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# Perspectives for study of Brachypodium distachyon as a model object for genetic and biochemical study in Kazakhstan

**Abstract**

The species so far the most used as model plant, Arabidopsis thaliana, has provided a wealth of useful information and valuable tools for understanding plant biology. However, Arabidopsis is too phylogenetically distant from the temperate cereals to be used as a model system for cereal-specific metabolisms and responses to the environment. For this reason, Brachypodium distachyon has been recently proposed as a new model for grasses and temperate cereals. It has many qualities that make it an excellent model organism for functional genomics research in temperate grasses, cereals like barley and wheat, and dedicated biofuel crops such as switchgrass. These attributes include small genome, which is fully sequenced, a small physical stature, self-fertility, a short lifecycle, simple growth requirements, and an efficient transformation system.

*Key words*: model plant object, genomics, metabolomics, sequencing, genome, phylogenetics, morphology, biofuel, biochemistry, lifecycle, transformation, wheat, rice, barley, switchgrass.

## Main part

Grasses provide the bulk of human nutrition, and highly productive grasses are promising sources of sustainable energy [1]. The grass family (Poaceae) comprises over 600 genera and more than 10,000 species that dominate many ecological and agricultural systems [2, 3].

So far, genomic efforts have largely focused on two economically important grass subfamilies, the Ehrhartoideae (rice) and the Panicoideae (maize, sorghum, sugarcane and millets). The rice [4] and sorghum [5] genome sequences and a detailed physical map of maize [6] showed extensive conservation of gene order [7] and both ancient and relatively recent polyploidization. Most cool season cereal, forage and turf grasses belong to the Pooideae subfamily, which is also the largest grass subfamily. Some of the representatives are presented on Figure 1.
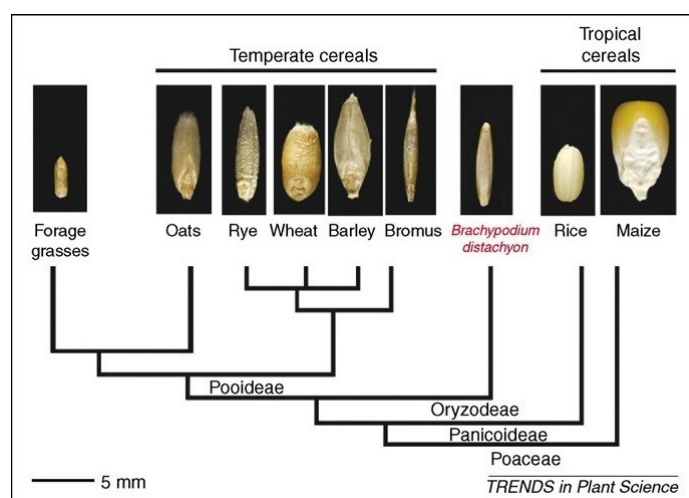


**Figure 1** – Phylogenetic relationships between Brachypodium and the small grain cereals. From: M.Opanowicz, Ph.Vain, J.Draper, D.Parker, J.H.Doonan. Brachypodium distachyon: making hay with a wild grass. Trends in Plant Science, 2008, Vol. 13, No. 4, pp. 172-177.

The genomes of many pooids are characterized by daunting size and complexity. For example, the bread wheat genome is approximately 17,000 Mb and contains three independent genomes [8]. This has prohibited genome-scale comparisons spanning the three most economically important grass subfamilies. Brachypodium, a member of the Pooideae subfamily, is a wild annual grass endemic to the Mediterranean and Middle East that has promise as a model system [9].

Brachypodium P. Beauv is a genus of about 26 annual or perennial bunch grasses, and is propagated through seed or rhizomes. Brachypodium is named for its features. Brachy is Greek for «short» and podion is Greek for «little foot». «Short footed» refers to the small pedicels of the spikelets. Flimsy upright stems form tussocks. Flowers appear in compact spike-like racemes with 5-25 flowers on each short-stalked spikelet in summer. Leaves are flat or curved [10].

According to an October 18, 2010 issue of «Nature Online» Laura Longo, an archeologist at University of Siena in Italy found evidence of Brachypodium and cattail residues on prehistoric human grinding tools dated 28,000 years ago from Bilanco in central Italy [11].

Although Brachypodium distachyon has little or no direct agricultural significance, it has several advantages as an experimental model organism for understanding the genetic, cellular and molecular biology of temperate grasses.

Brachypodium distachyon has been selected as a model organism in part because it is the only annual member, similar to crop grasses of agronomic importance.

The relatively small size of its genome makes it useful for genetic mapping and sequencing. At about 272 million base pairs and with five chromosomes, it has a small genome for a grass species (Table 1).

**Table 1** – Comparison of Brachypodium distachyon with other cereals and Arabidopsis thaliana

|  | Brachypodium distachyon | Arabidopsis thaliana | Triticum aestivum | Zea mays | Oryza sativa | Hordeum vulgare |
|---|---|---|---|---|---|---|
| Number of chromosomes | 10 (2 n) | 10 (2 n) | 42 (2 n) | 20 (2 n) | 24 (2 n) | 14 (2 n) |
| Genome size, 1C | 300Mb | 164Mb | 16 700Mb | 2400Mb | 441Mb | 5000Mb |
| Reproductive strategy | Self-fertilizing | Self-fertilizing | Self-fertilizing | Cross-pollination | Self-fertilizing | Self-fertilizing |
| Life cycle, weeks | 10-18 | 10-11 | 12 (spring wheat) 40+ (winter wh.) | 10+ | 20-30 | 16+ |
| Height at maturity, m | 0.3 | 0.2 | Up to 1 | Up to 2 | 1.2 | Up to 1.2 |
| Transformation | Facile | Facile | Possible | Facile | Facile | Facile |
| Growth requirements | Very simple | Very simple | Simple | Simple | Specialized | Simple |

The small Brachypodium genome has already been invaluable as an aid for cloning wheat and barley genes of agronomic importance. Early studies had indicated that gene order (or synteny) is largely conserved between Brachypodium and the small grain cereals [12-14], and this synteny was instrumental in the characterization of the wheat gene Ph1 [15] and the barley gene Ppd-H1 [16]. Ph1 is particularly interesting in the context of genome evolution. This complex locus is required for correct pairing of homologous chromosomes in hexaploid and tetraploid wheats, but the absence of allelic variation at the Ph1 locus precluded conventional mapping. Markers (based on synteny between the wheat genome and the smaller genomes of rice and B. sylvaticum) were used to map deletion lines and to pinpoint functional aspects of the Ph1 locus [15, 16]. Sequence analysis of the region revealed a complex of repeated versions of a novel cyclin-dependent protein kinase, the functional implications of which are currently being investigated.

Brachypodium distachyon's small size and apid life cycle are also advantages.

For early-flowering accessions it takes about three weeks from germination to flower (under an appropriate inductive photoperiod). The small size of some accessions makes it convenient for cultivation in a small space. As a weed it grows easily without specialized growing conditions (Fig. 2).
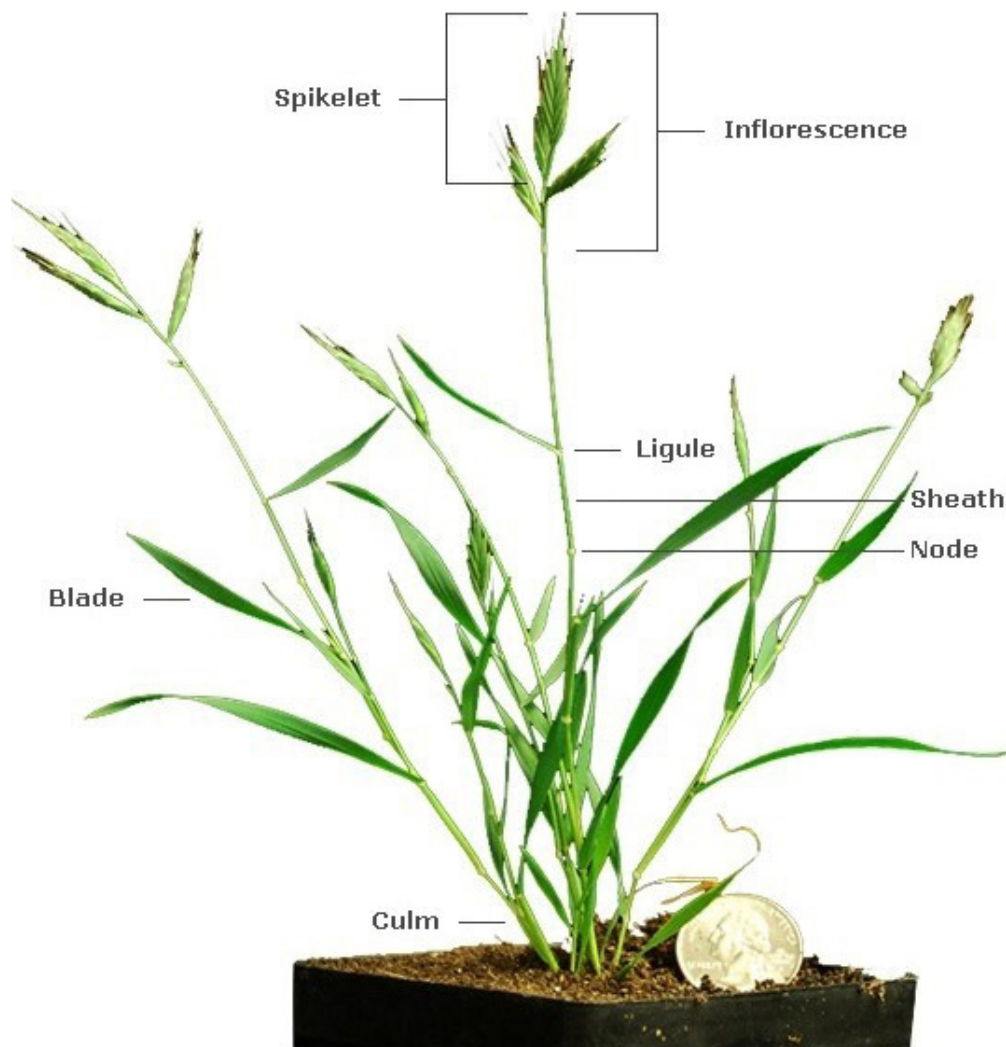


**Figure 2 –** Model object Brachypodium distachyon, commonly called purple false brome, is a grass species native to southern Europe, northern Africa and southwestern Asia east to India. It is related to the major cereal grain species wheat, barley, oats, maize, rice, rye, sorghum, and millet. From: Botanica systematica (http://www.homolaicus.com/scienza/erbario/utility/botanica_sistematica), http://bti. cornell.edu/home.php?page=Brachypodium&section=AnatomyClassification

Brachypodium distachyon may also prove itself as a model for metabolomics. Short genes likely beget relatively small metabolomes, and the absence of any marked drug effects from this simple grass makes it ideal for use in experiments to test teasing of plants to produce immunnologically customized, secondary metabolites as putative therapy for specific and varied human medical problems. The secondary metabolites that plants produce for their own purposes have underlined herbal and nutritional treatments for millenia. Many of these compounds, most notably resveratrol, are recognized as "phytoalexins" and have been and are the subject of ongoing "translational research" and clinical investigations [17, 18]. For instance, their effects against fungi like Magnaporthe grisea may provide

Homo sapiens, as well as Brachypodium distachyon, a competitive edge for survival [19]. New is the use of computing power sufficient for revealing the complexity of the plant's chemical signatures under particular environmental assaults. With the derivative metabolomics, it may even now be possible to expose previously unappreciated inter-kingdom metabolic networks connecting the stress-specific immunochemical output of plants to human biochemical byways for containing disease and promoting health.

Since 2005, the International Brachypodium Initiative has promoted the development of resources in Brachypodium [20]. The BrachyTAG project is part of the International Brachypodium Initiative, and aims to identify and tag key genes involved in all aspects of plant development, reproduction, environmental adaptation, biomass and yield of cereals and grasses.

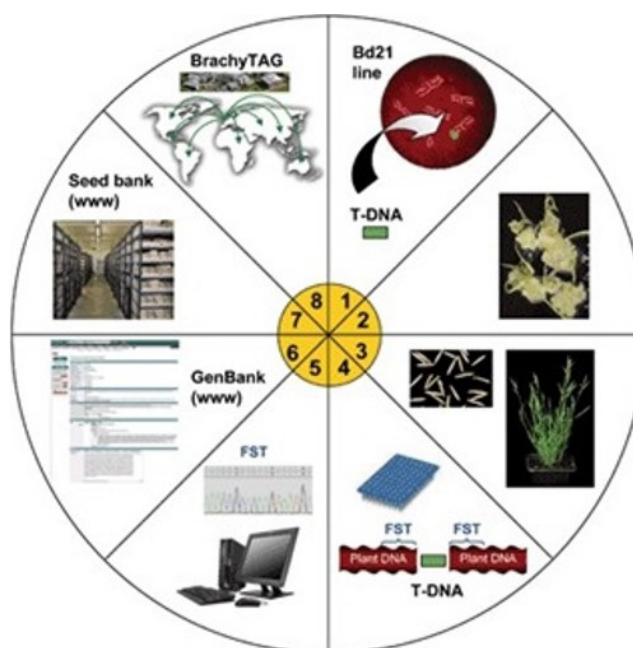This knowledge will allow scientists to unlock the potential of wheat and grasses for food and energy (Fig. 3).



**Figure 3.** Overview of the BrachyTAG project (John Innes Centre). Notes: 1. Insertion of a T-DNA (tag) in Brachypodium plant genome; 2. Regeneration of T-DNA plant; 3. Seed production from T-DNA plant; 4. Retrieval of plant sequences (FST) flanking the T-DNA insert; 5. Sequencing and analysis of FST; 6. Release of FST sequence to on-line public databases (GenBank); 7. Seed collection of T-DNA lines available online (www.BrachyTAG.org); 8. Dissemination of seeds with T-DNA insert to the scientific community.

The main output of the BrachyTAG project is to produce, characterise, preserve and distribute a collection of Brachypodium plants (also called "T-DNA insertion lines") containing a "gene tag" and to make the seed and DNA sequence available to the world. Some genes with a known or putative function have already been tagged and the corresponding T-DNA plant lines have been disseminated to the international scientific community since June 2008.

Brachypodium distachyon is a strategic model to increase our understanding of cereal crops (such as wheat) and biomass grasses (such as switchgrass or Miscanthus, research project No. 5325-21000-017-00 "Genetic Foundations for Bioenergy Feedstocks", sponsored by the United States Department of Agriculture). The BrachyTRAP project aims to identify and characterize "promoter" elements, which are driving the expression of key genes involved in all aspects of plant development, reproduction, environmental adaptation, biomass and yield of cereals and grasses. The BrachyGEN community resource was developed in 2012/2013 and complements the BrachyTAG and BrachyTRAP projects aiming at understanding the function of key genes involved in all aspects of plant development, reproduction, environmental adaptation, biomass and yield of cereals and grasses.

During the past decade, Brachypodium distachyon has emerged as an attractive experimental system and genomics model for grass research. Numerous molecular tools and genomics resources have already been developed. Functional genomics resources, including mutant collections, expression/tiling microarray, mapping populations, and genome re-sequencing for natural accessions, are rapidly being developed and made available to the community. Large collections of T-DNA-tagged lines are being generated by a community of laboratories in the context of the International Brachypodium Tagging Consortium [21]. To date, >13 000 lines produced by the BrachyTAG programme and USDA-ARS Western Regional Research Center are available by online request. The utility of these mutant collections is illustrated with some examples from the BrachyTAG collection at the John Innes Centre-such as those in the eukaryotic initiation factor 4A (*eIF4A*) and brassinosteroid insensitive-1 (*BRI1*) genes [22, 23].

The diploid inbred line Bd21 was sequenced using whole-genome shotgun sequencing. The ten largest scaffolds contained 99.6% of all sequenced nucleotides. Comparison of these ten scaffolds with a genetic map detected two false joins and created a further seven joins to produce five pseudomolecules that spanned 272 Mb, within the range measured by flow cytometry [24, 25]. The assembly was confirmed by cytogenetic analysis and alignment with two physical maps and sequenced BACs. More than 98% of expressed sequence tags (ESTs) mapped to the sequence assembly, consistent with a near-complete genome.

Compared to other grasses, the Brachypodium genome is very compact, with retrotransposons concentrated at the centromeres and syntenic breakpoints. DNA transposons and derivatives are broadly distributed and primarily associated with gene-rich regions. A series of other mutants exhibiting growth phenotypes are also available. These examples highlight the value of *Brachypodium* as a model for grass functional genomics.

This has led to the development of highly efficient transformation [26, 27], germplasm collections [28, 29], genetic markers [30], a genetic linkage map [31], bacterial artificial chromosome (BAC) libraries [32, 33], physical maps [34], mutant collections, microarrays and databases that are facilitating the use of Brachypodium by the research community. Comparison of the Brachypodium, rice and sorghum genomes shows a precise history of genome evolution across a broad diversity of the grasses, and establishes a template for analysis of the large genomes of economically important grasses, such as wheat [24, 25]. There are interdisciplinary consortium and research groups working on the Brachypodium at the leading universities of the world, like in the University of Massachusetts Amherst with the International Brachypodium Conference to be held in summer, 2015.

Currently, biochemical, morphometric and genetic studies on Brachypodium distachyon responses to biotic and abiotic stress (as model object for better understanding of mechanisms underlying Kazakhstani wheat varieties) are held at the Department of Molecular Biology and Genetics, al-Farabi Kazakh National University. Preliminary results were presented on the 1st Int. Brachypodium Conference, June 2013 in Italy [35] and in SEB 2013, July 2013 [36] in Spain.

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