Trends in Plant Science

CellPress OPEN ACCESS

Feature Review

Brachypodium: 20 years as a grass biology model system; the way forward?

Robert Hasterok,[1,*] Pilar Catalan,[2,3] Samuel P. Hazen,[4] Anne C. Roulin,[5] John P. Vogel,[6,7] Kai Wang,[8] and Luis A.J. Mur[9,10,*]

It has been 20 years since *Brachypodium distachyon* was suggested as a model grass species, but ongoing research now encompasses the entire genus. Extensive *Brachypodium* genome sequencing programmes have provided resources to explore the determinants and drivers of population diversity. This has been accompanied by cytomolecular studies to make *Brachypodium* a platform to investigate speciation, polyploidisation, perenniality, and various aspects of chromosome and interphase nucleus organisation. The value of *Brachypodium* as a functional genomic platform has been underscored by the identification of key genes for development, biotic and abiotic stress, and cell wall structure and function. While *Brachypodium* is relevant to the biofuel industry, its impact goes far beyond that as an intriguing model to study climate change and combinatorial stress.

Highlights

Brachypodium offers a unique combination of biological, ecological, phylogenetic, and cytological features. Many features of this model genus depend on its nondomesticated status.

These features are combined by well-developed resources such as diverse germplasm collections, sequenced genomes and pangenomes, and extensive mutant collections.

Brachypodium provides a solid foundation for diverse research on plant biodiversity, speciation, genome organisation at the cytomolecular level, cell wall organisation, response to abiotic and microbial interactions, including beneficial associations. These features are relevant to understanding ecological responses to environmental change.

Thus, despite improvements in genomic sequencing technologies and gene editing technologies in large-genome cereals, *Brachypodium* retains its value as a model nondomesticated grass genus.

Brachypodium: how it started

At the turn of the millennium, the plant science world was different. *Arabidopsis thaliana* (arabidopsis) was one of the major discovery workhorses based on the use of forward and reverse genetic approaches [1] and the genomes of arabidopsis and rice (*Oryza sativa*) were sequenced around that time [2,3]. However, the strategies used would not lend themselves to the sequencing of larger genome cereals that typically are rich in repetitive DNA. *Brachypodium distachyon* is phylogenetically related to the temperate cereals but has a small and compact nuclear genome with a low amount of repetitive DNA, which led to it being suggested as a genomic bridge to facilitate the assembly of larger genome cereals and as a functional genomics platform [4]. Driven by support from the US Department of Energy, the *B. distachyon* whole genome sequence was generated and it emerged as a bioenergy model, spurring significant interest in cell wall research [5]. The high collinearity of grass genomes [6] is in contrast to dicots, which do not show the same level of macrosynteny [7], and allowed *B. distachyon* to make a significant contribution to genome assembly in a range of species and was pivotal for the functional annotation of genes and proteins [8]. Thus, the *B. distachyon* **reference genome** (see Glossary) was an indispensable resource for mapping and cloning many important genes from the complex polyploid wheat genome (e.g., [9–13]). Moreover, the highly accurate *B. distachyon* genome played a vital role as a scaffold for the assembly of the barley [14] and wheat family (e.g., *Triticum urartu*-2x (AA) [15], *Aegilops tauschii*-2x (DD) [16], *Triticum durum*-4x (AABB) [17], *Triticum aestivum*-6x (AABBDD) [9,18] genomes. Since 2010, *B. distachyon* genomic resources have been used for comparative genomics, functional genomics, and comparative developmental analysis in, for example, other grasses (foxtail millet [19], sugar cane [20], *Panicum hallii* [21]).

Interest in *Brachypodium* has now expanded to the entire genus [8]. *B. distachyon* was initially considered to exist as three cytotypes of 2n = 10, 20, and 30 chromosomes, but these were later classified as three different species, respectively, *B. distachyon*, *Brachypodium stacei,* and *Brachypodium hybridum* [22]. The latter was shown to have arisen from allotetraploidisation events

[1]Plant Cytogenetics and Molecular Biology Group, Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Katowice 40-032, Poland
[2]Department of Agricultural and Environmental Sciences, High Polytechnic School of Huesca, University of Zaragoza, Huesca 22071, Spain
[3]Grupo de Bioquímica, Biofísica y Biología Computacional (BIFI, UNIZAR), Unidad Asociada al CSIC, Zaragoza E-50059, Spain
[4]Biology Department, University of Massachusetts Amherst, Amherst, MA 01003, USA
[5]Department of Plant and Microbial Biology, University of Zürich, Zürich 8008, Switzerland

between *B. distachyon* and *B. stacei*. Now multi-omic resources are available for other *Brachypodium* species offering opportunities to investigate a range of processes, such as perenniality, that are not easily studied in other systems [8]. Therefore, 20 years after the publication of the first paper to systemically assemble a case for *B. distachyon* as a model grass [4], ongoing developments are underlying the continuing relevance of the genus to plant science. In this review, we highlight some significant avenues of ongoing *Brachypodium* research and concentrate on insights being gained into genotypic diversity, responses to environmental stress, and the structure and function of the cell wall. These subjects are directly relevant to research assessing the potential impact of climate change or responding to this challenge.

[6]DOE Joint Genome Institute, Berkeley, CA 94720, USA
[7]University California, Berkeley, Berkeley, CA 94720, USA
[8]School of Life Sciences, Nantong University, Nantong 226019, Jiangsu, China
[9]Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Edward Llwyd Building, Aberystwyth SY23 3DA, UK
[10]College of Agronomy, Shanxi Agricultural University, Taiyuan 030801, Shanxi, China

*Correspondence:
robert.hasterok@us.edu.pl (R. Hasterok) and lum@aber.ac.uk (L.A.J. Mur).

Brachypodium: a model to study biodiversity and speciation in grasses

The *Brachypodium* genus is quite small (~18 recognised species) and has emerged as one of the best test beds for investigating natural diversity, evolution, and speciation in plants [23] and, crucially, environmental adaptation (Box 1). The small genome sizes of the various *Brachypodium* taxa with low repetitive contents [22] have facilitated the assembly and annotation of reference genomes for nearly one-third of its species (https://phytozome-next.jgi.doe.gov/).

Further, **pangenomes** for the three annuals, *B. distachyon, B. stacei,* and their derived allotetraploid *B. hybridum*, as well as the perennial *Brachypodium sylvaticum*, have been generated, which

Box 1. Exploiting genetic diversity of *Brachypodium* to study locally adapted traits

With grasses covering more than 40% of the world land area, they naturally play a pivotal role in the function of a range of agroecosystems. Hence, characterising how this ubiquitous plant family adapt to challenging environmental conditions is of prime interest for fundamental and applied research. As it occurs naturally in the circum-Mediterranean region [23,31], which is a recognised hotspot for climatic change [142], *Brachypodium* is well placed to be the pre-eminent model to study how grasses may adapt to arid and oligotrophic habitats. After years of collaborative work, the genotypic tools are available to do this with a large diversity panel of genotypes spanning from Spain to Iraq (Figure I) [27,36–78], enabling better comprehension of population structure in *B. distachyon* and its drivers. As of today, five genetic clades whose divergence backdates to the upper Pleistocene have been described (Figure I) [37]. By exploiting niche modelling, genome-wide association studies, and genome-wide scans of selection analyses, recent works have defined how selection shaped genetic diversity at multiple temporal and spatial scales [31,143–145] and identified genes under selection and involved in climate adaptation [143–145]. This ever-growing diversity panel will undoubtedly allow further dissection of how gene–environment interactions shape fitness-related traits in *B. distachyon* and, therefore, more widely in grasslands. For instance, there is still an active debate on whether the difference in flowering times is an important driver of *B. distachyon* population structure and the relative contribution of stressors such as drought tolerance [37,38,78,146]. In this context, exploring diversity of flowering genes [147–151] in the diversity panel will allow testing of whether flowering time evolved through neutral processes or selection. Newer possible research avenues could be the relationship between stomata development [152,153] and their relationship to the environment, which could define stress resilience traits of agronomical interest.

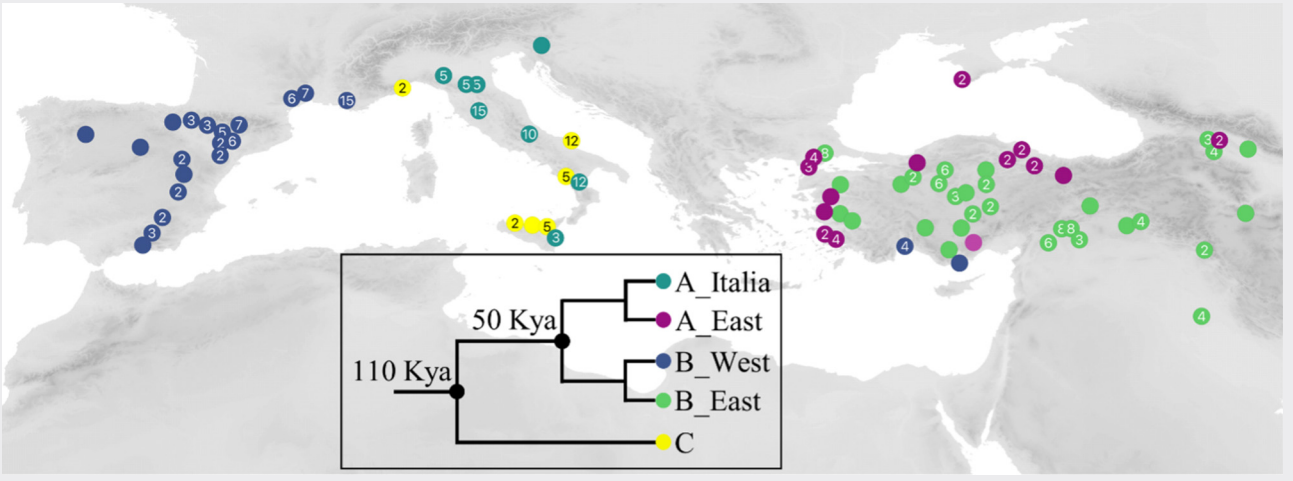

Figure I. *Brachypodium distachyon* as a model to study adaptation to local environments. Distribution and phylogeny of the sequenced genotypes (modified from [37]). Numbers indicate how many accessions were sampled per site (dots without numbers indicate that one accession was sampled from that site).

represents an enormously valuable resource in defining the origins and consequences of plant polyploidy and perenniality [24]. Important features such as **dysploidy**, **recurrent allopolyploidisation,** and extended **reticulation** [25] have made this genus an ideal model to identify the known and unknown diploid progenitor genomes of its polyploid species. Research into these could ultimately define generic features governing these processes. The basis for such studies are six polyploid species and cytotypes (*B. hybridum*-4x, *Brachypodium mexicanum*-4x, *Brachypodium boissieri*-6x, *Brachypodium retusum*-4x, *Brachypodium phoenicoides*-4x, *Brachypodium rupestre*-4x), which were shown to harbour three known and four **orphan ('ghost') progenitor subgenomes** (Figure 1A) [26]. Most important are reference genomes of accessions of the three annual *Brachypodium* species, *B. distachyon* Bd21, *B. stacei* ABR114, and *B. hybridum* ABR113 [5,27], which correspond to type specimens with distinctive morphological features and ecological traits [22,23]. Research has allowed their divergence, hybridisation, and polyploidisation events to be defined in other genera/lineages. This has included the analysis of artificially recreated autotetraploid progenitor species and allotetraploid *B. hybridum*-like lines [28]. Pangenomic and coalescence dated analyses detected multiple and bidirectional origins for the wild allotetraploid *B. hybridum*, with ancestral [*B. distachyon*-type (D) plastotypes] and recent [*B. stacei*-type (S) plastotypes] allotetraploids, originating 1.4 and 0.4 Ma, respectively (Figure 1B) [27]. These two different hybridisation and genome doubling events reflected a gradual polyploid genome evolution. Recent hybrids showed progenitor species subgenomes highly collinear with current *B. distachyon* and *B. stacei* genomes and old hybrids showing few rearrangements. This would not align with the 'polyploid genomic shock' hypothesis where allopolyploidisation has been suggested to lead to transposable element (TE) mobilisation and genome reshuffling in some plants [29]. Repetitive k-mer class analysis has indicated k-mers present only in the nuclear D and S subgenomes of the ancient hybrids and absent from both the remaining genomes of progenitor species and subgenomes of recent hybrids (Figure 1B) [27]. This suggested an exchange of k-mers between the two progenitor subgenomes in the old hybrids and postpolyploidisation evolution. This stated, the failure of these two types of stable allotetraploids to interbreed [27] may be indicative of a reproductive isolation barrier between these two potentially cryptic species. Thus, recurrent allopolyploidisations need not lead to the same speciation outcome but could end in separate polyploid species, as observed in other plant polyploids [30].

Several population-level studies have been conducted in *Brachypodium* that link ecology to phylogenetics. The mesic and drought-avoider *B. distachyon* differs from the aridic *B. stacei*. In contrast, the drought-escaper *B. hybridum* ecologically resembles its *B. stacei* progenitor species, although its climate niche overlaps with both parents whilst being statistically distinct [31,32]. This aligned with a niche competition hypothesis [33] that suggests the favouring of the newly formed allotetraploids in non-native habitats. Nonetheless, population analyses indicate that climate niche parameters and functional and phenotypic eco-physiological responses to environmental stress are more genotype-dependent within each species [32,34,35]. **Phylogenomics** of the pangenome has revealed up to five highly divergent Mediterranean lineages, which split from the common ancestor 0.16 Ma [27,36,37], showing some signs of geographic structure coupled with frequent long-distance dispersals. However, clear signatures of genome admixture and chloroplast capture between the highly diverging *B. distachyon* lineages [36,38] indicate that, despite its highly selfing nature, introgression is still acting within this species, preventing speciation [36]. Nonetheless, the extraordinary natural diversity of *B. distachyon* and its robust pangenome has served to launch a variety of studies into the sources of variation. These include potential interlineage isolation caused by different flowering times [36], the influence of the individual TEs in the genetic and epigenetic constitution of the host genomes [39] (Box 2), and drought stress [34]. Such studies illustrate ongoing intraspecific evolution of this *Brachypodium* species and how shifts in biodiversity could show the impact of environmental change.

Glossary

Chromosome painting (CP): a variant of FISH, which enables selective visualisation of individual chromosomes or chromosome regions using chromosome-specific probes.

Chromosome territories (CT): areas in the nucleus that are preferentially occupied by particular chromosomes.

CRISPR: (clustered regularly interspaced short palindromic repeats): DNA sequences that, together with specialised proteins, for example, Cas9, provide some bacteria with a defence against alien DNA. In genetic engineering, it allows genome editing via targeted mutagenesis to obtain mutants of choice.

Dysploidy: a change in basic chromosome number caused by chromosome rearrangements such as chromosome fusions and fissions. It leads to a gradual decrease (descending dysploidy) or increase (ascending dysploidy) of basic chromosome number but usually does not affect the genome size.

Fluorescence *in situ* hybridisation (FISH): kinetically controlled renaturation of fluorescently labelled or detected particles (probes) with a complementary substrate in cytological preparation. In its DNA:DNA variant, it detects various DNA sequences directly in biological material such as chromosomes, interphase nuclei, extended chromatin fibres, and their visualisation using epifluorescence microscopy.

Microbiome: the whole microbial population found in a given environment.

Orphan ('ghost') progenitor subgenome: in allopolyploids, a subgenome originated from an unknown diploid ancestor. This ancestor may not yet be discovered or may have gone extinct.

Pangenome: the sum of the genomes of different genotypes from the same species or taxon.

Phylogenomics: reconstruction of the evolutionary relationships of any group of organisms using genome sequence data or other -omic data related to the genome or its functional expression (genome, transcriptome, repeatome, SNPs, etc.).

Recurrent allopolyploidisation: the occurrence of more than one hybridisation and genome doubling event(s) between the same progenitor species. Recurrent allopolyploidisations usually produce several derived allopolyploids of the same species but occasionally lead to different species.

Brachypodium to study plant genome organisation at the cytomolecular level

One near unique feature of *Brachypodium* research is the key role that cytomolecular analyses have played and demonstrated the importance of such approaches in understanding speciation along with genome sequence assessments. Thus, *Brachypodium* karyotype structure and evolution has been extensively elucidated using **fluorescence *in situ* hybridisation (FISH)** with chromosome-specific low-repeat BAC clones as probes, known as **chromosome painting (CP)** or, sometimes, chromosome barcoding [27,40,41]. Together with phylogenomics, this approach identified some putative segmental allopolyploids and orphan genomes of some likely extinct ancestors in *Brachypodium* [41]. Although many kinds of DNA sequences make useful FISH probes [42], CP is particularly informative due to its high specificity (Figure 2A,B). The recent advent of single-copy oligonucleotides as probes (oligo-FISH) [43] made CP technically feasible in more plants, enabling comparative analyses not only of those with small genomes, like rice [44] but, to some

Reference genome: a fully assembled and annotated genome of an organism (including its nuclear and organellar genomes in eukaryotes) that serves as a reference for the species or taxon.
Reticulation: derivation of a lineage from the total or partial merging of two ancestral lineages.
Shell genes: genes that are shared by most or several of the genomes in a pangenome.

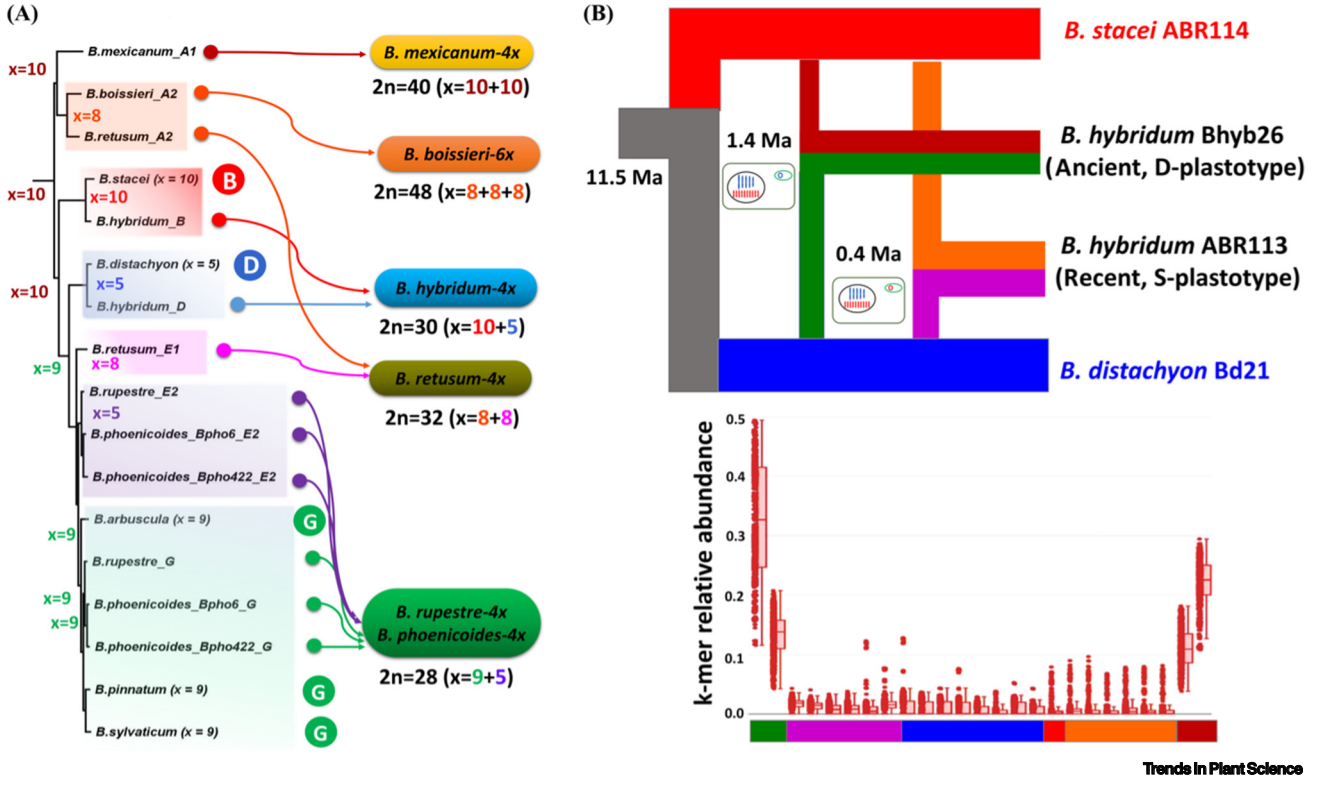

Figure 1. Inferred evolutionary speciation events of annual and perennial *Brachypodium* species. (A) Comprehensive evolutionary framework for the origins of *Brachypodium* diploids and polyploids based on the combined phylogenomic and comparative chromosome barcoding analyses [26]. Colours indicate the different types of (sub)genomes retrieved in the phylogenomic analysis using 322 gene-based phylogeny and subgenome detection algorithms; letters designate the karyotype profiles found in the diploids and polyploids. Allopolyploid species show different types of known (B, D, G) or orphan (A1, A2, E1, E2) diploid progenitor subgenomes [*Brachypodium hybridum*-4x (B, D), *Brachypodium retusum*-4x (A2, E1), *Brachypodium phoenicoides*-4x (E2, G), *Brachypodium rupestre*-4x (E2, G)], whereas putative autopolyploid (or segmental allopolyploid) species show the same type of orphan progenitor subgenome [*Brachypodium mexicanum*-4x (A1A1), *Brachypodium boissieri*-6x (A2A2A2)] (modified from [26]). (B) Evolutionary allopolyploidisation scenarios for the origins of the two types of *B. hybridum* lines; ancient *B. hybridum* (reference genome Bhyb26, showing maternal plastome D inherited from *B. distachyon*) and recent *B. hybridum* (reference genome ABR113 showing maternal plastome S inherited from *B. stacei*) were formed from distinct hybridisation and genome doubling events 1.4 and 0.4 Ma, respectively. Stylised plant cell diagrams with colour coding indicating the origins of the plastomes (circles) and nuclear genomes (haploid chromosome numbers) of ancient and recent *B. hybridum* lines (*B. distachyon*-type D genomes, blue; *B. stacei*-type S genomes, red). The datings were inferred from the coalescence-based and cross-bracing analyses of Gordon *et al.* 2020 (modified from [27]). Abundance of a repeat k-mer class that expanded in the D and S subgenomes of the ancient but not the recent hybrids, nor in the progenitor genomes, indicates a postpolyploidisation evolutionary novelty. Genomes and subgenomes are indicated by their respective colour codes in the bar below the x axis and correspond to those indicated in the above cladogram: from left to right, ancient *B. hybridum* D subgenome (green), recent *B. hybridum* D subgenome (purple); *B. distachyon* D genome (blue); *B. stacei* S genome (red); recent *B. hybridum* S subgenome (orange); ancient *B. hybridum* S subgenome (brown) (modified from [27]).

Box 2. *Brachypodium* to study transposable elements and genome dynamics

Despite its small genome size, *Brachypodium distachyon* constitutes an ideal system to investigate genome and, more specifically, transposable element (TE) evolution. The near base perfect genome sequence of the reference Bd21 genotype has allowed the comprehensive annotation of the TEs [5,39]. As with many plant genomes, TEs in *B. distachyon* are represented mainly by LTR-retrotransposons [39], but their distribution along the chromosomes are much more pervasive than in Brassicaceae [154]. Their detailed characterisation revealed that many families display ongoing transpositional activity [39,155] and suggested that TE dynamics mediate **shell gene** genesis and regulation in this species [36]. However, methylation spreading around TEs is limited in *B. distachyon* [154,156], implying that the impact of TEs on nearby gene expression might involve additional regulatory mechanisms. Assessing TE insertion polymorphisms (TIPs) in genomes originating from Spain and Turkey further suggested that TE dynamics is shaped by a complex interplay between purifying selection and population demographic history in the wild [155]. Although relatively scarce in genic regions, TIPs also alter nearby gene expression and may contribute to accession-specific regulation patterns [154]. Therefore, with the current development of a large diversity panel spanning from Spain to Iraq (Box 1), *B. distachyon* is well-established as a model to understand plant genome evolution in changing environments.

extent, also in previously intractable large-genome cereals, such as some Triticeae [45]. However, future research in *Brachypodium* is likely to focus on chromosomal localisation, genomic distribution, and the abundance of various satellites and TEs (Figure 2C1,C2). Repetitive DNA plays an essential role in organising any eukaryote genome and contributes to chromosome segregation, chromosome end protection, karyotype evolution, and reproductive isolation among species [46,47]. Recently, Li *et al.* [48] provided the first comprehensive insight into the cytomolecular characterisation of *Brachypodium* centromeres, the most repetitive chromosome regions [5]. Based on the presence or absence of *B. distachyon*-originated centromeric retrotransposons, species were classified into two distinct lineages. It will be interesting to test if the composition and organisation of various repeats within *Brachypodium* links to previous phylogenetic studies made using different approaches.

The spatial arrangement of chromosomes inside the interphase nucleus is another fascinating field due to its importance for gene regulation and genome stability [49], which could differ in responses to environmental change. Early studies in mammals revealed that individual decondensed chromosomes occupy **chromosome territories (CT)** (e.g., [50]). Their structure and distribution vary among vertebrates and often correlate with a given chromosome size and, in particular, its gene density [51,52]. Corresponding studies in plants are scarce, with the first demonstrating a predominantly random arrangement of CT in arabidopsis [53] but may depend on morphological features of a nucleus [54] that are conserved between related species [55]. Until only recently [56], *B. distachyon* was the only monocot for which the interphase arrangement of individual chromosomes had been studied. Using 3D preserved nuclei isolated from roots, four kinds of interactions between homologous CTs were observed, the frequency of which depended on morphometric parameters of chromosomes and the nuclear shape [57]. For example, the complete association of homologues was the most common for CTs of the large and metacentric chromosomes, chromosome Bd1 in particular. In contrast, in considerably shorter and more asymmetric chromosomes, such as Bd4, CTs were usually completely separated (Figure 2D,E). Whether CT arrangement varies among different organs or different representatives of the genus and could change with shifts in biodiversity with environmental change, remain open questions.

A novel spin-off from the studies on *B. distachyon* CTs concerns spontaneous or induced plant nuclear genome instability. Many physical and chemical agents cause micronuclei (MN) formation and assessing their frequency was commonly used to test genotoxicity [58]. It is probable that such stress could also be imposed as a result of climate change [59]. However, until our studies [60,61], there was no [62,63] or only a little [64] information about the MN composition. Our CP-based approach enabled better elucidation of mechanisms of their formation and, more indirectly, allowed us to infer on diverse susceptibility of specific genomic regions to genotoxic agents

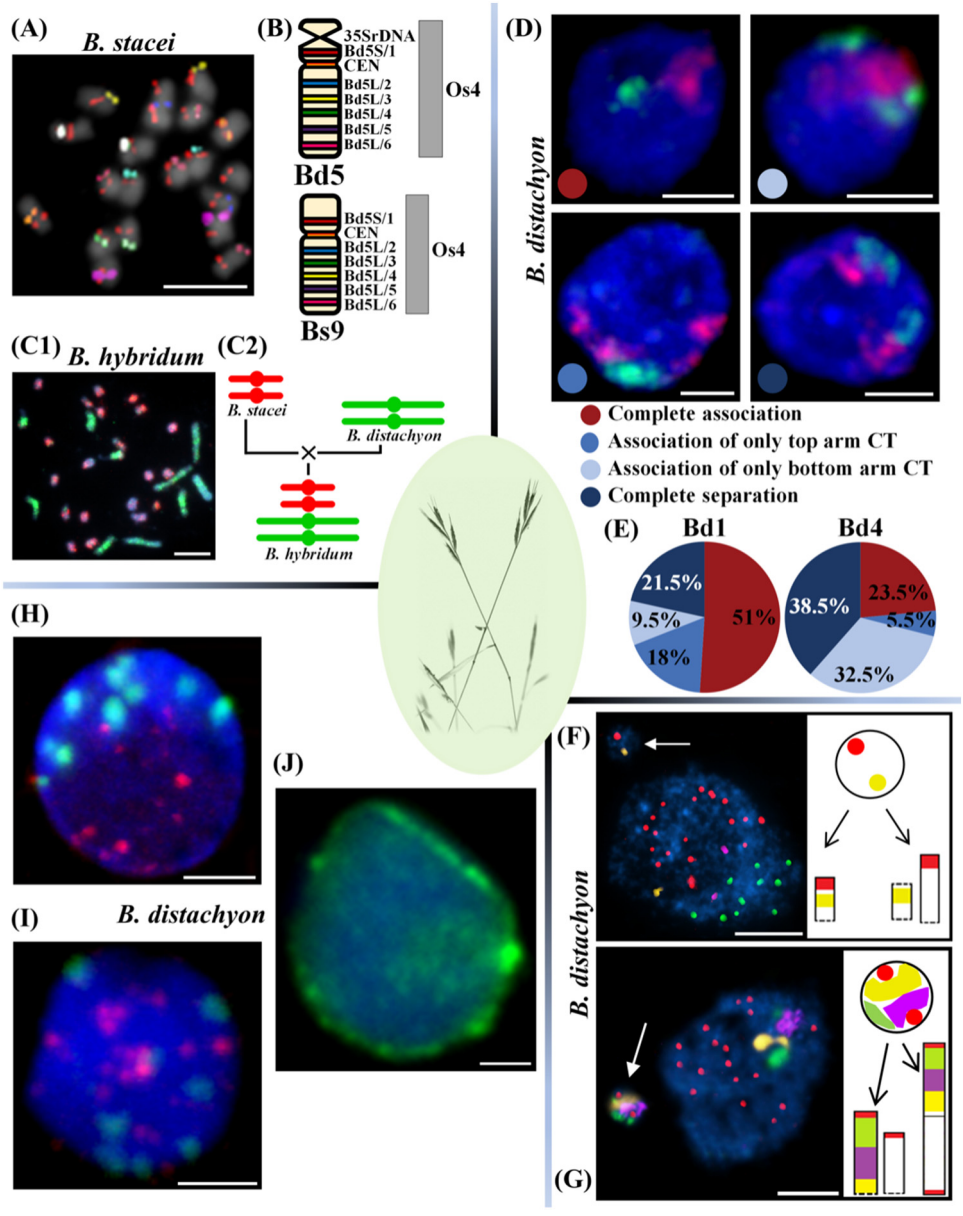

Figure 2. A simplified overview of main directions of recent cytomolecular research in *Brachypodium*. (A) Chromosome painting (CP) in *Brachypodium stacei* using six low-repeat BACs, the centromeric BAC, and two universal (5S and 25S) rDNA sequences as probes (adapted from [40]). (B) All BACs originate from *Brachypodium distachyon* genomic libraries, but they hybridise with chromosomes of other *Brachypodium* species, allowing dissection of the structure of individual chromosomes across the species, as exemplified by comparing chromosome Bd5 of *B. distachyon* and Bs9 of *B. stacei*. The chromosomes in extant *Brachypodium* species can be linked to so-called ancestral rice chromosome equivalents, Os4 in the exemplified case, enabling us to infer the evolution of entire karyotypes (modified from [27]). (C1) Dual-colour fluorescence *in situ* hybridisation discrimination of the *B. distachyon* (green) and *B. stacei* (red) subgenomes in *Brachypodium hybridum* using Bd genome-specific clone ABR1-63-E6 and Bs genome-specific clone 8P20, respectively. (C2) Schematic visualisation of *B. hybridum* subgenome discrimination shown in (C1) (both modified from [42]). (D) Four scenarios of interactions between homologous chromosome territories (CT) in root-tip interphase nuclei of *B. distachyon*, as demonstrated by CP using Bd4 short-arm- (green) and Bd4 long-arm-specific probes (modified from [57]). (E) Different frequencies of various interactions between homologues are shown for Bd1 and Bd4 chromosomes (modified from [57]).

(*Figure legend continued at the bottom of the next page.*)

(Figure 2F,G) [60,61]. In practical terms, it creates the opportunity for *B. distachyon* to become a standard in plant mutagenesis or in assessing environmental stress.

An intriguing aspect of 3D nuclear architecture is linked with the distribution of telomeric and centromeric domains. One well-known pattern is shown when all telomeres and centromeres are localised on opposite poles of the interphase nucleus. This so-called **Rabl configuration** is a natural consequence of the chromatid orientation at anaphase but is usually exhibited only in plants with large genomes and may act to reduce chromatin entanglement [65,66]. However, the studies in *Brachypodium* revealed that most of the root-tip nuclei in *B. distachyon* display the Rabl configuration (Figure 2H), while not in *B. stacei* and *B. hybridum* [67], despite small genomes in all species [22]. Furthermore, in *B. distachyon*, the proportion of the Rabl- to non-Rabl-arranged root-tip nuclei seems linked with nuclear shape and cell cycle phase. It cannot be ruled out that the Rabl configuration is also tissue/organ-specific since the leaf nuclei in *B. distachyon* do not display the polar arrangement of their telomeres and centromeres (Figure 2I) [67]. It is unknown why *B. hybridum* inherits the non-Rabl configuration found in the root-tip nuclei of *B. stacei* and not the Rabl configuration found in their counterparts in *B. distachyon*. Addressing this question could involve the 3D analysis of interphase nuclei using genome-specific probes, which efficiently cover the chromosomes at their entire length (Figure 2C1,C2) [42] together with telomeric and genome-specific centromeric probes [48]. The recent development of chromosome conformation capture techniques [49], Hi-C in particular [68], provides an attractive alternative to the cytomolecular approach in this respect. Alternatively, it may be that a fluorescence protein-tagged **CRISPR** system with deficient Cas9 could be used to track the telomeres *in vitro*, as demonstrated in *Nicotiana benthamiana* [69]. This would provide unprecedented insight into the dynamics of these particular chromosome domains by adopting both telomeric and (peri)centromeric sequences. However, the attempts to visualise telomeres in arabidopsis have not proven successful [70], so that live-cell CRISPR imaging could be problematic in small-genome plants. Considering underlying mechanisms, key nuclear envelope components (e.g., SUN proteins, Figure 2J) could be guiding the distribution of telomeric and centromeric regions during meiosis and at premeiotic interphase [71,72]. *Brachypodium* represents an excellent platform to assess the role of various nuclear envelope proteins through the availability of an efficient CRISPR-Cas9-based mutagenesis protocol [73] to dissect the key determinants of nuclear architecture, including the Rabl configuration.

Brachypodium to elucidate plant responses to environmental stress

As a nondomesticated plant genus, growing in climate change hotspots, *Brachypodium* lends itself to research into the mechanisms of responses to stress. This could be used for information on the development of new stress-tolerant major crops but also to report on the impact of environmental stress. Environmental change is likely to increase abiotic stresses such as drought, heat, and salinity in plants as well as changing pathogen dispersal patterns [74].

(F) The micronucleus (MN; marked by arrow) in *B. distachyon* root-tip cells shows the presence of one centromeric (red) and one 25S rDNA (yellow) signal. (G) The composition of MN in the same species was visualised using the centromeric probe (red) and pools of low-repeat BACs, specifically painting the distal (green), intercalary (purple), and proximal (yellow) segments of the top arm of chromosome Bd1. Diagrams on the right show putative ways of the MN formation (both adapted from [60]). (H) Spherical nucleus of *B. distachyon* root tip showing the Rabl configuration of centromeres (green) and telomeres (red). (I) Spherical nucleus of *B. distachyon* leaf without the Rabl configuration (both modified from [67]). (J) Immunolocalisation of the SUN1/2 nuclear envelope proteins (green) in the *B. distachyon* leaf nucleus. Chromatin stained with DAPI [blue, grey in (A)]. All bars, 5 μm. Photomicrograph (C1) is courtesy of Joanna Lusinska, (H–I) courtesy of Ewa Robaszkiewicz, and (J) courtesy of Dominika Idziak-Helmcke (Plant Cytogenetics and Molecular Biology Group, Institute of Biology, Biotechnology and Environmental Protection, Faculty of Natural Sciences, University of Silesia in Katowice, Poland). The photograph of the *B. distachyon* plant is courtesy of Anthony Pugh and Gwen Jenkins (Aberystwyth University, UK). Clone 8P20 is courtesy of Boulos Chalhoub (Institut national de la Recherche agronomique (INRA), Université d'Evry Val d'Essonne (UEVE), Evry, France, Institute of Crop Science, Zhejiang University, Hangzhou, China).

Although the literature describing drought responses in plants is extensive, *Brachypodium*-based research has made a contribution. At its most basic, an examination of *Brachypodium* inbred lines grown under range of soil % moisture contents indicated a nonlinear relationship, so simple pairwise comparisons between droughted and fully watered plants could be insufficient [75]. The variation in responses to drought in *Brachypodium* accessions has been assessed [34,76,77] and, in one case, the degree of drought tolerance related to rainfall in the original sampling sites [78]. The transcriptomic and metabolomic responses of *B. distachyon* to drought have been characterised and, amongst many changes, suggested the importance of the cell wall [76,79]. A proteomic approach described a network of drought-responsive proteins in leaves and roots and this highlighted changes in the bioenergetic and stress metabolism but also the cell wall [80]. *B. distachyon* is also an ideal test bed to identify and characterise the roles of genes linked to drought responses. Recent examples have included a large family of late embryogenesis-abundant (LEA) proteins and their responses to the important drought hormone abscisic acid (ABA) [81]. The impact of ABA on epigenetic changes under drought was revealed by overexpression of the histone deacetylases, BdHD1 (Bradi3g08060) [82], which interacts with the drought-responsive transcription factors, BdWRKY24, and BdMYB22 [83]. Drought stress generates many harmful metabolites and their detoxification by glutathione-*S*-transferases (GST) can be associated with tolerance. The small *B. distachyon* genome encodes a surprisingly large number of GST (a similar number to wheat) and it may be that BdGSTF8, BdGSTU35, and BdGSTU42 were influencing the relative tolerance to osmotic stress [84]. A link between physiology and drought tolerance was made in *B. distachyon* when the microtubule protein MAP20 was shown to be important in the function of pits in the cell walls of plant tracheary elements Here, researchers worked initially on the hybrid aspen (*Populus tremula* × *Populus tremuloides*), but when investigating tMAP20 in an annual species, the functional genomic properties of *B. distachyon* proved to be useful [85]. Other studies have adopted *Brachypodium* for similar reasons to characterise common responses to a wide range of abiotic stresses; for example, mechanical stress [86], heat shock proteins [87], the cystatin protease family [88], or phospholipase C genes [89]. With an increased appreciation of the need to undertake combinational stress studies, the value of relatively small *Brachypodium* plants as a valuable platform to assess such complex traits will surely increase.

The utility of *B. distachyon* in characterising responses to biotic stresses in cereals was one of the first suggested targets for research [4]. A range of important *Brachypodium*-based pathosystems have been developed and focused on *Magnaporthe oryzae,* the causal agent of rice blast disease [90], a range of rust species (*Puccinia graminis, Puccinia striiformis*, *Puccinia triticina*) [91,92], root rot caused by *Rhizoctonia* [93], head blight-forming *Fusarium* species [94], *Parastagonospora nodorum* causing *Septoria nodorum* blotch [95], or barley stripe mosaic virus [96]. Important development includes the definition of single, major gene-related host resistance [91,96], whilst resistance wheat-adapted *P. striiformis* f. sp. *tritici* was mapped to two loci, *Yrr1* and *Yrr2* [97]. Single gene-mediated resistance is notoriously ephemeral under field conditions. Therefore, it is of considerable importance that a recent study has dissected one example of multigenetic, non-host resistance [98]. Screening a population of recombinant inbred lines allows the differential responses to wheat stem rust (caused by *P. graminis* f. sp. *tritici*) to be rationalised into six quantitative trait loci. Grass genomic synteny could aid the translations of such findings into temperate cereals, as demonstrated when the synteny between wheat and *B. distachyon* not only allowed the fine mapping of resistance against powdery mildew (*Blumeria graminis*), but its introgression into bread wheat from a wild relative [99]. Equally, mutants of *B. distachyon* can be screened for increased susceptibility to non-host pathogens as with yAL6.2, which was mapped to *TIME FOR COFFEE* (*TIC*), a circadian rhythm gene, which has also been shown to influence pathogen resistance in arabidopsis [100,101].

B. distachyon can also be used to dissect features of the host and pathogen interaction. An early example of this was the detoxification of the *Fusarium* head blight toxin, deoxynivalenol, by host uridine diphosphate glycosyltransferases [102]. More recently, fungal small RNAs encoded by *Fusarium graminearum* were shown to suppress host defence gene expression [103] and a similar approach could also be a feature of the *Magnaporthe grisea infections* [104]. *B. distachyon* also lent itself to the development of an infection system with *Rhizoctonia solani*, based on which 61 secreted protein effectors were identified that could manipulate the host [105] via WRKY transcription factors BdWRKY38 and BdWRKY44 [106,107].

A recent comprehensive review of *B. distachyon*–viral interactions has highlighted the value of this model in elucidating complex virus–virus host interactions [108]. Viral interactions can be synergistic, interfering, cooperative, or attenuating and some have been described in panicum mosaic virus (PMV) infections of *B. distachyon*. Importantly, *B. distachyon* was used to show that the PMV satellite virus is modified at its 3′ end by the host to reduce disease symptoms [109].

The importance of beneficial microbial interactions is well appreciated in, for example, mobilising nutrients to the plant as well as influencing plant physiology. Using controlled mesocosms and stable isotopes showed surprisingly large amounts of fungal and plant N had moved below ground as gaseous ammonia, with 6–9% of total plant N having moved by this mechanism [110]. Importantly, these interactions will also be influenced by climate change [111] and require characterisation in a nondomesticated model that retains the richness of rhizospheric associations [112], hence the value of *Brachypodium.* Several studies demonstrated that *B. distachyon* growth could be enhanced by previously described beneficial bacteria [113–115]. Two studies focused on transcriptomic or metabolomic responses provided data to hypothesise mechanisms involved in two beneficial plant–bacterial interactions [116,117]. The beneficial effects of mycorrhizal associations with *B. distachyon* have been used to show differential responses, depending on plant or fungal genotype [118,119]. *B. distachyon* mutants have been used to explore the role of auxin in mycorrhizal associations [120] and to show the importance of the GRAS transcription factor, RAM1, previously shown to be essential for arbuscule formation in three dicots and a grass [121]. Given the importance of reducing synthetic fertiliser use with crops such as cereals, such *Brachypodium*-based studies will increase. This is especially the case with the development of approaches to describe the **microbiome** (Box 3), both in its characteristics and function, in soil and rhizospheric interactions.

Brachypodium to study the grass cell wall

The cell wall plays a central role in plant structure and function and its complexity has been extensively assessed in *B. distachyon.* The primary and secondary cell walls in grasses are distinct in several ways from eudicots (Figure 3). The cellulose fraction is equivalent, with (1,4)-beta-D-glucan polymers assembled in parallel at the plasma membrane to form microfibrils, but not so with the hemicellulose fractions. In eudicots, hemicellulose tends to be comprised of (1,4)-beta-D-glucans that are considerably shorter than cellulose and amended with xylose decorations. Grasses are most abundant in heteroxylans with a (1,4)-beta-D-xylose backbone decorated with xylose, arabinose, glucuronic acid, and phenolic side chains of hydroxycinnamates [122]. *B. distachyon* has helped to characterise enzymes that elongate the xylan chain, including BdGT43A [123] and XYLOSYL ARABINOSYL SUBSTITUTION OF XYLAN such as BdGT61 [124].

A qualitative distinction in grasses from eudicots is the presence of mixed linkage glucan (MLG), (1,3;1,4)-beta-D-glucan. It is synthesised by three classes of the cellulose synthase (Ces) superfamily, CslF, CslJ, and CslH [125]. Biochemical and cytological techniques have localised MLG synthase activity and the most active isoform CslF6 to the maize Golgi membrane alone [126] and to the plasma membrane in wheat and barley [127]. In *B. distachyon*, yellow fluorescent

Box 3. *Brachypodium* to study plant–environment–microbiome interactions

The importance of the microbiome for plant and animal health and productivity has become increasingly apparent over the past few years and *Brachypodium* is making a significant contribution to such studies. *B. distachyon* root microbiome and root exudate profiles are similar to wheat [157] and the location along the root greatly affected microbiome composition [158]. While these microbiome studies provide correlations between microbiome composition and plant phenotypes, they cannot definitively assign function to either microbes or genes. To address this, researchers have developed controlled systems that maintain sterility and allow for nondestructive imaging, which, when synthetic microbial communities are used to test the effects of relatively small numbers of microbes, begin to uncover mechanisms. One such system developed using *B. distachyon* is called EcoFAB and consists of a small chamber attached to a microscope slide, such that roots grow into a chamber containing liquid or solid growth media, where they can be visualised from below. Ports on either end allow media to be added and samples removed while maintaining sterility [159]. While this system can be used for any small plant, it has been optimised using *B. distachyon* and is large enough for *Brachypodium* plants to nearly complete their lifecycle under controlled conditions. The reproducibility of plant growth and root exudation in EcoFABs in response to nutrient deficiency and soils extracts was demonstrated by a ring trial among four laboratories [160] and the EcoFAB has been adapted for high-resolution microscopy [161]. To increase throughput and reproducibility, a robotic system has been developed that can move individual EcoFABs between a growth chamber, a liquid handler to change media and remove samples, a microscope that allows for imaging of entire root system as well as individual bacteria, and a hyperspectral camera to measure above-ground growth and composition (https://ecofab-teams.lbl.gov/the-ecobot/). When coupled with novel systems like the EcoFAB, the small size and experimental tractability of *B. distachyon* set the stage for rapid discovery.

protein-tagged BdCSLF6 localised to the Golgi membrane [128,129]. The relative ease of stable genetic transformation in *B. distachyon* compared with other grasses was key in establishing this study and identifying the first protein to directly regulate the transcription of a *CSLF* gene [130]. *BdTHX1* binds the GT-element in the first intron of *BdCSLF6* and the promoter region of *BdXTH8*, a grass-specific endotransglucosylase/hydrolase. While characterisation was facilitated by generating *BdTHX1* overexpression lines, 50 plants regenerated from calli died after 1–2 weeks, which was an outcome attributed to the transgene, another example of easy transformation, facilitating discovery. An example of a grass cell wall regulator not present in arabidopsis, but characterised in *B. distachyon*, is *SECONDARY WALL ASSOCIATED MYB1* (*SWAM1*) [131], which binds the AC-element to activate secondary wall-associated gene expression. Another regulatory factor identified in *B. distachyon* is the diurnal regulation of secondary cell wall-associated transcripts, which are controlled by daily rhythms of temperature change [132].

Another prevailing component of plant secondary cell wall is lignin. Among the enzymes in the lignin biosynthesis pathway, more than half have been characterised in *B. distachyon* [phenylalanine ammonia lyase (PAL), phenylalanine tyrosine ammonia-lyase (PTAL), C3H, 4CL, COMT, F5H, CAD, and PMT], and these included the first *bona fide* PTAL [133]. Grass lignins tend to have a greater proportion of S- to G-unit monolignols than eudicots and analysis of *BdPTAL1* downregulation by RNAi was found to be mainly involved in the biosynthesis of S-unit lignins. Another PAL output that is a defining aspect of grass walls is the presence of *p*-coumaric acid and ferulic acid. These hydroxycinnamic acids provide linkages between arabinoxylans and lignins and hemicelluloses via acylation by the plant-specific BAHD family of acyl-CoA-dependent acyltransferases [134]. The analysis of overexpression and RNAi transgenic plants suggests that BdBAHD01/BdAT9 and BdBAHD05/BdAT1 are involved in the feruloylation of arabinoxylan [135,136]. *p*-Coumaroylation by BAHDs was first demonstrated by genetic gain-of-function in rice for *OsBAHD10*/*OsAT10* [137] and by genetic loss-of-function in *B. distachyon* for *BdBAHD09*/*BdAT3*/*BdPMT1* [138]. These studies serve as another example where *B. distachyon* facilitated the dissection of salient traits in grasses.

Looking forward, these excellent mechanistic studies will inform applied science focusing on aspects such as cell wall digestibility. Such studies have been initiated in *B. distachyon* with, for example, manipulations of enzymes in the UDP–sugar interconversion pathway, which alter

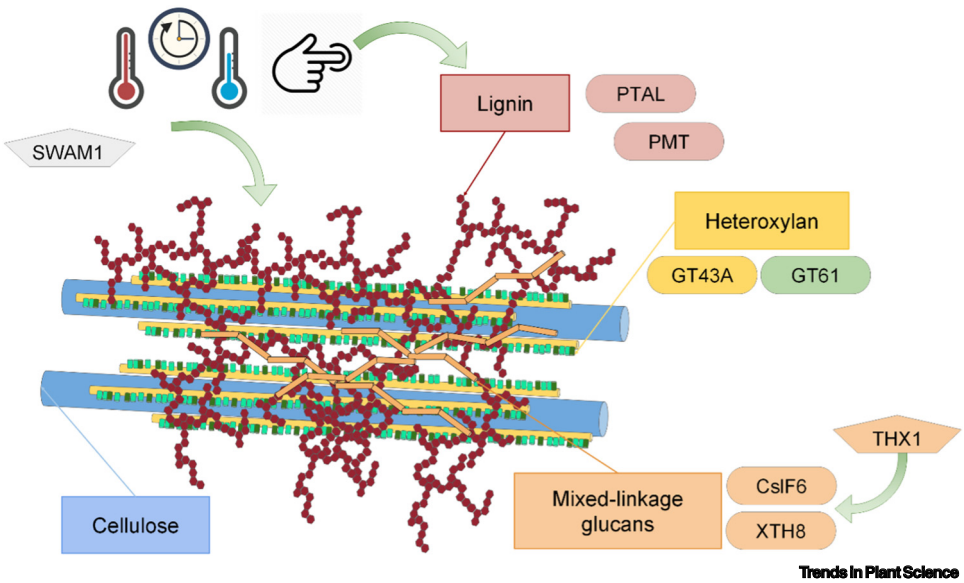

Figure 3. Schematic of grass secondary cell wall polymers highlighting enzymatic and transcriptional components uniquely defined using *Brachypodium distachyon*. Transcription factors are pentagons and cell wall biosynthesis genes are overalls. Hand denotes mechanical stress and thermometers denote the daily thermocycles that regulate wall thickening.

cell wall sugars [139]. Targeting a UDP-arabinopyranose mutase through an RNAi approach resulted in a twofold greater release of total carbohydrate with xylanase. Similarly, through knockdown and mutation of *PAL*, *LACCASE5* and *8*, *PMT*, and *COMT6*, cell walls were more digestible [140]. Furthermore, the genetic resources available for *Brachypodium* have been exploited to characterise natural genetic variation within accessions, which varied in the composition of most cell wall components, the determinants of which could be identified with availability of recombinant inbred lines to help in the derivation of more digestible biofuel crops [123,140,141].

Concluding remarks and future perspectives

This overview has highlighted aspects of *Brachypodium* research that show its continuing relevance. The features of *B. distachyon* that led it to be first suggested as a grass model have now been extensively studied. Important developments not envisaged in the 2001 paper [4] were the extensive characterisation of wild *B. distachyon* populations and expansion of the model to represent entire the genus. Crucially, the opportunity to do such studies arose directly from *Brachypodium* being a nondomesticated genus. Going forward, *Brachypodium* populations will undoubtedly continue to provide the resources of indicating evolutionary impacts on genomes and physiology and, perhaps most importantly, some of the drivers of environmental adaptation. *Brachypodium* will also continue to be a 'characterisation platform' for, for example, genes linked to responses to stress, the cell wall development, and influencing the microbiome. Set against a background of climate change, such studies continue to be vital. With improvements in genomic sequencing and gene editing technologies, crops are taking on some of the properties of model species; however, *Brachypodium* has now emerged as the prime model because it is a nondomesticated genus (see Outstanding questions).

Acknowledgements

R.H. is grateful to the National Science Centre Poland (2018/31/B/NZ3/01761 and 2015/18/M/NZ2/00394) and acknowledges the support under the Research Excellence Initiative of the University of Silesia in Katowice. P.C. thanks the Spanish

Outstanding questions

Can mechanisms of environmental stress tolerance defined in *Brachypodium* be translated to major crops?

How do the environment, stress, and nonstress conditions, influence cell elongation and wall thickening in the grasses?

What is the functional relevance of mixed linkage glucan in grass cell walls?

Could *Brachypodium* serve as a model genus to identify orphan subgenomes in polyploid plant groups with unknown ancestral diploid progenitor species?

Could the annual *Brachypodium* polyploid complex be used as a functional system for other plant polyploids?

Could the *Brachypodium* microbiomes indicate mechanisms that improve nutrient uptake in cereals through improved interaction of the rhizosphere?

Does the chromosome territory arrangement vary among different organs or different species of *Brachypodium*, or even alter in response to the environment?

Could changes in the diversity of *Brachypodium* populations be an indicator of environmental change, so that they could be considered 'sentinel' species?

Ministry of Science and Innovation (PID2019-108195GB-I00), the Spanish Aragon Government and European Social Fund (Bioflora grant A01-20R), and the JGI Community Science Program (503504). S.H. acknowledges the National Science Foundation Division of Integrative Organismal Systems (NSF IOS-2049966). A.C.R. thanks the University of Zurich Research Priority Programs (URPP) *Evolution in Action* and the Swiss National Science Foundation (SNSF 31003A_182785). The work conducted by J.P.V. at the US DOE Joint Genome Institute is supported by the Office of Science of the US Department of Energy (DE-AC02-05CH1123). K.W. acknowledges the support from the National Natural Science Foundation of China (32070544) and National Key R&D Program of China (2019YFD1000500). LA.J.M. recognises the support of the UK Biotechnology and Biological Sciences Research Council (BB/M027945/1).

Declaration of interests

The authors declare no conflict of interest.

References

1. Somerville, C. and Koornneef, M. (2002) A fortunate choice: the history of *Arabidopsis* as a model plant. *Nat. Rev. Genet.* 3, 883–889
2. The Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* 408, 796–815
3. International Rice Genome Sequencing Project (2005) The map-based sequence of the rice genome. *Nature* 436, 793–800
4. Draper, J. *et al.* (2001) *Brachypodium distachyon*. A new model system for functional genomics in grasses. *Plant Physiol.* 127, 1539–1555
5. International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature* 463, 763–768
6. Sun, S. *et al.* (2017) Alignment of common wheat and other grass genomes establishes a comparative genomics research platform. *Front. Plant Sci.* 8, 1480
7. Pei, L. *et al.* (2019) Tracing the origin and evolution history of methylation-related genes in plants. *BMC Plant Biol.* 19, 307
8. Scholthof, K.B.G. *et al.* (2018) *Brachypodium*: a monocot grass model genus for plant biology. *Plant Cell* 30, 1673–1694
9. Pfeifer, M. *et al.* (2014) Genome interplay in the grain transcriptome of hexaploid bread wheat. *Science* 345, 1250091
10. Choulet, F. *et al.* (2014) Structural and functional partitioning of bread wheat chromosome 3B. *Science* 345, 1249721
11. International Wheat Genome Sequencing Consortium (2014) A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. *Science* 345, 1251788
12. Gatti, M. *et al.* (2018) Identification, molecular cloning, and functional characterization of a wheat UDP-glucosyltransferase involved in resistance to *Fusarium* head blight and to mycotoxin accumulation. *Front. Plant Sci.* 9, 1853
13. Ma, G. *et al.* (2018) Cloning and characterization of the homoeologous genes for the Rec8-like meiotic cohesin in polyploid wheat. *BMC Plant Biol.* 18, 224
14. International Barley Genome Sequencing Consortium *et al.* (2012) A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491, 711–716
15. Ling, H.Q. *et al.* (2018) Genome sequence of the progenitor of wheat A subgenome *Triticum urartu*. *Nature* 557, 424–428
16. Luo, M.C. *et al.* (2017) Genome sequence of the progenitor of the wheat D genome *Aegilops tauschii*. *Nature* 551, 498–502
17. Maccaferri, M. *et al.* (2019) Durum wheat genome highlights past domestication signatures and future improvement targets. *Nat. Genet.* 51, 885–895
18. International Wheat Genome Sequencing Consortium (2018) Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Science* 361, eaar7191
19. Bennetzen, J.L. *et al.* (2012) Reference genome sequence of the model plant *Setaria*. *Nat. Biotechnol.* 30, 555–561
20. Garsmeur, O. *et al.* (2018) A mosaic monoploid reference sequence for the highly complex genome of sugarcane. *Nat. Commun.* 9, 2638
21. Lovell, J.T. *et al.* (2018) The genomic landscape of molecular responses to natural drought stress in *Panicum hallii*. *Nat. Commun.* 9, 5213
22. Catalan, P. *et al.* (2012) Evolution and taxonomic split of the model grass *Brachypodium distachyon*. *Ann. Bot.* 109, 385–405
23. Catalan, P. *et al.* (2016) Phylogeny and evolution of the genus *Brachypodium*. In *Genetics and Genomics of Brachypodium* (Vogel, J.P., ed.), pp. 353, Springer
24. Catalan, P. and Vogel, J.P. (2020) Advances on genomics, biology, ecology and evolution of *Brachypodium*, a bridging model grass system for cereals and biofuel grasses. *New Phytol.* 227, 1587–1590
25. Diaz-Perez, A. *et al.* (2018) Reconstructing the origins and the biogeography of species' genomes in the highly reticulate allopolyploid-rich model grass genus *Brachypodium* using minimum evolution, coalescence and maximum likelihood approaches. *Mol. Phylogenet. Evol.* 127, 256–271
26. Sancho, R. *et al.* (2022) Tracking the ancestry of known and 'ghost' homeologous subgenomes in model grass *Brachypodium* polyploids. *Plant J.* 109, 1535–1558
27. Gordon, S.P. *et al.* (2020) Gradual polyploid genome evolution revealed by pan-genomic analysis of *Brachypodium hybridum* and its diploid progenitors. *Nat. Commun.* 11, 3670
28. Dinh Thi, V.H. *et al.* (2016) Recreating stable *Brachypodium hybridum* allotetraploid by uniting the divergent genomes of *B. distachyon* and *B. stacei*. *PLoS One* 11, e0167171
29. Burns, R. *et al.* (2021) Gradual evolution of allopolyploidy in *Arabidopsis suecica*. *Nat Ecol Evol* 5, 1367–1381
30. Doyle, J.J. and Sherman-Broyles, S. (2017) Double trouble: taxonomy and definitions of polyploidy. *New Phytol.* 213, 487–493
31. Lopez-Alvarez, D. *et al.* (2015) Environmental niche variation and evolutionary diversification of the *Brachypodium distachyon* grass complex species in their native circum-Mediterranean range. *Am. J. Bot.* 102, 1073–1088
32. Martinez, L.M. *et al.* (2018) Variation in functional responses to water stress and differentiation between natural allopolyploid populations in the *Brachypodium distachyon* species complex. *Ann. Bot.* 121, 1369–1382
33. Rey, P.J. *et al.* (2017) The interplay between aridity and competition determines colonization ability, exclusion and ecological segregation in the heteroploid *Brachypodium distachyon* species complex. *New Phytol.* 215, 85–96
34. Decena, M.A. *et al.* (2021) Comparative genomics, evolution, and drought-induced expression of dehydrin genes in model *Brachypodium* grasses. *Plants* 10, 2664
35. Des Marais, D.L. *et al.* (2017) Interactive effects of water limitation and elevated temperature on the physiology, development and fitness of diverse accessions of *Brachypodium distachyon*. *New Phytol.* 214, 132–144
36. Gordon, S.P. *et al.* (2017) Extensive gene content variation in the *Brachypodium distachyon* pan-genome correlates with population structure. *Nat. Commun.* 8, 2184
37. Stritt, C. *et al.* (2022) Migration without interbreeding: evolutionary history of a highly selfing Mediterranean grass inferred from whole genomes. *Mol. Ecol.* 31, 70–85
38. Sancho, R. *et al.* (2018) Comparative plastome genomics and phylogenomics of *Brachypodium*: flowering time signatures, introgression and recombination in recently diverged ecotypes. *New Phytol.* 218, 1631–1644
39. Stritt, C. *et al.* (2020) Diversity, dynamics and effects of long terminal repeat retrotransposons in the model grass *Brachypodium distachyon*. *New Phytol.* 227, 1736–1748

40. Lusinska, J. *et al.* (2018) Chromosome identification and reconstruction of evolutionary rearrangements in *Brachypodium distachyon*, *B. stacei* and *B. hybridum*. *Ann. Bot.* 122, 445–459
41. Lusinska, J. *et al.* (2019) Comparatively barcoded chromosomes of *Brachypodium* perennials tell the story of their karyotype structure and evolution. *Int. J. Mol. Sci.* 20, 5557
42. Hasterok, R. *et al.* (2020) Progressive refinement of the karyotyping of *Brachypodium* genomes. *New Phytol.* 227, 1668–1675
43. Liu, G. and Zhang, T. (2021) Single copy oligonucleotide fluorescence *in situ* hybridization probe design platforms: development, application and evaluation. *Int. J. Mol. Sci.* 22, 7124
44. Liu, X. *et al.* (2020) Dual-color oligo-FISH can reveal chromosomal variations and evolution in *Oryza* species. *Plant J.* 101, 112–121
45. Li, G. *et al.* (2021) An efficient oligo-FISH painting system for revealing chromosome rearrangements and polyploidization in Triticeae. *Plant J.* 105, 978–993
46. Thakur, J. *et al.* (2021) Sequence, chromatin and evolution of satellite DNA. *Int. J. Mol. Sci.* 22, 4309
47. Shahid, S. and Slotkin, R.K. (2020) The current revolution in transposable element biology enabled by long reads. *Curr. Opin. Plant Biol.* 54, 49–56
48. Li, Y. *et al.* (2018) Centromeric DNA characterization in the model grass *Brachypodium distachyon* provides insights on the evolution of the genus. *Plant J.* 93, 1088–1101
49. Han, J. *et al.* (2018) 3C and 3C-based techniques: the powerful tools for spatial genome organization deciphering. *Mol. Cytogenet.* 11, 21
50. Pinkel, D. *et al.* (1988) Fluorescence *in situ* hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. *Proc. Natl. Acad. Sci. U. S. A.* 85, 9138–9142
51. Boyle, S. *et al.* (2001) The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells. *Hum. Mol. Genet.* 10, 211–219
52. Federico, C. *et al.* (2006) Gene-rich and gene-poor chromosomal regions have different locations in the interphase nuclei of cold-blooded vertebrates. *Chromosoma* 115, 123–128
53. Pecinka, A. *et al.* (2004) Chromosome territory arrangement and homologous pairing in nuclei of *Arabidopsis thaliana* are predominantly random except for NOR-bearing chromosomes. *Chromosoma* 113, 258–269
54. Berr, A. and Schubert, I. (2007) Interphase chromosome arrangement in *Arabidopsis thaliana* is similar in differentiated and meristematic tissues and shows a transient mirror symmetry after nuclear division. *Genetics* 176, 853–863
55. Berr, A. *et al.* (2006) Chromosome arrangement and nuclear architecture but not centromeric sequences are conserved between *Arabidopsis thaliana* and *Arabidopsis lyrata*. *Plant J.* 48, 771–783
56. Albert, P.S. *et al.* (2019) Whole-chromosome paints in maize reveal rearrangements, nuclear domains, and chromosomal relationships. *Proc. Natl. Acad. Sci. U. S. A.* 116, 1679–1685
57. Robaszkiewicz, E. *et al.* (2016) The arrangement of *Brachypodium distachyon* chromosomes in interphase nuclei. *J. Exp. Bot.* 67, 5571–5583
58. Evans, H.J. *et al.* (1959) The relative biological efficiency of single doses of fast neutrons and gamma-rays on *Vicia faba* roots and the effect of oxygen. Part II. Chromosone damage: the production of micronuclei. *Int J Radiat Biol Relat Stud Phys Chem Med* 1, 216–229
59. Macovei, A. *et al.* (2016) Plant response to genotoxic stress: a crucial role in the context of global climate change. In *Abiotic Stress Response in Plants* (Tuteja, N. and Gill, S.S., eds), pp. 428, John Wiley & Sons
60. Kus, A. *et al.* (2018) Dissecting the chromosomal composition of mutagen-induced micronuclei in *Brachypodium distachyon* using multicolour FISH. *Ann. Bot.* 122, 1161–1171
61. Kus, A. *et al.* (2019) Detecting *Brachypodium distachyon* chromosomes Bd4 and Bd5 in MH- and X-ray-induced micronuclei using mcFISH. *Int. J. Mol. Sci.* 20, 2848
62. Pesnya, D.S. and Romanovsky, A.V. (2013) Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the *Allium cepa* test. *Mutat. Res.* 750, 27–33
63. Khadra, A. *et al.* (2012) Assessment of the genotoxicity of quinolone and fluoroquinolones contaminated soil with the *Vicia faba* micronucleus test. *Ecotoxicol. Environ. Saf.* 76, 187–192
64. Juchimiuk-Kwasniewska, J. *et al.* (2011) FISH in analysis of gamma ray-induced micronuclei formation in barley. *J. Appl. Genet.* 52, 23–29
65. Dong, F. and Jiang, J. (1998) Non-Rabl patterns of centromere and telomere distribution in the interphase nuclei of plant cells. *Chromosom. Res.* 6, 551–558
66. Pouokam, M. *et al.* (2019) The Rabl configuration limits topological entanglement of chromosomes in budding yeast. *Sci. Rep.* 9, 6795
67. Idziak, D. *et al.* (2015) Spatial distribution of centromeres and telomeres at interphase varies among *Brachypodium* species. *J. Exp. Bot.* 66, 6623–6634
68. Dong, P. *et al.* (2020) Plant and animal chromatin three-dimensional organization: similar structures but different functions. *J. Exp. Bot.* 71, 5119–5128
69. Dreissig, S. *et al.* (2017) Live-cell CRISPR imaging in plants reveals dynamic telomere movements. *Plant J.* 91, 565–573
70. Fujimoto, S. and Matsunaga, S. (2017) Visualization of chromatin loci with transiently expressed CRISPR/Cas9 in plants. *Cytologia* 82, 559–562
71. Murphy, S.P. *et al.* (2014) A dynamic meiotic SUN belt includes the zygotene-stage telomere bouquet and is disrupted in chromosome segregation mutants of maize (*Zea mays* L.). *Front. Plant Sci.* 5, 314
72. Varas, J. *et al.* (2015) Absence of SUN1 and SUN2 proteins in *Arabidopsis thaliana* leads to a delay in meiotic progression and defects in synapsis and recombination. *Plant J.* 81, 329–346
73. Hus, K. *et al.* (2020) A CRISPR/Cas9-based mutagenesis protocol for *Brachypodium distachyon* and its allopolyploid relative, *Brachypodium hybridum*. *Front. Plant Sci.* 11, 614
74. Raza, A. *et al.* (2019) Impact of climate change on crops adaptation and strategies to tackle its outcome: a review. *Plants (Basel)* 8, 34
75. Monroe, J.G. *et al.* (2021) Diversity in non-linear responses to soil moisture shapes evolutionary constraints in *Brachypodium*. *G3 (Bethesda)* 11, jkab334
76. Fisher, L.H. *et al.* (2016) Linking dynamic phenotyping with metabolite analysis to study natural variation in drought responses of *Brachypodium distachyon*. *Front. Plant Sci.* 7, 1751
77. Luo, N. *et al.* (2011) Natural variation of drought response in *Brachypodium distachyon*. *Physiol. Plant.* 141, 19–29
78. Skalska, A. *et al.* (2020) Genetic and methylome variation in Turkish *Brachypodium distachyon* accessions differentiate two geographically distinct subpopulations. *Int. J. Mol. Sci.* 21, 6700
79. Lenk, I. *et al.* (2019) Transcriptional and metabolomic analyses indicate that cell wall properties are associated with drought tolerance in *Brachypodium distachyon*. *Int. J. Mol. Sci.* 20, 1758
80. Bian, Y. *et al.* (2017) Integrated proteomic analysis of *Brachypodium distachyon* roots and leaves reveals a synergistic network in the response to drought stress and recovery. *Sci. Rep.* 7, 46183
81. Ma, L. *et al.* (2021) Genome-wide identification, phylogenetic analysis and expression profiling of the late embryogenesis-abundant (LEA) gene family in *Brachypodium distachyon*. *Funct. Plant Biol.* 48, 386–401
82. Song, J. *et al.* (2019) *Brachypodium* histone deacetylase BdHD1 positively regulates ABA and drought stress responses. *Plant Sci.* 283, 355–365
83. Song, J. *et al.* (2020) BdHD1, a histone deacetylase of *Brachypodium distachyon*, interacts with two drought-responsive transcription factors, BdWRKY24 and BdMYB22. *Plant Signal. Behav.* 15, 1774715
84. Galle, A. *et al.* (2019) Genome-wide identification of the glutathione transferase superfamily in the model organism *Brachypodium distachyon*. *Funct. Plant Biol.* 46, 1049–1062

85. Smertenko, T. *et al.* (2020) *Brachypodium distachyon* MAP20 functions in metaxylem pit development and contributes to drought recovery. *New Phytol.* 227, 1681–1695
86. Gladala-Kostarz, A. *et al.* (2020) Mechanical stimulation in *Brachypodium distachyon*: Implications for fitness, productivity, and cell wall properties. *Plant Cell Environ.* 43, 1314–1330
87. Wen, F. *et al.* (2017) Genome-wide survey of heat shock factors and heat shock protein 70s and their regulatory network under abiotic stresses in *Brachypodium distachyon*. *PLoS One* 12, e0180352
88. Subburaj, S. *et al.* (2017) Molecular characterization and expression profiling of *Brachypodium distachyon* L. cystatin genes reveal high evolutionary conservation and functional divergence in response to abiotic stress. *Front. Plant Sci.* 8, 743
89. Wang, X. *et al.* (2020) Expression and evolution of the phospholipase C gene family in *Brachypodium distachyon*. *Genes Genomics* 42, 1041–1053
90. Routledge, A.P. *et al.* (2004) *Magnaporthe grisea* interactions with the model grass *Brachypodium distachyon* closely resemble those with rice (*Oryza sativa*). *Mol. Plant Pathol.* 5, 253–265
91. Ayliffe, M. *et al.* (2013) Infection of *Brachypodium distachyon* with selected grass rust pathogens. *Mol. Plant-Microbe Interact.* 26, 946–957
92. Figueroa, M. *et al.* (2015) Pushing the boundaries of resistance: insights from *Brachypodium*-rust interactions. *Front. Plant Sci.* 6, 558
93. Schneebeli, K. *et al.* (2015) *Brachypodium distachyon* is a pathosystem model for the study of the wheat disease rhizoctonia root rot. *Plant Pathol.* 64, 91–100
94. Peraldi, A. *et al.* (2011) *Brachypodium distachyon:* a new pathosystem to study *Fusarium* head blight and other *Fusarium* diseases of wheat. *BMC Plant Biol.* 11, 100
95. Falter, C. and Voigt, C.A. (2014) Comparative cellular analysis of pathogenic fungi with a disease incidence in *Brachypodium distachyon* and *Miscanthus* x *giganteus*. *Bioenerg Research* 7, 958–973
96. Cui, Y. *et al.* (2012) Fine mapping of the *Bsr1* barley stripe mosaic virus resistance gene in the model grass *Brachypodium distachyon*. *PLoS One* 7, e38333
97. Gilbert, B. *et al.* (2018) Components of *Brachypodium distachyon* resistance to nonadapted wheat stripe rust pathogens are simply inherited. *PLoS Genet.* 14, e1007636
98. Della Coletta, R. *et al.* (2019) Genomic dissection of nonhost resistance to wheat stem rust in *Brachypodium distachyon*. *Mol. Plant-Microbe Interact.* 32, 392–400
99. Zhang, H.T. *et al.* (2010) Genetic and comparative genomics mapping reveals that a powdery mildew resistance gene MI3D232 originating from wild emmer co-segregates with an NBS-LRR analog in common wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 121, 1613–1621
100. Shin, J. *et al.* (2012) TIME FOR COFFEE represses accumulation of the MYC2 transcription factor to provide time-of-day regulation of jasmonate signaling in *Arabidopsis*. *Plant Cell* 24, 2470–2482
101. Della Coletta, R. *et al.* (2021) A homolog of the *Arabidopsis* TIME FOR COFFEE gene is involved in nonhost resistance to wheat stem rust in *Brachypodium distachyon*. *Mol. Plant-Microbe Interact.* 34, 1298–1306
102. Schweiger, W. *et al.* (2013) Functional characterization of two clusters of *Brachypodium distachyon* UDP-glycosyltransferases encoding putative deoxynivalenol detoxification genes. *Mol. Plant-Microbe Interact.* 26, 781–792
103. Werner, B.T. *et al.* (2021) *Fusarium graminearum* DICER-like-dependent sRNAs are required for the suppression of host immune genes and full virulence. *PLoS One* 16, e0252365
104. Zanini, S. *et al.* (2021) Comparative analysis of transcriptome and sRNAs expression patterns in the *Brachypodium distachyon-Magnaporthe oryzae* pathosystems. *Int. J. Mol. Sci.* 22, 650
105. Abdelsalam, S.S.H. *et al.* (2020) Identification of effector candidate genes of *Rhizoctonia solani* AG-1 IA expressed during infection in *Brachypodium distachyon*. *Sci. Rep.* 10, 14889
106. Kouzai, Y. *et al.* (2018) Salicylic acid-dependent immunity contributes to resistance against *Rhizoctonia solani*, a necrotrophic fungal agent of sheath blight, in rice and *Brachypodium distachyon*. *New Phytol.* 217, 771–783
107. Kouzai, Y. *et al.* (2020) BdWRKY38 is required for the incompatible interaction of *Brachypodium distachyon* with the necrotrophic fungus *Rhizoctonia solani*. *Plant J.* 104, 995–1008
108. Scholthof, K.B.G. (2020) *Brachypodium* and plant viruses: entwined tools for discovery. *New Phytol.* 227, 1676–1680
109. Pyle, J.D. and Scholthof, K.B.G. (2018) *De novo* generation of helper virus-satellite chimera RNAs results in disease attenuation and satellite sequence acquisition in a host-dependent manner. *Virology* 514, 182–191
110. Hestrin, R. *et al.* (2021) Plants and mycorrhizal symbionts acquire substantial soil nitrogen from gaseous ammonia transport. *New Phytol.* 231, 1746–1757
111. Classen, A.T. *et al.* (2015) Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: what lies ahead? *Ecosphere* 6, art130
112. Perez-Jaramillo, J.E. *et al.* (2016) Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant Mol. Biol.* 90, 635–644
113. do Amaral, F.P. *et al.* (2016) Differential growth responses of *Brachypodium distachyon* genotypes to inoculation with plant growth promoting rhizobacteria. *Plant Mol. Biol.* 90, 689–697
114. Gagne-Bourque, F. *et al.* (2015) Accelerated growth rate and increased drought stress resilience of the model grass *Brachypodium distachyon* colonized by *Bacillus subtilis* B26. *PLoS One* 10, e0130456
115. Schillaci, M. *et al.* (2021) Time-resolution of the shoot and root growth of the model cereal *Brachypodium* in response to inoculation with *Azospirillum* bacteria at low phosphorus and temperature. *Plant Growth Regul.* 93, 149–162
116. Schillaci, M. *et al.* (2021) The metabolic response of *Brachypodium* roots to the interaction with beneficial bacteria is affected by the plant nutritional status. *Metabolites* 11, 358
117. Yang, X. *et al.* (2021) Differential gene expression of *Brachypodium distachyon* roots colonized by *Gluconacetobacter diazotrophicus* and the role of *BdCESA8* in the colonization. *Mol. Plant-Microbe Interact.* 34, 1143–1156
118. Hong, J.J. *et al.* (2012) Diversity of morphology and function in arbuscular mycorrhizal symbioses in *Brachypodium distachyon*. *Planta* 236, 851–865
119. Riley, R.C. *et al.* (2019) Resource allocation to growth or luxury consumption drives mycorrhizal responses. *Ecol. Lett.* 22, 1757–1766
120. Buendia, L. *et al.* (2019) *Brachypodium distachyon tar2l*^{hypo} mutant shows reduced root developmental response to symbiotic signal but increased arbuscular mycorrhiza. *Plant Signal. Behav.* 14, e1651608
121. Muller, L.M. *et al.* (2020) Constitutive overexpression of *RAM1* leads to an increase in arbuscule density in *Brachypodium distachyon*. *Plant Physiol.* 184, 1263–1272
122. Scheller, H.V. and Ulvskov, P. (2010) Hemicelluloses. *Annu. Rev. Plant Biol.* 61, 263–289
123. Whitehead, C. *et al.* (2018) A glycosyl transferase family 43 protein involved in xylan biosynthesis is associated with straw digestibility in *Brachypodium distachyon*. *New Phytol.* 218, 974–985
124. Marriott, P.E. *et al.* (2014) Range of cell-wall alterations enhance saccharification in *Brachypodium distachyon* mutants. *Proc. Natl. Acad. Sci. U. S. A.* 111, 14601–14606
125. Little, A. *et al.* (2018) Revised phylogeny of the cellulose synthase gene superfamily: insights into cell wall evolution. *Plant Physiol.* 177, 1124–1141
126. Carpita, N.C. and McCann, M.C. (2010) The maize mixed-linkage (1->3),(1->4)-beta-D-glucan polysaccharide is synthesized at the Golgi membrane. *Plant Physiol.* 153, 1362–1371
127. Wilson, S.M. *et al.* (2015) Determining the subcellular location of synthesis and assembly of the cell wall polysaccharide (1,3;1,4)-beta-D-glucan in grasses. *Plant Cell* 27, 754–771
128. Kim, S.J. *et al.* (2015) The cytoplasmic localization of the catalytic site of CSLF6 supports a channeling model for the biosynthesis of mixed-linkage glucan. *Plant J.* 81, 537–547
129. Kim, S.J. *et al.* (2018) In the grass species *Brachypodium distachyon*, the production of mixed-linkage (1,3;1,4)-beta-glucan (MLG) occurs in the Golgi apparatus. *Plant J.* 93, 1062–1075

130. Fan, M. *et al.* (2018) A trihelix family transcription factor is associated with key genes in mixed-linkage glucan accumulation. *Plant Physiol.* 178, 1207–1221
131. Handakumbura, P.P. *et al.* (2018) SECONDARY WALL ASSOCIATED MYB1 is a positive regulator of secondary cell wall thickening in *Brachypodium distachyon* and is not found in the Brassicaceae. *Plant J.* 96, 532–545
132. MacKinnon, K.J. *et al.* (2020) Changes in ambient temperature are the prevailing cue in determining *Brachypodium distachyon* diurnal gene regulation. *New Phytol.* 227, 1709–1724
133. Barros, J. *et al.* (2016) Role of bifunctional ammonia-lyase in grass cell wall biosynthesis. *Nat Plants* 2, 16050
134. Mitchell, R.A. *et al.* (2007) A novel bioinformatics approach identifies candidate genes for the synthesis and feruloylation of arabinoxylan. *Plant Physiol.* 144, 43–53
135. Buanafina, M.M. *et al.* (2016) Functional testing of a PF02458 homologue of putative rice arabinoxylan feruloyl transferase genes in *Brachypodium distachyon*. *Planta* 243, 659–674
136. de Souza, W.R. *et al.* (2018) Suppression of a single BAHD gene in *Setaria viridis* causes large, stable decreases in cell wall feruloylation and increases biomass digestibility. *New Phytol.* 218, 81–93
137. Bartley, L.E. *et al.* (2013) Overexpression of a BAHD acyltransferase, OsAt10, alters rice cell wall hydroxycinnamic acid content and saccharification. *Plant Physiol.* 161, 1615–1633
138. Petrik, D.L. *et al.* (2014) p-Coumaroyl-CoA:monolignol transferase (PMT) acts specifically in the lignin biosynthetic pathway in *Brachypodium distachyon*. *Plant J.* 77, 713–726
139. Rancour, D.M. *et al.* (2015) Cell wall composition and digestibility alterations in *Brachypodium distachyon* achieved through reduced expression of the UDP-arabinopyranose mutase. *Front. Plant Sci.* 6, 446
140. Cass, C.L. *et al.* (2015) Effects of *PHENYLALANINE AMMONIA LYASE (PAL)* knockdown on cell wall composition, biomass digestibility, and biotic and abiotic stress responses in *Brachypodium*. *J. Exp. Bot.* 66, 4317–4335
141. Lee, S.J. *et al.* (2012) Biological conversion assay using *Clostridium phytofermentans* to estimate plant feedstock quality. *Biotechnol Biofuels* 5, 5
142. Giorgi, F. (2006) Climate change hot-spots. *Geophys. Res. Lett.* 33, L08707
143. Bourgeois, Y. *et al.* (2018) Genome-wide scans of selection highlight the impact of biotic and abiotic constraints in natural populations of the model grass *Brachypodium distachyon*. *Plant J.* 96, 438–451
144. Dell'Acqua, M. *et al.* (2014) Targeting environmental adaptation in the monocot model *Brachypodium distachyon*: a multifaceted approach. *BMC Genomics* 15, 801
145. Wilson, P.B. *et al.* (2019) Global diversity of the *Brachypodium* species complex as a resource for genome-wide association studies demonstrated for agronomic traits in response to climate. *Genetics* 211, 317–331
146. Tyler, L. *et al.* (2016) Population structure in the model grass *Brachypodium distachyon* is highly correlated with flowering differences across broad geographic areas. *Plant Genome* 9, 2
147. Lomax, A. *et al.* (2018) An ortholog of CURLY LEAF/ENHANCER OF ZESTE like-1 is required for proper flowering in *Brachypodium distachyon*. *Plant J.* 93, 871–882
148. Qin, Z. *et al.* (2019) Divergent roles of FT-like 9 in flowering transition under different day lengths in *Brachypodium distachyon*. *Nat. Commun.* 10, 812
149. Ream, T.S. *et al.* (2014) Interaction of photoperiod and vernalization determines flowering time of *Brachypodium distachyon*. *Plant Physiol.* 164, 694–709
150. Woods, D.P. *et al.* (2017) Genetic architecture of flowering-time variation in *Brachypodium distachyon*. *Plant Physiol.* 173, 269–279
151. Woods, D.P. *et al.* (2017) Establishment of a vernalization requirement in *Brachypodium distachyon* requires *REPRESSOR OF VERNALIZATION1*. *Proc. Natl. Acad. Sci. U. S. A.* 114, 6623–6628
152. Nunes, T.D.G. *et al.* (2020) Form, development and function of grass stomata. *Plant J.* 101, 780–799
153. Raissig, M.T. *et al.* (2016) Grasses use an alternatively wired bHLH transcription factor network to establish stomatal identity. *Proc. Natl. Acad. Sci. U. S. A.* 113, 8326–8331
154. Wyler, M. *et al.* (2020) Impact of transposable elements on methylation and gene expression across natural accessions of *Brachypodium distachyon*. *Genome Biol Evol* 12, 1994–2001
155. Stritt, C. *et al.* (2018) Recent activity in expanding populations and purifying selection have shaped transposable element landscapes across natural accessions of the Mediterranean grass *Brachypodium distachyon*. *Genome Biol Evol* 10, 304–318
156. Eichten, S.R. *et al.* (2016) DNA methylation profiles of diverse *Brachypodium distachyon* align with underlying genetic diversity. *Genome Res.* 26, 1520–1531
157. Kawasaki, A. *et al.* (2016) Microbiome and exudates of the root and rhizosphere of *Brachypodium distachyon*, a model for wheat. *PLoS One* 11, e0164533
158. Wei, S. *et al.* (2021) Spatial analysis of the root system coupled to microbial community inoculation shed light on rhizosphere bacterial community assembly. *Biol. Fertil. Soils* 57, 973–989
159. Gao, J. *et al.* (2018) Ecosystem fabrication (EcoFAB) protocols for the construction of laboratory ecosystems designed to study plant-microbe interactions. *J. Vis. Exp.* 10, 57170
160. Sasse, J. *et al.* (2019) Multilab EcoFAB study shows highly reproducible physiology and depletion of soil metabolites by a model grass. *New Phytol.* 222, 1149–1160
161. Jabusch, L.K. *et al.* (2021) Microfabrication of a chamber for high-resolution, in situ imaging of the whole root for plant-microbe interactions. *Int. J. Mol. Sci.* 22, 7880