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FLOWERING NEWSLETTER REVIEW

Dissecting the role of MADS-box genes in monocot floral development and diversity

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Abstract

Many monocot plants have high social and economic value. These include grasses such as rice (*Oryza sativa*), wheat (*Triticum aestivum*), and barley (*Hordeum vulgare*), which produce soft commodities for many food and beverage industries, and ornamental flowers such ase lily (*Lilium longiflorum*) and orchid (*Oncidium* Gower Ramsey), which represent an important component of international flower markets. There is constant pressure to improve the development and diversity of these species, with a significant emphasis on flower development, and this is particularly relevant considering the impact of changing environments on reproduction and thus yield. MADS-box proteins are a family of transcription factors that contain a conserved 60 amino acid MADS-box motif. In plants, attention has been devoted to characterization of this family due to their roles in inflorescence and flower development, which holds promise for the modification of floral architecture for plant breeding. This has been explored in diverse angiosperms, but particularly the dicot model *Arabidopsis thaliana*. The focus of this review is on the less well characterized roles of the MADS-box proteins in monocot flower development and how changes in MADS-box proteins throughout evolution may have contributed to creating a diverse range of flowers. Examining these changes within the monocots can identify the importance of certain genes and pinpoint those which might be useful in future crop improvement and breeding strategies.

Keywords: Arabidopsis, barley, floral development, inflorescence, lily, MADS-box, monocots, rice, transcription factors, wheat, orchid.

Introduction

The grass family, Poaceae, diverged from other Poales ~55–70 million years ago (Bommert *et al.*, 2005). The inflorescence morphology of grasses is one of the major determinants of yield, and is thus a key breeding target (Bommert *et al.*, 2005). Identifying genes and proteins that are involved in flower development and their behaviour in high-yielding varieties and varieties that are resistant to biotic and abiotic stresses may help to identify pathways that can be targeted for the improvement of important crops.

Much of our knowledge of flower structure, morphology, and genetics has been gained through study of the model dicotyledonous plants *Arabidopsis thaliana* and *Antirrhinum majus*. Arabidopsis flowers contain four concentric whorls of organs including four sepals, four petals, six stamens, and two fused carpels. In general, flowers in the grasses share a similar structure, but exhibit some key differences. The rice spikelet comprises a single fertile floret that contains a lemma and palea in whorl 1, two lodicules in whorl 2, six stamens in

whorl 3, and a pistil in whorl 4 (Fig. 1A). In addition, there are two pairs of repressed bracts: rudimentary glumes and sterile lemmas (Zhang *et al.*, 2013). The identity of the palea and lemma has caused a lot of debate (Clifford, 1987; Bell, 1991). Their morphology is very similar except for three vascular strands in the lemma compared with two in the palea (Ambrose *et al.*, 2000), and a higher density of trichomes and more stomata in the lemma compared with the palea (Ambrose *et al.*, 2000). The palea is considered a prophyll in whose axil the grass flower arises (Bell, 1991). Many mutant phenotypes support the interpretation that the palea and lemma are equivalent to the sepals of most other flowers (Bowman, 1997; Ambrose *et al.*, 2000; Kyozuka *et al.*, 2000; Prasad *et al.*, 2001; Xu *et al.*, 2017). Their function is to protect the florets and kernels from pathogen and insect attack and supply carbohydrates to the developing seeds (Zhang *et al.*, 2013). Lodicules play a role in opening the florets and aid in co-ordination of stamen extrusion, pollination, and fertilization (Bommert *et al.*, 2005; Yoshida, 2012). They are believed to be equivalent to petals in other flowers (Ambrose *et al.*, 2000; Kyozuka *et al.*, 2000; Nagasawa *et al.*, 2003). Wheat, barley, and rye have spikelets that are directly attached to the main axis (Fig. 1B), while other grasses have long, branched

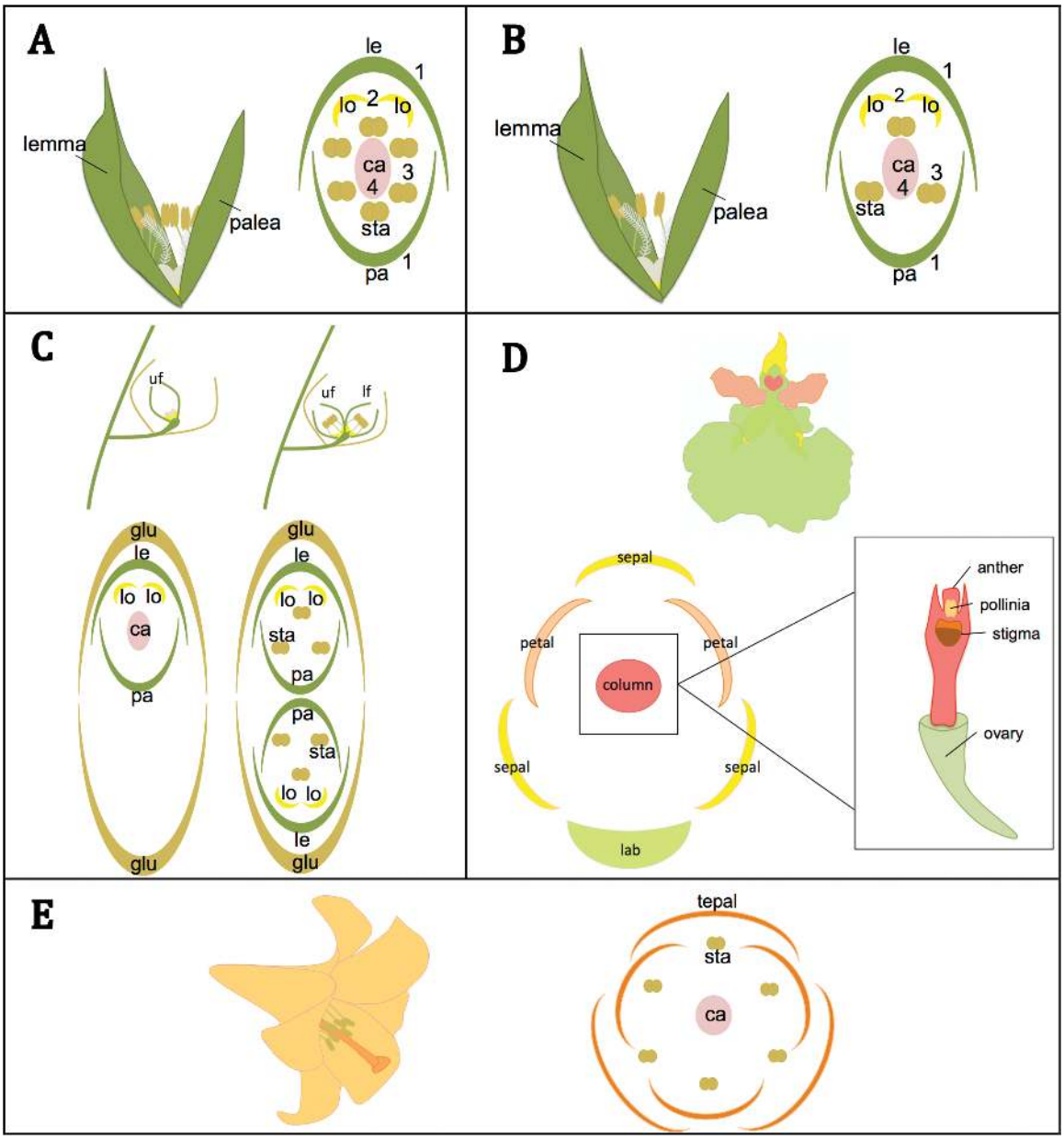

Fig. 1. Rice, maize, wheat, barley, orchid, and lily floral structures. (A) A rice floret has four whorls: a lemma (le) and palea (pa) in whorl 1 that protect the floret, two lodicules (lo) in whorl 2, six stamens (sta) in whorl 3, and a carpel (ca) in whorl 4. (B) Barley and wheat florets are very similar, but only have three stamens. (C) Maize has two separate inflorescences, one male (tassel) and one female (ear). Spikelets consist of a pair of florets: the upper floret (uf) and lower floret (lf). Female florets (C, left) have a lemma, palea, two lodicules, and a carpel, but no stamens. Male florets (C, right) have a lemma, palea, two lodicules, and three stamens, but no carpel. Both are protected by glumes (glu). (D) Orchids have three sepals in the first whorl and two petals and a labellum (lab) in the second whorl. The third and fourth whorl are located in the column. (E) Lily has five tepals in the first and second whorl, six stamens in the third whorl, and a carpel in the fourth whorl.

inflorescences and spikelets that are attached to lateral inflorescence branches (Zhang and Yuan, 2014). A spike can contain up to 40 florets (Bommert *et al.*, 2005).

In rice, the inflorescence meristem produces several primary branch meristems and they produce secondary branch meristems. Both of these in turn produce spikelet meristems (Hoshikawa, 1989). The spikelet meristem turns into a terminal spikelet meristem and produces the flowers (Kellogg, 2007). Maize has distinct male (tassel) and female (ear) inflorescences (Zhang and Yuan, 2014) that are physically separated (Fig. 1C), and each spikelet has a pair of florets, an upper and lower one (Dreni and Zhang, 2016). The shoot apical meristem (SAM) gives rise to the terminal tassel, which has long branches and develops male flowers. The first branches that are produced by the apical meristem are long branches, which produce a large number of short branches. Each short branch produces a single lateral branch that terminates in a spikelet (Kellogg, 2007). Ears are derived from axillary shoot meristems, have no long branches, and develop female flowers (Bommert *et al.*, 2005). Male and female flowers initiate one pistil, three stamens, two lodicules, a palea, and a lemma. The carpel primordia in the male florets and the stamen primordia in the female florets are aborted after initiation to produce unisexual florets (Bommert *et al.*, 2005).

Orchids are also members of the monocotyledons, in the family Orchidaceae, but are distinct from the true grasses. Orchid flowers have a zygomorphic structure, which is very different from any of the grass floret structures, and within the orchid family there is also great diversity (Pan *et al.*, 2014). *Oncidium* Gower Ramsey, the variety that has been frequently used for floral characterization, has three types of perianth organs. In the first whorl, three small sepals can be identified, while in the second whorl, two petals and the very distinctive lip, or labellum, are found (Fig. 1D); because the sepals and petals are not significantly different in some plant species, they are often called tepals. The labellum is particularly interesting from an evolutionary perspective since it represents a unique floral structure that may indicate a shift in protein function and interactions in the highly conserved MADS-box family (Mondragón-Palomino and Theissen, 2008). It is essential for the interaction with pollinators, and different models have been proposed to describe the protein interactions leading to labellum development (Mondragón-Palomino and Theissen, 2008).

Lily (*Lilium longiflorum*) from the monocot family Liliaceae produces flowers that have three sepals in the first whorl, three petals in the second whorl, six stamens in the third whorl, and three fused carpels in the fourth whorl (Fig. 1E). In *L. longiflorum*, most parts of the sepals and petals are still connected to each other, giving the lily flowers their distinct trumpet form and distinguishing them from other lily species. Similar to orchids, the sepals and petals are almost identical, which earned them the general name tepals (Tzeng and Yang, 2001). Orchid flowers probably originated from a flower with lily-like actinomorphic perianth with undifferentiated whorls of tepals (Mondragón-Palomino and Theissen, 2008).

The MADS-box protein family

The MADS-box acronym is derived from MCM1 (yeast), AG (Arabidopsis), DEFICIENS (Antirrhinum), and SRF (mammals), the first four proteins discovered in the transcription factor family (Shore and Sharrocks, 1995; Lawton-Rauh *et al.*, 2000). The MADS-box proteins are involved in diverse developmental processes in flowering plants, cardiac muscle development in animals, and pheromone response in yeast (Schwarz-Sommer *et al.*, 1990; Pelucchi *et al.*, 2002; Becker and Theissen, 2003).

In plants, the MADS-box genes have been proposed to be the driving force behind much floral diversity (Theissen and Saedler, 2001; Yamaguchi and Hirano, 2006). Therefore, better insight into their expression and function, and their conservation in different species is important to inform breeding strategies targeting alterations in floral architecture. The MADS-box domain is highly conserved across different species in dicots and monocots, which makes the functional diversity of the proteins extremely interesting. In this review, the expression patterns and functions of MADS-box genes relative to flower development in six different monocot species, namely barley, wheat, maize (*Zea mays*), rice, orchid, and lily, have been compared. The cereals barley, wheat, maize, and rice are mainly cultivated for food purposes, while orchid and lily have economic value as ornamental plants and flowers.

MADS-box protein structure is conserved between diverse plant species

The MADS-box genes have been divided into two groups: Type I and Type II (Becker and Theissen, 2003). Type I genes seem to have a faster evolutionary rate than Type II genes. The number of duplications of Type I genes is higher, however, even in the shorter time frame (Gramzow and Theissen, 2013). In plants, the Type II MADS-box genes are called MIKC-type genes, an acronym of the four different domains that have been identified in all genes of this type (Becker and Theissen, 2003).

The MIKC-type MADS-box genes consist of a MADS-box domain, an intervening domain (I), a K-box (K), and a C-terminal domain (C) (Fig. 2) (Theissen *et al.*, 1996). The highly conserved MADS-box motif has 60 amino acids for a sequence-specific DNA binding activity that also plays a role in dimerization and accessory factor binding. The weakly conserved intervening domain is a regulatory determinant for formation of DNA-binding dimers. The keratin-like K-box is defined by conserved regular spacing of hydrophobic residues and can form amphipatic helices involved in protein dimerization, which mediate protein–protein interactions. The most variable domain is located at the C-terminal end. It is involved in transcriptional activation and formation of multimeric transcription factor complexes (Shore and Sharrocks, 1995; Becker and Theissen, 2003; Fornara *et al.*, 2003; Zhao *et al.*, 2006).

Dependent on the structure of the I-domain and K-box, the MIKC-type MADS-box proteins can be further subdivided into two categories: the MIKC^c-type and the MIKC*-type proteins. The I-domain in the MIKC^c-type proteins is only

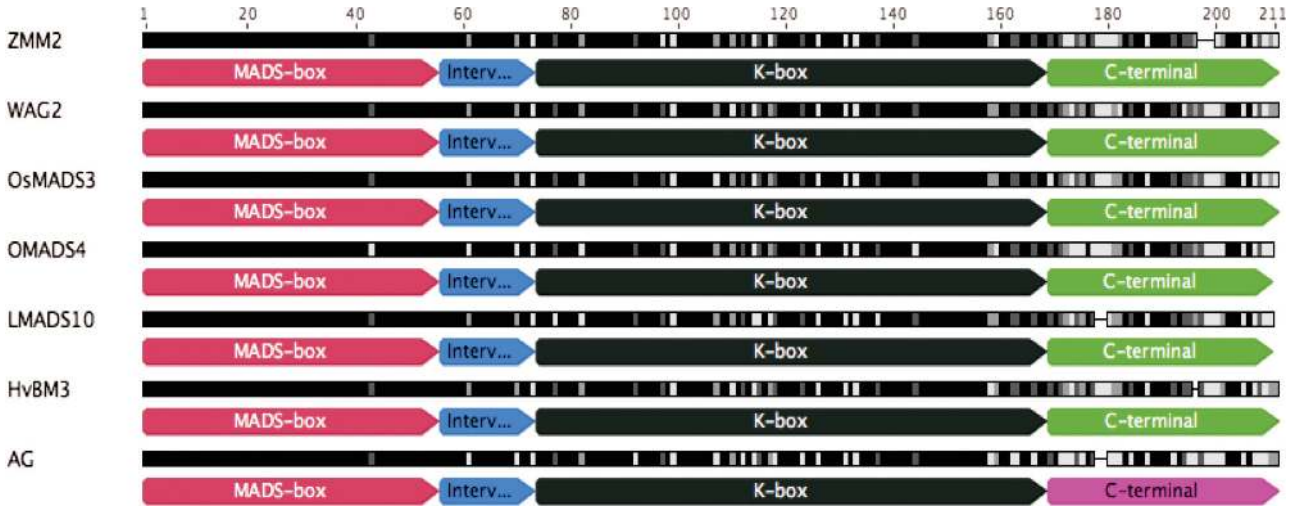

Fig. 2. Structure of MIKC-type MADS-box proteins. MIKC-type MADS-box proteins consist of a highly conserved MADS-box domain, responsible for DNA binding, dimerization, and accessory factor binding. The intervening domain is weakly conserved and is a regulatory determinant for the formation of DNA-binding dimers. The K-box is a keratin-like domain that mediates protein–protein interactions. The C-terminal domain is the most variable domain and is involved in transcriptional activation and formation of transcription factor complexes. As an example, MIKC-type proteins from maize (ZMM2), wheat (WAG2), rice (OsMADS3), orchid (OMADS4), lily (LMADS10), barley (HvBM3), and Arabidopsis (AG), all C-class genes, were aligned and their domains were highlighted. The C-terminal domain for AG was significantly different in sequence from that of the monocots and is therefore highlighted in a different colour. MUSCLE multiple alignment of protein sequences from the NCBI, IPK, and MSU rice databases.

encoded by one exon, while that in the MIKC*-type proteins is longer, with four or five exons (Becker and Theissen, 2003; Zhao *et al.*, 2006).

Gene duplication within the MADS-box gene family is believed to be a key process during flower evolution (Theissen and Saedler, 2001). After gene duplication, a gene can have several different fates. If a gene is duplicated in its entirety, this frequently leads to functional redundancy (Tautz, 1992; Pickett and Meeks-Wagner, 1995). On the other hand, one duplicated gene can retain the ancestral function, while the other acquires a mutation or a series of cumulative mutations and becomes a pseudogene. In another scenario, one gene retains the ancestral function, while the other gains a beneficial mutation that will be positively selected for, which results in a new function. Another possibility is that both genes acquire complementary loss-of-function mutations that result in the preservation of both genes as they now together retain the original functions of their single ancestor (Lynch and Force, 2000). This is also referred to as the duplication–degeneration–complementation (DDC) model (Force *et al.*, 1999; Prince and Pickett, 2002). These are called non-functionalization, neo-functionalization, and sub-functionalization, respectively (Schilling *et al.*, 2015). Most major difference in the MADS-box gene family between species are thought to have arisen from gene duplications.

The role of MIKC^c-type MADS-box proteins in the ABCDE model of flower development

The floral organ identity MADS-box genes of the MIKC^c type have been divided into five different classes based on their homeotic function: class A, B, C, D, and E genes (Bowman *et al.*, 1989, 1991; Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994; Theissen, 2001). The A- and E-class protein complexes specify sepals in the first whorl (Fig. 3). Complexes of A-, B-, and E-class proteins specify petals in the second whorl (Honma and Goto, 2001). B-, C-, and E-class complexes specify stamens in the third whorl, and C- and E-class protein complexes specify carpels in the fourth whorl (Coen and Meyerowitz, 1991; Honma and Goto, 2001). D-class proteins specify ovules together with E-class genes (Fig. 3) (Colombo *et al.*, 1995; Angenent and Colombo, 1996; Theissen and Saedler, 2001; Becker and Theissen, 2003; Li *et al.*, 2011; H. Wang *et al.*, 2015). Another group of genes, phylogenetically related to the B-class genes, was identified and was named the B_{sister} or B_s genes (Becker *et al.*, 2002). Genes in this class are mainly expressed in female reproductive organs, especially in the ovules (Münster *et al.*, 2001; Becker *et al.*, 2002; Becker and Theissen, 2003). All of these genes also fall into separate clades, named after the first proteins identified (Fig. 4). The genes in the SQUA-clade all determine either inflorescence or floral meristem identity, and some have additional A-type functions, while genes in the DEF/GLO clade have class B functions (Theissen *et al.*, 1996). The AG-clade consists of an AG- and an AGL11 (or STK)-lineage, and the class E genes are all part of the SEP/AGL2-clade. Alignments of all proteins in the different subfamilies can be found in Supplementary Figs S1–S7 at *JXB* online

The ABCDE model in monocots

MADS-box genes involved in flower development have been studied in a wide variety of species. In monocots, most research has been undertaken in rice, wheat, and maize. Comparing the expression patterns and functions of MADS-box floral genes in different monocot species provides information on the differences in their morphology and how evolution may have

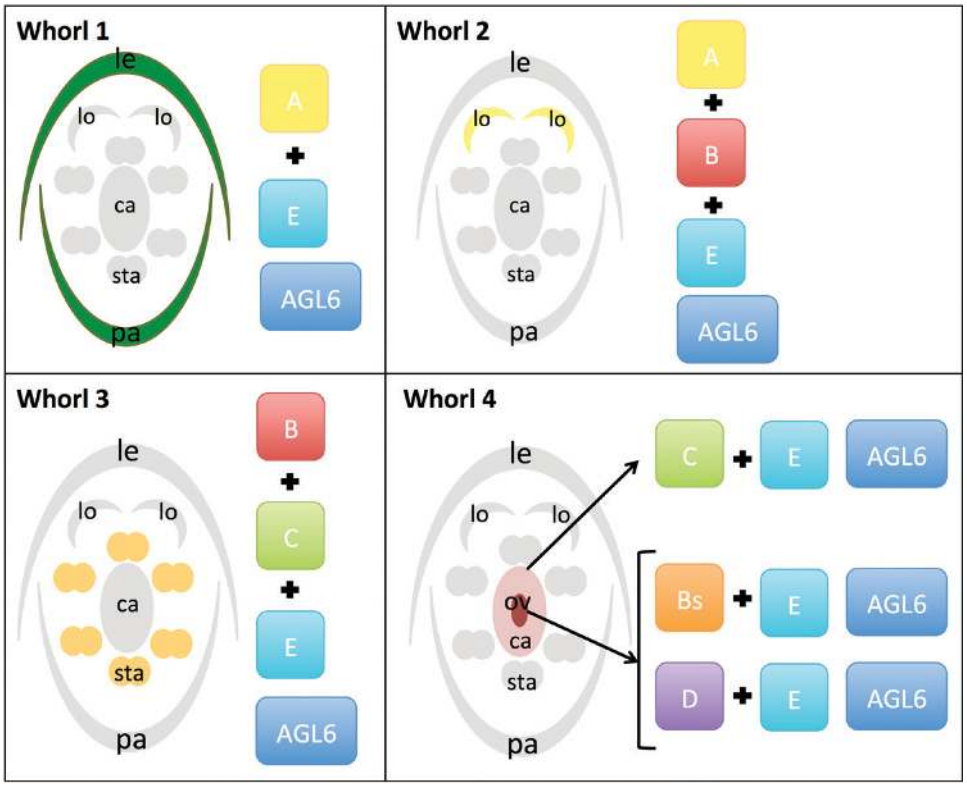

Fig. 3. The ABCDE model in rice florets. The model depicts the pattern of gene expression required for normal whorl development. The MIKC^c-type MADS-box proteins are divided into different classes: A, B, C, D, and E-class. The B_{sister} proteins are classified as B-class proteins, but have a distinct function. AGL6-like proteins are often classified together with the E-class proteins because they have similar functions. These proteins form complexes to determine the identity of floral organs shown here in a rice floret: lemma (le), palea (pa), lodicules (lo), stamen (sta), carpel (ca), and ovule (ov).

affected different floral structures and floral diversity among these species. While rice, wheat, and barley have a similar floral pattern, the flowers in orchid and lily are very different. The emergence of unique organs such as the labellum in orchid and the differentiation between male tassels and female ears in maize are also interesting to be elucidated. Comparing the expression and function of the ABCDE MADS-box genes within these monocot species provides an interesting opportunity to elucidate more about their role in shaping these different floral structures.

A-class genes

In Arabidopsis and *Antirrhinum*, the A-class genes *AP1* and *SQUA* are responsible for the transition from vegetative to reproductive growth, determination of floral organ identity, and the regulation of fruit maturation (Fornara *et al.*, 2004). Their orthologues in monocots have some level of conservation, but there is some divergence in sequence, expression pattern, and function (Zhang and Yuan, 2014). In the core eudicots, there are two different gene clades within the class A genes: euAP1 and euFUL, which have arisen from a duplication event that coincided with the origin of this angiosperm group (Litt and Irish, 2003; Shan *et al.*, 2007). In non-core eudicots and monocots, only sequences that are similar to those of euFUL genes have been found, and these have been termed 'FUL-like' genes (Litt and Irish, 2003). The monocot FUL-like genes fall into two successively branching clades, which indicates another duplication in the gene lineage (Litt and Irish, 2003).

The FUL-like and the euFUL sequences have a highly conserved motif in the C-terminus (Fig. 5), the FUL-like or paleoAP1 motif (L/MPPWML), which has not been found in the euAP1 sequences (Litt and Irish, 2003). euAP1 sequences have two distinct conserved motifs in their C-terminus: RRNaLaLT/NLa and CFAT/A. These motifs contain an acidic transcription activation domain and a farnesylation signal (Litt and Irish, 2003; Fornara *et al.*, 2004; Chen *et al.*, 2008). Neither of these motifs has been observed in FUL-like and euFUL sequences. It is suggested that the euAP1 motif has arisen via a translational frameshift from the euFUL/FUL-like motif. This frameshift may have resulted in different functions for the euAP1 proteins (Litt and Irish, 2003).

The rice genome contains four A-class genes, *OsMADS14*, *OsMADS15*, *OsMADS18*, and *OsMADS20*. Northern blot and *in situ* hybridization analysis showed that *OsMADS15* is expressed in the apical region of the floral meristem and subsequently accumulates in the developing lemma and palea (Kyozuka *et al.*, 2000). Expression becomes restricted to the palea, lemma, and lodicules after differentiation of the spikelet organs (Fig. 5B) (Kyozuka *et al.*, 2000), which is similar to *AP1* (Fornara *et al.*, 2003). T-DNA insertional lines that lead to loss-of-function mutants of *OsMADS15* show smaller paleas, while a single nucleotide mutation in *OsMADS15* leads to degenerative paleas and occasional pseudovivipary (Wang *et al.*, 2010; Wu *et al.*, 2017). Overexpression of *OsMADS15*

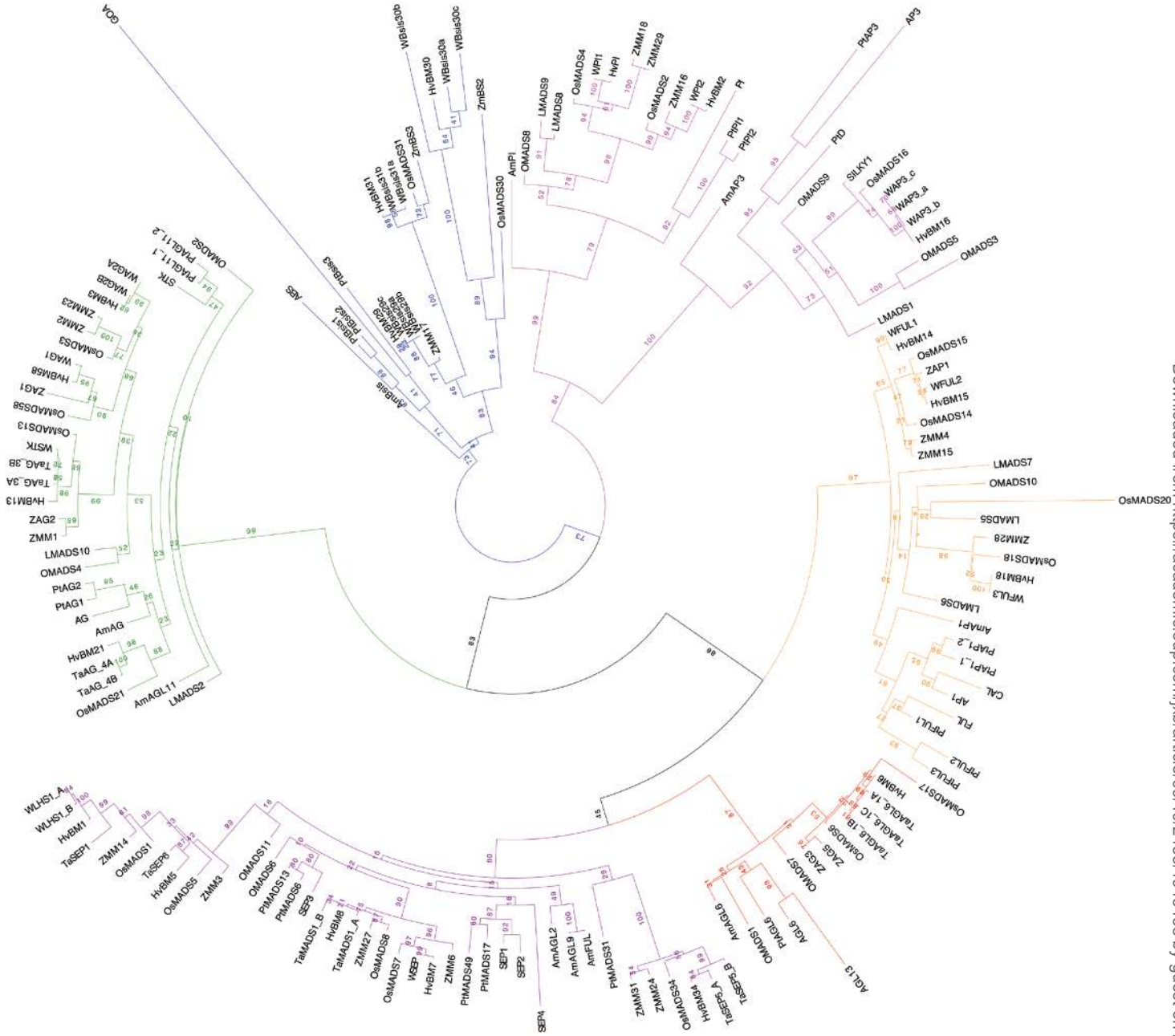

Fig. 4. Phylogenetic analysis of ABCDE MADS-box genes from Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily. Phylogenetic tree obtained with RAxML tree building through Geneious version 8.0 by Biomatters. Available from http://www.geneious.com. Maximum likelihood tree from 1000 bootstrap replicates. MUSCLE multiple alignment of protein sequences from the NCBI, IPK, and MSU databases was used. BMGE clean up of the multiple aligment via Galaxy@pasteur (https://galaxy.pasteur.fr). The different subfamilies are represented by different colours: SQUA (orange), DEF/GLO (pink), GMM13 (blue), AG (green), AGL2 (purple), AGL6 (red). Alignments of all proteins in the different subfamilies can be found in Supplementary Figs S1–S7.

causes early internode elongation, shoot-born crown root development, reduced plant height, and early flowering (Lu *et al*., 2012). Northern blot and *in situ* hybridization analysis showed that *OsMADS14* expression is similar to that of *OsMADS15*, and is initially detectable in the whole region of the floral meristem during flower development, and subsequently becomes restricted to the primordia of glumes, lemma, and palea (Pelucchi *et al*., 2002). In mature flowers, the expression of *OsMADS14* is detectable in the reproductive organs (Fig. 5B) (Moon *et al*., 1999*b*; Pelucchi *et al*., 2002). A loss-of-function T-DNA insertion mutant in *OsMADS14* showed no phenotype in the field, while ectopic expression leads to early flowering at the callus stage (Jeon *et al*., 2000*b*; Wu *et al*., 2017). Double mutant *osmads14osmads15* plants fail to produce secondary branches and spikelets, and only leaf-like organs are observed (Wu *et al*., 2017). The single mutant phenotype of *OsMADS14* and that of the double mutant suggest that its function is largely redundant with other genes, such as *OsMADS15*. Analysis of heterozygous double mutants suggests that OsMADS14 and OsMADS15 went through sub-functionalization and acquired partially overlapping functions (Wu *et al*., 2017). They work together in a dose-dependent manner

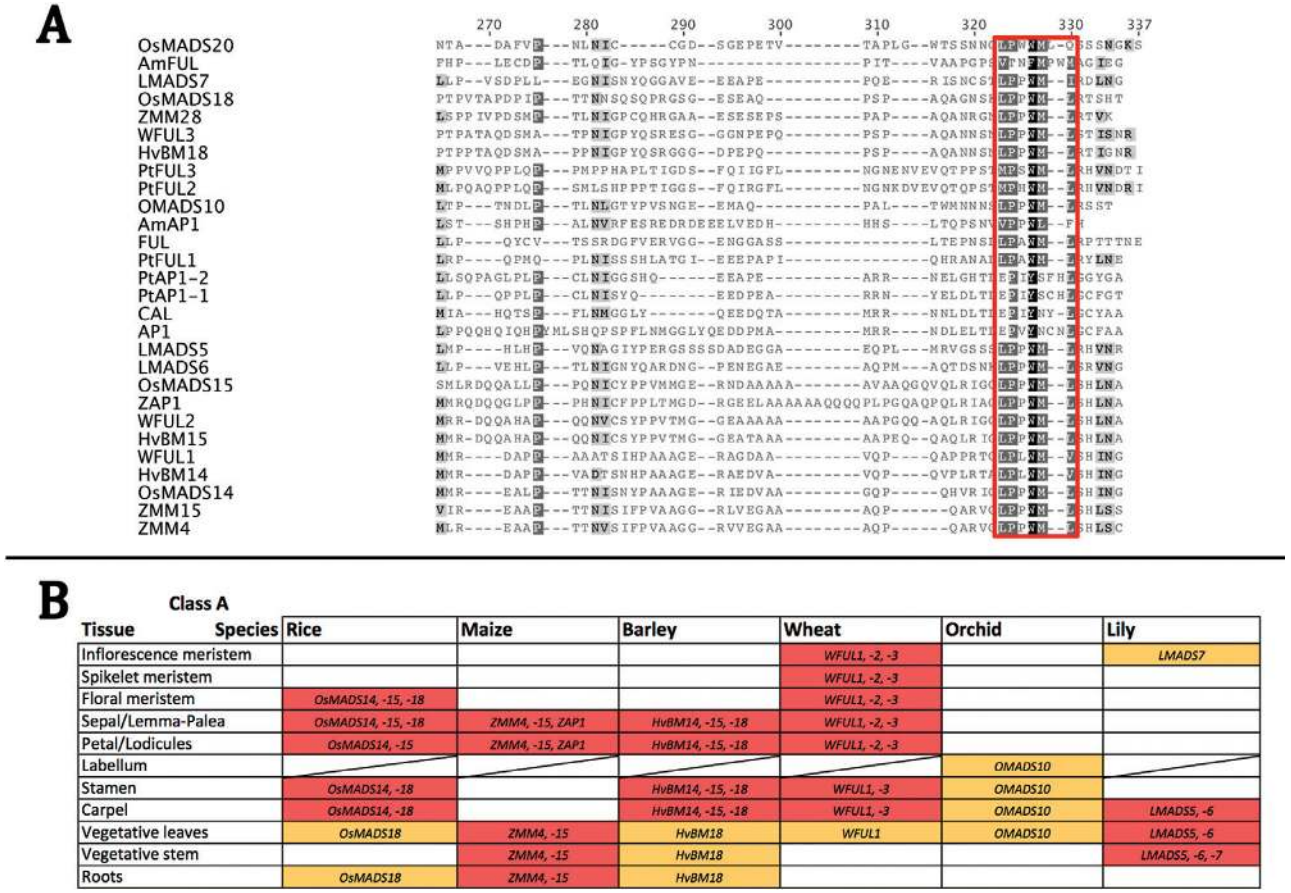

Fig. 5. Sequence alignment and expression patterns of A-class MADS-box genes in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, barley, wheat, orchid, and lily. (A) The conserved FUL-like motif (LPPWML) can be found in all the monocot A-class MADS-box genes, with only minor differences. In HvBM5 and WFUL1, the proline at the third position has been substituted by a leucine, while the leucine at the sixth position has been substituted by a valine. In OsMADS20, the proline at the third position has been substituted by a tryptophan and in LMADS7 the leucine at the sixth position has been substituted by an isoleucine. (B) The expression patterns appear conserved in the grasses, with some diversity in orchid and lily. Red squares indicate that multiple genes are expressed in this tissue, while yellow indicates that only one gene is expressed in this tissue. White squares indicate that there is no expression, and grey squares indicate that no data are available regarding the expression in these tissues.

by antagonizing C-class genes and both determine floral meristem fate (Wu *et al.*, 2017). OsMADS14 mainly regulates the identities of the lodicule and stamens, while OsMADS15 is mainly responsible for the empty glumes, palea, and lemma (Wu *et al.*, 2017). *OsMADS18* has a different expression pattern compared with the other *AP1* orthologues. Northern blot and *in situ* hybridization analysis revealed expression in roots, leaves, and flowers, with a strong signal in the inflorescence (Masiero *et al.*, 2002; Pelucchi *et al.*, 2002; Fornara *et al.*, 2003). *OsMADS18* expression levels are maximal when the plant reaches the reproductive stage (Fornara *et al.*, 2003), but are absent from the lodicules and the sterile glumes in mature flowers (Pelucchi *et al.*, 2002). Fornara *et al.* (2004) described an RNAi line of *OsMADS18* that showed no visible phenotype, while a recent RNAi line described by Wu *et al.* (2017) showed only a low seed setting rate. Overexpression of *OsMADS18* induces precocious initiation of axillary shoot meristems and early transition to flowering (Fornara *et al.*, 2004). These results suggest that OsMADS18 is possibly not required for specifying floral organ identity but may be involved in promoting the differentiation of the vegetative shoot or seed development together with OsMADS14 and OsMADS15 (Fornara *et al.*, 2004; Wu *et al.*, 2017). Yeast two-hybrid and bimolecular fluorescence complementarion (BiFC) experiments have shown that OsMADS18 forms heterodimers with OsMADS14, OsMADS15, OsMADS8, OsMADS7, OsMADS6, and OsMADS47 (Masiero *et al.*, 2002; Wu *et al.*, 2017), but does not form homodimers (Wu *et al.*, 2017), revealing a conserved aspect between monocots and dicots (Fornara *et al.*, 2004). Both OsMADS14 and OsMADS15 have been shown to interact with each other and with OsMADS1, and can also form homodimers (Lim *et al.*, 2000; Wu *et al.*, 2017). The expression of *OsMADS20* was detected in shoots and seeds by RT-PCR (Lee *et al.*, 2003*b*), but RNAi lines show no observable phenotype (Wu *et al.*, 2017). The quadruple mutant of *osmads14 osmads15 osmads18 osmads20* does not display a more severe phenotype than the double mutant *osmads14 osmads15*, suggesting that OsMADS14 and OsMADS15 are sufficient for specifying palea, lemma, and lodicule identity in rice florets (Wu *et al.*, 2017).

In maize, *ZAP1* was identified as the *AP1* orthologue because of the sequence similarities and the similar expression pattern to Arabidopsis (Mena *et al.*, 1995). *ZAP1* mRNA was detected in male and female inflorescences and the husk

leaves that surround the developing ear using northern blot analysis (Fig. 5B) (Mena *et al.*, 1995). *ZAP1* is expressed in lemma, palea, and lodicules, similar to *OsMADS14* and *OsMADS15* (Li *et al.*, 2014). *ZMM4* and *ZMM15* have also been identified as orthologues of rice *OsMADS14*; *ZMM28* is the orthologue of rice *OsMADS18* (Table 1) (Zhao *et al.*, 2011; Li *et al.*, 2014). *ZMM4* and *ZMM15* are not expressed in young tissues, but accumulate after the transition from vegetative to reproductive growth in developing apical and lateral inflorescences (Danilevskaya *et al.*, 2008). Expression of *ZMM4* and *ZMM15* was not found in any of the embryonic tissues, but low levels of expression in husk, stalk, mature leaf, and root were detected by massively parallel signature sequencing (MPSS) analysis, *in situ* hybridization, and promotor:GUS (β-glucuronidase) analysis (Danilevskaya *et al.*, 2008). The expression profile of *ZMM15* is similar to that of *ZMM4* but overall has a low expression level (Danilevskaya *et al.*, 2008). When both genes are overexpressed, only ZMM14 mediates early flowering, which may suggest that ZMM15 has a function similar to but weaker than ZMM14 (Danilevskaya *et al.*, 2008).

The expression patterns of the barley A-class genes do not correspond to those of *SQUA* and *AP1*, implying that they are not functional equivalents (Schmitz *et al.*, 2000). *In situ* hybridization, RT-PCR, and northern blot analysis showed that at the awn primordium stage, the expression of *HvBM18* (also known as *BM3*) and *HvBM14* (also known as *BM5*) is hardly detectable, while *HvBM15* (also known as *BM8*) expression is strong (Schmitz *et al.*, 2000). Subsequently the three genes are expressed in all organ primordia and the vascular system of the

Table 1. *The ABCDE genes in Arabidopsis and monocot species*

	Clade	Core eudicot clade	Arabidopsis	Monocot clade	Orchid	Lily	Grasses clade	Rice	Maize	Barley	Wheat
SQUA	**AP1**	**euAP1**	AP1 CAL								
	FUL	**euFUL**	FUL								
	FUL-like	**FUL-like**		**FUL-like**	OMADS10	LMADS5	**FUL1**	OsMADS14	ZMM4 ZMM15	HvBM14	WFUL1
						LMADS6	**FUL2**	OsMADS15	ZAP1	HvBM15	WFUL2
						LMADS7	**FUL3**	OsMADS18	ZMM28	HvBM18	WFUL3
							FUL4	OsMADS20			
DEF/GLO	**DEF**	**euAP3**	AP3	**paleoAP3**	OMADS3 OMADS5 OMADS9 OMADS12	LMADS1	**paleoAP3**	OsMADS16	SILKY1	HvBM16	WAP3
	GLO	**GLO**	PI	**GLO**	OMADS8	LMADS8 LMADS9	**GLO**	OsMADS2 OsMADS4	ZMM16 ZMM18 ZMM29	HvBM2 HvBM4	WPI2 WPI1
GMM13	**B_{sister}**	**B_{sister}**	ABS GOA	**B_{sister}**			**OsMADS29**	OsMADS29	ZMM17	HvBM29	WBsis
							OsMADS30	OsMADS30	ZmBS2	HvBM30	TaBS2
							OsMADS31	OsMADS31	ZmBS3	HvBM31	TaBS3
AG	**AG**	**euAG**	AG	**AG**	OMADS4	LMADS10	**AG**	OsMADS3	ZMM2 ZMM23	HvBM3	WAG2
		PLENA	SHP1 SHP2					OsMADS58	ZAG1	HvBM58	WAG1
	AGL11	**AGL11**	STK	**AGL11**	OMADS2	LMADS2	**AGL11**	OsMADS13 OsMADS21	ZAG2 ZMM1	HvBM13 HvBM21	WSTK Ta-AG4
AGL2	**LOFSEP**	**SEP1/2**	SEP1 SEP2	**LOFSEP**	OMADS11	LMADS4	**OsMADS1**	OsMADS1	ZMM14 ZMM8	HvBM1	WLHS1 TaSEP1
		FBP9/23					**OsMADS5**	OsMADS5	ZMM3	HvBM5	TaSEP6
		SEP4	SEP4				**OsMADS34**	OsMADS34	ZMM24 ZMM31	HvBM34	TaSEP5
	SEP3	**SEP3**	SEP3	**SEP3**	OMADS6	LMADS3	**OsMADS7**	OsMADS7	ZMM6	HvBM7	WSEP
							OsMADS8	OSMADS8	ZMM27	HvBM8	TaMADS1
AGL6	**AGL6**	**euAGL6**	AGL6 AGL13	**AGL-I**			**ZAG3/OsMADS6**	OsMADS6	ZAG3 ZAG5	HvBM6	TaAGL6
		AGL6-like					**OsMADS17**	OsMADS17			
				AGL-2							
				AGL-3	OMADS7 OMADS1						
				AGL-4							

Listed are the genes in the model organism Arabidopsis and the orthologues in monocots rice (*Oryza sativa*), maize (*Zea mays*), barley (*Hordeum vulgare*), wheat (*Triticum aestivum*), orchid (*Oncidium* Gower Ramsey), and lily (*Lilium longiflorum*) that have been identified to date

barley floret throughout inflorescence development (Schmitz *et al.*, 2000). *HvBM14* and *HvBM15* are specific for these tissues, while *HvBM18* is also expressed in all other tissues, similar to its orthologue in rice, *OsMADS18* (Fig. 5B) (Schmitz *et al.*, 2000). *HvBM14* shows a marked increase in transcript abundance during the induction of the reproductive phase, similar to *OsMADS18* (Fornara *et al.*, 2004). *HvBM14* is the equivalent of the *VRN1* gene in other temperate cereals and is generally not expressed in non-vernalized winter barleys, but is induced by vernalization (Trevaskis *et al.*, 2003). Spring barley lines carrying dominant spring *VRN-H1* alleles or with homozygous recessive *VRN-H2* alleles have low levels of *HvBM14* expression (Trevaskis *et al.*, 2003). Trevaskis *et al.* (2003) suggest that *HvBM14* expression might be controlled by activation and repression to respond to vernalization, which has been suggested previously in wheat (Tranquilli and Dubcovsky, 2000; Yan *et al.*, 2003; Sasani *et al.*, 2009).

Orthologues of the rice genes *OsMADS14*, *OsMADS15*, and *OsMADS18* have been found in wheat and have been termed *WFUL1* (corresponding to *VRN1*), *WFUL2*, and *WFUL3*, respectively (Table 1) (Kinjo *et al.*, 2012). *In situ* hybridization, RT-PCR, and qRT-PCR determined that *WFUL3* is expressed in the spikelet primordia and throughout the spikelet meristem. *WFUL1* and *WFUL2* are only expressed in the basal part of the spikelet meristem. *WFUL1* is expressed in leaves at the vegetative phase, in young spikes, and in all floral organs after floral organ development, while the expression of *WFUL2* is reduced in stamens and undetectable in pistils (Fig. 5B) (Kinjo *et al.*, 2012). This corresponds to the expression pattern and function of *OsMADS14* and *OsMADS15* in rice and *ZAP1* in maize, indicating that this diversification of function has also occurred in the common ancestor of all the mentioned grasses (Murai, 2013). Overexpression of *WFUL1* and *WFUL2* leads to early flowering phenotypes (Adam *et al.*, 2007; Kinjo *et al.*, 2012). *WFUL1* has been suggested to have a function in phase transition in leaves and providing flowering competency (Murai *et al.*, 2003; Murai, 2013). *WFUL3* seems to have a function in floral meristem development together with *WFUL2*, while *WFUL2* has a specialized function in development of the outer floral organs (Kinjo *et al.*, 2012). Yeast two- or three-hybrid analysis showed that WFUL2 interacts with the B-class proteins WAP3 and WPI and the E-class proteins WSEP and WLHS1, while WFUL1 and WFUL2 both interact with WSEP (Kinjo *et al.*, 2012).

OMADS10, the *AP1* orthologue in orchid, is almost undetectable in flower buds of early developmental stages and during flower maturation, as shown by RT-PCR (Chang *et al.*, 2009). In mature flowers, *OMADS10* is expressed in the labellum, carpel, anther cap, and stigmatic cavity (Fig. 5B) (Chang *et al.*, 2009). It is also strongly detected in vegetative leaves. This expression pattern is different from those of A-function genes in Arabidopsis, *Antirrhinum*, and the grasses, but is similar to that found in the *AP1* orthologues in lily, *LMADS5* and *LMADS6* (Chang *et al.*, 2009). Ectopic expression of *OMADS10* in Arabidopsis induced an early flowering phenotype, but no homeotic conversions of floral organs (Chang *et al.*, 2009). Apart from *LMADS5* and *LMADS6*, there is one more A-class MADS-box gene in lily: *LMADS7*. Northern blot analysis showed that *LMADS5* and *LMADS6* were strongly expressed in vegetative stem, and leaves and carpels, and weakly in the other three floral organs (Chen *et al.*, 2008). *LMADS7* expression was absent in vegetative leaves and in any of the four organs of the flower, but was detected in the vegetative stem and the inflorescence meristem (Chen *et al.*, 2008). The expression pattern of *LMADS5*, *6*, and 7 is mostly different from that of other genes in the SQUA clade, with the exception of the A-class MADS-box genes in orchid (Fig. 5B). Ectopic expression of the A-class lily genes in Arabidopsis results in early flowering phenotypes and floral organ conversions such as carpelloid sepals and staminoid petals (Chen *et al.*, 2008). Functional complementation analysis showed that ectopic expression of these genes could rescue an *ap1* mutant phenotype in Arabidopsis (Chen *et al.*, 2008). Based on their expression pattern and ectopic expression analysis, it was suggested that they have a function in flower induction, initiation, and formation (Chen *et al.*, 2008).

In rice, only *OsMADS18* shows a different expression pattern compared with other A-class genes, whereas all the A-class genes in barley have a different expression pattern. There is also no *OsMADS20* orthologue in barley, maize, or wheat. In maize, there has been a duplication event resulting in *ZMM4* and *ZMM15*, and both appear to be orthologues of *OsMADS14*. In wheat, only *WFUL2* has the ascribed A-class function. *WFUL1* and *WFUL3* have a different expression pattern and function. The A-class genes in orchid and lily have a expression patterns completely different from those of their orthologues in grasses and Arabidopsis. Loss-of-function or knock-down mutants are currently missing for most of the A-class genes in maize, barley, wheat, orchid, and lily, and they could lead to a better understanding of their function.

B-class genes

B-class genes determine the identity of petals and stamens in Arabidopsis (Fornara *et al.*, 2003), and increasing evidence suggests that this is an ancestral function (Becker and Theissen, 2003; Münster *et al.*, 2001). Similar to the A-class genes, the B-class genes have been shaped by a gene duplication event close to the base of the crown group angiosperms, creating two lineages: the DEF-like lineage, which consists of AP3-like proteins, and the GLO-like lineage, which consists of PI-like proteins (Fig. 6B) (Winter *et al.*, 2002*a*; Becker and Theissen, 2003; Zahn *et al.*, 2005*b*).

AP3-like genes

In higher eudicots, an euAP3 motif is found in the AP3-like proteins, but is absent in non-core eudicots and non-eudicots. Instead a highly conserved paleoAP3 motif (YGxHDLRLA) is observed in their sequences (Fig. 6A) (Kramer *et al.*, 1998). AP3-like proteins also have a highly conserved sequence motif in the K-box (Q/HYExM) (Kramer *et al.*, 1998; Tzeng and Yang, 2001). Only one DEF-like gene has been found in most monocots, so it is presumed that no gene duplication event happened here, except for orchids, where the gene duplication seems to have occurred in the DEF-clade instead of the GLO-clade (Table 1) (Chen *et al.*, 2012). The

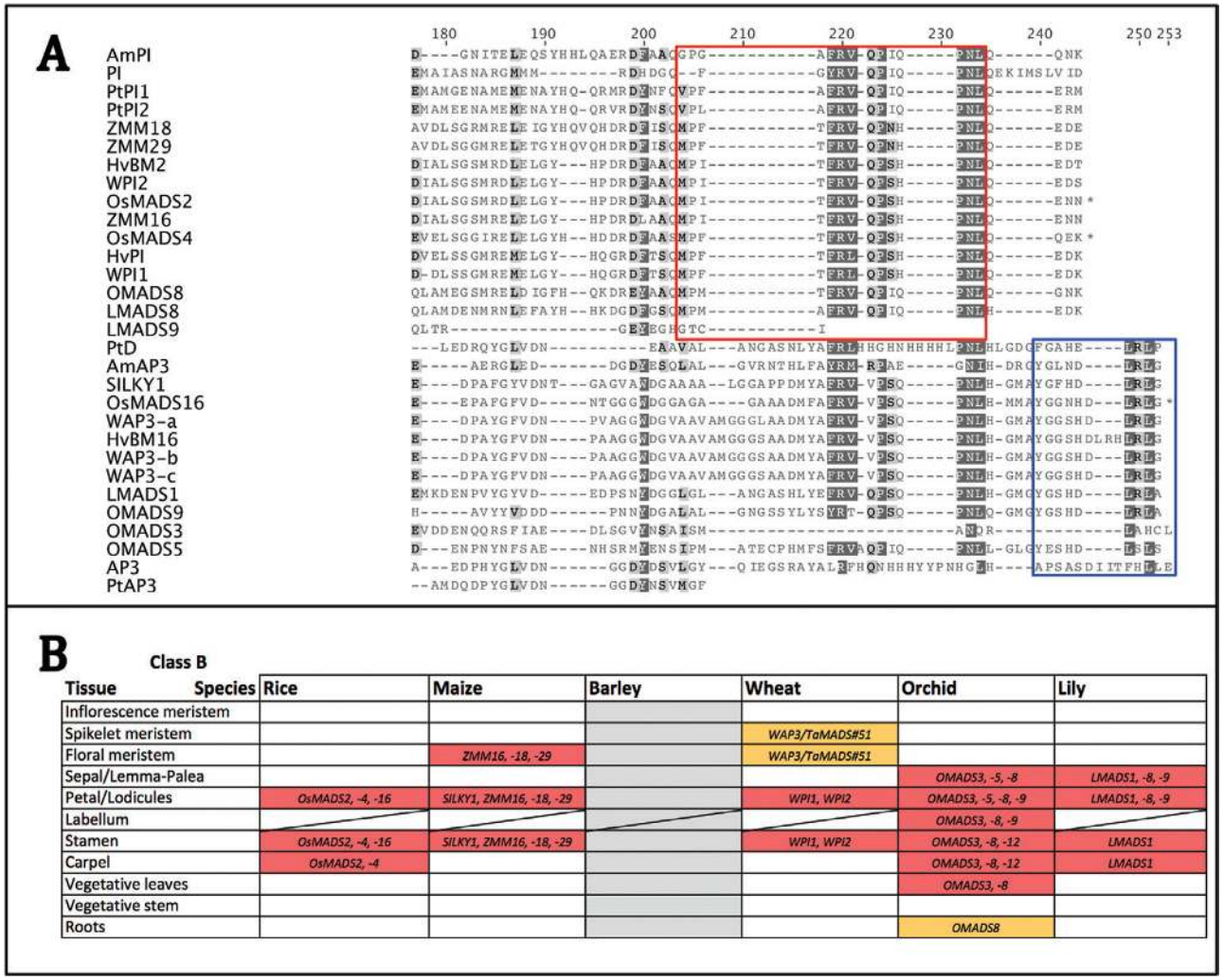

Fig. 6. Sequence alignment and expression patterns of B-class MADS-box genes in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, barley, wheat, orchid, and lily. The B-class genes can be subdivided into two different clades: the DEF- and the GLO-clade. (A) Multiple alignment of protein sequences from the NCBI, IPK, and MSU rice databases. Both clades have different motifs, a paleoAP3-motif (YGxHDLRLA) or a PI-motif (MPFTFRVQPSHPNL), respectively. HVPI and WPI1 have similar differences in the motif, as have LMADS8 and OMADS8. HvBM2, WPI2, OsMADS2, and ZMM16 also have similar differences, identifying them as homologues. LMADS9 is a truncated version of LMADS8 and does not have the PI-motif. All members of the monocot DEF-clade have a variation of the motif, except OMADS3. (B) The expression patterns of the grasses are conserved and have diversified in orchid and lily. Red squares indicate that multiple genes expressed in this tissue, while yellow indicates that only one gene is expressed in this tissue. White squares indicate that there is no expression, and grey squares indicate that no data are available regarding the expression in these tissues.

paleoAP3 motif seems to have significant sequence diversification in the GLO-like lineage after duplication, where it has been termed a PI-like motif (Fig. 6A) (Kramer *et al.*, 1998; Moon *et al.*, 1999*a*). The observation of these different motifs in the monocot B-class MADS-box genes shows that AP3 homologues were highly conserved in most monocots during evolution and that they are more closely related to the lower eudicots than to the higher eudicots (Tzeng and Yang, 2001).

In rice *OsMADS16* is a member of the DEF-clade, and expression is detected in lodicule and stamen primordia from initiation onwards, as revealed by RNA blot analysis and *in situ* hybridization (Fig. 6B) (Moon *et al.*, 1999*a*; Fornara *et al.*, 2003; Nagasawa *et al.*, 2003). DEF- and GLO-like proteins, like AP3 and PI in Arabidopsis, form obligate heterodimers, which might have originated after the gymnosperm–angiosperm split but before the monocot–eudicot split (Goto and Meyerowitz, 1994; Davies *et al.*, 1996; Winter *et al.*, 2002*b*). The interaction between proteins of the GLO- and the DEF-clade is conserved, as shown by the interaction of OsMADS16 with OsMADS4 and OsMADS2 by yeast two-hybrid analysis (Moon *et al.*, 1999*a*; Yao *et al.*, 2008). They form a heterodimer and may auto-regulate their own expression (Yadav *et al.*, 2007), similar to AP3 and PI in Arabidopsis (Krizek and Meyerowitz, 1996). The function of *OsMADS16* seems to be well conserved between rice and Arabidopsis (Yamaguchi and Hirano, 2006). A loss-of-function mutant of *OsMADS16*, known as *spw1* (*superwoman1*), shows the homoetic transformation of stamens into carpels and lodicules into palea-like organs (Nagasawa *et al.*, 2003). Similarly, *SILKY1*, the *AP3* orthologue in maize, is required for the normal development of lodicules and stamens. *SILKY1* is expressed in the centre of the floral meristem after the lemma and palea primordia have initiated, as

well as in lodicules and stamens throughout their development (Ambrose *et al.*, 2000). A loss-of-function mutation of *SILKY1* results in homeotic transformations of stamens to carpels and lodicules to lemma- or palea-like organs (Ambrose *et al.*, 2000). OsMADS16 also seems to interact with OsMADS3 (C-class), OsMADS15 (A-class), OsMADS8 (E-class), and OsMADS6 (AGL6-like) (Lee *et al.*, 2003*a*).

In wheat, two homeologous genes of *WAP3* (*TaMADS#51* and *TaMADS#82*) on chromosomes 7B and 7D, respectively, were identified as AP3-like B-class genes (Table 1) (Hama *et al.*, 2004). *WAP3/TaMADS#51* expression is only detected in young spikes at the floral organ development stage, while *WAP3/TaMADS#82* expression was lower in young spikes, but higher in spikes at the heading stage (Fig. 6B) (Hama *et al.*, 2004).

The DEF-like genes in orchid are subdivided into four different clades (Mondragón-Palomino and Theissen, 2008). *OMADS3* (clade 2), one AP3-like gene in orchid, does not contain the C-terminal motif, which differs from the other B-class genes found so far (Fig. 6) (Hsu and Yang, 2002). The conserved K-box sequence (QYQRM), however, is present (Hsu and Yang, 2002; Tsai and Chen, 2006). Its expression can be detected in all four floral organs as well as in vegetative leaves, as shown by a combination of RT-PCR and northern analysis (Hsu and Yang, 2002) which is different from other B-class genes that show specific expression in flowers (Fig. 6B). Yeast two-hybrid analysis showed that OMADS3 is able to form strong homodimers (Hsu and Yang, 2002; Tsai and Chen, 2006). Three other DEF-like genes are found in orchid; *OMADS12* (clade 4), *OMADS5* (clade 1) with expression in sepals and petals, and *OMADS9* (clade 3) which is highly expressed in petals and absent in vegetative tissues (Fig. 6B) (Chang *et al.*, 2010; Hsu *et al.*, 2015). OMADS5 and OMADS9 may play a different role in the formation of the sepal, petal, and labellum (Chang *et al.*, 2010). The difference for petal and lip formation may be due to the expression of *OMADS5* in the petal and its absence in the lip. OMADS5 may have a negative role in regulating labellum formation (Chang *et al.*, 2010), which was further supported by the reduced expression of *OMADS5* in lip-like sepals and lip-like petals of peloric orchid mutants of *O.* Gower Ramsey (Chang *et al.*, 2010). OMADS5 and OMADS9 are able to form homodimers and heterodimers with each other and with OMADS3 (Chang *et al.*, 2010). *OMADS12* is weakly expressed in stamen, but strongly expressed in the carpel (Hsu *et al.*, 2015). Its expression is completely absent in the sepal, petal, and labellum (Hsu *et al.*, 2015). This indicates that clade 4 in *O.* Gower Ramsey does not appear to affect perianth differentiation (Hsu *et al.*, 2015).

In lily, the *LMADS1* gene is the functional counterpart of *AP3* in Arabidopsis (Table 1) (Tzeng and Yang, 2001), with conserved function in regulating petal and stamen development. *LMADS1* is expressed in all four floral whorls, but the protein is only detected in petals and stamens, as revealed by western blot analysis, suggesting post-transcriptional regulation (Tzeng and Yang, 2001). *LMADS1* transcripts were also strongly detected in late-developing carpels (Tzeng and Yang, 2001). Yeast two-hybrid analysis showed that LMADS1 can form strong homodimers, similar to OMADS3 (Hsu and Yang, 2002; Tzeng *et al.*, 2004; Tzeng and Yang, 2001; Tsai and Chen, 2006). The highly conserved paleoAP3 motif (YGSHDLRLA) was found at the C-terminus of LMADS1 (Fig. 6A). Within the K-box, the highly conserved sequence (QYEKM) was also identified (Tzeng and Yang, 2001).

Briefly, wheat has two *AP3* homeologues showing different expression patterns, possibly indicating divergent functions. A series of duplication events in orchid are proposed to form four different clades of AP3-like B-class genes with functional diversification, which may contribute to the development of the unique orchid floral structure, the labellum. Unlike the A-class genes, lily AP3-like genes now show more similarity with the AP3-like genes in grasses and Arabidopsis than with those in orchid.

PI-like genes

Several GLO-like genes have been identified in rice, barley, wheat, maize, and lily (Chung *et al.*, 1995; Münster *et al.*, 2001; Hama *et al.*, 2004; Chang *et al.*, 2010; Chen *et al.*, 2012); proteins of the GLO-like lineage have a conserved PI-motif in their C-terminal domain (Fig. 6).

In rice, the PI-like genes *OsMADS2* and *OsMADS4* are mainly expressed in lodicules, stamens, and carpels (Fig. 6B) (Chung *et al.*, 1995; Kyozuka *et al.*, 2000; Fornara *et al.*, 2003). The function of *OsMADS2* is similar to that of PI in Arabidopsis, based upon RNAi analysis (Prasad and Vijayraghavan, 2003; Kang and An, 2005; Yadav *et al.*, 2007; Yao *et al.*, 2008). RNAi knock-down lines of *OsMADS2* showed continued growth of the distal region of second whorl organs forming an elongated bract-like structure, but no apparent changes in stamen shape (Yadav *et al.*, 2007; Yoshida *et al.*, 2007; Yao *et al.*, 2008). *OsMADS2* is transiently expressed early in all floral tissues and later strongly expressed in early stamen primordia, as shown by *in situ* hybridization (Kyozuka *et al.*, 2000; Yadav *et al.*, 2007). Similar expression levels are detected in developing lodicules and stamens, but are later substantially reduced in differentiating stamens (Kyozuka *et al.*, 2000; Yadav *et al.*, 2007). *OsMADS4* transcription activation occurs very early and uniformly during spikelet meristem initiation (Chung *et al.*, 1995; Yadav *et al.*, 2007). During floret organ development, high levels of *OsMADS4* expression occur in the stamen and carpel, with reduced expression in differentiating lodicules (Yadav *et al.*, 2007). RNAi lines of *OsMADS4* showed no phenotypic alterations, indicating that OsMADS4 and OsMADS2 might be acting redundantly in stamen specification (Yoshida *et al.*, 2007; Yao *et al.*, 2008). Supporting this, in the double knock-down mutants of *OsMADS2* and *OsMADS4*, the stamens were transformed into carpel-like organs (Yoshida *et al.*, 2007; Yao *et al.*, 2008). Moreover, the lodicules in these double mutants also showed a complete homeotic conversion to bract-like organs, suggesting that OsMADS4 plays a minor role in determining lodicule identity (Yoshida *et al.*, 2007; Yao *et al.*, 2008).

The *PI* orthologues *ZMM18*, *ZMM29*, and *ZMM16* in maize show an expression pattern similar to that of *OsMADS2* and *OsMADS4* (Fig. 6B) (Fornara *et al.*, 2003). *ZMM16* is the orthologue of *OsMADS2*, while *ZMM18* and *ZMM29* are orthologous to *OsMADS4* (Table 1) (Münster *et al.*, 2001).

These maize genes are expressed in lodicules, stamens, and carpel primordia in male and female inflorescences and later are restricted only to the stamen and lodicules (Whipple *et al.*, 2004). *ZMM16* was also weakly detected in vegetative organs (Münster *et al.*, 2001). The observation of some different expression patterns of *ZMM16* from *ZMM18* and *ZMM29* suggest that different degrees of selection pressures led to a functional diversification of the genes (Münster *et al.*, 2001). The gene pair *ZMM18* and *ZMM29* appears to have originated by a gene duplication event (Münster *et al.*, 2001). Using an EMSA, Whipple *et al.* (2004) showed that ZMM16 forms obligate heterodimers to bind DNA. They also showed that neither SILKY1 nor ZMM16 alone could bind DNA, while SILKY1 and ZMM16 together could bind DNA, indicating that the heterodimer is necessary for DNA binding. *WPI1* and *WPI2* in wheat are orthologous to *OsMADS4* and *OsMADS2*, respectively. *WPI1* is expressed in the primordia of the stamen and lodicules, as shown by *in situ* analysis (Table 1; Fig. 6B) (Hama *et al.*, 2004). The alloplasmic wheat with a deficiency of *WPI1* showed pistillody, the change of stamens into pistil-like structures, suggesting that WPI1 plays a role in floral organ identity (Hama *et al.*, 2004).

OMADS8 is the only GLO-like gene identified in *O.* Gower Ramsey (Table 1), with expression detected in vegetative leaves, roots, and all floral organs (Fig. 6B) (Chang *et al.*, 2010; Hsu *et al.*, 2015). OMADS8 was unable to form homodimers or heterodimers with OMADS5 or OMADS9, while it does, however, form heterodimers with OMADS3 (Chang *et al.*, 2010). Ectopic expression of *OMADS8* in Arabidopsis converted sepals into petal-like organs (Chang *et al.*, 2010). Based on these findings in *O.* Gower Ramsey, Chang *et al.* (2010) proposed that the presence of at least OMADS3/8/5 and/or OMADS9 is required for sepal and petal formation, whereas the presence of OMADS3/8/9 and the absence of OMADS5 are likely to be required for labellum formation (Chang *et al.*, 2010).

LMADS8 and *LMADS9* were identified as the *PI* orthologues in *L. longiflorum* (Table 1) (Chen *et al.*, 2012). qRT-PCR analysis revealed that *LMADS8* is highly expressed in the first and second whorl tepals in young and mature flowers, but is absent in vegetative leaves, roots, and stem (Chen *et al.*, 2012). The expression pattern of *LMADS9* is very similar to that of *LMADS8* (Fig. 6B). As seen in Arabidopsis AP3 and PI, and OsMADS4 and OsMADS16 in rice, LMADS8 and LMADS9 are able to form heterodimers with the AP3-like LMADS1 proteins, and can also form homodimers and heterodimers with each other, as shown by yeast two-hybrid analysis (Chen *et al.*, 2012). LMADS8 and LMADS9 seem to be involved in tepal formation and to a minor extent in early stamen formation (Chen *et al.*, 2012). Interestingly, *LMADS9* is a truncated version of *LMADS8*, missing the PI-motif in the C-terminal region (Fig. 6A) (Chen *et al.*, 2012). Ectopic expression of *LMADS8* and *LMADS9* in Arabidopsis partially converts sepals into petal-like organs (Chen *et al.*, 2012). Overexpression of *LMADS8* in the *pi* mutant of Arabidopsis completely rescued the phenotype, while overexpression of *LMADS9* only partially rescued the phenotype (Chen *et al.*, 2012).

Overall, the PI-like B-class genes in the grasses seem to have a conserved expression pattern and function. Only one PI-like gene is found in orchid, with a different protein–protein interaction pattern and function, indicating that the B-class genes are essential for the unique floral structure of orchids (Chang *et al.*, 2010). Even though LMADS9 does not have the defining PI-motif at its C-terminus, it does not seem to have lost its interaction possibilities and, possibly, may have retained its function (Chen *et al.*, 2012).

The B_{sister} genes are phylogenetically closely related to the B-class genes but have different functions

Close relatives of B-class genes have been identified in various species including rice, maize, barley, and wheat, and have been termed the B_{sister} (B_s) genes. They are mainly expressed in female reproductive organs, especially ovules. The two lineages were most probably generated by gene duplication (Münster *et al.*, 2001; Becker and Theissen, 2003). Compared with the B-class genes, B_{sister} genes share a shorter I domain, a subterminal PI-motif-derived sequence, and in some cases a paleoAP3 motif in the C-terminal region (Fig. 7A) (Becker *et al.*, 2002). In Arabidopsis, two B_{sister} genes have been identified, *ABS* and *GOA* (Becker *et al.*, 2002; Nesi *et al.*, 2002; Mizzotti *et al.*, 2012). *ABS* is expressed in the endothelial layer of the inner integuments of mature ovules and is necessary for inner integument differentiation (Nesi *et al.*, 2002). *GOA* has a broad expression pattern in ovule primordia and in ovules, which later is restricted to the outer integuments (Prasad *et al.*, 2010). It has functions in ovule outer integument development and the regulation of fruit longitudinal growth (Prasad *et al.*, 2010; Yang *et al.*, 2012).

The B_{sister} genes form three subclades in monocots: OsMADS29, OsMADS30, and OsMADS31 (Yang *et al.*, 2012), which are named after the three B_{sister} genes found in the rice genome (Table 1). Expression analysis showed that *OsMADS29* expression is restricted to developing seeds, while *OsMADS30* is expressed throughout all organs in the plant (Fig. 7B) (Yang *et al.*, 2012). Suppressed expression of *OsMADS29* by an antisense construct results in reduced and delayed cell degradation of the nucellar projection, abnormal endosperm development, and altered seed morphology (Yin and Xue, 2012), indicating that *OsMADS29* is important for the degradation of the nucellar projection and the nucellus. Yeast two-hybrid analysis showed that OsMADS29 interacts with all five E-class MADS-box genes and both AGL6-like MADS-box genes (Nayar *et al.*, 2014). It also interacts with A-class OsMADS14 and OsMADS18, C-class OsMADS3 and B_{sister} protein OsMADS31, and forms homodimers (Nayar *et al.*, 2014). *OsMADS30* lacks the characteristic B_{sister} motifs (Becker *et al.*, 2002; Yang *et al.*, 2012) and has a different C-terminus due to the insertion of a mobile element (OsME), which has an altered function and expression profile (Fig. 7A) (Schilling *et al.*, 2015). In maize, *ZMM17* has been identified as a B_{sister} gene; *ZMM17* is expressed in all organ primordia of the female spikelet, but later is restricted to the ovule and the developing silk, as determined by northern hybridization

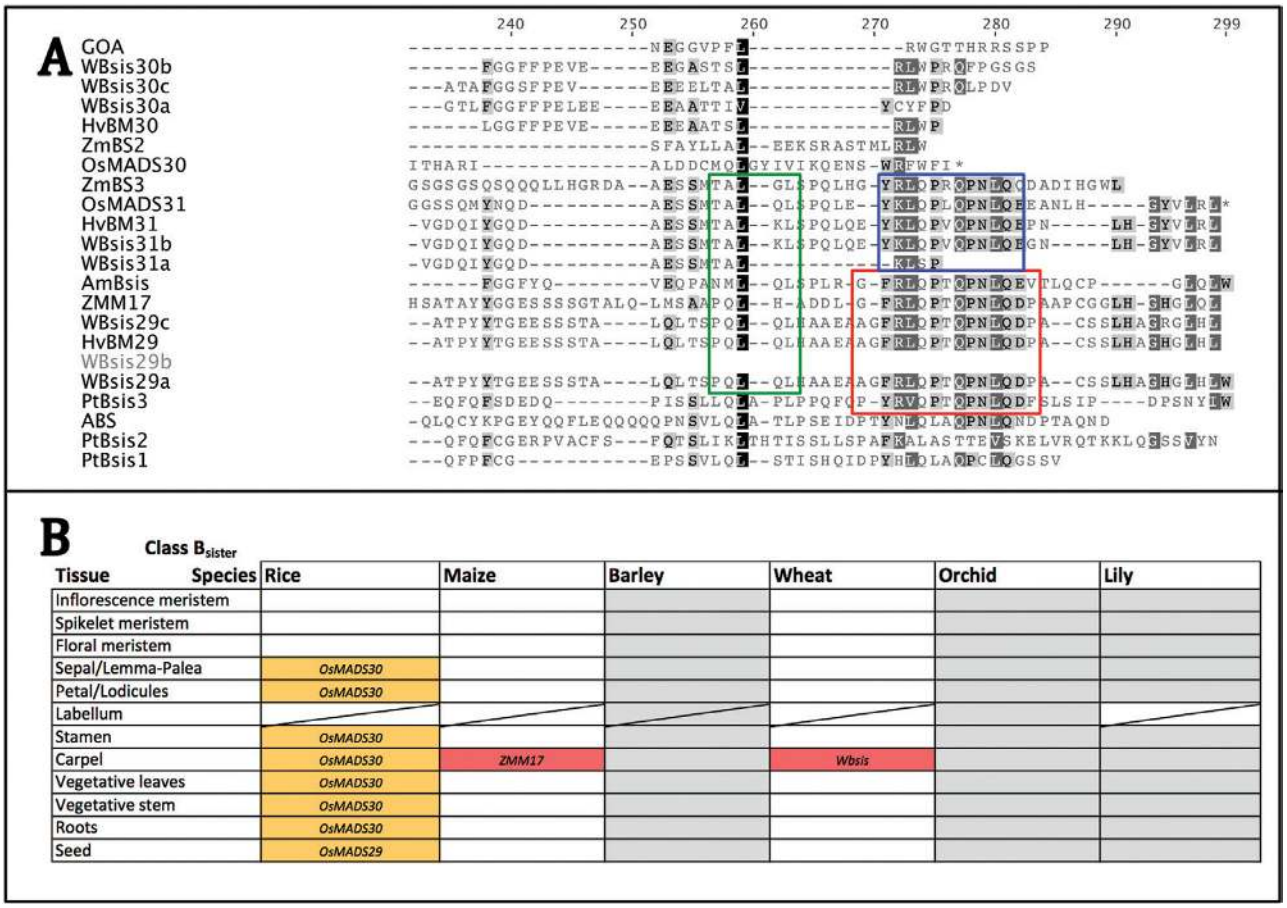

Fig. 7. Sequence alignment and expression patterns of B_{sister}-class MADS-box genes in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, barley, wheat, orchid, and lily. (A) Multiple alignment of protein sequences from the NCBI, IPK, and MSU rice databases. A conserved PI-derived motif can be found in the B_{sister} genes together with another unidentified motif downstream of the PI-derived motif. Variations in the PI-derived motif seem to divide the B_{sister} genes into two groups. One group consisting of ZMM17, OsMADS29, WBsis, and HvBM29 has GFRLQPTQPNLQDP as the PI-derived motif. The other group consisting of OsMADS31 and HvBM31 has YKLQPL/VQPNLQE as the PI-derived motif. An unidentified TALQL motif can be found in all monocot B_{sister} genes, which is remarkably similar to the motif found in the C-class MADS-box genes (see Fig. 8). OsMADS30 contains neither of the two motifs. (B) The expression patterns of B_{sister} genes that have been investigated show conservation in the female reproductive organs. Red squares indicate that multiple genes are expressed in this tissue, while yellow indicates that only one gene is expressed in this tissue. White squares indicate that there is no expression, and grey squares indicate that no data are available regarding the expression in these tissues.

analysis (Becker *et al.*, 2002; Yang *et al.*, 2012). *WBsis* was classified as a B_{sister} gene and part of the *OsMADS29*-like clade in wheat because of the high sequence similarity to *OsMADS29* and *OsMADS31* (Yamada *et al.*, 2009). *WBsis* is expressed in the endothelial layer of the inner integument of the ovule, similar to *ABS* in Arabidopsis; weak expression is also detected in the nucellus and the outer integument (Yamada *et al.*, 2009; Mizzotti *et al.*, 2012; Yang *et al.*, 2012).

All B_{sister} genes discussed here show a similar expression pattern, except *OsMADS30* which also has a diverged function. No B_{sister} genes have been thoroughly investigated in barley, orchid, and lily.

C- and D-class genes

C-class genes in eudicots specify the plant reproductive organs alone (carpels) or together with the B-class genes (stamens) (Fornara *et al.*, 2003). They also seem to be involved in the negative regulation of A-class MADS-box genes (Gustafson-Brown *et al.*, 1994; Wang *et al.*, 2015). Upon the discovery of the function of the MADS-box genes *FBP7* and *FBP11* in *Petunia* in regulating ovule organ identity, the ABC model was extended to incorporate a D function (Angenent *et al.*, 1995; Colombo *et al.*, 1995). D-gene function is involved in the determination of the identity of the central meristem, the progenitor tissue of the placenta, and the ovules (Angenent and Colombo, 1996). Both C- and D-class genes belong to the AG-like subfamily and have arisen through a gene duplication event close to the base of the angiosperm emergence (Becker and Theissen, 2003).

C- and D-class proteins can be distinguished by the structure of the N-terminal part of the K-box. In the D-lineage, a glutamine at position 105 is conserved, while this residue is not found in the C-lineage (Figs 7, 8) (Kramer *et al.*, 2004; Dreni *et al.*, 2007). Most D-lineage proteins also have a non-polar hydrophobic residue at position 106, whereas C-lineage proteins have a polar residue at that position (Dreni *et al.*, 2007). Monocot D-lineage proteins have a specific single amino acid insertion at position 90, and

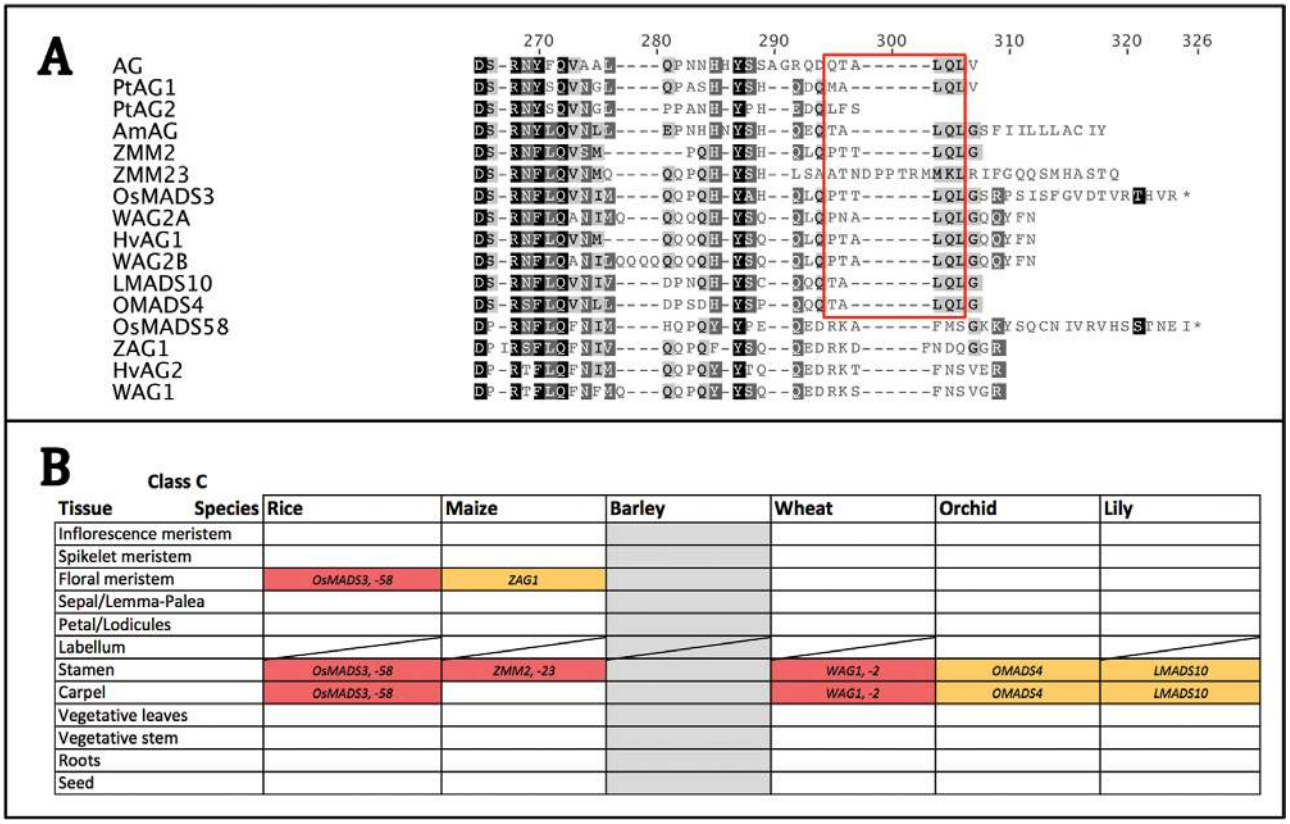

Fig. 8. Sequence alignment and expression patterns of C-class MADS-box genes in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily. (A) The C-class genes are very conserved throughout the entire sequence. A small distinction can be made at the C-terminus where the TALQL motif, that is also present in the B_{sister} genes, can be found in some of the homologues. Expression of C-class genes seems to be conserved in all species. (B) The expression patterns of C-class genes are conserved across all species that have been investigated to date. Red squares indicate that multiple genes are expressed in this tissues, while yellow indicates that only one gene is expressed in this tissue. White squares indicate that there is no expression, and grey squares indicate that no data are available regarding the expression in these tissues.

at position 113 there is a histidine residue. Both of these are not present in C-lineage proteins (Dreni *et al.*, 2007). Furthermore there is a conserved AG motif I and AG motif II in the C-terminal region of AG-like proteins, which can be found in C- and D-class proteins (Kramer *et al.*, 2004). A nine amino acid motif downstream of the AG motif II is specific for D-class proteins (Hsu *et al.*, 2010) (Figs 8, 9).

In rice, two duplicated C-class genes *OsMADS3* and *OsMADS58* have partially sub-functionalized (Table 1) (Kang *et al.*, 1995; Yamaguchi *et al.*, 2006). *OsMADS3* shows high sequence and expression similarity to Arabidopsis *AG* (C-class gene). *In situ* hybridization showed that *OsMADS3* is strongly expressed in stamen primordia, while *OsMADS58* is expressed at a lower level uniformly throughout the floral meristem (Dreni *et al.*, 2011). After the differentiation of the third whorl organ, both *OsMADS3* and *OsMADS58* have a similar expression profile in the filament and the anther wall, and a stable expression level in the carpel and ovule primordia (Dreni *et al.*, 2011). *OsMADS3* plays a predominant role in stamen specification, with knock-out mutants by T-DNA insertion (*mads3-3*) exhibiting stamens completely or incompletely transformed into lodicules while carpels developed normally (Yamaguchi *et al.*, 2006; Dreni *et al.*, 2011). Even though *osmads58* insertional mutants showed no drastic phenotype (Dreni *et al.*, 2011), *osmads3-3 osmads58* double mutants showed a complete loss of reproductive organ identity and floral meristem determinacy (Dreni *et al.*, 2011). The size of the floral meristem also strongly increased, and the combination of these features resulted in an enlarged third whorl. In half of the florets, the carpel was replaced by a small green lemma/palea-like structure (Dreni *et al.*, 2011). Based on these results, it seems that OsMADS3 and OsMADS58 work redundantly, with the contribution of OsMADS3 being more important (Dreni *et al.*, 2011). OsMADS3 and OsMADS58 genetically interact with the B-class gene OsMADS16 and together they play a key role in suppressing indeterminate growth within the floral meristem in the third whorl primordia (Yun *et al.*, 2013).

WAG1 and *WAG2* are classified as C-function genes in *Triticum aestivum* (Table 1) (Meguro *et al.*, 2003; Zhao *et al.*, 2006; Shitsukawa *et al.*, 2007; Hirabayashi and Murai, 2009; Murai, 2013). Although they share high level sequence similarity to rice *OsMADS58* and *OsMADS3*, respectively, they have different expression patterns and functions (Wei *et al.*, 2011; Murai, 2013). Meguro *et al.* (2003) detected three homeologues of *WAG1* in the wheat genome on the group one chromosomes (1A, 1B, and 1D) by Southern blot analysis, while Wei *et al.* (2011) found three homeologues of *WAG2* on the group two chromosomes (2A, 2B, and 2D). *WAG1* expression is low during initiation of floral organ primordia, but transcripts accumulate in developing spikes at the booting to

Fig. 9. Sequence alignment and expression patterns of D-class MADS-box genes in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily. (A) Multiple alignment of protein sequences from the NCBI, IPK, and MSU rice databases The C- and D-class MADS-box genes in monocots can be distinguished by a conserved glutamine at position 105 and a single amino acid insertion at position 90 in the D-lineage. Remarkably, HvBM21 does not have a glutamine, but a leucine at position 105. It seems that most monocot genes have a glutamine insertion at position 90, except OsMADS21, that has a histidine. (B) Expression of D-class genes seems to be conserved among all species. Red squares indicate that multiple genes are expressed in this tissue, while yellow indicates that only one gene is expressed in this tissue. White squares indicate that there is no expression, and grey squares indicate that no data are available regarding the expression in these tissues.

heading stage seen by northern blot analysis, suggesting that it is involved in floral organ development rather than differentiation (Meguro *et al.*, 2003). *In situ* hybridization showed that *WAG1* and *WAG2* are detected in the stamen, carpel, and ovule (Fig. 8B) (Yamada *et al.*, 2009). Ectopic expression of the *WAG1* and *WAG2* genes induced pistilloid stamens in alloplasmic wheat, which suggests they participate in ectopic ovule formation in these structures (Yamada *et al.*, 2009).

The maize orthologues of rice *OsMADS3* are *ZMM2* and *ZMM23*, and *OsMADS58* is *ZAG1* (Table 1) (Schmidt *et al.*, 1993; Theissen *et al.*, 1995; Münster *et al.*, 2002; Li *et al.*, 2014). *ZAG1* is expressed early in stamen and carpel primordia, as shown by RNA blot analysis and *in situ* hybridization (Schmidt *et al.*, 1993). *ZMM2* is mainly expressed in the anthers (Fig. 8B) (Mena *et al.*, 1996; Li *et al.*, 2014). Analysis of loss-of-function mutants showed that *ZAG1* determines the floral meristem, while *ZMM2* participates in regulating the formation of stamens and carpels (Mena *et al.*, 1996; Wei *et al.*, 2011). The orchid genes *OMADS4* and *OMADS2* are both placed in the AG-clade, with *OMADS4* having a C-class function and *OMADS2* a D-class function (Table 1) (Hsu *et al.*, 2010). qRT-PCR analysis showed that *OMADS4* is expressed in stamens, the stigmatic cavity, and the ovule (Fig. 8B) (Hsu *et al.*, 2010), which is similar to the expression pattern of *AG* in Arabidopsis (Yanofsky *et al.*, 1990). Yeast two-hybrid analysis showed that OMADS4 and OMADS2 can form homodimers and heterodimers with each other (Hsu *et al.*, 2010). *LMADS10*, the C-class gene in lily, is expressed in stamens and carpels (Hsu *et al.*, 2010). This is very similar to the expression pattern in *O.* Gower Ramsey (Fig. 8B). Ectopic expression of *LMADS10* in Arabidopsis caused early flowering and produced small, curly leaves and floral organ conversions such as carpelloid sepals (Hsu *et al.*, 2010). Overexpression of *OMADS4* in Arabidopsis only showed a moderate early flowering phenotype with no homeotic floral organ changes (Hsu *et al.*, 2010).

Rice has two duplicated D-lineage genes: *OsMADS13* and *OsMADS21* (Table 1) (Kramer *et al.*, 2004; Dreni *et al.*, 2007). *OsMADS13* is expressed in the ovule primordium and the inner cell layer of the carpel wall. Its expression persists during development of the ovule, mainly in the integuments (Lopez-Dee *et al.*, 1999). In a *Tos17* insertion mutant of *OsMADS13*, ovule primordia developed into carpelloid structures that grew out of the carpel, giving rise to ectopic styles and stigmas (Dreni *et al.*, 2007; Yamaki *et al.*, 2011). The *osmads3-3 osmads13* double mutant showed a complete loss of floral meristem determinacy inside the fourth whorl, while the *osmads13 osmads58* double mutant showed a similar but milder phenotype (Dreni *et al.*, 2011; Li *et al.*, 2011).

OsMADS13 interacts with the E-class MADS-box proteins, OsMADS7 and OsMADS8, and is involved in ovule specification and floral meristem determinacy (Fornara *et al.*, 2003; Yamaguchi and Hirano, 2006; Dreni *et al.*, 2007). RT-PCR and *in situ* hybridization showed that *OsMADS21* is expressed at low levels in the inner two whorls of the flower and ovules; its expression overlaps with that of *OsMADS13* (Arora *et al.*, 2007; Dreni *et al.*, 2007). The *OsMADS21* expression is in two whorls of the flower which differs from other D-lineage genes, which are ovule specific (Fig. 9B) (Dreni *et al.*, 2007); it is also highly expressed in developing kernels (Arora *et al.*, 2007; Dreni *et al.*, 2007). T-DNA insertional mutants of *OsMADS21* show no aberrant phenotype, while os*mads13 osmads21* double mutants showed no more severe phenotypes than the *osmads13* single mutant, and up-regulation of *OsMADS21* resulted in partial complementation of the *osmads13* phenotype, but ovule development was not completely restored (Dreni *et al.*, 2007, 2011). These results suggest that *OsMADS21* has lost its function in determining ovule identity, presumably because of its redundancy with *OsMADS13* (Fornara *et al.*, 2003; Yamaguchi and Hirano, 2006; Dreni *et al.*, 2007).

The closest relative of the Arabidopsis D-function gene *STK* in wheat is *WSTK*, also known as TaAG-3 (Table 1) (Zhao *et al.*, 2006; Paolacci *et al.*, 2007). Yeast two-hybrid analysis has shown that WSTK forms a complex with the E-class protein WSEP (Shitsukawa *et al.*, 2007; Yamada *et al.*, 2009; Murai, 2013). RT-PCR assays showed that it is expressed in pistils, with strong expression in the developing ovule (Yamada *et al.*, 2009). *In situ* hybridization showed WSTK mRNA in the ectopic ovules and pistil-like stamens of alloplasmic wheat, suggesting a role in ovule formation (Yamada *et al.*, 2009). There are presumably three homeologues of *WSTK* in the wheat genome (Zhao *et al.*, 2006; Yamada *et al.*, 2009). The closest relative to *OsMADS21* in wheat has been identified as *TaAG-4* (Paolacci *et al.*, 2007). *TaAG-4* has weak expression in stamens and very high expression in pistils, as shown by RT-PCR (Paolacci *et al.*, 2007). *ZAG2* and *ZMM1* have been identified as D-class genes in maize (Schmidt *et al.*, 1993; Theissen *et al.*, 1995; Li *et al.*, 2014). *ZAG2* is a floral specific gene, but is expressed later in floral primordia than the C-class gene *ZAG1*. Expression of *ZAG2* is largely restricted to the developing ovules and the inner carpel face, as determined by *in situ* hybridization (Schmidt *et al.*, 1993). qRT-PCR showed that *OMADS2* in *O.* Gower Ramsey is expressed in the stigmatic cavity and the ovary, but is undetectable in sepals, petals, the labellum, and stamens (Fig. 9B) (Hsu *et al.*, 2010). Ectopic expression of *OMADS2* shows the same phenotype as *LMADS10*, except that there are no floral organ conversions (Hsu *et al.*, 2010). *LMADS2* was identified as the D-class protein in *L. longiflorum* (Tzeng *et al.*, 2002). It was exclusively expressed in the carpel, more specifically in the ovule, as seen by RNA blot analysis (Tzeng *et al.*, 2002). LMADS2 can form heterodimers with LMADS10 and both can also form homodimers, as shown by yeast two-hybrid analysis (Hsu *et al.*, 2010). Ectopic expression of *LMADS2* in Arabidopsis caused early flowering and floral organ conversion of sepals and petals to carpel- and stamen-like structures (Tzeng *et al.*, 2002).

The gene duplication event of C-class genes is also seen in some grasses, for instance in maize, leading to three different C-class genes and possible sub-functionalization (Dreni and Kater, 2014). In contrast, only one C-class gene and one D-class gene have currently been found in *O.* Gower Ramsey and *L. longiflorum*, but their expression patterns are highly conserved compared with those of Arabidopsis and rice.

E-class genes

E-class genes belong to the *AGL2*-subfamily and specify flower organ identity by forming higher order protein complexes with the class A, B, or C proteins (Pelaz *et al.*, 2000; Theissen, 2001; Becker and Theissen, 2003). This ability to form tetrameric complexes also contributes to the development of floral quartets to control sepal, petal, stamen, and carpel formation or their equivalents in grasses (Theissen and Saedler, 2001; Becker and Theissen, 2003; Fornara *et al.*, 2003;). In Arabidopsis, *SEP1/2/3/4* have been identified as E-class genes (Ma *et al.*, 1991; Huang *et al.*, 1995; Mandel and Yanofsky, 1998). *SEP1*, *SEP2*, and *SEP4* are expressed in all four whorls of the flower, with SEP4 showing higher expression in the central dome (Flanagan and Ma, 1994; Savidge *et al.*, 1995; Ditta *et al.*, 2004). *SEP3* is only expressed in the inner three whorls (Mandel and Yanofsky, 1998).

AGL2-like genes were deduced to have undergone a gene duplication event before the origin of the extant angiosperms, and after the divergence between extant gymnosperms and angiosperms, creating the SEP3- and LOFSEP-lineages (Malcomber and Kellogg, 2005; Zahn *et al.*, 2005*a*). Furthermore, SEP3- and LOFSEP-lineages may have undergone more gene duplication events in the grasses, leading to three LOFSEP lineages: OsMADS1-, OsMADS5- and OsMADS34-clades and two SEP3-lineages: OsMADS7- and OsMADS8-clades (Malcomber and Kellogg, 2005; Zahn *et al.*, 2005*a*). In addition, two motifs (SEPI and SEPII) that consist of hydrophobic and polar residues were observed in AGL2-like proteins (Vandenbussche *et al.*, 2003; Zahn *et al.*, 2005*a*). Clade-specific changes in these motifs can be seen; for instance, the OsMADS5-clade in grasses have lost the final 12–15 amino acids within the SEPII motif, possibly caused by a recent gene duplication followed by a frameshift mutation (Vandenbussche *et al.*, 2003; Zahn *et al.*, 2005*a*).

LOFSEP-lineage

OsMADS1-clade. *OsMADS1*, one well-characterized E-class gene in rice, plays an important role in floral meristem determination and controls the differentiation and proliferation of palea- and lemma-specific cell types (Jeon *et al.*, 2000*a*; Prasad *et al.*, 2005). The expression of *OsMADS1* is detected in the floral meristem during early flower development, and later in the palea, lemma, and weakly in the carpel, shown by northern blot analysis, RT-PCR, and *in situ* hybridization (Fig. 10B) (Chung *et al.*, 1994; Prasad *et al.*, 2001; Kobayashi *et al.*, 2010). Overexpression of

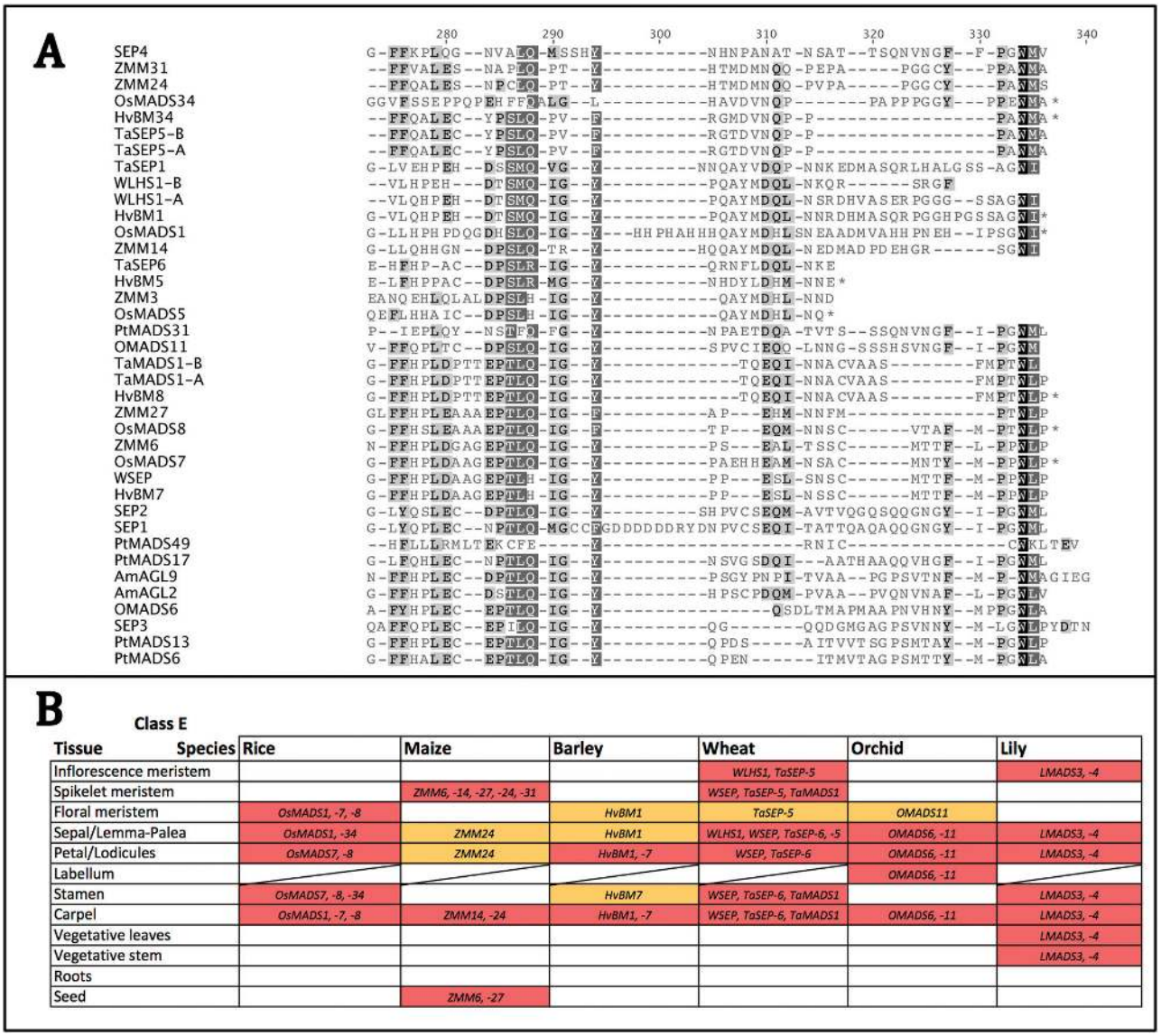

Fig. 10. Sequence alignment and expression patterns of E-class MADS-box genes in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily. (A) Multiple alignment of protein sequences from the NCBI, IPK, and MSU rice databases. The distinction between the two subgroups can clearly be seen, with the OsMADS1 group less related to the Arabidopsis SEP genes, and the OsMADS7 group more closely related to the SEP genes. (B) Expression of E-class genes in very diverse, but seems to be mostly conserved among the different species. Maize seems to have distinct genes with specified expression. Red squares indicate that multiple genes are expressed in this tissue, while yellow indicates that only one gene is expressed in this tissue. White squares indicate that there is no expression, and grey squares indicate that no data are available regarding the expression in these tissues.

OsMADS1 caused stunted panicles, irregularly positioned branches and spikelets, and the rudimentary glumes were transformed into palea/lemma-like structures (Prasad *et al.*, 2001, 2005). Different mutants of *OsMADS1* have been investigated. Jeon *et al.* (2000*a*) reported that *lhs-1* (*leafy hull sterile 1*), which contains two missense mutations in the *OsMADS1* MADS-domain, showed a loss of floral meristem determination and transformation of palea and lemma into leaf-like structures. Similarly, other *OsMADS1* mutants such as *osmads1-z* and *nsr* (*naked seed rice*) showed the transformation of the lemma, palea, and lodicules into leaf-like structures (Chen *et al.*, 2006; Gao *et al.*, 2010). OsMADS1 was shown to interact with the A-class proteins OsMADS14 and OsMADS15, the B-class protein OsMADS16, the C-class proteins OsMADS3 and OsMADS58, the D-class protein OsMADS13, the E-class proteins OsMADS7 and OsMADS8, and the AGL-like protein OsMADS6 (Moon *et al.*, 1999*b*; Lim *et al.*, 2000; Cui *et al.*, 2010; Hu *et al.*, 2015). Two maize homologues of *OsMADS1*, *ZMM8* and *ZMM14*, are thought to determine the alternative identity of the upper versus the lower floret within each spikelet primordium (Cacharrón *et al.*, 1999; Becker and Theissen, 2003). Their expression was only detectable in the upper floret, but not in the lower floret of the developing spike, shown by *in situ* hybridization (Fig. 10B) (Cacharrón *et al.*, 1995, 1999). *ZMM14* expression is lower than that of *ZMM8*, and is stronger in the carpels than in the other tissues (Cacharrón *et al.*, 1999). The function of barley *HvBM1* (also known as *BM7*) remains to be elucidated. The expression of *HvBM1* is seen in the floret meristem at the distal part of the awn primordium. As floret development continues, expression is detected in the lemma and palea, in the lodicules and the ovule, but not in the anther (Schmitz *et al.*, 2000).

Wheat has three homeologues of *OsMADS1* called *WLHS1* located on chromosomes 4A, 4B, and 4C (Shitsukawa *et al.*, 2007). *In situ* hybridization analysis showed that the expression of *WLHS1* is initially detectable in the inflorescence axis at inflorescence meristem initiation (Shitsukawa *et al.*, 2007). During floral organ differentiation, their expression signals are detected in the spikelet axis at the most proximal position (Shitsukawa *et al.*, 2007). Later, their expression was observed in the glume, lemma, and palea until maturity of the floral organs (Shitsukawa *et al.*, 2007). Shitsukawa *et al.* (2007) showed that expression of *WLHS1-B* is much lower than that of *WLHS1-A* and *-D*. WLHS1-B and WLHS1-D interact with B-class WAP3 and WPI2 and all E-class genes, with the exception of WLHS1-A (Shitsukawa *et al.*, 2007). It has been suggested that the lack of interaction with WLHS1-A is due to the loss of the K-box in WLHS1-A (Davies *et al.*, 1996; Shitsukawa *et al.*, 2007). Overexpression of *WLHS1* homeologues in Arabidopsis showed no phenotype for *WLHS1-A*, and early flowering and late production of terminal flowers for *WLHS1-B* and *-D* (Shitsukawa *et al.*, 2007).

OsMADS5-clade. The function of the *LOFSEP* gene *OsMADS5* has remained a mystery because of no detectable phenotype in either panicles or vegetative organs in loss-of-function mutants, except for the lodicules being more tightly attached to the lemma and palea upon spikelet dissection (Agrawal *et al.*, 2005). Recent findings using genetic and molecular approaches suggest that one role of *OsMADS5* is to regulate spikelet morphogenesis together with *OsMADS1* and *OsMADS34* redundantly by positively regulating the other MADS-box floral homeotic genes. Furthermore, OsMADS1, OsMADS5, and OsMADS34 can form protein–protein interactions with other MADS-box floral homeotic members, which is a typical, conserved activity of plant SEP proteins (Wu *et al.*, 2018).

ZMM3 (maize) was classified as a member of the OsMADS5-clade in the LOFSEP-lineage with unknown function (Malcomber and Kellogg, 2005). Paolacci *et al.* (2007) identified *TaSEP-6* as an orthologue of *OsMADS5*, located on chromosomes 7A, 7B, and 7D in the wheat genome. Northern blot analysis, RT-PCR, and qRT-PCR showed that it is expressed in all floral organs, but at very high levels in glumes, lemma, and palea (Paolacci *et al.*, 2007).

OsMADS34-clade. Unlike other *SEP*-like genes involved in controlling flower development, *OsMADS34* [*PANICLE PHYTOMER2* (*PAP2*)], one *LOFSEP* gene, is required for rice inflorescence and spikelet development (Gao *et al.*, 2010; Kobayashi *et al.*, 2010; Lin *et al.*, 2014). *osmads34-1* showed altered inflorescence shape with increased primary branch number and decreased secondary branch number. In addition, *osmads34-1* showed fewer spikelets and changed spikelet morphology, containing elongated sterile lemmas with lemma/palea-like features (Gao *et al.*, 2010). Recently *OsMADS34/PAP2* was shown to be involved in the transition from vegetative to reproductive development via specifying inflorescence meristem identity together with three *AP1/FUL*-like genes *OsMADS14*, *OsMADS15*, and *OsMADS18* (Kobayashi *et al.*, 2012). These findings clearly show that OsMADS34 is a positive regulator of inflorescence meristem identity and spikelet meristem identity, as well as a suppressor of elongation of the glumes (Kobayashi *et al.*, 2010, 2012).

In maize and wheat, the functions of *OsMADS34* homologues have not been elucidated, and only expression data are reported. Two maize homologues of *OsMADS34*, *ZMM24* and *ZMM31*, are expressed in early developing tassels and ears, and *ZMM24* shows high expression throughout ear development (Danilevskaya *et al.*, 2008). *TaSEP-5* was identified as the orthologue of *OsMADS34* in wheat, and its three homeologues are located on chromosomes 5A, 5B, and 5D, with a high expression level at the early spike developmental stages, which decreases, but increases again in spikes at the booting and heading stages (Paolacci *et al.*, 2007). Notably, *TaSEP-5* is highly expressed in the glumes, lemma, and palea (Paolacci *et al.*, 2007).

Orchid and lily. To date there is no direct genetic evidence showing the function of the *OsMADS1*-like gene *OMADS11* in orchid. *OMADS11* is highly expressed in the sepal, petal, lip, carpel, anther cap, and stigmatic cavity, and has no expression signal in vegetative leaves and stamens, as was shown by RT-PCR. Ectopic expression of *OMADS11* in Arabidopsis showed an early flowering phenotypes and smaller, curled leaves (Chang *et al.*, 2009). In lily, *LMADS3* and *LMADS4* were identified as E-class genes (Table 1) (Tzeng *et al.*, 2003). *LMADS4* is a *SEP1/2* orthologue, which is expressed in the inflorescence meristem, floral buds of different developmental stages, and in all four whorls of the flower (Tzeng *et al.*, 2003; Chang *et al.*, 2009). *LMADS4* is also expressed in the vegetative leaf and in the inflorescence stem (Tzeng *et al.*, 2003). Arabidopsis plants with ectopic expression of *LMADS4* were indistinguishable from the wild-type plants (Tzeng *et al.*, 2003).

SEP3-lineage

OsMADS7-clade. *OsMADS7* has a redundant function in specifying rice flower development with *OsMADS8*, as suggested by the observation that *OsMADS7* and *OsMADS8* share almost identical expression patterns (Kang *et al.*, 1997; Pelucchi *et al.*, 2002). *OsMADS7* and *OsMADS8* are expressed early in the floral meristem where the lodicule and stamen primordia develop (Kang *et al.*, 1997; Pelucchi *et al.*, 2002). Subsequently they are expressed in lodicules, developing stamen, and carpel primordia throughout floret development (Fig. 10B) (Kang *et al.*, 1997; Pelucchi *et al.*, 2002). Overexpression and knock-down of *OsMADS7* shows similar phenotypes to that of *OsMADS8* (Kang *et al.*, 1997; Jeon *et al.*, 2000*b*; Cui *et al.*, 2010). Knock-down of both *OsMADS7* and *OsMADS8* resulted in late flowering and homeotic transformation of lodicules, stamens, and carpels into palea/lemma-like structures, while knock-down of *OsMADS7* or *OsMADS8* using RNAi only showed mild phenotypes (Cui *et al.*, 2010). *In vitro* and *in vivo* assays showed that OsMADS7 interacts with OsMADS8 and OsMADS1, and can form homodimers (Cui *et al.*, 2010).

ZMM6 in maize is weakly expressed in all organs of the upper and lower floret during inflorescence development, and is strongly expressed in the endosperm transfer cell region and the embryo during maize kernel development (Fig. 10B) (Cacharrón *et al.*, 1995, 1999; Lid *et al.*, 2004). Loss of function of *ZMM6* with a *Mutator* insertion showed no obvious developmental defects in the kernel (Lid *et al.*, 2004).

In barley, *HvBM7* (also known as *BM9*) expression has been found in anthers, but not in the lemma or palea, and later also in lodicules and the carpel (Fig. 10B) (Schmitz *et al.*, 2000). The wheat SEP-like protein WSEP has three homeologues in the wheat genome on chromosomes 7A, 7B, and 7D (Paolacci *et al.*, 2007; Shitsukawa *et al.*, 2007). Just before initiation of the lodicule, and stamen and carpel formation, *WSEP* expression was detected in whorls 2, 3, and 4 (Shitsukawa *et al.*, 2007). In all subsequent stages, expression was also detected in the palea of the floret (Fig. 10B). qRT-PCR showed that there is no difference in expression between the three homeologues (Shitsukawa *et al.*, 2007). Overexpression of *WSEP* in Arabidopsis showed early flowering and 4–5 curled leaves phenotypes for all three homeologues (Shitsukawa *et al.*, 2007). The strong expression of *WSEP* not only during floral organ differentiation but also after floral organ determination suggests that *WSEP* genes are involved in both floral organ differentiation and their subsequent development (Shitsukawa *et al.*, 2007; Chang *et al.*, 2009; Murai, 2013). WSEP interacts with the A-class WAP1, the B-class WAP3 and WPI2, the C-class WAG1 and WAG2, the D-class WSTK, and all E-class genes, except WLHS1-A (Shitsukawa *et al.*, 2007).

OsMADS8-clade. The expression pattern of the *OsMADS8* homologue in maize *ZMM27* is similar to that of *ZMM6*, showing weak expression during development of the inflorescence and strong expression during maize kernel development (Lid *et al.*, 2004). Further, loss of function of *ZMM27* in a *Mutator* insertional mutant did not induce obvious defects and neither did the double mutant with *ZMM6* (Lid *et al.*, 2004). *TaMADS1* was identified as the *OsMADS*8 orthologue in wheat, with the three homeologues located on chromosomes 5A, 5B, and 5D (Paolacci *et al.*, 2007). Northern blot analysis and *in situ* hybridization showed that they are uniformly expressed in the spikelet primordia and later confined to the carpels and stamens (Zhao *et al.*, 2006). Overexpression of *TaMADS1* in Arabidopsis showed mild to severe phenotypes, with early flowering and abnormal floral organs (Zhao *et al.*, 2006).

Orchid and lily. Expression of the *OsMADS7*-like gene in orchid, *OMADS6*, is abundant in the sepal, petal, labellum, carpel, anther cap, and stigmatic cavity, and weak in the stamen, as shown by RT-PCR (Fig. 10B) (Chang *et al.*, 2009). Overexpression of *OMADS*6 in Arabidopsis resulted in early flowering, 2–4 small curled leaves, terminal flowers composed of 2–3 flowers, and homeotic conversions of sepals into carpel-like structures and petals into stamen-like structures (Chang *et al.*, 2009). In lily, *LMADS3* is a *SEP3* orthologue, which shows almost identical expression to that of the *OsMADS*1-like gene in lily, *LMADS4* (Tzeng *et al.*, 2003). Northern blot analysis showed that *LMADS3* is expressed in the inflorescence meristem and later in all four floral organs, but is absent in vegetative leaves (Tzeng *et al.*, 2003). Overexpression of *LMADS3* in Arabidopsis resulted in early flowering, 2–3 small curled rosette leaves, and two curled cauline leaves (Tzeng *et al.*, 2003). Inflorescence determinacy was lost, as was production of terminal flowers at the end of the inflorescence that had 2–3 carpels.

AGL6-*like genes*

The *AGL6* subfamily is thought to be sister to the E-class AGL2-like genes (Becker and Theissen, 2003). Rijpkema *et al.* (2009) proposed adding *AGL6*-like genes to the E-class of the ABCDE model. Arabidopsis has two AGL6-like genes: *AGL6* and *AGL13*, both of which have various divergent functions in the plant, although no loss-of-function mutants have been described so far (Dreni and Zhang, 2016). AGL6 in Arabidopsis can interact with some type I MADS proteins, which is unusual for MIKC^c-type MADS proteins (Dreni and Zhang, 2016). AGL6-like proteins have a C-terminus with two short, but highly conserved regions named AGL6-I and AGL6-II motifs (Ohmori *et al.*, 2009).

In monocots, the *AGL6* family has four well-defined clades: *AGL6*-I to *AGL6*-IV (Dreni and Zhang, 2016). Orchid sequences are part of the AGL6-III and AGL6-IV clade (Dreni and Zhang, 2016). The *AGL6*-I clade in grasses can be further subdivided into two branches: *ZAG3/OsMADS6* and *OsMADS17* (Dreni and Zhang, 2016). Li *et al.* (2010) proposed that a duplication event that gave rise to these clades may have occurred before the diversification of grasses. The *OsMADS17* clade is characterized by 25 amino acid substitutions, most of them located in the K-domain and the C-terminal domain. *OsMADS6*-like sequences in grasses have a highly conserved motif (MLGWVL) that is different in *OsMADS17*-like genes (VMGWPL) (Fig. 10A) (Reinheimer and Kellogg, 2009).

The expression pattern of *AGL6*-like genes in plants shows clear differences reflecting evolutionary changes (Reinheimer and Kellogg, 2009). Their expression in the inner integument of the ovule is ancestral, and is also seen in the gymnosperms. Expression in the floral meristem was acquired in angiosperms, and expression in the second whorl organs was acquired in monocots. Early in grass evolution, a new expression domain emerged in the palea (Reinheimer and Kellogg, 2009).

Rice has two *AGL6*-like genes, *OsMADS6* and *OsMADS17*, which have different expression patterns (Ohmori *et al.*, 2009; Reinheimer and Kellogg, 2009). RT-PCR and *in situ* hybridization showed that *OsMADS6* is expressed in the floral meristem at early stages and later in the emerging palea primordium (Li *et al.*, 2010). It is also detected in developing palea, lodicules, ovule integuments, and carpels, and weakly in lemma (Fig. 11B) (Li *et al.*, 2010; Dreni and Zhang, 2016). Mutants of *OsMADS6* (also called *mfo1*) showed disturbed palea and lodicule identities, and had extra carpels or spikelets (Ohmori *et al.*, 2009). The *mfo1 lhs1* double mutant resulted in a severe phenotype including the loss of spikelet meristem determinacy, suggesting that together with OsMADS1, OsMADS6 determines floral organ and meristem identities (Ohmori *et al.*, 2009; Li *et al.*, 2010). This also suggests that OsMADS6 has a very similar function to the E-class genes, which regulate the development of all four whorls and floral meristem determinacy (Li *et al.*, 2010). OsMADS6 can also form protein complexes with rice B-, D-, and E-class proteins in yeast two-hybrid assays, which resemble the complexes formed by E-class genes with A-, B-, and C-class proteins in Arabidopsis (Moon *et al.*, 1999*b*; Lee *et al.*, 2003*a*; Seok *et al.*, 2010). OsMADS6 also interacts with the D-class protein OsMADS13 and the B_{sister}-class protein OsMADS29 (Favaro *et al.*, 2002; Nayar *et al.*, 2014). Together with B-class proteins, it specifies lodicule identity (Dreni and Zhang, 2016). OsMADS6 also represses the A-class genes *OsMADS14* and *OsMADS15*. *OsMADS17* is expressed in the floral meristem and later becomes restricted to the lodicule

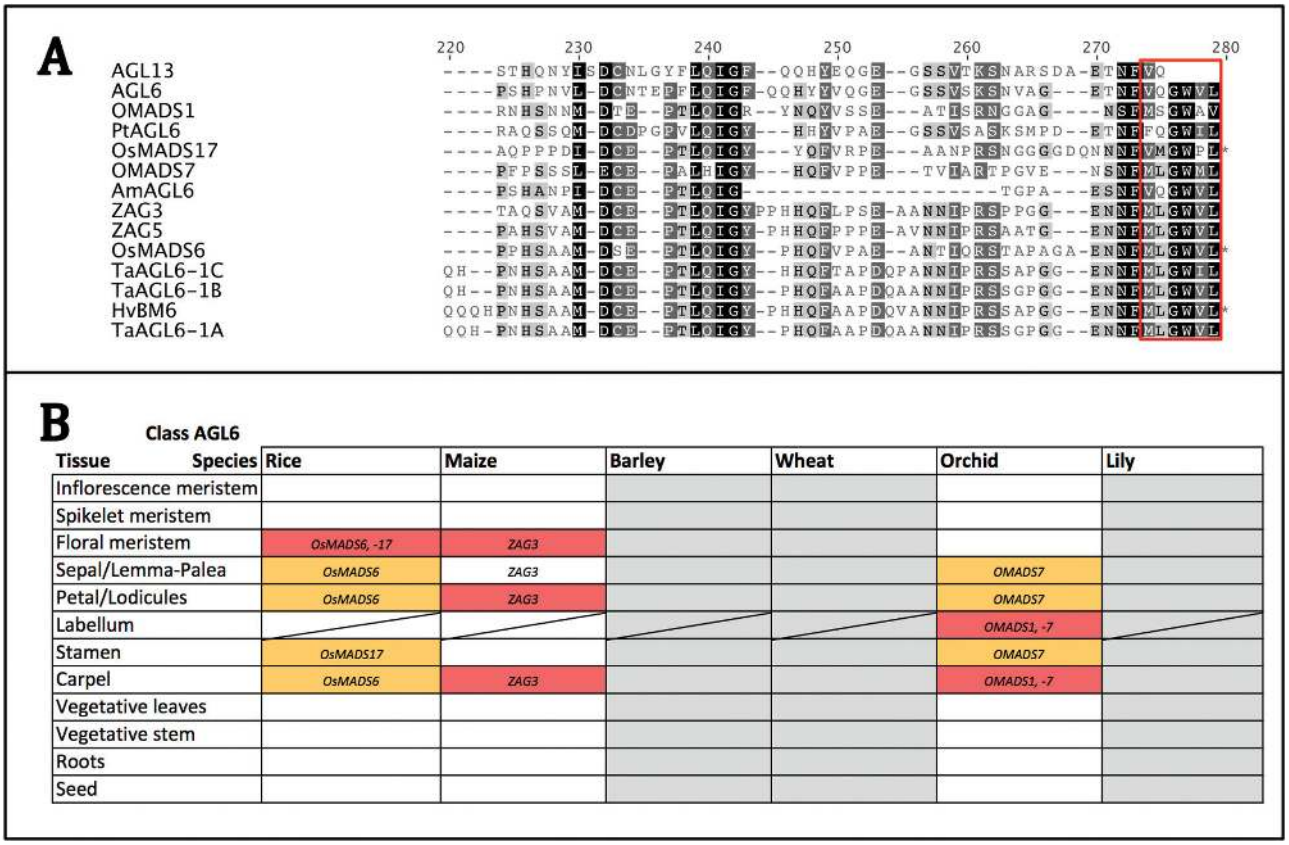

Fig. 11. Sequence alignment and expression patterns of AGL6-like MADS-box genes in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily. (A) Multiple alignment of protein sequences from the NCBI, IPK, and MSU rice databases. The AGL6-like genes are very conserved throughout the entire sequence. At the C-terminus (A), the motif for the OsMADS6-like genes (MLGWVL) can be distinguished, while the OsMADS17-like genes have a different motif (VMGWPL). (B) The expression pattern of AGL6-like genes seems to be conserved among the different species, with the exception of the labellum in orchid. Red squares indicate that multiple genes are expressed in this tissue, while yellow indicates that only one gene is expressed in this tissue. White squares indicate that there is no expression, and grey squares indicate that no data are available regarding the expression in these tissues.

primordia and is also detected in the anther wall (Fig. 11B) (Reinheimer and Kellogg, 2009). Suppression of *OsMADS17* by RNAi did not result in any morphological abnormalities (Ohmori *et al.*, 2009). In the *mfo1* background, however, it enhanced the *mfo1* phenotype (Ohmori *et al.*, 2009).

Maize also has two *AGL6*-like genes: *ZAG3* and *ZAG5* (Table 1) (Mena *et al.*, 1995; Reinheimer and Kellogg, 2009). It was suggested that maize had lost the AGLI/OsMADS17-clade and that both *ZAG3* and *ZAG5* are orthologues of *OsMADS6* (Dreni and Zhang, 2016). *In situ* hybridization showed that *ZAG3* is expressed in both the upper and lower floral meristems, but not in the lemma and stamens (Thompson *et al.*, 2009). Later in development, it was observed in developing lodicules, palea, carpel, and the inner integument of the ovule (Fig. 11B). ZAG3 interacts with the C-class protein ZAG1 (Reinheimer and Kellogg, 2009; Thompson *et al.*, 2009). Loss of function of *ZAG3*, known as the *bearded-ear* (*bde*) mutant, resulted in spikelets that produce more florets with more floral organs in the tassels (Thompson *et al.*, 2009). In the ear of the mutant, the spikelets also produce more florets, which have more palea/lemma-like organs and sterile ovaries.

Similar to rice and maize, orchid also has two AGL6-like genes: *OMADS7* and *OMADS1*. The expression pattern of *OMADS7* is extremely similar to that of the E-class gene *OMADS6* and of AGL6-like genes in other species, for example *AGL6* in Arabidopsis and *ZAG3* in maize (Chang *et al.*, 2009). Overexpression of *OMADS7* in Arabidopsis resulted in early flowering, producing small curled leaves and homeotic conversion of sepals into carpel-like structures with stigmatic papillae (Chang *et al.*, 2009). *OMADS1* shows a different expression, only in the apical meristem, the labellum, and carpel of the flowers (Hsu *et al.*, 2003). Yeast two-hybrid analysis showed that OMADS1 can interact with OMADS3 (Hsu *et al.*, 2003). Ectopic expression of *OMADS1* in Arabidopsis and tobacco resulted in reduced plant size, early flowering, and loss of inflorescence determinacy (Hsu *et al.*, 2003). Homeotic conversions of sepals into carpel-like structures and petals into staminoid structures were also observed (Hsu *et al.*, 2003).

AGL6-like genes seem to be involved in diverse processes in all four whorls, with conserved expression and function in most of the species. In orchid there seems to be a specialized function for these genes in labellum formation.

Conclusions and perspectives

MADS-box ABCDE genes are crucial for floral development, and their evolutionary changes with gene duplication, sub-functionalization, and neo-functionalization led to novel morphological forms in plants. Understanding the function of

these MADS-box genes can provide information on how different floral structures originated and identify targets for future crop improvement.

In grasses, the A-class genes underwent more gene duplications and acquired functions in specifying the grass-specific flower organs such as the palea and lodicule. Clearly the whole picture of A-class genes in grasses still remains to be elucidated.

As in other species, the function of B-class genes is relatively conserved in most grasses, even though there may has been gene duplication and sub-functionalization. Exceptionally, in orchids, two separate duplication events have led to some remarkable changes in floral structure. *OMADS3* in orchid lost the C-terminal motifs of MADS-box proteins and has the expression signal in the vegetative leaves (Hsu and Yang, 2002; Tsai and Chen, 2006). It is speculated that *LMADS1* in lily may represent an ancestral form of the B function gene, which retains the ability to form homodimers and regulates petal and stamen development (Tzeng and Yang, 2001). Notably, the *OsMADS30* B_{sister} gene has gone through neo-functionalization, giving it a function in vegetative development instead of ovule and seed development (Schilling *et al.*, 2015). Until now, little is known about the B_{sister} genes in most of the species described.

Despite gene duplication events the C- and D-class genes seem to have retained most of their function and expression patterns in monocots. Sub-functionalization has led to genes working redundantly, and the rice D-class gene *OsMADS21* has lost its ability to determine ovule development because of redundancy with *OsMADS13* (Fornara *et al.*, 2003; Prasad *et al.*, 2005; Yamaguchi and Hirano, 2006; Dreni *et al.*, 2007). Its higher expression in developing kernels might suggest that OsMADS21 has gone through neo-functionalization and has a function after fertilization (Arora *et al.*, 2007).

The E-class genes are more difficult to compare than the other classes of genes from the ABCDE model as they have diversified with a function in inflorescence and spikelet development during evolution. The expression of *OsMADS1* homologues in grasses varies from species to species with the developmental pattern of florets in the spikelet. *OsMADS1*-like genes may have been involved in morphological diversification of inflorescences during the evolution of grass species (Yamaguchi and Hirano, 2006).

Expression of *AGL6*-like genes in the palea is conserved in all spikelet-bearing grasses. This could indicate that AGL6-like genes might play a conserved role in palea development (Reinheimer and Kellogg, 2009). It has been proposed that AGL6-like genes may have played an important role in the evolution of unique flower features, such as the labellum in orchids (Dreni and Zhang, 2016).

Characterization of these genes, their structure, their expression pattern, and their function will give greater insight into their role in flower development. Importantly, phylogenetic analysis can sometimes be misleading, and data from functional analysis experiments are needed to confirm whether genes belong in specific clades and still retain a function in flower development. In line with this, neo-functionalization probably plays a relatively important and unexplored role in monocot floral diversity. The identification of orthologues is currently heavily reliant on sequence similarities, but, due to the many gene duplication events that have shaped the MADS-box family, some MADS-box genes in monocots have gained new roles, or lost their ancestral function. It must also be noted that most of these sequences are extracted from reference genomes, and therefore a much greater level of diversity may be present in the pangenome that is not represented here. Since flower development is one of the major determinants for yield in important crops, improving our understanding about the genes and networks involved in flower development is an essential tool to help towards devising new strategies for crop improvement.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. Sequence alignment of A-class proteins in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily.

Fig. S2. Sequence alignment of B-class proteins in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily.

Fig. S3. Sequence alignment of B_{sister}-class proteins in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily.

Fig. S4 Sequence alignment of C-class proteins in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily.

Fig. S5. Sequence alignment of D-class proteins in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily.

Fig. S6. Sequence alignment of E-class proteins in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily.

Fig. S7. Sequence alignment of AGL6-class proteins in Arabidopsis, *Amborella trichopoda*, *Populus trichocarpa*, rice, maize, wheat, barley, orchid, and lily.

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