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Commentary

Advances on genomics, biology, ecology and evolution of Brachypodium, a bridging model grass system for cereals and biofuel grasses

In 2019 the Fourth International Brachypodium Conference took place in Huesca (Spain), celebrating a decade of tremendous progress in the development and use of species in the genus *Brachypodium* as a model system. Since the publication of the sequenced genome of its flagship species (*B. distachyon*) in 2010 (International Brachypodium Initiative, 2010), the temperate grass genus *Brachypodium* has become an indispensable tool to investigate many aspects of functional genomics and biology of grasses and its translation to cereals and biomass crops, paralleling the role of its counterpart dicot *Arabidopsis*. This collection uncovers some of the most outstanding scientific discoveries presented in that meeting or obtained in recent years using *Brachypodium* and other plant species as targets for the development of scientific knowledge in plants.

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The Letter by Spoelhof *et al.* (2020) published in *New Phytologist* highlights a hotly debated question on the evolutionary significance of polyploidy, examining hypotheses that may explain its high prevalence in plants and its low frequency in animals. In addition to the discussion of previous hypotheses on the disruptive effect of polyploidy in development and sex chromosome function, the authors propose a complementary reproductive assurance hypothesis that better supports the success of polyploids in higher eukaryotes. Reproductive assurance, exerted through asexual reproduction or self-fertilization ensures the establishment of the new polyploid at this critical step of its emergence and, when followed with reversion to (or maintenance of) sexual reproduction and outcrossing, led to the long-term persistence and diversification of polyploid species. The authors support their hypothesis with a wealth of reproductive assurance examples found in the plant, animal and fungal kingdoms.

Stritt *et al.* (2020, in this issue) exquisitely illustrates the landscape genomics of highly dynamic long terminal repeat (LTR) transposable elements (TEs) and how individual TE lineages change the genetic and epigenetic constitution of the host. A thorough inspection of the Copia and Gypsy LTR retrotransposon families identified the three elements that most profoundly affect the evolution of the *B. distachyon* genome. The Copia Angela family shows the highest turnover and shortest half-life (66 kya) and so contribute the most to genome dynamics; it constitutes the main source of intraspecific TE polymorphisms, many of which are solo LTRs. Their high plasticity and potential inter-element recombination are viewed as plausible mechanisms that keep the genome small despite ongoing activity. An active nonautonomous centromeric Gypsy family is interpreted as the causal agent of the dynamic nature of the *B. distachyon* centromeres. By contrast, the less active, oldest and heavily methylated Gypsy Retand copies account for much of the GC bias detected across the genome. Their abundance in pericentromeric positions may explain the negative correlation observed between GC content and recombination rates in the species, whereas the variation of GC content and methylation levels along their copies could influence genetic and epigenetic regulation.

VanWallendael *et al.* (2020, in this issue) describes the C_4 biofuel switchgrass to investigate the ecogenomics of local adaptation to *Puccinia* spp leaf-rust resistance along a large north-to-south geoclimatic cline in the United States. In their elegant experiment the authors analyse the association of quantitative trait loci (QTLs) with rust severity across cross-designed mapping population genotypes from north-sensitive and south-tolerant parental ecotypes in multiple sites and years. Their results indicate that rust infection severity mostly results from the interaction of two large-effect genetic loci conferring plant resistance and abiotic factors covarying with latitude, especially in northern sites. The data also show a negative synergistic epistatic interaction of southern and northern alleles at those loci, suggesting that segregating genetic variation in epistatically interacting loci plays a role in determining responses to infection across geographic space.

Smertenko *et al.* (2020, in this issue) is an excellent example of reverse genomics using transgenic *Brachypodium* and tobacco plants and optical and electronic immunology applied to the study of metaxylem pit formation in *B. distachyon* and its potential contribution to the acquisition of drought tolerance. Through expression analysis of the TPX2-like microtubule MAP20 protein, its artificial microRNA (amiRNA)-mediated knockdown mutants and immunolocalization approaches, the authors demonstrate the role of MAP20 in determining cell wall composition in phloem, metaxylem pit formation during the late stages of vascular bundle development and in inhibition of microtubule depolymerization. Their study of a knockdown mutant also supports a role for MAD20 in drought adaptation by modulating pit size and pit membrane thickness in the metaxylem.

The paper by Yu *et al.* (2019) shows, through an excellent and comprehensive evo-devo study, how formation and placement of abscission zones (AZs) vary in three divergent grasses (*B. distachyon*, *Oryza sativa*, *Setaria viridis*). Their phylogenetic analysis reconstructs the ancestral AZ rachilla position in *B. distachyon* and *O. sativa*, whereas in *S. viridis* it has a derived pedicel position. Their gene expression and co-expression network analysis demonstrates that the set of genes involved in AZ formation is conserved across species, although developmental patterns of expression and networks are very different. Through differential gene expression (DGE) and transmission electron microscopy (TEM) analysis they confirm that lignification is not a prerequisite for abcission. Furthermore, their *in situ* hybridization studies in developing floral tissues corroborates the position of AZs in each species and their different genetic regulation during AZ development. The authors conclude that when genes in the same pathway shift together to a new position, their interaction with genes in the new position also differs and therefore cause distinct co-expression networks. Their data support the role of a few key genes in determining heterotopy in AZ location.

The work of MacKinnon *et al.* (2020, in this issue) presents a thorough transcriptome-based analysis that identifies and functionally characterizes the genes and regulatory elements involved in the rhythmic circadian clock of *B. distachyon*. The study reveals that thermocycles rather than photocycles are the prevailing signals controlling diurnal gene regulation. Notably, they find that internal circadian clock circle regulators are smaller in *B. distachyon* (3.6%) than in other plant species. This suggests that those genes have a smaller role in circadian gene regulation, and that the circadian clock period in constant conditions is slower in *Brachypodium* (26–28 h) than in other plants (23–25 h), although it may be influenced by light intensity and genetic background. The study unveils new regulatory motifs involved in diurnal gene expression or circadian clock output (VNS, W-box, Dof), among them possible drivers of thermocycle rhythms, suggesting also that night temperature causes rhythmic cell wall thickening. The authors conclude that although either photocycles and thermocycles (or both) affect rhythmic gene expression, temperature effects appear to be prevalent. This is consistent with the lack of elongation growth rhythms in grasses in constant conditions or in the presence of photocycles alone.

Woods *et al.* (2020, in this issue) highlight work that advances our knowledge of the regulatory processes that control flowering time in *B. distachyon*. Through a combination of forward and reverse genetics, ChIP and histone immunoblotting, the authors demonstrate that a single mutation in the third subunit of DNA polymerase (POLD3), a previously uncharacterized gene in plants, is responsible for a vernalization-independent rapid-flowering phenotype. Genome-wide analysis of the mutant phenotype in a segregating mapping population identified a gene orthologous to POLD3 as its causative effect, and its expression was characterized across developmental stages and tissues. Using transcriptome analysis the authors show that when the mutant is grown under long-day conditions FT is precociously induced, VRN1 is constitutively expressed and the active chromatin mark H3K4me3 is significantly enriched around flowering induction gene regions, thus causing the rapid-flowering phenotype.

The Tansley insight by Scholthof (2020, in this issue) directs us to four main research areas that are revealing new discoveries in grass–virus interactions. Analysis of RNA modification of the host and viruses during infection has detected a plethora of alternative splicing events from which certain types of messenger RNAs (mRNAs) involved in resistance response have been identified in *B. distachyon* and other grasses, as well as polyadenylations of viral RNAs that may reflect a host response to viral infection. Although evolutionary studies of virus–host interactions in grasses are still in their infancy, primary findings on long-term relationships of barley stripe mosaic viruses (BSMVs) with barley and of genes related to BSMV resistance in close *B. distachyon* lines reveal the possibilities of potential coevolution. The author stresses how basic and new virology methodologies have set the way for synthetic biology as a tool for understanding host–virus interactions using *B. distachyon* as a model and how a more holistic approach, the dissection of environment–host–multiple viral infections using also *Brachypodium*, may reveal the complexity of beneficial non-pathogenic interactions. These include effects within the ecological context, and the immunity created in susceptible plants by some mild virus that prevent more severe infections.

Another Tansley insight by Hasterok *et al.* (2020, in this issue) reviews the cytomolecular methods used to disentangle the karyotype evolution of *Brachypodium*, one of the few genera of angiosperms which allows the intra-chromosomal characterization of its chromosomes due to its low content of intersticial broad repetitive DNA. The paper describes the technological advances and scientific achievements attained during the last two decades of chromosomal study and their parallel insights with those provided by phylogenetic methods. Initial genomic *in situ* hybridization (GISH) analysis first detected the existence of three cytotypes within the *B. distachyon* complex, which were later described as separate species. Ribosomal DNA (rDNA) mapping of 5S genes reveals the ploidy level of *Brachypodium* species whereas the use of bacterial artificial chromosome (BAC)-based probes in fluorescence *in situ* hybridization (FISH) analysis allowed the characterization of known and orphan subgenomes of *Brachypodium* allopolyploids. Refinements in advanced comparative chromosome painting (CCP) and of comparative chromosome barcoding (CCB) methods have served to map and characterize the chromosomes and to infer the karyotype evolution of the studied species and their putative ancestors, as well as to reconstruct the evolutionary rearrangements of allotetraploid *B. hybridum* lines with respect to those of its diploid progenitors *B. distachyon* and *B. stacei*. FISH analyses targeted to centromeric retrotransposons have also been used to characterize the karyotypes and to infer evolutionary relationships among *Brachypodium* species.

Becana *et al.*'s Tansley review (2020, in this issue) presents an updated and exhaustive revision of the globin family in green organisms and their symbiotic N_2-fixing bacteria. The paper describes the structure of the proteins (3/3, 2/2 distribution of α-helices), their axial coordination to the heme group (hexacoordination, pentacoordination) and their known or accepted functions in the main Chlorobionta (and cyanobacteria) lineages. The globins of plants and algae have been classified into three classes; Class 1 (3/3) and Class 3 (2/2) are present in all angiosperms, whereas Class 2 (3/3) are absent in monocots, probably due to secondary loss. The 3/3 globins likely evolved from a bacterial flavoHb gene transferred to an ancestral eukaryote through horizontal gene transfer, whereas the 2/2 globins were transferred from a bacterium ancestral to Chloroflexi before the evolution of land plants. The origin of Class 2 (3/3) globins is probably related to the whole-genome-duplication that occurred at the emergence of the angiosperms. Symbiotic globins derive from Class 1 or Class 2 globins and originated more recently. A thorough review of physiological and functional genomic studies conducted on globins indicates that Class 1 globins are involved in hypoxia responses and modulate NO concentration, thus playing roles in plant morphogenesis, hormone signalling, cell fate determination, nutrient deficiency, nitrogen metabolism and plant–microorganism symbioses, whereas leghemoglobins of legumes and other symbiotic globins transport O_2 in the nodules.

A Tansley review by Coomey *et al.* (2020, in this issue) is an encyclopaedic revision of grass secondary cell walls that also highlights novel discoveries found in the model plant *B. distachyon*. The paper describes the importance of the secondary cell wall as a physical protective barrier against pathogens, promoting tolerance to abiotic stresses and as crucial for water transport and support of plant structures. A thorough comparative review of the synthesis, deposition and function of the main components of the secondary cell wall in *Arabidopsis* (dicots) and *Brachypodium* (grasses), such as cellulose, mixed-linkage glucans (MLGs), heteroxylans, lignin, hydroxycinnamic acids, tricin and silicon, reveals special features of the grasses. Among the untapped characteristics of grass cell walls in *B. distachyon* are the discovery of xylosyl and arabinosyltransferases, a MLG-synthase and the identification of novel substrates and enzyme activities in the synthesis of lignin monolignols like phenylalanine tyrosine ammonia lyase (PTAL), hydroxycinnamate acyltransferases, *p*-coumaryl-coenzyme A:monolignol transferase (PMT), caffeic acid/5-hydroxyferulic acid *O*-methyltransferase (COMT) and laccases. The authors have found a nearly complete overlap between the regulatory network components of eudicots and grasses, though some transcription factors are likely to be unique to grasses. One example is THX1, a protein that binds to a CSLF gene encoding a predominant MLG-synthase, which regulates a hemicellulose gene not present in eudicots.

The comprehensive Tansley review by McKown & Bergmann (2020, in this issue) splendidly describes the recently clarified paradox of differential stomata formation in dicots and monocots through the comparative study of *Arabidopsis* and *Brachypodium* (and crop cereal) model plants. Starting from the striking developmental differences that produce two-cell stomata from scattered leaf epidermis meristemoid cells at the base of the leaf in dicots, to the regular alternate lineal dispositions of precursor cells in the longitudinally elongated leaves of grasses that ends in its typical four-cell stomata (two stomata and two subsidiary cells) that differentiate towards the tip of the leaf; the authors review the genetic regulation of these processes. They point to an evolutionary 'rewiring' involving novel roles, shifted functions, and altered regulation of the Ia (SPCH, MUTE, FAMA) and IIIb (ICE1, SCRM2) basic helix-loop-helix (bHLH) gene expression patterns and protein behaviour that render stomatal organization and patterning in grasses distinct from those of dicots. The authors also illustrate the epidermal patterning factor (EPF)-mediated peptide signalling cascade that is responsible for the leaf stomatal density, and how this density can be engineered in both axes across the grass leaf. This can be done by modulating the number of stomatal files that flank the veins, or by modulating the distance between and within stomatal files and veins, to improve water-use-efficiency – although the mechanistic understanding of this regulation is still lacking. Genetic studies show that adaxial/abaxial stomatal patterning may be a trait that has been subject to evolutionary selection for specific ecosystems, and vein-derived stomatal signals may have a larger effect on the surface closest to the vein. The authors highlight the importance of the well-known bHLH stomatal developmental framework of *Brachypodium* as an optimal model system to characterize genetic candidates linked to known pathways that may uncover how environmental inputs are integrated into stomatal regulation.

The collection of papers united in this issue highlights the importance of *Brachypodium* as a model system for almost any research topic relevant to monocots and grasses including physiology, development, plant–pathogen interactions, evolution, and ecology. Although most of the studies used the better-studied and more resource-rich annual *B. distachyon* species, considerable new biological and genomic resources are available for other *Brachypodium* species, especially for the allotetraploid *B. hybridum* and the perennial *B. sylvaticum*. This will lead to new outstanding discoveries in many fields of research and particularly to those related to polyploidy and perenniality.

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