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journal homepage: [www.elsevier.com/locate/jcs](http://www.elsevier.com/locate/jcs)A comparative view of grain development in *Brachypodium distachyon*

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## ABSTRACT

Cereal grains are both at the forefront of agriculture as a food source and in basic botanical terms are unique fruit forms. Analyses and characterisation of their development and composition are therefore invaluable in both applied and basic biological research. A key approach in driving forward this research is a comparative one and this approach is facilitated when there are sequence, gene expression and functional resources available for a variety of species. In this article we review and assess the current status of resources for *Brachypodium distachyon* as a model system and we then focus specifically on recent studies characterising and comparing grain development, organisation and composition in this species.

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## 1. Introduction

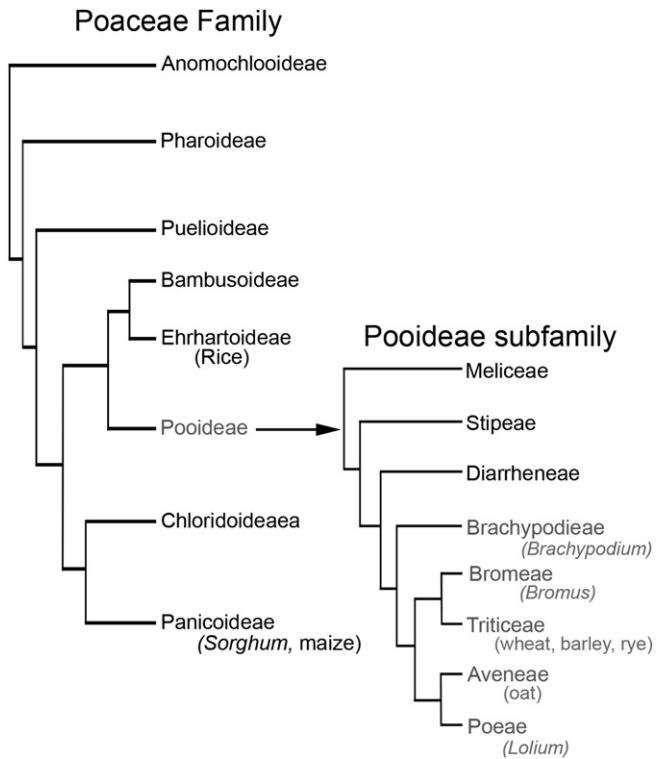
1.1. *Brachypodium* – what is it and why are we studying it?

As monocots and dicots last shared a common ancestor over 150 million years ago and significant biological differences exist between these two groups, it is clear that we need an alternative model to *Arabidopsis* for the study of grasses. Much modern cereal grain research has the objective of increased genetic understanding or genetic manipulations designed to influence traits such as grain quality and disease resistance but in the temperate cereals this research is often hampered by the genetic size and complexity of these species. Prior to the availability of the *Brachypodium* genome, rice has formed the main monocotyledonous model system in terms of genomic resources while maize provided an extensive resource of developmental genetics. However, rice as a semi-aquatic tropical species with specialised cultivation requirements and lacking many important temperate cereal traits does not form an ideal model for the temperate grasses. As a consequence a small wild grass within the Pooideae, *Brachypodium distachyon*, has been developed as a new model system for the temperate cereals though we are still learning how its basic development compares to that of the domesticated cereals. This small, fast-growing species is native to the Middle East and Southern Europe but well established in temperate areas of Australasia, America and Asia. The community standard line, Bd21, originates from a region close to Salukadin in

Iraq (Vogel and Bragg, 2009). Though perhaps regarded initially as little more than as a minor weed of areas of cereal production, it has much to offer not only as a model for study of the temperate grasses but also as an interesting specimen in its own right (Draper et al., 2001; Garvin et al., 2008; Huo et al., 2009).

Diploid *B. distachyon* offers one of the smallest genomes to be found in the entire grass family, ~272 Mbp along with many other physical and phylogenetic attributes that make it suitable as a temperate grass model (Brkljacic et al., 2011; Draper et al., 2001; Garvin et al., 2008; Huo et al., 2009; Opanowicz et al., 2008). A small stature, rapid life cycle and simple growing conditions are particularly useful features for research, especially where extensive or field-based growth facilities may not be available. Densities of up to 1000 plants/m<sup>2</sup> can be achieved in growth chambers or glasshouses, a space which by comparison may accommodate just 50 wheat plants, or around 36 rice plants (Vogel and Bragg, 2009). The small genome of Bd21 favours insertional mutagenesis and efficient positional cloning of genes whilst the fact that *B. distachyon* is inbreeding facilitates the maintenance of homozygous lines (Vogel and Bragg, 2009). *B. distachyon* is the only annual member of the tribe Brachypoideae (Khan and Stace, 1998) which is sister to the “Core pooids”, a sub-group that includes wheat, barley and rye and so is expected to exhibit a strong physiological and genetic resemblance to these species (Huo et al., 2009; Fig. 1). Wheat and *Brachypodium* diverged around 32–39 million years ago whilst wheat and rice diverged more than 50 million years ago (Mur et al., 2011; Vogel et al., 2006). *Brachypodium* displays many of the agronomic traits that are of great importance in the temperate cereals, such as freezing tolerance, resistance to certain pathogens and dormancy mechanisms that

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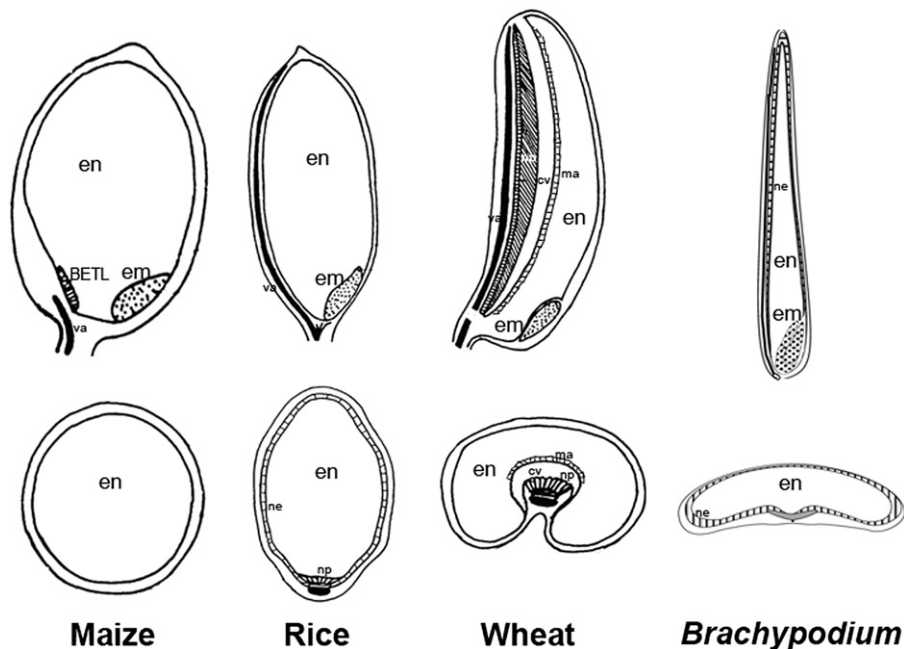
**Fig. 1.** Simplified representation of phylogenetic position of *Brachypodium distachyon*. Based on Catalan et al. (1997), GPWG (2001) and Kellogg (2001).

cannot be seen in rice (Ozdemir et al., 2008; Peraldi et al., 2011). Significantly, *Brachypodium* offers a more similar general grain structure to that of the temperate species (Opanowicz et al., 2011; Ozdemir et al., 2008; Fig. 2) than does rice. Furthermore, the *Brachypodium* genome offers the first example of that of a wild grass from within the Pooideae, unaltered or shaped by

domestication pressures that have driven other species such as wheat (Shewry, 2009).

### 1.2. *Brachypodium* toolkit status

In 2006, the US Department of Energy (DOE) in conjunction with the Joint Genome Institute (JGI) embarked upon a project to sequence the genome of the diploid ecotype, Bd21. Much of the initial funding for this sequencing project came from the USDA and centred on the potential of *Brachypodium* as a model for the bio-energy crops (Bevan et al., 2010). In 2010, the complete and annotated *Brachypodium* genome was released (Vogel et al., 2010) and a sophisticated and growing collection of tools and facilities are already available, with more becoming available in the near future. With the development of efficient stable transformation methods for both the reference line Bd21 and other genotypes (Alves et al., 2009; Vogel and Hill, 2008), the production of a growing collection of insertion lines is underway (Thole et al., 2009). Research groups at both JIC and USDA–ARS are generating thousands of insertion lines in an effort to emulate the SALK Arabidopsis T-DNA insertion line collection. Many of these insertions have now been assigned to unique locations in the genome and associated with ESTs, and are already available to the research community (Thole et al., 2010). The first characterisation of one of these T-DNA insertion lines affecting the *elif4A* gene resulted in a dwarfing phenotype correlating with expected roles in both cell division and cell growth (Vain et al., 2011). Tilling populations have been generated by INRA in France and by the Boyce Thompson Institute and are being phenotyped. These resources will be available to the research community through a searchable database (<http://urgv.evry.inra.fr/UTILLdb>; Brkljacic et al., 2011). In addition to stable transformation methods, VIGS (virus induced gene silencing) techniques have been optimised, paving the way for rapid functional testing of candidate genes (Demircan and Akkaya, 2010; Pacak et al., 2010). The availability of Affymetrix microarrays (NASC) will facilitate gene expression and transcriptomic analyses (Brkljacic et al., 2011). Germplasm collections, although initially



**Fig. 2.** Schematic comparison of basic grain shapes in longitudinal and transverse orientations for maize, rice, wheat and *Brachypodium* – adapted from Krishnan and Dayanandan (2003) with permission – copyright Springer Press. Drawings are not to scale. em, embryo; en, endosperm; ma, modified aleurone; cav, cavity; va, vasculature; ne, nucellar epidermis; np, nucellar projection; BETL, basal endosperm transfer layer.

quite poor, have recently increased significantly with a large collection of 195 diploid lines from diverse sites in Turkey and numerous collections from varied environments in Northern Spain (Filiz et al., 2009; Mur et al., 2011). Researchers report considerable variation in agronomically important traits such as seed size and set, plant architecture and flowering time within these collections (Filiz et al., 2009; Vogel et al., 2009). With the advent of efficient and relatively simple crossing protocols (<http://brachypodium.pw.usda.gov>) overcoming the difficult cleistogamy of these diploid lines, the production of recombinant inbred lines and mapping families can move forward and will add to those already developed (Garvin et al., 2008).

Comparative genomics has revealed evidence of synteny at the whole genome level (Hasterok et al., 2006; Huo et al., 2009; Ma et al., 2010) and patterns of gene loss or duplication in groups of genes involved in starch synthesis and flowering time (Comparot-Moss and Denyer, 2009; Higgins et al., 2010). However, there are some challenges to the significance of the syntenic relationship between *Brachypodium* and the Triticeae as many genomic rearrangements and duplications occurring in the wheat/barley lineage after the divergence of the Brachypoideae appear to have made alignment at the macro level somewhat fragmentary (Bossolini et al., 2007; Mur et al., 2011; Wicker et al., 2011). Although gene order appears more highly conserved between rice and *Brachypodium* at the nucleotide level sequence conservation between *Brachypodium* and the Pooids is generally much higher than can be seen in rice (Bossolini et al., 2007; Mur et al., 2011; Vogel et al., 2006). This similarity favours *Brachypodium* in functional genomic experimentation and markers created in *Brachypodium* have a higher conversion rate to temperate species (Mur et al., 2011). Even so, gene family sequence similarity across the Poaceae has been shown to be comparatively high and recent, rapid evolutionary divergence such as may occur under domestication could be influential; as a consequence care must be taken to ensure *Brachypodium* will offer the best source of genomic information for any particular trait under investigation, and to utilise other grass genomes in a comparative approach (Mur et al., 2011).

*B. distachyon* is already a demonstrably valuable and successful functional genomic model for temperate grass research. *Brachypodium* sequence data have also proved invaluable in the identification and cloning of agronomically important genes in wheat and barley (Opanowicz et al., 2008) and the syntenic relationship to wheat was instrumental in the characterisation and functional analysis of the *PH1* locus in wheat, as the rice sequence, although available, was too divergent in this area (Griffiths et al., 2006). Another prominent example of this value is a study of globulin gene conservation amongst temperate and tropical grasses (Gu et al., 2010). A globulin gene duplication in the Triticeae resulted in the HMW glutenin gene, whose encoded protein is critical to the unique bread-making properties of wheat. This duplication cannot be seen in any of the tropical grass genomes, but it can be identified in *Brachypodium* (Gu et al., 2010) and is a good example of the insights and features that *Brachypodium* can add. A recent in-depth review by Mur et al. (2011) critically assesses the current status of the *Brachypodium* toolbox.

## 2. Grain biology in the temperate grasses

Much work has been done on the evolution and development of floral organ identity and inflorescence architecture in cereals, and these analyses extend to both uncultivated and wild relatives (Vollbrecht et al., 2005; Whipple et al., 2007). However, less is known about the evolution of grain form and function. There is a particular paucity of information with regard to tissue organisation in the grain and the ability of the endosperm to store reserves,

especially true in species outside of the cultivated cereals. For the economically important cereal crops, grain morphology and organisation can have important practical implications – for example, the wheat grain has a distinct crease that is missing in rice, with profound consequences for processing (Evers and Millar, 2002; Kent and Evers, 1994).

The grain (caryopsis) is a single seeded fruit characteristic of the grasses. It is a composite organ with three genetically distinct components, the pericarp and associated maternal tissues, the embryo and a prominent and persistent triploid endosperm for which cereal species have been domesticated. The cereal endosperm is typically rich in starch and protein and one of the main sources for human nutrition. The ability of endosperm to store starch so proficiently is a character associated with the family Poaceae, which is synonymous with “The Grasses” or “Grass Family”, and contains important grain crops such as wheat, rice, maize and sorghum (Fig. 1). Within this family, the Pooideae subfamily has diversified in cooler climates and contains the cereals of most value to the UK economy, wheat, barley, oats and rye. In addition, it includes genera of important forage grasses such as *Lolium* and *Festuca* and invasive weed genera such as *Elymus*, *Bromus* and *Brachypodium* itself.

In most cultivated and wild grasses, the endosperm is the largest component of the grain and demonstrates certain developmental features that appear well conserved (in studies to date centred primarily on wheat, barley, maize and rice) (Becraft, 2001; Brown et al., 1996; Olsen, 2001; Wegel et al., 2005). For example, early development progresses in the post-fertilisation phase via the division of the central cell-derived triploid nucleus. The descendants of this division form a syncytial ring of nuclei around the central vacuole that divide and cellularise in a concerted sequence of anticlinal and periclinal cell divisions to eventually fill the central vacuole. The endosperm subsequently differentiates into functionally distinct subdomains, which include the aleurone, modified aleurone or transfer layer, central starchy endosperm and embryo-surrounding region. Temporal and spatial development can vary depending on the species and our studies also indicate that the number of subdomains is not fixed.

The organisation of the maternal tissue also varies, affecting the possible routes by which sugars and amino acids are supplied to the developing endosperm. For example, the generally accepted conduit for maternal nutritional supplies in wheat grain is through the nucellar projection and modified aleurone (Wang et al., 1994), but differences can be seen in other grains. In maize the vascular tissue supplying the grain terminates at the junction of funiculus and ovule, and a pronounced transfer cell layer exists within the endosperm. No endosperm cavity exists and this basal endosperm transfer layer or BETL facilitates nutritional supply to the rest of the endosperm (Becraft, 2001; Costa et al., 2004; Olsen, 2001). Rice has two pathways involved in the transport of nutrients within the developing caryopsis: one via a pathway analogous to the nucellar projection pathway of wheat and the other via the nucellar epidermis (Ellis and Chaffey, 1987; Krishnan and Dayanandan, 2003; Oparka and Gates, 1981). Reminiscent of the temperate grains, the vascular system in rice grains extends along the length of the grain (Fig. 2). Though there is no distinctive transfer layer in the endosperm, a region of cells in the rice aleurone adopts a typical transfer layer morphology and there is a pronounced transfer area in the maternal domain overlying the vasculature analogous to the nucellar projection (Kuwano et al., 2011; Li et al., 2008). Rice may utilise an alternative nucellar epidermis-mediated transport route whereby the prominent, plasmodesmata-rich cells of the nucellar epidermis form a nutrient transport pathway into the endosperm, at least until the nucellar epidermis becomes compressed during later development. Despite the apparent functional homology of

some of these transfer tissues, they may not be genetically homologous tissues in the evolutionary sense. For example, one of the few genes shown to specify the identity of particular tissues in the grain is *ZmMRP-1*, a determinant of the BETL layer in maize (Gomez et al., 2009). Whilst the BETL would appear to be a direct functional homologue of the wheat modified aleurone, we identified no orthologues in any of the temperate cereals (no sequence homology extended beyond the conserved myb-domain of the protein). We also found no orthologues in rice, which lacks the BETL layer. This supports the idea that *ZmMRP-1* is the key regulator specifying BETL cell fate but also suggests that, though functionally homologous, the modified aleurone of temperate cereals is genetically distinct and has evolved independently (Fig. 2).

In barley, a host of transcription factors have been identified in the peripheral aleurone as playing key roles in controlling the expression of genes required for germination processes (Rubio-Somoza et al., 2006; Zou et al., 2008) and it will be interesting to assess the role of their orthologues in *Brachypodium*, especially in light of the differences identified in grain development, structure and composition described in the sections below.

### 3. How the *Brachypodium* grain compares with grains of cultivated cereals

As a relatively new model system even basic developmental information on the species is rare (Watt et al., 2009). We have recently constructed a detailed structural and molecular description of domains within the developing *Brachypodium* grain and compared it to that of the cultivated cereals, mainly wheat (Opanowicz et al., 2011). A number of distinctive features of grain morphology, anatomy and development were identified that place it as intermediate between wheat and rice. Our results reveal significant differences in endosperm development and differentiation that may reflect its phylogenetic position or, alternatively, effects of domestication.

#### 3.1. Grain development and organisation

The *Brachypodium* grain is relatively large compared to plant size, approximately 17% of the size of a wheat grain (Garvin et al., 2008), and is almost comparable to a typical wheat grain in length but smaller in width and depth. The *Brachypodium* grain has a long but flat profile and is crescent-shaped in cross-section. While the grain has a crease morphology, the adaxial side crease is notably less re-entrant than that in cultivated barley or hexaploid wheat grains (Fig. 2). The endosperm accounts for ~75% of the dehulled grain weight in *Brachypodium* (Guillon et al., 2011), slightly less than for the other domesticated cereals at about 80% (Kent and Evers, 1994).

Whilst broadly similar, we found key informative differences in *Brachypodium* grain development and tissue structure as compared to wheat (Opanowicz et al., 2011). The endosperm cavity is absent in *Brachypodium* and the endosperm is not as clearly differentiated into discrete regions of different cell types as in wheat and barley. The aleurone layer is not regionally differentiated into distinct peripheral and modified aleurone regions and the modified aleurone layer, implicated as a major transfer tissue in wheat, is completely absent as judged by vital staining and gene expression analyses (in situ hybridisation; ISH). The peripheral aleurone is distinct and continuous around the entire endosperm but is irregular and poorly organised, being from one to three or more cells deep, in contrast to the very distinct and uniform aleurone layers typical of the Triticeae and other domesticated cereals such as rice and maize. The aleurone does show some distinction between the adaxial and abaxial sides of the grain in terms of

depth. It was also noted that the *Brachypodium* aleurone is fused to the central endosperm and does not separate with physical disruption to the grain, an important characteristic in wheat processing. An orthologue of a wheat aleurone-specific transcript *BdPPDK* also shows differences in expression indicating that aleurone differentiation may occur later than in wheat and a distinct peripheral aleurone was only detectable at a correspondingly later stage in *Brachypodium* (Opanowicz et al., 2011).

Other features in grain development also vary between species – Fig. 2 and Table 1. The nucellar projection in *Brachypodium* grains is very much reduced throughout development (as compared to wheat) while the nucellar epidermis is extensive and persistent throughout development, supporting the idea that nutrient transport in *Brachypodium* grain resembles that in rice. As in rice, the lack of a transfer cell layer in *Brachypodium* is coupled with the persistence of a functional nucellar epidermis whose cross walls are rich in plasmodesmata (unpublished). Therefore, it could play a major part in assimilate transport into the caryopsis. Finally, the timing of cell differentiation relative to overall grain development differs markedly between the species. We have found that maternal integument layers differentiate earlier in *Brachypodium* than in wheat. Cell wall thickness in the central endosperm was a striking feature of the *Brachypodium* grain with central/storage endosperm cell walls being notably thicker than those of the aleurone. At approximately 4.4  $\mu\text{m}$  the central endosperm cell walls are ~3 times the thickness of those in the aleurone (Opanowicz et al., 2011).

Other groups have performed in-depth preliminary analyses of the biochemical features of the *Brachypodium* grain's composition including storage protein deposition and cell wall characteristics that are reviewed below.

#### 3.2. Storage proteins

Cereal grains utilise several different types of storage protein and the occurrence and relative amounts of these different proteins is an important and distinctive feature of different species. Amongst the Triticeae species and maize, the prolamins form the dominant protein class and are highly significant to the processing and qualities of the grain, giving wheat flour its viscoelastic properties (Shewry and Halford, 2002). In oat and rice, globulins are the dominant storage proteins, with prolamins forming only a minor component of this fraction (Bewley and Black, 1994). In *Brachypodium*, globulins account for the majority of total extractable grain protein, prolamins representing less than 12% (Larre et al., 2010; Laudencia-Chingcuanco and Vensel, 2008).

Globulins are widely distributed amongst the flowering plants, both monocots and dicots, where they are typically embryo storage proteins but in cereal grains occur in both embryo and endosperm

**Table 1**  
Summary of *Brachypodium* grain characteristics compared to other grains.

	Grain crease	Cell wall profile <sup>a</sup>	Storage protein	Starch reserve <sup>b</sup>
<i>Brachypodium</i>	Yes	+++	Globulin	+
Wheat	Yes	+	Prolamin	+++
Barley	Yes	++	Prolamin (Hordein)	+++
Oat	Yes	+	Globulin	++
Rice	No	+	Globulin	+++
Maize	No	+	Prolamin (Zein)	+++
Domestic species most similar	Wheat	Barley	Oat/rice	n/a

<sup>a</sup> Similarity level.

<sup>b</sup> Amount.

(Bewley and Black, 1994; Heck et al., 1993; Larre et al., 2010). In cereal grains, the globulins can be divided into two major classes, the 7S and 11–12S globulins. The 7S globulins have limited homology to the vicilin-like proteins of legumes and other dicots and have been identified in all of the major cereal grains (Shewry and Halford, 2002). 7S globulins appear to have an exclusive storage function in the grain and their role does not appear crucial in all species, as a null mutant in maize shows normal grain development and germination (although globulins are not the dominant storage protein in maize) (Kriz and Wallace, 1991). In all cereal grains, the 7S globulins are the dominant class of storage protein in the embryo and aleurone tissues, their presence making these tissues protein rich (Shewry and Halford, 2002). Protein levels in *Brachypodium* are amongst the highest seen in cereal grains close to that of oat in both protein amount and composition (Guillon et al., 2011; Larre et al., 2010). *BdGLO1* (*Bradi1g13040*) was identified as encoding the main 7S globulin in the *Brachypodium* grain (Larre et al., 2010; Laudencia-Chingcuanco and Vensel, 2008) and as the orthologue of *barley embryo globulin 1* (*BEG1*) and wheat *globulin 3-A* (*TaGLO3A*) (Heck et al., 1993; Loit et al., 2009). We have shown that expression of *BdGLO1* is restricted to the embryo and aleurone layers in *Brachypodium* and the expression pattern of this gene in the endosperm highlighted the more irregular organisation of the peripheral aleurone as described in the previous section (Opanowicz et al., 2011).

7S globulins account for around a third of the globulins identified in *Brachypodium*, the remainder comprising 11S proteins with similar properties and solubility to the globulins that dominate in oat and rice where they can account for 70–80% of total protein (Larre et al., 2010; Laudencia-Chingcuanco and Vensel, 2008; Shewry and Halford, 2002). The 11–12S globulins are related to legumin proteins that can be found in most dicot seeds and in many other dicotyledonous plants (Bewley and Black, 1994). A related protein group is present in wheat, triticins, but accounts for only 2% of total grain protein (Dupont et al., 2011). *BdGLO2* (*Bradi2g38060*) was identified as the most represented member, at the protein level, of a family of 12 putative proteins of which five were identified as expressed in *Brachypodium* grains (Larre et al., 2010). mRNA ISH of *BdGLO2* showed transcripts localised to the central endosperm region and excluded from the aleurone layer (Opanowicz et al., 2011).

The details of storage protein deposition and subcellular localisation are not yet well detailed in *Brachypodium* and this area will benefit from further investigation. Observations by Larre et al. (2010) show the accumulation of 11S proteins in discreet protein bodies, sometimes associated with starch granules, but that deposition may also be occurring in a protein matrix. Globulins accumulate in protein storage vacuoles generally – in rice globulins and prolamins exist in two distinctive populations of protein bodies, from vacuolar and ER origins respectively; while in oats the globulins and prolamins exist in the same protein bodies. For wheat and barley the predominant prolamins may be of ER or vacuolar origin and form protein bodies that subsequently fuse to form a matrix (reviewed in Shewry and Halford, 2002).

Xu and Messing (2009) defined three groups of the prolamin gene families based upon amino acid sequence homology with group I representing a very young prolamin group and largely present only in the Panicoideae; group II as the largest and spanning all of the Poaceae and showing conservation in both chromosomal location and sequence at the family level; and group III which contains the Pooideae specific HMW-like prolamins, a very old group that could be the precursors to all of the other prolamins. Larre et al. (2010) note that no “group I” prolamins were identified in *Brachypodium*, these being the youngest of the prolamin group which arose after the split of the Poaceae and strengthens the

placement of *Brachypodium* in the Pooideae and as a close relation of wheat, barley and oat. The presence of large amounts of 11S proteins in the *Brachypodium* grain supports suggestions that *Brachypodium* may be evolutionary closer to *Avena* than to wheat (Draper et al., 2001; Kellogg, 2001; Larre et al., 2010; Opanowicz et al., 2008).

### 3.3. Starch biosynthesis and deposition

The central endosperm of *Brachypodium* is relatively starch-poor (Guillon et al., 2011; about 10% of total grain weight as opposed to 35–40% in primitive wild wheats and 50–70% in domesticated cereals) and the starch granules are small, with uniform size distribution and one or a few granules per plastid in contrast to the bimodal distribution typical of the Triticeae (Opanowicz et al., 2011). In grass endosperms the starch precursor, ADPglucose, is also synthesised in the cytosol and genes for components of its synthesis and transport into plastids are unique to grasses (Comparot-Moss and Denyer, 2009). The cytosolic pathway requires three genes, two for the large and small subunits of AGPase (ADPglucose pyrophosphorylase) and one for the ADPglucose transporter. The alternate pathway is dominant in both wild and domesticated grains which suggests an evolutionary advantage, possibly that cytosolic production of ADPglucose is energetically more efficient than plastidial starch synthesis. *Brachypodium* endosperm cells appear to synthesise ADPglucose in the cytosol as well as inside the plastids, like other grass species (K. Trafford, pers. comm.). The basis of the low starch content in *Brachypodium* grains (Guillon et al., 2011) is yet to be discovered and cannot yet be linked to a simple absence of key genes or to more complex issues of gene expression or protein function differences.

### 3.4. Cell walls in the *Brachypodium* grain

*Brachypodium* may provide an informative model to study cell wall biosynthesis and degradation (Buckeridge, 2010; Christensen et al., 2009). Indeed, much of the initiative for developing the species was to do with its potential as a model for biofuel grasses (Bevan et al., 2010). The Poaceae are distinguished for their deposits of (1,3;1,4)- $\beta$ -D-glucan (hereafter referred to as  $\beta$ -glucan) and comparative genomics has played an important role in identifying the  $\beta$ -glucan synthase genes in grasses (Fincher, 2009).

Seedling cell walls of *Brachypodium* have been found to be compositionally similar to those of wheat and barley (Christensen et al., 2009) whilst endosperm cell walls appear to be quite different (Guillon et al., 2011; Opanowicz et al., 2011). To a large extent, the arabinoxylans and  $\beta$ -glucan in the primary cell walls of grasses perform the roles of pectin and xyloglucans in dicots.  $\beta$ -glucan deposition occurs in a developmental stage specific manner in seedlings, occurring during cell elongation with levels then decreasing during cell maturation through the activities of glucanases (Christensen et al., 2009).  $\beta$ -glucan appears therefore to primarily have transient structural function during cell elongation. Glucose from hydrolysed  $\beta$ -glucan was shown to play a role in the sugar-recycling mechanism during *Zea mays* seedling growth (Gibeaut and Carpita, 1991) and suggests that  $\beta$ -glucan can function as a readily convertible storage compound in addition to its structural role (Burton et al., 2010). This may offer an explanation as to their origins and abundance in the endosperm cell wall.

Cell walls have major impacts upon the nutritional quality and end use properties of cereal grains and, as with storage proteins, the cell wall profile in grains is often distinctive to the genus, species and even cultivar (Toole et al., 2011). In grains of the major domesticated cereals, the cell walls typically account for around 3–8% of the total grain weight. Guillon et al. (2011) reported that the cell wall

polysaccharide content of dehulled *Brachypodium* grains was 60% of the dry weight compared to 10–20% for wheat, barley and oat (Barron et al., 2007; Manthey et al., 1999; Oscarsson et al., 1996). With relatively little starch, it seems that much of the carbohydrate material in the grain is contained within the very thick cell walls of the endosperm (Guillon et al., 2011; Opanowicz et al., 2011). This feature is distinctive when compared to the grains of barley, wheat, maize and rice characterised in detail to date. While *Brachypodium* contains the same types of cell wall polysaccharides as in other cereals, the relative amounts differ (Guillon et al., 2011).

The relative proportions of  $\beta$ -glucan and arabinoxylan in grains vary significantly according to species. Barley and oat are rich in  $\beta$ -glucan whilst wheat is rich in arabinoxylan. The ratio of ferulic acid to arabinoxylan is higher in *Brachypodium* and there are high levels of cellulose and xylan relative to pectin and mannan. Though starch content is low, levels of  $\beta$ -glucan are unusually high and similar to that seen in barley, 80% in *Brachypodium* and ~75% in barley. Oat has similar levels, ~70%, whilst levels in wheat are much lower, just 25%. *Brachypodium* also shows an even distribution of  $\beta$ -glucan in the storage endosperm, as can also be seen in barley. The  $\beta$ -glucan in the cell walls of *Brachypodium* contributes about 74% of the total glucose in the whole endosperm with only 12% being derived from starch. Guillon et al. (2011) suggest that  $\beta$ -glucans in the *Brachypodium* grain have a storage function and are mobilised during germination. During germination, the cell walls can provide a source of easily mobilised storage reserves to the germinating grain (Buckeridge, 2010) and we found evidence of endosperm cell wall degradation as early as 3 days after germination (Opanowicz et al., 2011). Arabinoxylan levels in *Brachypodium* are similar to that reported for wheat and barley whilst cellulose levels are higher, accounting for around 7% of the endosperm cell walls compared to a typical 2–4% in domesticated temperate cereals (in rice it is higher; Fincher and Stone, 1986). Guillon et al. (2011) showed that cell walls of the storage endosperm region are richer in cellulose than the cells of the aleurone layer.

The molecular genetic basis of this variation in endosperm cell wall composition has yet to be identified and characterised and comparative genomics should prove an important tool in such cases with the future availability of fully annotated barley and wheat genome sequences (IWGSP, 2010; Schulte et al., 2009). This high proportion of cell wall material in the uncultivated *Brachypodium* endosperm may also reflect ecological adaptation against insect predation. *Brachypodium* seeds are large and prominent in their natural habitat and the hard endosperm may offer some mechanical defence against predation by granivorous ant species, and may even aid their dispersal (Jacob et al., 2009).

#### 4. Conclusions

Analysis of *Brachypodium* grains to date has revealed both similarities and significant differences to our knowledge of grain development, based primarily on work in cultivated wheat, barley, rice and maize (Table 1). Various features are related to these species such as the presence of a shallow crease (wheat); a persistent nucellar epidermis (rice); a high globulin content (oat) and a high  $\beta$ -glucan content in endosperm cell walls (barley); as well as features that may reflect its status as an undomesticated grass species such as small grain size and low starch content. For all these reasons, further research on aspects of grain biology should be of interest to researchers with basic and applied interest. *Brachypodium* has become established as a valuable model system and with completed genomes and a host of associated resources for barley and wheat already available or imminent (Allen et al., 2011; Druka et al., 2011; IWGSP, 2010; Schulte et al., 2009), the comparative genomics approach in cereals has much to offer.

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