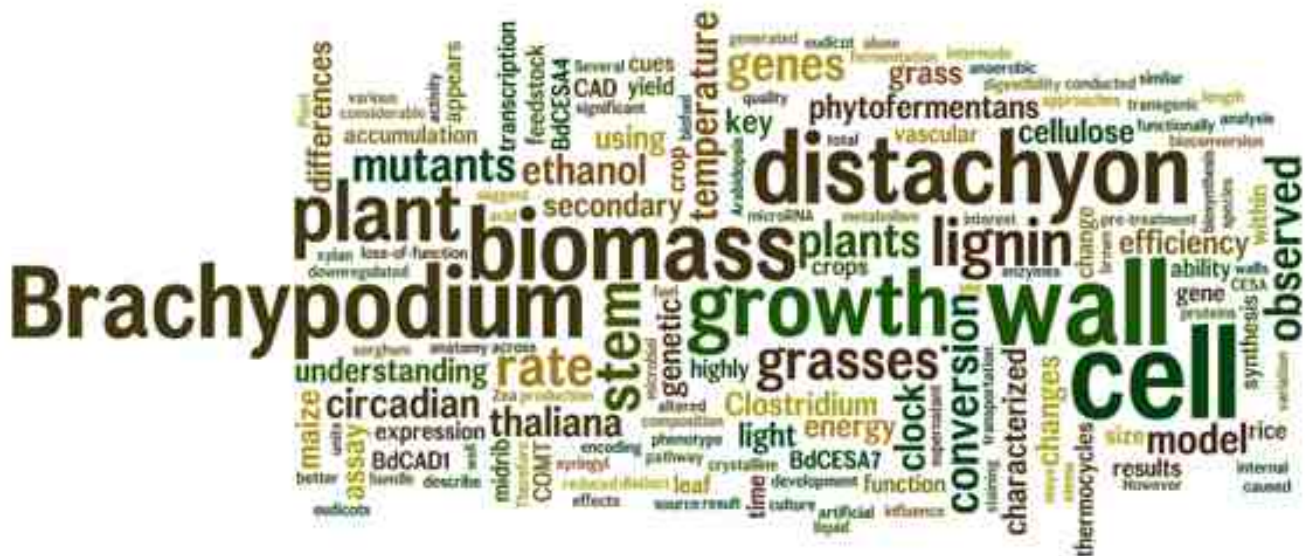
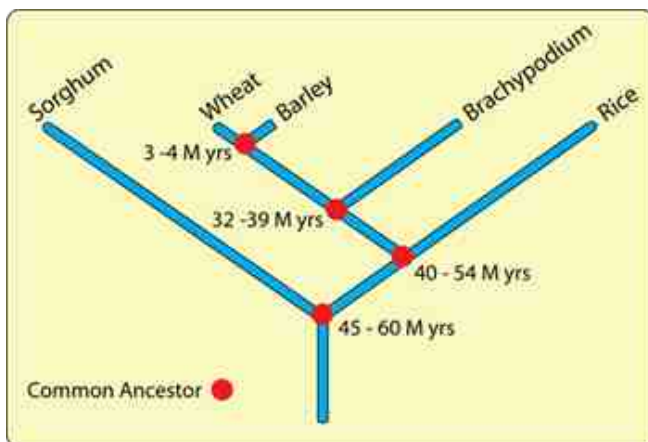


# General



## *Brachypodium distachyon*

Biologists rely on a plethora of model systems to study an exceptionally broad range of questions in biology. These model systems range from simple prokaryotes to complex animal species. attributes required for use as a model system



# General

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Cellular organisms +  
Eukaryota +  
Viridiplantae +  
Streptophyta +  
Streptophytina  
Embryophyta +  
Tracheophyta +  
Euphyllophyta +  
Spermatophyta +  
Magnoliophyta +  
Liliopsida +  
Commelinids +  
Poales +  
Poaceae +  
Bepclade +  
Pooideae +  
Brachypodieae +  
Brachypodium +  
Brachypodium distachyon



In plants, the most powerful model system (for plants) has been *Arabidopsis thaliana*\* (*Arabidopsis*), a small dicotyledonous weed with an ensemble of features that make it particularly amenable as a model system. While a staggering amount of biological knowledge has been gained from *Arabidopsis*, there has been limited translation to crop improvement due to fundamental differences between dicots and domesticated grasses, the most important crops for human existence. Rice (*Oryza sativa*) has afforded many opportunities for translational genomics for crop improvement. However, it falls short as a truly tractable monocot model system because not only is rice a large aquatic tropical species, but it is evolutionarily distant from cool season grasses.

The rapid development of *Brachypodium distachyon*

(*Brachypodium*) as a new model system reflects the serendipitous convergence of access to high throughput sequencing at U.S. Department of Energy research laboratories, the rapid emergence of interest in cellulosic ethanol production from herbaceous perennial grasses, and an appreciation for the potential that a small genome cool season grass. The wild annual grass *Brachypodium distachyon* belongs to Pooideae subfamily. This subfamily habited to Mediterranean and Middle East. The development of *Brachypodium* as a model system is inverted compared to previous model systems, with genome sequencing emerging nearly simultaneously with typical core research resources. *B. hybridum* is now considered to be an allopolyploid resulting from a natural interspecific hybridisation between *B. distachyon* and *B. stacei*, and the structure of its karyotype is to a large extent an amalgam of features observed in its putative ancestors.

The utility of the small annual grass *Brachypodium distachyon* (hereafter referred to as *Brachypodium*) as a model system for the study of the Triticeae was first discussed in a 2001 paper that pointed out that *Brachypodium* displays all of the biological, physical and genomic attributes required for use as a model system (Draper et al. 2001). The small size and rapid generation time of *Brachypodium* enables high-throughput studies. Densities of 1,000 plants/m<sup>2</sup> can be easily achieved in growth chambers or greenhouses allowing growth of large numbers of plants under controlled environmental conditions. Furthermore, *Brachypodium* is self-fertile and does not typically outcross. This feature is useful for breeding and maintaining homozygous lines for many applications that require the maintenance of large numbers of independent genotypes (e.g. mapping experiments, mutant analysis, and studies of natural diversity).

## **The *B. distachyon* genome**

Genome draft was first published Feb 11, 2010 in *Nature*, by The International *Brachypodium* Initiative. Diploid Ecotypes of *B. distachyon* has the smallest reported genome size in the Poaceae family. The diploid inbred line Bd21 was sequenced using whole-genome shotgun sequencing. A total of 25,532 protein-coding ORFs was predicted with in genome size of ~ 355 Mbp. The *Brachypodium* genome contains 21.4% retrotransposon sequences of the genome, compared to 26% in rice, 54% in sorghum, and more than 80% in wheat genome. According to *Brachypodium* genome report former was diversified from wheat 32–39 Million year ago, rice 40–53 Million year ago, and from sorghum 45–60 Million year ago, while genome was duplicated about 56–72 Million year ago, even before the diversification of the grasses.

The genome assemble database for *Brachypodium* is in public domain and can be access from [Brachypodium.org](http://www.brachypodium.org) (<http://www.brachypodium.org/>). The foresaid server offers all versions of BLAST against *Brachypodium* genome assemble as well as one can manually download and use these genome assemblies release on their own local network. Further these analytical suits and accession are quite easily accessible on common plant genome database servers like NCBI, EnsemblePlants and PlantGDB.

## **Relationship to Other Grasses**

The phylogenetic relationship between the genus *Brachypodium* and the other grasses has been evaluated a number of times with increasing amounts of data. *Brachypodium* has consistently been placed approximately halfway between rice and wheat. Reports based on internal transcribed spacer (ITS) and 5.8 s rDNA sequence (Hsaio et al. 1994), genomic RFLP and RAPD markers (Catala'n et al. 1995), and ITS sequence plus the chloroplast *ndfH* gene (Catala'n and Olmstead 2000) all placed *Brachypodium* between rice and a clade containing temperate grains like wheat, barley and Secale.

## **Growth Requirements and Flowering**

One of the strengths of *Brachypodium* as a model system is its ease of culture under laboratory conditions. This is in contrast to rice whose demanding growth requirements, large size, and long generation time are a barrier to many researchers. Generally, *Brachypodium* can be grown in growth chambers or greenhouses used for *Arabidopsis*, wheat or barley. Our standard conditions for growth chambers are: 20 h light: 4 h dark photoperiod, 24°C during the day and 18°C at night with cool-white fluorescent lighting.

## **Transformation and T-DNA Tagging**

Efficient transformation is a keystone of any modern model system and the extremely efficient floral dip method of *Arabidopsis* transformation is a key reason behind the tremendous success of this dicot model. Fortunately, *Brachypodium* has proven to be very responsive to in vitro culture and current transformation efficiencies are on par with rice. Embryogenic callus is a preferred target for transformation due to its highly regenerable nature. Thus, the development of a method for the induction of embryogenic callus from *Brachypodium* seeds and the regeneration of fertile plants from embryogenic callus was a major step toward developing *Brachypodium* transformation.

# General

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Both particle bombardment and *Agrobacterium tumefaciens* have been used to transform *Brachypodium* and each offers unique advantages and disadvantages. *Brachypodium* has proven amenable to *Agrobacterium* mediated transformation and the first report of *Agrobacterium*-mediated transformation was published in 2006 (Vogel et al. 2006a). In this study, 16 polyploid accessions and three diploid accessions were evaluated for transformability. The highest transformation efficiency (14% of the callus pieces co-cultivated with *Agrobacterium* produced transgenic plants) was achieved with the polyploid line.

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

## Germplasm Resources and Natural Diversity

A number of germplasm collections and several inbred lines are available to researchers. The USDA National Plant Germplasm System (NPGS) has almost 30 accessions that are freely available ([www.ars-grin.gov/npgs/](http://www.ars-grin.gov/npgs/)). These collections were made many years ago and are population samples rather than inbred lines. Detailed passport data about these accessions can be found at ([www.brachypodium.org](http://www.brachypodium.org)).

Variability for several interesting morphological traits also has been observed among these diploid accessions including: vernalization requirements, flower initiation by long days, size, seed pubescence, and inflorescence architecture. The level of molecular and phenotypic diversity observed in *Brachypodium* collections indicate that *Brachypodium* can be used to identify genes responsible for natural variation in economically important traits. Furthermore, the level of polymorphism observed in SSR markers indicates that the generation of molecular markers will not be a limitation.

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## The applications of Brachypodium research

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. The exponential increase in publications using Brachypodium as a model system indicates that Brachypodium is on a very strong trajectory, similar to Arabidopsis in the early years. 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.

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. 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

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.

