., 2016). By contrast, overexpression of *VRN2* resulted in delayed flowering and reduced expression of *VRN1* and *FT*, suggesting that VRN2 is the flowering repressor in *B. distachyon* (Woods et al., 2016).

Cold and freezing temperatures can severely limit grain yields and cereal productivity. As a protective mechanism, vernalization helps to delay the transition from the vegetative to reproductive phase and thus protects the floral meristem from freezing temperatures. Additionally, "hardy" cereals adapt to freezing temperatures by precisely sensing their environment and modulating multiple physiological, biochemical, and molecular parameters, resulting in a freezing tolerance (Thomashow, 1999; Dhillon et al., 2010). Some of these changes include—but are not limited to—the upregulation of cold-regulated (COR) genes, accumulation of carbohydrates, organic acids, proline, and enzymes, and modification of the cell wall, lipid membranes, and photosynthetic apparatus.

B. distachyon was recently employed to evaluate the responses of temperate cereals to cold and freezing temperatures. The freezing tolerance and cold-hardiness of seven *B. distachyon* accessions were evaluated using a strategy that included comparisons at the phenological, metabolic, and molecular levels (Colton-Gagnon et al., 2014). This study demonstrated that *B. distachyon* accessions acclimate to the cold in a manner similar to winter cereals and that they can also become freezing tolerant. For instance, when subjected to low and freezing temperatures throughout the plant, *B. distachyon* "winter" genotypes (Bd1-1, Bd18-1, and Bd29-1) showed the expected accumulation of osmoprotectants such as proline and sugars, as well as activated *COR* gene expression. Surprisingly, accessions that were thought of as "spring" genotypes (Bd2-3, Bd3-1, Bd21, and Bd30-1) also exhibited responses typical of "winter" genotypes, thus indicating that these spring genotypes are actually "facultative" genotypes that are tolerant of low temperatures, yet do not demand vernalization (von Zitzewitz et al., 2005; Colton-Gagnon et al., 2014). Nevertheless, this study demonstrated the existence of molecular and metabolic wiring that is needed for cold adaptation and freezing tolerance in *B. distachyon*. It will be interesting to extend these experiments using (wild and laboratory) perennial and annual species of *Brachypodium*. Taken together, these studies exemplify the value of *Brachypodium* as a model system to study flowering, vernalization, and cold-acclimation dynamics relevant to agronomic grasses and cereals.

Perenniality and Polyploidy

Plant perenniality is an outstanding issue in biological research aimed at identifying the mechanisms causing the allocation of resources and the subsequent life strategy (Fjellheim et al., 2014). As it pertains to applied science, with possible environmental benefits, some perennial grasses have been developed to produce cellulosic biofuels (e.g., *Miscanthus* and switchgrass), and other grasses (e.g., intermediate wheatgrass) are being studied with the goal of developing perennial cereal crops (Gordon et al., 2016). The perennial-annual transition (or its converse) has occurred several times across the evolution of the Poaceae (Kellogg, 2015a). This suggests that either an overarching mechanism controls the life cycle or that more than one mechanism directs perenniality-annuality, if perenniality is considered to be a syndrome (Fjellheim et al., 2014). Some authors have identified "rhizomatosity" genes responsible for the perennial cycle in wild species of *Oryza* and *Sorghum* (e.g., Rh2 and Rh3) and suggested that convergent mutations have resulted in the evolution of the respective annual species (Hu et al., 2003, 2011).

The genetic control of the life cycle transition likely shares molecular components with the developmental networks that control the floral transition in annual grasses; some of these mechanisms and the associated genes (vernalization, photoperiod, and flowering) have been identified in *B. distachyon* and in several cereals (Higgins et al., 2010; Fjellheim et al., 2014; Woods et al., 2016) but have not yet been analyzed in perennial grasses. Two evolutionary switches from more ancestral annual lineages to more recently evolved perennial lineages (*B. stacei* → *B. mexicanum* and *B. distachyon* → core perennial clade) have occurred along the *Brachypodium* phylogeny (Catalán et al., 2016a), framing an exceptional evolutionary scenario for the ongoing investigation of annuality/perenniality switches in the model genus (JGI CSP503006; https://jgi.doe.gov/brachypodium-model-grass-genus-bioenergy/). The construction of the *B. sylvaticum* pan-genome will allow for comparative pan-genomic studies between this perennial species and the annual *B. distachyon*.

Allopolyploidy is recognized as a major evolutionary event in angiosperms, which are all considered descendants of paleopolyploid ancestors, and with some subsequently diploidized

lineages accumulating more recent mergings (hybridization) and genome doublings in meso- and neopolyploid species (Soltis and Soltis, 2016). This is especially evident in the grass family, where allopolyploids account for 70 to 80% of all species (Stebbins, 1949; Kellogg, 2015a), including allopolyploid wheats, a primary cereal for human consumption (Marcussen et al., 2014). Despite the enormous biological and economic importance of allopolyploid grasses, relatively few studies have investigated the regulation and functional expression of homoeologous genes in the allopolyploids, mostly due to the complexity, large genome sizes, and abundance of repetitive DNA of their subgenomes (Pfeifer et al., 2014; Gordon et al., 2016).

Brachypodium again serves as an optimal model genus, in this case to explore the evolution and functional outcomes of allopolyploidy (Catalán et al., 2014; Gordon et al., 2016). Of the ~20 species in this genus, half are diploids and half are allopolyploids, with the latter showing differentially inherited (more ancestral to more recently evolved) compact subgenomes that are phylogenetically and syntenically close to those of the wheats and other allopolyploid crop and forage grasses.

The three annual *Brachypodium* species were selected as a tractable grass polyploid system to investigate the effects of subgenome dominance and origin in the phenotypic, physiological, and adaptive responses of the allotetraploid hybrids (Catalán et al., 2016a; Gordon et al., 2016; Takahagi et al., 2018) (Table 2). These species are the allotetraploid *B. hybridum* and its diploid parents *B. stacei* and *B. distachyon*, which have repeatedly and bidirectionally crossed during the last million years in the wild. Furthermore, the recent development of a synthetic fertile allotetraploid hybrid (♀*B. distachyon* Bd3-1 × ♂*B. stacei* Bsta5 and genome doubling with colchicine) that phenotypically resembles the wild *B. hybridum* (Dinh Thi et al., 2016) will allow for a comprehensive comparative functional genomic and epigenomic analysis of ancestral versus recently produced allotetraploids, the estimation of the heterosis effects, and the analysis of mechanisms that allow the neopolyploid to stabilize and speciate soon after "allopolyploid shock" (drastic subgenomic changes). This integrative genomic approach to studying polyploidy and flowering is the basis of an ongoing JGI-CSP project (CSP503504; https://jgi.doe.gov/csp-2018-chalhoub-shaping-brachypodium-polyploid-model/).

Abiotic Stress

The study of plant abiotic stress tolerance mechanisms is of great importance to agriculture, perhaps allowing breeders to develop superior crops that can withstand climate change and environmental extremes. *Brachypodium* is a useful model to study the genetics and genomics of abiotic stress responses that affect major cereals and bioenergy grasses. As discussed in earlier sections, *B. distachyon* and its congeners thrive in a wide range of temperate environments, thus providing researchers the opportunity to use this genetic diversity to study adaptation traits to local climates and abiotic stressors. *B. distachyon* abiotic stress responses also closely reflect those of wheat (*Triticum*), barley (*Hordeum*), and rye (*Secale*), owing to similar adaptation to temperate climates and common C_3 photosynthetic mechanisms (Des Marais and Juenger, 2016).

With the development of high-throughput phenotyping and imaging technologies, screening for complex traits related to stress-tolerance has advanced in the past decade (Fahlgren et al., 2015). Several drought-tolerant wild *B. distachyon* accessions were recently identified by screening leaf temperature using thermal imaging techniques (Ruíz et al., 2016). The wild accessions were collected from different geographical regions with contrasting rainfall and temperature patterns in the Iberian Peninsula. The drought-adapted genotypes had higher leaf temperatures under drought stress, consistent with increased stomatal closure and decreased evapotranspiration (Jones, 1999), compared with lines not adapted to arid conditions. This study also supports the existence of stomatal-based mechanisms governing natural variation to drought adaptation among wild *B. distachyon* genotypes. Further molecular and genetic characterization of these drought-adapted and sensitive accessions will undoubtedly help decipher the underlying mechanisms.

B. distachyon exposed to four major abiotic stresses (heat, high salinity, drought, and cold stress) exhibited transcriptome changes in 22 gene modules, 10 of which belong to defined biological processes (Priest et al., 2014). Gene coexpression network analysis further revealed that drought and salinity trigger synergistic coexpression profiles, while heat and cold trigger antagonistic coexpression profiles. Interestingly, drought and salinity stress upregulated the expression of gene modules with unique Gene Ontology categories, while cold and heat stress activated transcription factors and putative genes involved in protein folding and stability, respectively. These stress-associated gene networks are important additions to the outstanding resources available to *Brachypodium* researchers for the dissection of abiotic stress signaling processes and mechanisms (Priest et al., 2014).

Priest et al. (2014) also determined that desiccation stress downregulates *B. distachyon* genes (~5000 genes over the 24-h sample collection period), many of which are involved in regulating the cell cycle and DNA replication, suggesting a gradual shutdown of cellular growth during dehydration stress (Priest et al., 2014). Yet, at the anatomical level, the responses of *B. distachyon* to desiccation appear to contradict those of Arabidopsis and other cereals. While most plants, including Arabidopsis, show reduced cell division and cell expansion under dehydration stress, *B. distachyon* showed no changes in cell number compared with the unstressed controls. The reduced leaf size in *B. distachyon* under dehydration stress appears to primarily result from decreased cell expansion (Verelst et al., 2013). These results point toward some unique aspects of *B. distachyon* with regards to the cellular and anatomical responses to dehydration stress.

A growing number of studies have used *B. distachyon* as a model system for genetic analysis of abiotic stress responses. Among the cellular processes, protein ubiquitination is a key process that plays an important role in plant adaptation to abiotic stress. Overexpression of the wheat *Ta-Ub* gene in tobacco was found to confer resistance to drought (Guo et al., 2008) and heat (Tian et al., 2014) stresses, demonstrating the role of Ta-UB in plant stress tolerance in dicots. However, little is known about its role in monocots. Kang et al. (2016) recently demonstrated that overexpression of *Ta-Ub2* under the control of the stress-inducible *RD29A* promoter conferred transgenic *B. distachyon* plants with

enhanced drought tolerance, enhanced water retention, and increased expression of reactive oxygen species-scavenging related genes. However, constitutive expression of *Ta-Ub2* driven by the CaMV35S promoter had negative effects on growth and development in *B. distachyon*, suggesting that stress-associated gene expression needs to be carefully regulated to avoid deleterious consequences to plant growth and yield. Sun et al. (2015) recently showed that *B. distachyon WRKY36* (a group IIe WRKY) positively regulates the drought tolerance response in transgenic *Nicotiana tabacum* (tobacco). *WRKY36* expression is induced during drought stress in *B. distachyon*, and overexpression of *BdWRKY36* in tobacco enhanced its drought tolerance, as characterized by reduced ion leakage and reactive oxygen species accumulation. Together, these results indicate that dicots and monocots share conserved stress tolerance mechanisms involving Ta-Ub2 and WRKY36.

Biotic Stress

Plant diseases and pests are economically devastating to growers, causing annual crop yield losses of 40 to 100%. Much of our knowledge of the plant immune system is based on studies using the dicot model plant Arabidopsis and several other plants with developed genetic systems (e.g., *M. truncatula*, *N. attenuata*, rice, maize, and tomato [*Solanum lycopersicum*]), as well as *N. benthamiana*. Grass-microbe interaction studies have been hindered by the lack of a tractable monocot model system. Of course, rice and maize have been invaluable tools for plant biologists, but they lack several key features of model systems (Table 1). Recently, as we have noted, *Brachypodium* has risen to model status for the investigation of diverse grass-microbe and grass-insect interactions, supporting the life cycles of a remarkable diversity of fungi, oomycetes, viruses, bacteria, insects, as well as other invertebrates (Mandadi and Scholthof, 2013) (Table 4).

B. distachyon is a host to several economically important viruses, including *Barley stripe mosaic virus* (BSMV), *Brome mosaic virus*, *Panicum mosaic virus* (PMV) and its satellites (satellite panicum mosaic virus and satellite RNAs [satRNAs]), *Foxtail mosaic virus*, *Wheat streak mosaic virus*, *Sorghum yellow banding virus*, and *Maize mild mottle virus* (Cui et al., 2012; Lee et al., 2012; Mandadi and Scholthof, 2012, 2015; Mandadi et al., 2014, 2015; Fitzgerald et al., 2015; Pyle et al., 2017; Irigoyen et al., 2018; Pyle and Scholthof, 2018).

In the first demonstration of the use of *B. distachyon* for pivotal investigations of *Brachypodium*-virus interactions, a set of geographically diverse accessions were screened for BSMV resistance. Through fine-mapping, *BARLEY STRIPE MOSAIC VIRUS RESISTANCE1* was identified as a resistance (*R*) gene, opening the possibility of identifying similar *R* genes in barley and other small grains that are negatively affected by BSMV infection (Cui et al., 2012).

Using *B. distachyon*, we previously characterized genome-wide changes in transcriptome and defense signaling networks, splicing landscapes, as well as virulence determinants critical for PMV infection in grasses (Mandadi and Scholthof, 2012, 2015; Mandadi et al., 2015). Mandadi et al. (2014) identified the conserved and unique defense responses triggered by grass-virus interactions. For this, comparative analyses of *B. distachyon* and *Setaria viridis* (a C_4 grass) immune responses to eight monocot-infecting viruses were performed (Table 4). These studies underscore the importance of *B. distachyon* as a model system to advance fundamental studies of grass-virus interactions (Mandadi et al., 2014).

B. distachyon has also been used to study of viral synergism and subviral agents, a largely understudied area of plant-virus interactions. *B. distachyon* supports the synergism between PMV and its satellite virus, satellite panicum mosaic virus, as well as replication of satRNAs. Pyle and colleagues recently demonstrated that a conserved PMV satRNA (satS) undergoes dynamic 3′-end modifications in *B. distachyon* and proso millet and attenuates PMV symptoms, resulting in less severe disease symptoms (Pyle et al., 2017; Pyle and Scholthof, 2018). These findings suggest that plant-virus interactions can be studied to understand the mechanisms by which the host machinery subverts the replicative ability of virus infections in grasses and to develop defense strategies to reduce virus infections in agronomic grass hosts.

Brachypodium is an excellent model to study the genetics and genomics of biotrophic, hemibiotrophic, and necrotrophic fungal pathogens of grasses such as *Puccinia* spp, *Fusarium* spp, *Rhizoctonia* spp, *Colletotrichum* spp, and *Magnaporthe* spp (Routledge et al., 2004; Ayliffe et al., 2013; Figueroa et al., 2013; Sandoya and de Oliveira Buanafina, 2014; Fitzgerald et al., 2015), as well as bacterial pathogens (*Xanthomonas* spp) (Fitzgerald et al., 2015). *B. distachyon* is also a host plant for several insect species, including aphids and the fall army worm (*Spodoptera frugiperda*) (Table 4). Aphids, in addition to causing extensive yield losses due to feeding on plant hosts, often transmit plant viruses. For example, *Barley yellow dwarf virus* GAV strain is transmitted by *Schizaphis graminum*. Using *Brachypodium* spp, it will now be possible to dissect the molecular genetics and biochemistry of aphid-virus and aphid-plant interactions (Tao et al., 2016). Similarly, the identification of *B. distachyon* as a host for two key pest species, fall army worm and Russian wheat aphid (*Diuraphis noxia*), will allow researchers to pursue studies related to plant-insect resistance mechanisms and to dissect the roles of green leafy volatiles in grass defense signaling (Sandoya and de Oliveira Buanafina, 2014). The results of these studies should be readily applicable to the development of field-based pest control measures.

Root Biology and the Microbiome: Holistic Studies to Guide Reductionist Laboratory Science

The study of root biology in *Brachypodium* is a relatively new field, but the features of this system make it an ideal model for temperate grass root biology, as well as comparative studies with dicot plants. Because *B. distachyon* is a wild grass, it also is an excellent choice for comparative studies of root traits that might have been modified or lost during crop domestication in field grasses and cereal crops. The natural variations in root morphology and physiology among *B. distachyon* accessions result in differential responses to nutrient deficiency or osmotic stress (Pacheco-Villalobos and Hardtke, 2012).

Angiosperms exhibit diverse root architecture; however, two distinct patterns can be found among monocot and dicot

Table 4. *Brachypodium* as a Model Organism for Investigations of Plant-Microbe and Plant-Invertebrate Interactions

Microbe or Invertebrate	References
MICROBES	
Viruses	
Bamboo mosaic virus (BaMV)	Liou et al. (2014)
Satellite RNA (BaMV satRNA)	Liou et al. (2014)
Barley stripe mosaic virus (BSMV)	Pacak et al. (2010); Yuan et al. (2011); Cui et al. (2012); Mandadi et al. (2014); Cheuk and Houde (2017); Wang et al. (2017)
Barley yellow dwarf virus (BYDV-GAV)	Tao et al. (2016)
Brome mosaic virus (BMV)	Mandadi et al. (2014)
Foxtail mosaic virus (FoMV)	Mandadi et al. (2014)
Maize dwarf mosaic virus	Rosenkranz (1978)
Maize mild mottle virus (MMMV)	Mandadi et al. (2014)
Panicum mosaic virus (PMV)	Mandadi and Scholthof (2012); Mandadi et al. (2015)
Satellite panicum mosaic virus (SPMV)	Mandadi and Scholthof (2012)
Satellite RNA (satRNA)	Pyle et al. (2017); Pyle and Scholthof (2018)
Sorghum yellow banding virus (SYBV)	Mandadi et al. (2014)
Sugarcane mosaic virus (SCMV)	Rosenkranz (1978)
Wheat steak mosaic virus (WSMV)	Mandadi et al. (2014)
Bacteria	
Agrobacterium tumefaciens, crown gall, transformation[a]	Vain et al. (2008); Steinwand et al. (2013); Bragg et al. (2015); Sogutmaz Ozdemir and Budak (2018)
Azospirillum brasilense, rhizobacteria, growth promoting	do Amaral et al. (2016)
Bacillus subtilis, endophyte	Gagné-Bourque et al. (2015)
Herbaspirillum seropedicae, rhizobacteria, growth promoting	do Amaral et al. (2016)
Rhizobium rhizogenes, biocontrol, transformation[a]	Collier et al. (2016)
Xanthomonas translucens, black chaff (wheat/barley)	Fitzgerald et al. (2015)
Fungi: Ascomycetes	
Alternaria sp, leaf spot[b]	Roy et al. (2011)
Ascochyta sp, leaf spot[b]	Roy et al. (2011)
Bipolaris sorokiniana	Falter and Voigt (2014)
Blumeria graminis, powdery mildew	Draper et al. (2001)
Claviceps purpurea, ergot	Kind et al. (2017)
Cochliobolus heterostrophus, southern corn leaf blight	Falter and Voigt (2014)
Cochliobolus sativus, root rot/leaf spot	Zhong et al. (2015)
Colletotrichum cereale, anthracnose (turf)	Sandoya and de Oliveira Buanafina (2014)
Epichloë sylvatica, endophyte[b]	Meijer and Leuchtmann (1999); Brem and Leuchtmann (2001)
Fusarium culmorum, head blight	Peraldi et al. (2014)
Fusarium graminearum, head blight	Peraldi et al. (2014)
Fusarium pseudograminearum, crown rot	Powell et al. (2017)
Gaeumannomyces graminis	Sandoya and de Oliveira Buanafina (2014)
Magnaporthe grisea, rice blast	Routledge et al. (2004)
Magnaporthe poae	Sandoya and de Oliveira Buanafina (2014)
Microdochium nivale, snow mold (turf)	Rioux et al. (2017)
Neotyphodium sp, endophyte	Brem and Leuchtmann (2001)
Oculimacula spp, eye spot (barley)	Peraldi et al. (2014)
Ophiosphaerella agrostis, dead spot (turf)	Sandoya and de Oliveira Buanafina (2014)
Ophiosphaerella korrae, necrotic ringspot (turf)	Sandoya and de Oliveira Buanafina (2014)
Pithomyces chartarum	Falter and Voigt (2014)
Pyrenophora erythrospila, leaf spot[b]	Halbritter et al. (2012)
Pyrenophora teres, net blotch	Brown et al. (1993)
Ramularia collo-cygni, Ramularia leaf spot (barley)	Peraldi et al. (2014)
Sclerotinia homeocarpa, dollar spot (turf)	Sandoya and de Oliveira Buanafina (2014); Rioux et al. (2017)
Stagonospora macropycnidia	Falter and Voigt (2014)
Stagonospora nodorum, glume blotch[b]	Halbritter et al. (2012)
Zymoseptoria tritici, Septoria tritici blotch	O'Driscoll et al. (2015)
Fungi: Basidiomycetes	
Puccinia brachypodii, leaf rust[a]	Barbieri et al. (2011)
Puccinia emaculata, switchgrass rust	Gill et al. (2015)
Puccinia graminis f. sp *lolii*	Figueroa et al. (2013)
Puccinia graminis f. sp *phlei-pratensis*	Figueroa et al. (2013)
Puccinia striiformis f. sp *avenea*, stripe rust (oat)	Ayliffe et al. (2013)
Puccinia striiformis f. sp *bromi*, stripe rust (brome grass)	Barbieri et al. (2011)
Puccinia striiformis f. sp *hordei*, stripe rust (barley)	Barbieri et al. (2011)

(Continued)

Table 4. (continued).

Microbe or Invertebrate	References
MICROBES	
Puccinia striiformis f. sp *tritici*, stripe rust (wheat)	Barbieri et al. (2011); Ayliffe et al. (2013)
Puccinia triticina	Ayliffe et al. (2013)
Rhizoctonia solani, brown patch (turf)	Schneebeli et al. (2015); Rioux et al. (2017)
Ustilago bromivora, loose smut	Rabe et al. (2016)
Fungal-like organisms: oomycetes	
Pythium aphanidermatum	Sandoya and de Oliveira Buanafina (2014)
Mycrorhizal fungi	
Glomus candidum	Hong et al. (2012)
Glomus versiforme	Hong et al. (2012)
Gigaspora decipiens	Hong et al. (2012)
Gigaspora gigantea	Hong et al. (2012)
Rhizophagus irregularis (*Glomus intraradices*)	Hong et al. (2012); Jakobsen et al. (2016)
Rhizosphere	
Ascomycetes	
Chaetomium globosum	Kawasaki et al. (2016)
Emericellopsis mirabilis	Kawasaki et al. (2016)
Chitridomycetes	
Rhizophlyctis rosea	Kawasaki et al. (2016)
Actinobacteria	
Arthrobacter sp	Staley et al. (2017)
Alphaproteobacteria	Kawasaki et al. (2016)
Betaproteobacteria	
Herbaspirillum seropedicae, endophyte	do Amaral et al. (2016)
Achromobacter xylosoxidans	Staley et al. (2017)
Deltaproteobacteria	
Sorangium, cellulose degradation; fungicide and bactericide production; biocontrol agent	Kawasaki et al. (2016)
Gammaproteobacteria	
Stenotrophomonas	Tkacz et al. (2015)
Firmicutes	Kawasaki et al. (2016); Staley et al. (2017)
INVERTEBRATES	
Nematodes	
Heterodera avenae, cereal cyst nematode[c]	Kong et al. (2016)
Earthworms	
Apporectodea caliginosa, endogeic earthworm	Agapit et al. (2017)
Insects	
Diuraphis noxia, Russian wheat aphid	Azhaguvel et al. (2014); Sandoya and de Oliveira Buanafina (2014)
Laodelphax striatellus, small brown leafhopper	Zhou et al. (2016)
Schizaphis graminum, greenbug (aphid)	Azhaguvel et al. (2014); Tao et al. (2016)
Spodoptera frugiperda, Fall army worm	Brem and Leuchtmann (2001); Sandoya and de Oliveira Buanafina (2014)

Within each grouping, microbes are listed in alphabetical order. Vernacular names and functions are indicated, as known.
[a]For both *B. distachyon* and *B. sylvaticum*.
[b]Host is *B. sylvaticum*.
[c]On *B. distachyon*, *H. avenae* did not progress beyond the J2 stage.

plants (Osmont et al., 2007). *B. distachyon* roots display a typical monocot system (Chochois et al., 2012; Pacheco-Villalobos and Hardtke, 2012), which starts with a single primary root, that persists throughout the plant life cycle. Typically, two coleoptile node roots emerge during the postembryonic stage, and several stem node roots can form, depending on nutrient availability (Ingram et al., 2012; Poiré et al., 2014). At the anatomical level, *B. distachyon* roots have one epidermis layer, five cortex layers that constitute the ground tissue, and a single endodermis layer (Watt et al., 2009; Pacheco-Villalobos and Hardtke, 2012). The stele is surrounded by a single pericycle layer containing the vascular tissues arranged in a radial pattern of alternating xylem and phloem poles. The ring of xylem and phloem poles surrounds the central metaxylem at the center of the root. This vasculature pattern is representative of most monocot root types, with slight variations (Watt et al., 2009; Pacheco-Villalobos and Hardtke, 2012).

At the genetic level, there appears to be some degree of conservation in gene function and signal transduction processes between *Brachypodium* and Arabidopsis, which might facilitate functional studies in *Brachypodium*. For example, the Arabidopsis *BREVIS RADIX* loss-of-function mutant, which displays shorter roots due to impaired cell growth in the elongation zone, is rescued by genetic transformation with members of the *B. distachyon BREVIS RADIX-LIKE* gene family (Mouchel et al., 2004; Beuchat et al., 2010). Another example is the Arabidopsis loss-of-function mutant for the brassinosteroid receptor BRASSINOSTEROID INSENSITIVE1, which displays reduced root growth. A mutation in the *B. distachyon* homolog also produces plants with reduced root size, although to a lesser degree (Thole et al.,

2012; Goddard et al., 2014). Like Arabidopsis, *B. distachyon* also responds to the exogenous application of brassinosteroids; however, different root phenotypes are observed in ethylene and auxin signaling mutants (Stepanova et al., 2008; Won et al., 2011; Liang et al., 2012; Pacheco-Villalobos and Hardtke, 2012; Tao et al., 2016).

The anatomy of *B. distachyon* and wheat roots and their environmental and nutritional requirements are comparable (Watt et al., 2009; Chochois et al., 2012), but *B. distachyon* roots are much smaller and more convenient than wheat for laboratory research, since the adult root system can be contained in a small volume of soil without negatively affecting root physiology (Watt et al., 2009; Chochois et al., 2012). Furthermore, *B. distachyon* root exudates and rhizosphere microbial communities are comparable to those of wheat (Kawasaki et al., 2016) and include bacteria in the orders Burkholderiales, Sphingobacteriales, and Xanthomonadales and fungal communities belonging to the phyla Ascomycota, Chytridiomycota, and Basidiomycota (Kawasaki et al., 2016).

Arbuscular mycorrhiza (AM) symbiosis is the most common type of mycorrhizal association found in vascular plants. Unlike Arabidopsis, *Brachypodium* forms associations with AM fungi, thus allowing researchers to explore this interaction in a model system. For *B. distachyon* and many other plants, AM enhance phosphorous (P) and nitrogen (N) uptake from the soil and promote shoot growth. The *B. distachyon*-AM association results in distinct morphologies and functional associations unlike those formed by rice and *M. truncatula* roots—two model systems that have been widely used for AM symbiosis studies (Smith et al., 2003; Cui et al., 2012; Jakobsen et al., 2016). The AM associations in *B. distachyon* roots are exclusively intracellular (*Paris-type*), while AM associations in *Medicago* and rice are primarily intercellular (*Arum-type*) (Hong et al., 2012). Similarly, under P-limiting conditions, *Medicago* displayed a positive AM growth response, while *B. distachyon*, wheat, and barley showed neutral to negative growth (Jakobsen et al., 2016). Furthermore, although multiple AM fungi (e.g., *Glomus candidum*, *G. intraradices*, and *G. versiformes*) promote shoot growth and P uptake in *B. distachyon*, a few species (e.g., *Gigaspora gigantea* and *G. decipiens*) do not, suggesting a degree of functional diversity among AM associations with *B. distachyon* (Hong et al., 2012). In summary, *Brachypodium* is a suitable system to dissect grass-AM interactions and the associated morphological and functional diversity.

With its ~20 species and well-defined evolutionary biology, wild *Brachypodium* species are being used to study complex host-microbe interactions. Of particular interest will be the investigation of naturally occurring interactions of microbes with *Brachypodium* strains aimed at defining the holobiont, which may have direct applications for increasing yields in grain crops as well as pasture grasses by promoting plant health. Similarly, investigating natural populations is key in determining the coevolution of host-microbe interactions in the identification of resistance genes and innate immune responses, which may be broadly beneficial for maintaining host homeostasis. For this rapidly advancing area of study, *Brachypodium* has become a key resource for understanding the broad implications of complex interactions between host genetics and the environment.

For example, within the broad topic of host microbiota, several studies have identified bacteria and fungi in the rhizosphere that can produce biopesticides (fungicides and bactericides), as well as biostimulants that abrogate plant dysbiosis (microbial imbalance). Thus far, three dozen microbes (viruses, fungi, and bacteria) have been approved by the European Union for crop application under organic or sustainable farm practices (Matyjaszczyk, 2015). Microbiota studies of *B. distachyon* have shown that *Bacillus subtilis* B26, which is vertically transmitted in the seed, reduces the impact of drought stress in subsequent generations of plants (Gagné-Bourque et al., 2015) (Table 4). From these initial studies, soil microbiota (much like human microbiota; Yong, 2016) will have specific species associated with plant health and others that are indicative of less fit plants (Ramírez-Puebla et al., 2013). For *B. distachyon*, increased fitness is associated with bacterial volatiles from *B. subtilis* GB03, resulting in increased total biomass (Delaplace et al., 2015). These findings provide a means by which new strategies and applications may be developed to improve grasses, reduce fertilizer and water inputs, and improve soil health.

OUTLOOK: EMBRACING AND ENHANCING THE *BRACHYPODIUM* MODEL SYSTEM

For *Brachypodium* to succeed as a model system for plant biology akin to Arabidopsis (Lyons and Scholthof, 2015, 2016), a dedicated stock center for the centralized maintenance and distribution of *Brachypodium* spp seeds and reagents is vital. Currently, the burden of this task is taken on by only a few individual laboratories around the world. Although the *Brachypodium* research community is ready to share this responsibility, many laboratories do not have the required resources. Moreover, when investigators retire, lose funding, or move into new areas of study, the germplasm and other resources are oftentimes no longer available to the community. A dedicated *Brachypodium* seed/reagent stock center with long-term financial support would alleviate these concerns.

Another avenue for the improvement of resources is data deposition, access, and mining tools (Table 3). To enable uniformity in data deposition and the mining of valuable data, the community needs an integrated *Brachypodium* data resource platform that enables data mining in a manner similar to Araport (Krishnakumar et al., 2015) and PlaNET (Ruprecht et al., 2017). This latter resource, PlaNET, does host some *B. distachyon* data sets, but it is limited to the visualization and analysis of coregulated gene networks. Another, and more well-developed example, is the *N. attenuata* data hub (*Na*DH); *N. attenuata*, like *Brachypodium*, spans the wild plant-laboratory divide and has a platform for genetic, transcriptomic, and metabolomics resources (Brockmöller et al., 2017).

A unique aspect of the *Brachypodium* scientific community, compared with that of Arabidopsis, is their interest in the ecology and evolution of grasses in temperate climates. With the recent developments in plant microbiome research, *Brachypodium* is poised to serve several additional roles, including long-term ecology studies (Hæggström and Skytén, 1996), evolution of host-microbe interactions, and speciation in nature (Smiley et al., 2016). Long-term field studies of *Brachypodium*-microbe interactions,

soil fertility, and insect and nematode ecology could prove to be valuable additions to the resources used for applied, fundamental, and translational research on perennial and annual C_3 grasses and small grains.

Lastly, grant funding is a major bottleneck for the *Brachypodium* research community. Future outcomes are, of course, difficult to predict, but one thing is certain: Progress is predicated on the continued development and sharing of community resources, which requires long-term financial support from federal agencies, private institutions, and educational institutions (particularly colleges of agriculture at land-grant universities) for research, seed bank, and collaborative efforts. With the advent of NGS and phenotyping resources and the emphasis on translational and applied research in crop plants, with a few exceptions, many agencies and programs have directed their limited resources away from fundamental research and model systems to crop plants. Although it is important to ultimately translate basic research findings to field crops, something that has been extremely successful for rice and maize, vast knowledge gaps in our understanding of plant biology persist, more so in monocots. Because of this, many researchers (new and established) are currently faced with the challenge of supporting their basic research programs and highly discouraged from initiating new projects leveraging model plants. If we want to meaningfully, and sustainably, carry out our research endeavors as a scientific enterprise, we need to balance the current grant funding landscape to support fundamental research in model plants, such as *Brachypodium* spp, that will have direct, positive outcomes in our understanding of allopolyploidy, speciation, host-microbe interactions, ecology, and improvements of (wild and forage) grasses and key cereal crops.

Note from the Authors

To share your knowledge and to learn more about *Brachypodium*, international collaborative research projects, and community goals—from the landscape to the laboratory—please join us in Spain in 2019 for the 4th International Brachypodium meeting (https://sites.google.com/view/Brachypodium/home).

We apologize for not covering any other research topics or studies due to space constraints.

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