Journal of Experimental Botany, Vol. 67, No. 6 pp. 1625–1638, 2016
doi:10.1093/jxb/erw046

FLOWERING NEWSLETTER REVIEW

Flower development: the evolutionary history and functions of the *AGL6* subfamily MADS-box genes

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Received 22 October 2015; Accepted 25 January 2016

Editor: Lars Hennig, Swedish University of Agricultural Sciences

Abstract

***AGL6* is an ancient subfamily of MADS-box genes found in both gymnosperms and angiosperms. Its functions remained elusive despite the fact that the MADS-box genes and the ABC model have been studied for >20 years. Nevertheless, recent discoveries in petunia, rice, and maize support its involvement in the 'E' function of floral development, very similar to the closely related *AGL2* (*SEPALLATA*) subfamily which has been well characterized. The known functions of *AGL6* span from ancient conserved roles to new functions acquired in specific plant families. The *AGL6* genes are involved in floral meristem regulation, in floral organs, and ovule (integument) and seed development, and have possible roles in both male and female germline and gametophyte development. In grasses, they are also important for the development of the first whorl of the flower, whereas in Arabidopsis they may play additional roles before floral meristem formation. This review covers these recent insights and some other aspects that are not yet fully elucidated, which deserve more studies in the future.**

Key words: ABC model, AGL2, AGL6, Arabidopsis, E function, floral organ development, lodicule, MADS-box genes, ovule, petunia, palea, rice.

Introduction

One fundamental feature of plant evolution and adaptation to the land environment is the seed, serving as a protective 'vessel' for the new generation of sporophytes. The seed derives from an ovule with a fertilized embryo sac inside. Despite no continuous fossil record describing their earliest ancestors, it is commonly accepted that seed plants are a monophyletic group which originated ~310 million years ago (MYA) based on morphological and molecular evidence (Schneider *et al.*, 2004). Among the existing seed plants, the 'old' term gymnosperms refers to a likely monophyletic group of four main taxa, namely Coniferophyta, Cycadophyta, Ginkgophyta, and Gnetophyta, the ovule/seed of which is neither developed nor protected by an ovary. Their sister lineage, referred to as angiosperms, or flowering plants, or Magnoliophyta, is a far larger taxon which arose ~140–198 MYA, between the Jurassic and Early Cretaceous (Bell *et al.*, 2005; Moore *et al.*, 2007). During the Early and Late Cretaceous, flowering plants radiated exponentially to become the dominant land plant taxon.

The character which distinguishes flowering plants from gymnosperm is the formation of flowers, bisexual structures typically composed of reproductive organs surrounded by

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vegetative perianth organs. Stamens are the male reproductive organs that produce pollen. The presence of an ovary enclosing and protecting the ovule(s) at the centre of the flower is the other major distinctive character of this taxon. After the embryo sac is fertilized, the ovule starts to develop into a seed, and the surrounding ovary generates the fruit tissues.

Compared with the more ancient groups of land plants, it might not be surprising that such a drastic increase in structural complexity of reproductive organs in flowering plants required the recruitment of new molecular regulatory networks, which are orchestrated by transcription factors (TFs). The MADS-box TFs are indeed master regulators of these floral structures (as well as of nearly all the plant body and life cycle aspects). The way in which they genetically interact with one other to regulate flower development was first understood in two of the core eudicot plants, *Arabidopsis thaliana* (L.) Heynh. and *Antirrhinum majus* L., upon which the ABC model was built (Coen and Meyerowitz, 1991). In the ABC model, a flower is composed of four concentric whorls bearing different kind of floral organs—sepals, petals, stamens, and carpels—sequentially arranged from the outer to the inner part of the flower. This floral structure is typical of core eudicots. There are three functional classes of TFs that regulate the organ identity in these four whorls: the A class genes alone specify sepal identity in the first whorl, and the combined activity of A and B genes is required for petal identity in the second whorl. Together, B and C genes initiate the establishment of stamen identity in the third whorl, and then C genes alone impose the termination and differentiation of the floral meristem (FM) into carpel. Later studies in petunia (*Petunia×hybrida* hort. ex E.Vilm.) revealed more genes regulating ovule identity and development inside the carpel, where a D function was introduced into the initial model (Angenent *et al.*, 1995; Colombo *et al.*, 1995). Finally, an E function was assigned to another class of genes, which is essential for the identity of all floral organs in combination with the A, B, C, and D genes (Pelaz *et al.*, 2000; Theissen, 2001; Ditta *et al.*, 2004).

With the exception of the Arabidopsis A function gene *APETALA2* (*AP2*), all of the genes involved in the ABC(DE) model encode type II MADS-box TFs from the large MIKC^C group (Henschel *et al.*, 2002). Since several yeast two-hybrid interaction studies suggested that MADS-box proteins can form higher order complexes, it was proposed that the MADS-box proteins encoded by ABC(DE) genes form different combination of tetramers or quartets, resulting in a 'quartet model' that can explain the ABC model at the molecular level (Honma and Goto, 2001; Theissen and Saedler, 2001; Favaro *et al.*, 2003). *In vitro* interaction studies suggest that DNA-bound MADS tetramers, or more properly double dimers, are the transcriptionally active form of the 'ABC' MADS-box TFs (Melzer and Theissen, 2009; Melzer *et al.*, 2009; Smaczniak *et al.*, 2012; Jetha *et al.*, 2014).

Compared with core eudicots, the monocot flowers are significantly different, in particular in the external organs. Within monocots, rice (*Oryza sativa* L.) and maize (*Zea mays* L.) are the main models to investigate the development of grass inflorescences and flowers, and its molecular regulation (reviewed by Zhang and Yuan, 2014). In grasses, the flower consists of two opposite bract-like structures arranged in adjacent whorls, the lemma and the palea; the latter is mostly regarded as the true first whorl of the grass flower. In rice, the lemma and the palea are interlocked at their margins. The rice palea is morphologically similar to the lemma, but has only three vascular bundles instead of five, and two distinctive membranous, semi-transparent margins (Prasad *et al.*, 2001; Prasad and Vijayraghavan, 2003). Enclosed by the lemma and the palea are two small lodicules in the second whorl, possibly homologous to petals; then six stamens in the third whorl and one ovary containing just one ovule in the centre. Maize produces two types of reproductive inflorescences, and in both of them spikelets are borne in pairs, with an upper and a lower floret in each spikelet. The apical male inflorescence (tassel) bears staminate flowers where pistil primordia are aborted during development. In the lateral female inflorescence (ear), the lower floret is aborted, whereas the upper floret aborts only the stamen primordia. Maize and rice flowers are similar, but in maize the lemma and palea have lost their protective function, and there are only three stamens.

The definition of A function, its conservation outside of the Brassicaceae family, as well as the homologous origin of the first floral whorl itself between different plant taxa are the subject of debate. Besides having a general function in the regulation of reproductive meristems, the *SQUAMOSA* (*SQUA*) subfamily MADS-box genes are considered to provide the A function in Brassicaceae at least (Shan *et al.*, 2007). It is not clear if the second whorl organ of core eudicots and grass monocots has a homologous or an independent origin. Despite this, the B function seems quite well conserved in specifying its identity. Another hypothesis is that B function genes might have been recruited independently in dicots and monocots to play a similar role in the second whorl (Whipple *et al.*, 2007). Current evidence reveals that the B, C, D, and E genes and their functions are highly conserved within flowering plants. In summary, the B function is provided by the *DEFICIENS*/*GLOBOSA* (*DEF*/*GLO*) subfamily, both C and D by the *AGAMOUS* (*AG*) subfamily, and E by the *AGAMOUS-LIKE 2* (*AGL2*; or *SEPALLATA*, *SEP*) subfamily (Zahn *et al.*, 2005*a*; Kater *et al.*, 2006; Cui *et al.*, 2010; Ciaffi *et al.*, 2011; Dreni *et al.*, 2011; Li *et al.*, 2011*b*; Yun *et al.*, 2013; Zhang *et al.*, 2013; Dreni and Kater, 2014). Studies conducted in petunia and Arabidopsis showed that the distinction of a true 'D' function from the C function is much more vague than initial assumptions, many *AG* C function genes being redundantly also involved in ovule development (Pinyopich *et al.*, 2003; Heijmans *et al.*, 2012).

The MIKC^C family includes several other genes which are not, or not yet, functionally included in the ABC model. Actually, 14 major subfamilies of MIKC^C MADS-box genes have been identified in flowering plants, seven among which also exist in gymnosperms, which also possess the gymnosperm-specific *GpMADS4* subfamily (Becker and Theissen, 2003; Gramzow *et al.*, 2014). However, some of these subfamilies share a close relationship with the aforementioned *DEF*/*GLO*, *AG*, *AGL2*, and *SQUA* involved in floral organ development. For example, the *GGM13* (B_{SISTER}, B_S) and

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OsMADS32 lineages form a superclade together with the B function *DEF*/*GLO*. The former is probably involved in the development of ovule and seed integument in all seed plants (Nesi *et al.*, 2002; Prasad and Ambrose, 2010; Prasad *et al.*, 2010; Mizzotti *et al.*, 2012; Yang *et al.*, 2012; Yin and Xue, 2012; Lee *et al.*, 2013; Lovisetto *et al.*, 2013; Nayar *et al.*, 2013), while the latter is found in monocots and in *Amborella trichopoda* Baill. (Amborella Genome Project, 2013; Gramzow *et al.*, 2014) and, at least in rice, is important for proper floral organ identity (Sang *et al.*, 2012; Wang *et al.*, 2015). The *AGL12* subfamily is sister to the *AG* subfamily which regulates stamen, carpel, and ovule development, and FM determinacy. However, studies conducted in Arabidopsis showed a major function of *AGL12*-like genes in both root development and floral transition (Tapia-López *et al.*, 2008).

In this review, we focus on the *AGL6* subfamily, sister to *AGL2* (*SEP*), whose functions in flower development and plant reproduction have only been recently elucidated. Investigations by yeast two-hybrid screenings, as well as their close sequence similarity with AGL2 proteins, suggest that AGL6 TFs might form multimeric complexes with several ABCDE proteins. Not until 2009 did the direct evidence that they are indeed involved in floral organ identity and patterning emerge, thanks to various loss-of-function studies first in petunia, and shortly after in rice and maize. Furthermore, molecular studies in several other species also provide functional clues about this interesting and ancient group of TFs, which are seemingly ubiquitous in seed plants.

The origin of the *AGL6* subfamily pre-dates the common ancestor of gymnosperms and angiosperms

In published phylogenies, the *AGL6* subfamily clusters as a sister group of the *AGL2* subfamily with high confidence. Both of them, plus the *SQUA* subfamily, are included in the so-called *AGL2*/*AGL6*/*SQUA* superclade (reviewed by Becker and Theissen, 2003). A recent discovery is that the *FLOWERING LOCUS C* (*FLC*) subfamily, a key player in the vernalization response in both eudicots and grasses, also belongs to this superclade. The possibility that the *TOMATO MADS 3* subfamily (*TM3* or *SOC1*-like, from *SUPPRESSOR OF CONSTANS 1*) may share the same origin requires further validation (Ruelens *et al.*, 2013). Through a combination of genome synteny and phylogenetic reconstructions, a single MIKC^{C}-type MADS-box gene has been shown at the origin of the whole superclade, which underwent a tandem duplication before the most recent common ancestor (MRCA) of seed plants. This ancestral tandem duplication can be considered the earliest event of the series which gave birth to both the *SQUA–FLC* sister subfamilies and the *AGL2–AGL6* sister subfamilies. These four subfamilies are all represented in angiosperms, but only *AGL6* has been maintained in the gymnosperm lineage.

The close phylogenetic relationship between *AGL2* and *AGL6* subfamilies is reflected by both the sequence and structure similarities of their encoded proteins. AGL6 proteins possess a divergent C-terminus with two short, but highly conserved regions, which are referred to as AGL6-I and AGL6-II motifs, respectively (Ohmori *et al.*, 2009). Both AGL6 motifs share consistent similarity to the corresponding SEP I and SEP II motifs that are typically found in the C-terminus of AGL2 proteins (Zahn *et al.*, 2005*a*), and could function as the transcriptional activation motifs of the floral identity quartets (Malcomber and Kellogg, 2005; Zahn *et al.*, 2005*a*). Indeed, in the rice AGL6 proteins OsMADS6 and OsMADS17, the C-terminal region spanning the two motifs induces strong transcriptional activation in yeast (Ohmori *et al.*, 2009). This, together with the functional studies described below, led to the hypothesis that *AGL6* subfamily genes mainly have an E function in floral development, similar to the well-characterized *AGL2*, *SEP*-like genes.

The evolutionary history of the *AGL6* subfamily in flowering plants reveals lineage duplications and losses

To date, the most informative phylogenetic analyses of *AGL6* subfamily genes have been conducted by Viaene *et al.* (2010) and Li *et al* (2010). Due to the release of several new genomes and transcriptomes recently, we are able to update our understanding of this subfamily in angiosperms, and in particular in monocots, from an evolutionary perspective (Fig. 1; Supplementary Figs S1–S5 at *JXB* online).

The subfamilies of MADS-box genes regulating floral development in angiosperms generally underwent a number of interesting duplication events which occurred before their radiation into the extant taxa, where they were subsequently maintained. In particular, many well-documented lineage duplications are observed in both core eudicots and grasses (Kramer *et al.*, 1998, 2004; Litt and Irish, 2003; Malcomber and Kellogg, 2005; Zahn *et al.*, 2005*a*, *b*; Shan *et al.*, 2007; Viaene *et al.*, 2010; Ciaffi *et al.*, 2011; Airoldi and Davies, 2012; Vekemans *et al.*, 2012; Ruelens *et al.*, 2013). Most of these duplications, if not all, derived from whole-genome duplication (WGD) events, probably because of the gene balance hypothesis (Edger and Pires, 2009; Airoldi and Davies, 2012).

Compared with these examples, the angiosperm *AGL6* did not expand much in paralogue lineages, despite more than one gene existing in this subfamily in most species. In general, where *AGL6* paralogue lineages are found, at least one of them is poorly conserved (Fig. 1). Based on the phylogenetic reconstructions, a duplication in the *AGL6* subfamily may have occurred before the MRCA of gymnosperms (Fig. 1) (Zahn *et al.*, 2005*a*; Li *et al.*, 2010), but probably a single *AGL6* lineage has been maintained in the putative polyploid ancestor of angiosperms. Within angiosperms, Viaene and collaborators (2010) found a lineage duplication in magnoliids and two paralogue clades in core eudicots, which are named *AGL6*-like and *euAGL6*. The former is frequently lost (also in Brassicaceae), as we could find it in less than half of the available rosid genomes, and it corresponds to the legume *PsMADS3* clade (Hecht *et al.*, 2005; Wong

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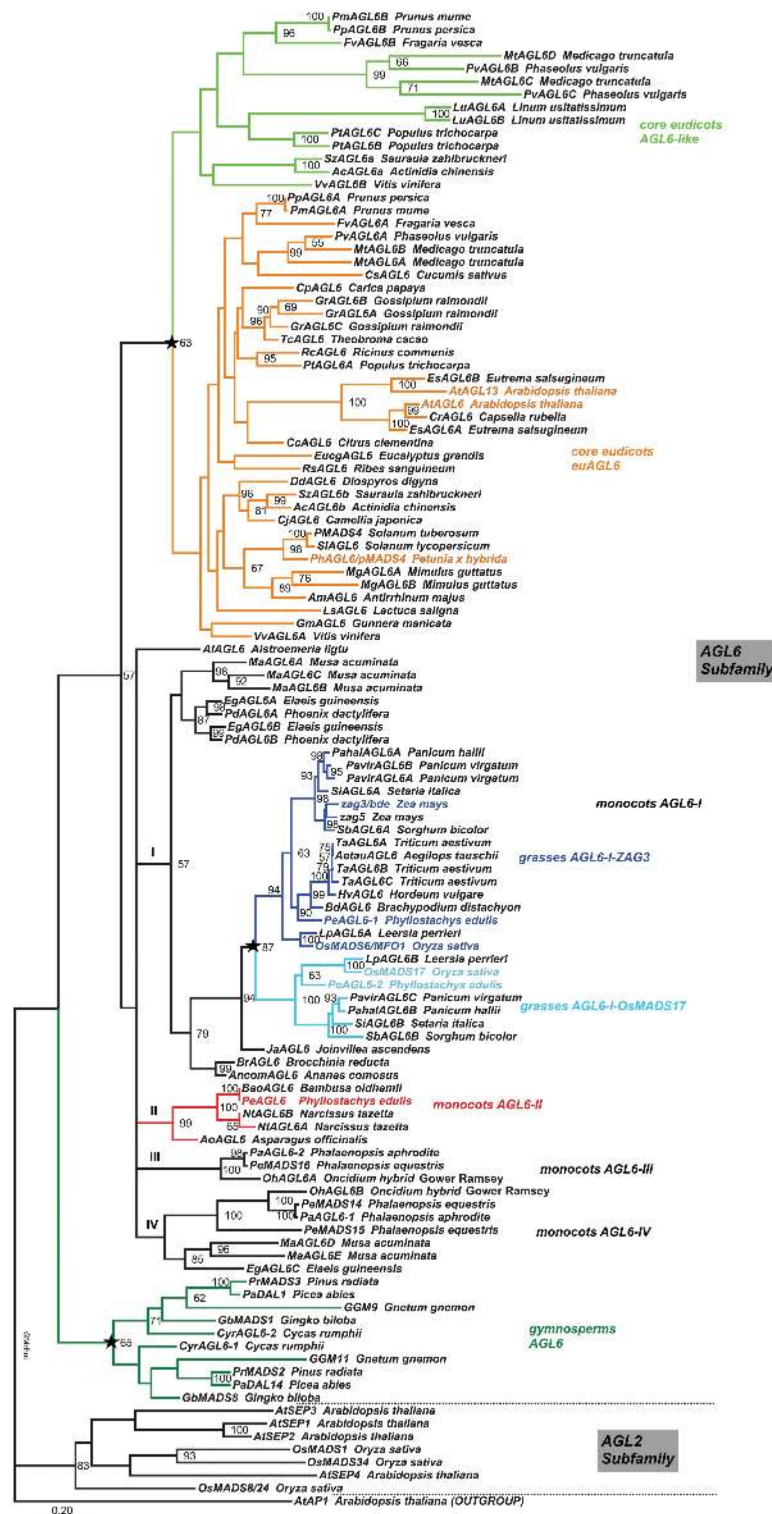

Fig. 1. ML phylogenetic tree of the *AGL6* subfamily. The tree was built from 100 subreplicates using gene coding sequences from gymnosperms, monocots, and core eudicots. The relevant previously reported lineage duplications are indicated with a black star. The scale bar indicates the number of nucleotide changes per site. Accession numbers are provided in Supplementary Table S1.

et al., 2013). In asterids, in agreement with previous analysis, *AGL6*-like sequences could be found only in Roridulaceae and Actinidiaceae, which are placed in the basal order Ericales. Furthermore, the function of core eudicot *AGL6*-like members is totally obscure. Interestingly, all the AGL6-like proteins show several amino acid substitutions in highly conserved residues, including the MADS-box domain, and in the C-terminus the AGL6 motifs I and II are nearly lost (Supplementary Fig. S1), suggesting that these genes underwent a process of neofunctionalization, or maybe pseudogenization. Conversely, the *euAGL6* clade is strongly conserved, and is only absent in the current flax (*Linum usitatissimum* L.)

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genome assembly and available transcriptomic data from the *Linum* genus, where only *AGL6*-like genes are found. The two Arabidopsis *euAGL6* genes, *AGL6* and *AGL13* (Fig. 1), are assumed to be derived from the WGD (named as α) that preceded the diversification of Brassicaceae (Vision *et al.*, 2000; Bowers *et al.*, 2003).

Our survey of sequences from monocots revealed a more complex scenario than the previous assumptions. Phylogenetic analysis by maximum likelihood (ML), parsimony, and Neighbor–Joining (NJ) methods identified four main, well-defined monocot clades, named *AGL6*-I to *AGL6*-IV, plus the only sequence available from the order Liliales, the *Alstroemeria ligtu* L. *AGL6* homologue. However, due to low bootstrapping and unclear relationship in the three analyses, we collapsed their basal nodes (Fig. 1; Supplementary Figs S2, S3).

The wide clade *AGL6*-I is commelinid specific (Arecales, Zingiberales, Poales), and in grasses it further divides into two well-defined branches that here we name, after their founder members, *ZAG3* (which is often referred to in the literature as the *OsMADS6* clade) and *OsMADS17*. Based on the current sequence and genomes, the *AGL6*-I/*ZAG3* clade is conserved in all grasses. Despite first being described as specific for the *Oryza* genus, we showed that the *AGL6*-I/*OsMADS17* clade was lost in the MRCA of Pooideae but retained in Panicoideae (Fig. 1). In Panicoideae, the *OsMADS17* clade is found in the genera *Panicum*, *Setaria*, and *Sorghum*, the latter of which has lost the *AGL6* motif II. In the ancient tetraploid maize, the *OsMADS17* clade was lost; however, there are two *OsMADS6* paralogues, *zag3* and *zag5*, which will be discussed in the next section.

The clade *AGL6*-II is represented by a few sequences from Asparagales and bamboo grasses, as already reported by Viaene *et al.* (2010), whereas the new clade *AGL6*-III is only represented by orchids. Finally, the new *AGL6*-IV clade is supported by a limited number of sequences from orchids in Asparagales and from the commelinids African oil palm (*Elaeis guineensis* Jacq., Arecales) and banana (*Musa acuminata* Colla, Zingiberales), suggesting that this clade is also poorly conserved.

Our detailed analysis of the moso bamboo, *Phyllostachys edulis* (Carrière) J. Houzeau (synonym *Phyllostachys heterocycla* Carrière), genome (Peng *et al.*, 2013) shows that it has all three clades, namely *AGL6*-I/*ZAG3*, *AGL6*-I/*OsMADS17*, and *AGL6*-II. This strongly supports the hypothesis of an ancient divergence of clades I and II, where clade I might have been retained only in commelinids and clade II might have experienced multiple independent losses during monocot evolution, in particular in commelinids. Furthermore, it reveals that all three clades were represented in the grass MRCA and that *AGL6*-II was lost only after the divergence of Bambusoideae from the main grass lineages.

In summary, in our phylogenetic analysis, the monocot *AGL6* subfamily divides into four main branches, two of which are reported for the first time in this study. They probably derived from putative duplications which occurred before the radiation of Asparagales, Liliales, and Commelinids. However, further studies are required to determine the precise relationship and the origin of these clades. Previously, a single early duplication event had been hypothesized in the monocot *AGL6* subfamily (Viaene *et al.*, 2010), which could fit well with the single WGD (τ) that has been predicted before the separation of commelinids (Jiao *et al.*, 2014). However, a simple duplication event does not seem to be enough to explain the complex *AGL6* phylogeny that we reveal in monocots, and this analysis is limited by the current lack of whole-genome sequences, especially outside commelinids.

In contrast, the further separation of clade *AGL6-I* in the grass-specific *OsMADS17* and *ZAG3* clades matches very well with the most recent WGD (ρ) which occurred before the MRCA of extant grasses (Paterson *et al.*, 2004; Wang *et al.*, 2005; Jiao *et al.*, 2014). In rice, *OsMADS6* and *OsMADS17* reside in the long arms of chromosomes 2 and 4, respectively, which are highly syntenic to each other (Thiel *et al.*, 2009). Furthermore, we have found consistent microsynteny around the two loci (not shown). How early this WGD occurred in the grass ancestor lineage is not clear. A sequence from *Joinvillea ascendens* Brongn. & Gris, which belongs to a grass sister lineage, clusters strongly with the *ZAG3* clade in the NJ analysis, but not in ML and parsimony (Fig. 1; Supplementary Figs S1, S2).

AGL6 genes may have been recruited to regulate the unique features of monocot flowers

The expression of *AGL6* subfamily genes in the perianth differs within flowering plant taxa (Fig. 2). Its expression in the FM, carpel primordium, and ovule integument seems ancestral and is highly conserved in grasses, but not in stamens. In grasses, its expression is conserved in the palea, which is regarded as an evolutionary novelty of the family due to its link to the acquisition of new functions in the first floral whorl. The expression of the *AGL6* subfamily genes in the second whorl is conserved in grasses and their close relatives (Reinheimer and Kellogg, 2009).

The rice *OsMADS6* gene, also called *MOSAIC FLORAL ORGANS1* (*MFO1*; Ohmori *et al.*, 2009), is probably the most well characterized *AGL6* subfamily gene in seed plants. It was isolated and characterized for its ability to interact with most of the other rice AGL2/AGL6/SQUA TFs (OsMADS1, 5, 45/7, 24/8, 14, 15, 17, and 18) >15 years ago (Moon *et al.*, 1999); however, functional characterization studies did not take place until 2009. There was some inconsistency in the numbering of published mutant alleles (Ohmori *et al.*, 2009; Li *et al.*, 2010; Zhang *et al.*, 2010), and here we follow the nomenclature given by Duan and co-workers (2012) (Table 1). *OsMADS6* is first expressed in the FM at stage Sp3 of spikelet development (Fig. 2), following the stages proposed by Ikeda *et al.* (2004) and Itoh *et al.* (2005). After the formation of the lemma primordium, it is expressed in the emerging palea primordium (Ohmori *et al.*, 2009; Li *et al.*, 2010). Its expression is also detected in developing palea and lodicules, and continues until the formation of the pistil primordium in the FM, where it soon becomes restricted to the

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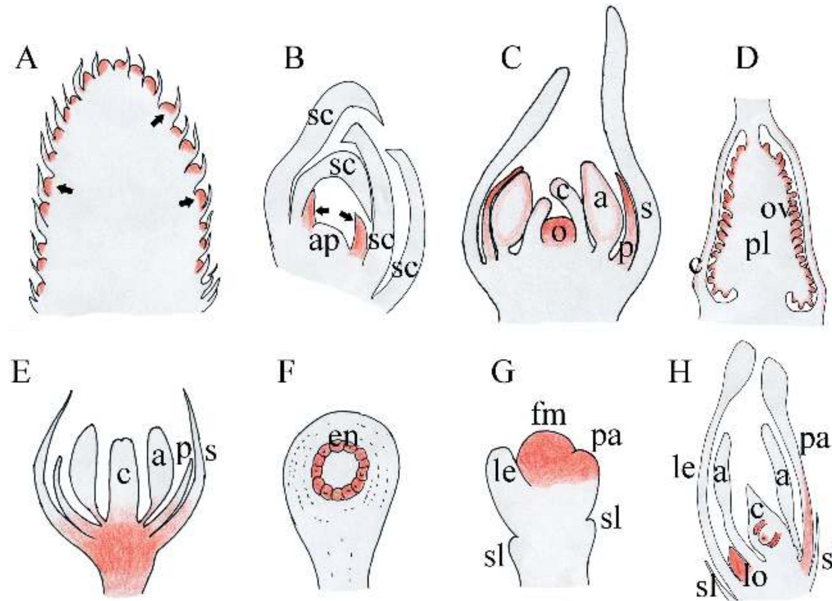

Fig. 2. Comparison of the expression domain of *AGL6* genes from representative species. (A) *Pinus radiata PrMADS3* is expressed in ovuliferous scale primordia (arrows) in stage 2 female seed cone buds. (B) In the vegetative dwarf shoot buds of *Pinus radiata*, *PrMADS3* is expressed in the initiating needle primordia (arrows). (C) In the young flower bud of petunia, *PhAGL6* is most expressed in petal and ovary primordia, but also in anthers. (D) In the petunia ovary at anthesis, *PhAGL6* is mainly expressed in ovule primordia. (E) In Arabidopsis floral buds, *AGL6* is expressed at the base of all floral whorls, whereas in the Arabidopsis ovule (F), it is expressed in the endothelium. (G) In rice, *OsMADS6* expression starts between spikelet stage Sp3 and Sp4, in the FM and palea primordium. (H) During mid stages of rice flower development, *OsMADS6* expression is strong in the lodicules; in the palea it becomes restricted to the margin tissues. Inside the carpel, strong expression is found in the ovule integuments, but it is also reported in the nucellus area where the MMC differentiates. The drawings and expression patterns are inspired by: Mouradov *et al.* (1998); Favaro *et al.* (2002); Pelucchi *et al.* (2002); Ohmori *et al.* (2009); Rijpkema *et al.* (2009); Schauer *et al.* (2009); Koo *et al.* (2010); Li *et al.* (2010); and Zhang *et al.* (2010). ap, apical meristem; sc, sterile cataphylls; o, developing ovary; c, carpel/carpel wall; a, anther; p, petal; s, sepal; pl, placenta; ov, ovule; en, endothelium; sl, sterile lemma; le, lemma; pa, palea; fm, floral meristem; lo, lodicule.

Table 1. *List of published rice OsMADS6 mutant alleles*

The nomenclature follows that used by Duan *et al.* (2012).

Gene	Mutant allele	Phenotype	Authors	Notes
OsMADS6	*mfo1-1*	Strong	Ohmori *et al.* (2009)	Arg to His single amino acid substitution of the conserved MADS-box domain Arg24, which is deeply conserved from human SERUM RESPONSE FACTOR (SRF) to MADS-box proteins of plants. Arg24 is involved in both DNA docking and protein dimerization (Pellegrini *et al.*, 1995), and is within the putative bipartite nuclear localization signal (NLS) (Gramzow and Theissen, 2010; Nayar *et al.*, 2014). Mutations in Arg24 and Gly27 also cause the *OSMADS1* strong mutant *lhs1* (Jeon *et al.*, 2000).
OsMADS6	*mfo1-2*	Weak	Ohmori *et al.* (2009)	Tos17 insertion in the 3′UTR, line NE4011 from NIAS, Ibaraki, Japan. Same allele published as *osmads-2* by Li *et al.* (2010) and Zhang *et al.* (2010).
OsMADS6	*osmads6-1*	Strong	Li *et al.* (2010)	Frameshift mutation in the seventh exon causing an abnormal translation termination of the C-terminus of the protein, which lacks the last 50 amino acids of the wild-type sequence.
OsMADS6	*osmads6-2*	Strong	Zhang *et al.* (2010)	Originally reported as *osmads6-1* by the authors. Knock-out allele generated by a T-DNA insertion in the first intron. Line AHJC09 from the Oryza Tag Line collection, Montpellier, France
OsMADS6	*osmads6-5*	Very strong, it differs from the other alleles	Duan *et al.* (2012)	Large insertion and deletion mutation in *OsMADS6* involving the promoter and part of the MADS-box. A chimeric RNA is still transcribed in this mutant.

ovule integuments (Favaro *et al.*, 2002; Pelucchi *et al.*, 2002; Ohmori *et al.*, 2009). Noticeably, *OsMADS6* is not expressed in developing stamen primordia.

Strong *osmads6* mutant alleles show floral defects in all tissues where the gene is expressed (Ohmori *et al.*, 2009; Li *et al.*, 2010). No defects are found in the lemma, whereas the marginal tissues of the palea are completely missing, resulting in the disappearance of the well-interlocked structure which normally exists between the lemma and palea (Fig. 3). Furthermore, the palea vascular bundles increased from

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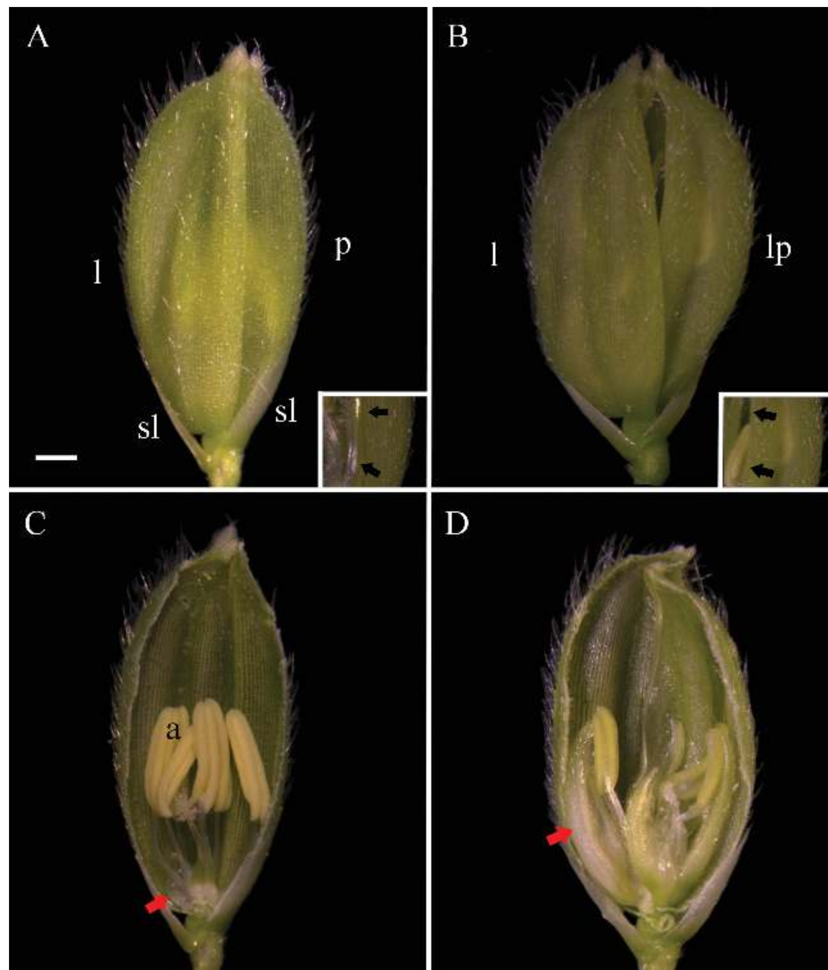

Fig. 3. Flower phenotype of the rice *osmads6-1* strong mutant. (A) Wild-type mature flower before anthesis. Bottom right: a papery, semi-transparent marginal tissue characterizes the palea margin (arrows). (B) In *osmads6-1*, the flower is enlarged as the palea is converted into a lemma-like organ, not interlocked with the lemma. Bottom right: the glume interlocking is lost because the marginal tissue is totally missing from the palea margins of the mutant (arrows). (C) Second, third, and fourth whorl organs of a rice wild-type flower. Second whorl lodicules are indicated with a red arrow. (D) In *osmads6-1*, lodicules are mostly transformed into glume-like organs (red arrow), and stamen/lodicule/glume chimeric organs develop in the third whorl. Scale bar=0.5 mm. a, anther; l, lemma; lp, lemma-like palea; p, palea; sl, sterile lemma.

three to five or six. Thus the palea is in fact homeotically converted into lemma in severe *osmads6* alleles. Lodicules were converted in elongated glume-like structures, and additional glume-like and lodicule/stamen chimeric organs appeared between whorls 2 and 3 (Fig. 3). The defects in the FM lead to a decrease in the average number of stamens in the third whorl. Depending on the mutant allele, two or more carpels or even new spikelets were often observed in the fourth whorl, suggesting a partial loss of FM determinacy, while ovules partially lost their identity within carpels, thus leading to strong female sterility (Li *et al.*, 2010).

Some of the floral defects found in *osmads6* mutants resemble those caused by mutants in the *AGL2*-like gene *OsMADS1* (Jeon *et al.*, 2000; Agrawal *et al.*, 2005). OsMADS1 and OsMADS6 are able to form heterodimers (Moon *et al.*, 1999). Interestingly, the *osmads1* and *osmads6* phenotypes increase dramatically in forms of double mutants (Ohmori *et al.*, 2009; Li *et al.*, 2010), suggesting that *OsMADS1* and *OsMADS6* might have partially redundant functions in regulating spikelet meristem determinacy and its transition to FM, as well as floral patterning. Further evidence for the redundancy between *OsMADS1* and *OsMADS6* is that they are both required for the proper expression of a common set of floral genes, such as B, C, and E floral homeotic MADS-box genes, especially during early floral stages (Chen *et al.*, 2006; Li *et al.*, 2011*a*; Hu *et al.*, 2015). Additionally, they are both required for the proper expression of genes involved in hormonal signalling and homeostasis like *OsMGH3*, even though at least for *OsMADS1* the regulation of this gene seems to be indirect (Prasad *et al.*, 2005; Zhang *et al.*, 2010; Yadav *et al.*, 2011).

Finally, *OsMADS6* and *OsMADS1* are likely to be targets of similar regulatory pathways. The expression of both is enhanced in RNAi knock-down lines of the LDB-like TF gene *OsIG1*, another regulator of floral development (Zhang *et al.*, 2015), and they are also putative targets of the Polycomb Repressor Complex 2 (PRC2), which seems essential for the regulation of FM determinacy and fate in rice (Conrad *et al.*, 2014).

In *osmads6*, the homeotic conversion of lodicules into membranous or glume-like organs is similar to the effect of B

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class loss-of-function mutants (Nagasawa *et al.*, 2003; Prasad and Vijayraghavan, 2003; Yao *et al.*, 2008), despite both AP3 and PI homologues still being expressed in the abnormal floral organs of the *osmads6* mutant (Ohmori *et al.*, 2009). Nevertheless, OsMADS6 has been shown to interact with the rice DEF/GLO heterodimers (OsMADS2–OsMADS16 and OsMADS4–OsMADS16) to form nuclear-localized ternary complexes (Seok *et al.*, 2010), suggesting that OsMADS6 is an important subunit of the B class complex which specifies lodicule identity in the second whorl. OsMADS6 seems to be required to repress the AP1/SQUA-like genes *OsMADS14* and *OsMADS15* in the second whorl (Ohmori *et al.*, 2009).

As *OsMADS6* is not expressed during the differentiation of the third whorl, it is not likely to be required for the formation of the B–C complex to determine stamen identity. Indeed, stamen identity is not affected completely in *osmads6* mutants, and the change in stamen number and the appearance of ectopic third whorl chimeric organs can be attributed to a role for *OsMADS6* in FM regulation and identity. However, *OsMADS6* expression was reported in the tapetum and in the male germline (Reinheimer and Kellogg, 2009; Tang *et al.*, 2010; Zhang *et al.*, 2010), implying possible function in male gametogenesis.

In the fourth whorl, the defects found in *osmads6* in terms of FM determinacy, and gynoecium and ovule development corroborate the role of OsMADS6 as an interactor of the D function protein OsMADS13 (Favaro *et al.*, 2002). In the ovule nucellus, *OsMADS6* expression was reported in the zone of the differentiating megaspore mother cell (MMC) (Zhang *et al.*, 2010). Interestingly, the weak mutant allele *mfo1-2* (Ohmori *et al.*, 2009) produced normal carpels but sometimes defective ovules with the outgrowth of outer integument and the absence of an embryo sac, which was replaced by an abnormally proliferated nucellus. Furthermore, in *Brachiaria brizantha* (Hochst. ex A.Rich.) Stapf, a tropical forage grass characterized by facultative aposporic apomixis, the *OsMADS6* homologue *BbrizAGL6* is expressed differentially in the nucellus of apomictic and sexual plants. In fact, it seems to mark the formation of the MMC in sexual plants and the aposporic initial (AI) precursor cells in apomictic plants (Guimarães *et al.*, 2013). Therefore, it will be interesting to test whether grass *AGL6*-like genes are involved in the differentiation of the MMC.

The function of *OsMADS6* in gynoecium is unlikely to be restricted to its formation, as it continues to be expressed for several days after pollination in the developing kernel, along with its paralogue *OsMADS17* (Arora *et al.*, 2007). It was reported that a minority of the *osmads6* mutant pistils are fertile; however, the kernels produced were rounder and defective in filling, with a reduced starch content and increased protein fraction (Zhang *et al.*, 2010). It was not clarified whether those kernel phenotypes are under maternal control, or if they depend on some *OsMADS6* functions in the progeny tissues. OsMADS6 is able to interact with the B_{SISTER} TF OsMADS29 (Nayar *et al.*, 2014) which has an essential conserved function for the development of the kernel, where they appear to be largely co-expressed (Yang *et al.*, 2012; Nayar *et al.*, 2013).

Interestingly, a new recessive allele of *OsMADS6*, namely *osmads6-5* (or the '*lemmata*' mutant), was reported recently (Duan *et al.*, 2012). This allele exhibits phenotypical abnormalities which are markedly different from those of any other *osmads6* mutant. In its homozygotes, the lemma remains unaffected. Similar to the other *osmads6* mutants, the palea loses its identity and is converted into a lemma-like organ, but with up to 10 vascular bundles. Notably, inside of the first whorl, the indeterminate FM continuously produces new lemma-like organs; hence the name '*lemmata*' is given to this mutant. Therefore, because of this novel phenotype, the authors argued that none of the previous mutants is a null allele, which, however, is questionable (Table 1). The *osmads6-5* mutant itself cannot be considered as a knock-out, as it is still able to transcribe a chimeric RNA with partial *OsMADS6* ORFs. Despite the authors performing a careful analysis to exclude possible semi-dominant or co-suppression effects from this transcript, we assume that this very interesting allele might have caused some unexpected molecular effect which warrants further studies.

In comparison with *OsMADS6,* its paralogue *OsMADS17* (Moon *et al.*, 1999) is far less well studied. The expression patterns of *OsMADS6* and *OsMADS17* are largely overlapping, and the transcript level of *OsMADS17* is increased in *osmads6* mutants (Ohmori *et al.*, 2009). Using an RNAi approach, Ohmori and colleagues (2009) observed that *OsMADS17* could play minor redundant roles that are only visible when the function of *OsMADS6* is severely compromised. We believe that the global elucidation of the rice *AGL6* subfamily genes requires stable knock-out mutants of *OsMADS17*, and its double mutants with *osmads6*.

In maize, the *OsMADS17* clade was lost whereas the *ZAG3* clade contains the two duplicated genes *zag3* and *zag5* (Mena *et al.*, 1995). The *bearded-ear* (*bde*) mutant is caused by the loss of function of *zag3* (Thompson *et al.*, 2009). In the mutant tassels, spikelets produce more florets with more floral organs, which are often silks. In the ear of the mutant, spikelets also contain multiple florets, which in turn produce more palea–lemma-like organs, while the ovaries are mostly sterile with extra silks. The *bde* mutants affect the upper and lower floret meristems differentially.

To our knowledge, no mutant of *AGL6* subfamily genes has yet been reported in non-grass monocots. In some species, the number of *AGL6*-like genes is expanded. For example, we found three loci from the African oil palm and a large expansion of five loci in the banana genome (Fig. 1). The genome of the orchid *Phalaenopsis equestris* (Schauer) Rchb.f. has three *AGL6*-like genes plus a putative pseudogene (Cai *et al.*, 2014). The authors argued that the expansion of *AGL2*, *AGAMOUS*, *DEF/AP3*, and *AGL6* clades might be the origin of the unique evolutionary novelties found in orchids. In line with this, the expression profiling of MADS-box genes conducted in the related species *Phalaenopsis aphrodite* Rchb.f. led to the proposition of a modified ABCDE model, where *PaAGL6-1* is being expressed only in the lip (Su *et al.*, 2013).

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In petunia, the function of the *euAGL6* gene *PhAGL6* is masked by its redundancy with the *AGL2* genes

Only one *euAGL6* gene is known in petunia, the *PETUNIA MADS BOX GENE4* (*pMADS4*) (Tsuchimoto *et al.*, 2000) which has been renamed as *Petunia hybrida AGL6* (*PhAGL6*) (Rijpkema *et al.*, 2009). This gene is specifically expressed in flowers, where its transcripts were detected at high levels first in the petal primordium and in the early ovary primordium, and later in the ovule primordia (Fig. 2). At significantly lower levels, the transcript was found even in the other floral organs. Despite this, *phagl6*, the first null mutant reported for a plant *AGL6*-like gene, did not show obvious phenotypes in flowers. *PhAGL6* is largely co-expressed with some *AGL2* subfamily genes, such as *FBP2* (Ferrario, 2003; Rijpkema *et al.*, 2009). In *fbp2* single mutants, the corolla was converted to green sepal-like organs at the edges of the petals and along the main veins at the abaxial side (Vandenbussche *et al.*, 2003). Indeed the *fbp2* phenotype was dramatically enhanced in a *fbp2 phagl6* double mutant, resulting in completely green petals and reduced corolla size. Moreover, the stamens of *phagl6 fbp2* double mutants were also affected, showing sepal/petal-like structures and occasionally stigma-like structures on top of the anthers (Rijpkema *et al.*, 2009). As further evidence of redundancy between petunia *AGL6* and *AGL2* genes, mutation of another *AGL2*-like gene, *FBP5*, further enhanced the *phagl6 fbp2* double mutant phenotype, causing a more severe conversion of petals and stamens into sepal- or leaf-like organs (Rijpkema *et al.*, 2009). The strong expression of *PhAGL6* in the ovary and ovule primordia, together with the ability of its protein product to interact with C and D proteins, suggests that *PhAGL6* may also have an E-like function in the fourth whorl. However, this is not supported by mutant analysis, suggesting complete redundancy with other *AGL2* genes (Rijpkema *et al.*, 2009). Despite the function of *AGL6* in petunia and Arabidopsis gynoecium not yet being clear, the fact that Arabidopsis AGL6 is one of the few MIKC^{C}-type proteins able to interact with some type I MADS proteins (de Folter *et al.*, 2005) led to the hypothesis that they might be involved together in the development of the ovule and gametophyte (Rijpkema *et al.*, 2009).

AGL6 and *AGL13* may have wide-ranging functions in Arabidopsis flowering time, inflorescence, and flower development

The Arabidopsis genome has two *euAGL6* genes, *AGL6* (Ma *et al.*, 1991) and *AGL13* (Rounsley *et al.*, 1995).

AGL6 is expressed during the vegetative phase at a similar level in different growth stages, under both long- and short-day conditions, suggesting that *AGL6* is regulated in a photoperiod-independent manner (Yoo *et al.*, 2011*b*). *AGL6* activation tagged mutants showed early flowering phenotypes, which are associated with the down-regulation of the floral repressor *FLC* and of its homologues *MAF4* and *MAF5*, and with the up-regulation of the floral promoter *FT*. These findings indicate that *AGL6* is a strong floral promoter (Yoo *et al.*, 2011*b*). Moreover, *AGL6* activation tagged mutants have altered leaf movement and expression of core oscillator genes. This suggests that *AGL6* can play a role in control of the circadian clock, although knock-down alleles suggested that there might be redundancy with other MADS TFs (Yoo *et al.*, 2011*a*).

Through the analysis of epistatic natural alleles, it was shown that *AGL6* specifically affects stem branching by promoting axillary bud formation (Huang *et al.*, 2012). *AGL6* expression was also found in the abaxial and proximal region of cauline leaf primordia, in the cryptic bracts subtending newly initiated floral primordia 6 d after transfer to long days, and later at the base of all floral organs (Fig. 2) (Koo *et al.*, 2010). Expression of a fusion of the *AGL6* genomic sequence to the strong transcriptional activation domain VP16 under the control of the *AGL6* promoter (*gAGL6::VP16*), led to the formation of ectopic organs on most flower pedicels, which were initially bract like. Together, these findings suggest a role for *AGL6* in suppressing the outgrowth of the cryptic bracts. Furthermore, the ectopic organs became increasingly carpel and stamen like in later developing flowers, and finally flowers were completely replaced by staminoid bracts, suggesting an additional role for *AGL6* in floral organ identity. This effect was caused by a *LFY*-independent activation of B and C homeotic genes (Koo *et al.*, 2010). Furthermore, the ectopic expression of either *AGL6* (*35S:AGL6*) or a fusion of *AGL6* with the EAR transcriptional repression domain (*35S:AGL6::EAR*) dramatically accelerated flowering time, suggesting the role of *AGL6* in regulating flowering time by repressing floral repressors (Koo *et al.*, 2010).

In flowers, *AGL6* and *AGL13* are co-expressed only in the developing chalaza of the ovule. *AGL6* is also expressed in the endothelium (Fig. 2), whereas *AGL13* is expressed in the innermost anther wall layer, the tapetum. In addition, an *AGL13* reporter line showed activity in the vasculature underlying the developing shoot apical meristem (Schauer *et al.*, 2009).

Recently, the characterization of reporter lines where β-glucuronidase (GUS) is driven by an *AGL13* genomic region comprising the putative regulatory elements (Hsu *et al.*, 2014) suggested a broader expression profile in the FM, anther, pollen up to stage 12, and ovules compared with the more cell type-specific pattern reported by Schauer and colleagues (2009). In *35S:AGL13* RNAi transgenic lines, about half of the ovules were aborted at early stages, and pollen was also affected, showing aberrations in the exine patterning of the outer wall; however, half of the pollen was still viable and able to germinate and fertilize the egg cell. Further genetic analysis showed that after meiosis and tetrad formation, the development of the two pollen grains of each tetrad carrying the RNAi transgene was arrested (Hsu *et al.*, 2014). In order to create dominant loss-of-function mutants, *AGL13* was fused to the SRDX suppressing motif and expressed ectopically. This resulted in similar phenotypes compared with RNAi experiments, suggesting that *AGL13* mainly functions as a transcriptional activator. In addition, the most severe *35S:AGL13::SRDX* lines produced

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considerably shorter sepals, petals, and anthers that were similar to *35S:SEP2::SRDX* plants (Hsu *et al.*, 2014), indicating that *AGL13* and *SEP2* may have a similar function and target similar genes during flower development. Furthermore, both AGL13 and SEP3 proteins can enhance the nuclear localization efficiency of AG. Fluorescence resonance energy transfer (FRET) experiments indicated that AGL13 and AG can form quartet-like complexes. Finally, the AG–AGL13 heterodimer could also interact with the AP3–PI heterodimer (Hsu *et al.*, 2014), which may be functional against known target genes of AG in the anther, such as *SPL/NZZ* (Ito *et al.*, 2004).

The *AGL6* subfamily genes are expressed in both vegetative and reproductive tissues of gymnosperms and core eudicots

The *AGL6* genes have been reported also to be expressed in the vegetative tissues of gymnosperms, but not within monocots, magnoliids, and Ranunculales. However, as they are frequently expressed in the vegetative organs in core eudicots, it has been proposed that their vegetative expression was lost in angiosperms but regained in core eudicots (Viaene *et al.*, 2010).

In gymnosperms, the *AGL6* subfamily divides into two clades (Fig. 1), which are represented by *DAL1* and *DAL14* in Norway spruce [*Picea abies* (L.) H.Karst.]. In *Pinus radiata* D.Don, *PrMADS2* and *PrMADS3* are orthologous to *DAL14* and *DAL1*, respectively. Current data strongly support that, at least in these two conifers, the *DAL14/PrMADS2* clade is active exclusively in male and female reproductive tissues, whereas the *DAL1/PrMADS3* clade is active in both reproductive and vegetative shoots (Tandre *et al.*, 1995; Mouradov *et al.*, 1998; Carlsbecker *et al.*, 2004). In female cones, all these genes are expressed in the ovuliferous scales and ovule primordia, with some differences in the precise pattern and timing. *DAL1* has been proposed as a possible mediator of the juvenile-to-adult transition in Norway spruce (Carlsbecker *et al.*, 2004). In *P. radiata*, the *PrMADS3* transcript was precisely localized in the peripheral zone of the vegetative dwarf shoot buds where the needle primordia are differentiating, and then in the needle primordia (Fig. 2) (Mouradov *et al.*, 1998). However in *Gnetum gnemon* L. (Gnetophyta), the expression of both the *AGL6*-like genes *GGM9* (*DAL1/PrMADS3* clade) and *GGM11* (*DAL14/PrMADS2* clade) has been reported only in male and female cones to date (Winter *et al.*, 1999).

In core eudicots where the *AGL6* subfamily has two major clades, the expression of *AGL6*-like genes is highest in vegetative tissues, and in tendrils of grape vine (*Vitis vinifera* L.), whereas *euAGL6* genes are predominantly expressed in floral tissues and occasionally in vegetative tissues (Viaene *et al.*, 2010).

Conclusion and perspective

To sum up, the AGL6-like proteins show a conserved and broad spectrum of interactions with other MIKC^C proteins in petunia (Rijpkema *et al.*, 2009), Arabidopsis (de Folter *et al.*, 2005; Hsu *et al.*, 2014), and rice (Moon *et al.*, 1999; Favaro *et al.*, 2002; Seok *et al.*, 2010; Nayar *et al.*, 2014), which is also very similar to the interactome of the AGL2-like proteins.

At least in petunia and rice, there is a significant functional redundancy between the AGL6 and AGL2 factors (Ohmori *et al.*, 2009; Rijpkema *et al.*, 2009; Li *et al.*, 2010). Regarding this, it is worth mentioning that single recessive loss-of-function mutants of Arabidopsis *AGL6* genes do not show abnormal phenotypes, which were mostly caused by the expression of its dominant activated or repressed forms (Koo *et al.*, 2010; Hsu *et al.*, 2014). Now that targeted mutagenesis, in particular CRISPR/CAS9, is becoming a standard technique, real null mutants for these genes in Arabidopsis should be generated, in order to test their function and the possible genetic interactions with *SEP* genes.

Therefore, based on the molecular characterization and mutant analyses carried out in petunia, rice, and maize, we can add the *AGL6* genes to the E function of the ABC model, as was first proposed and proven by Rijpkema and co-workers (2009). For example, their functions are evident in the second and third whorl of petunia, and very probably also in the fourth whorl. In rice, *OsMADS6* is active in the first whorl, which probably represents a neofunctionalization of *AGL6* genes of grasses, and in the second and fourth whorls. Based on the expansion in terms of gene number and on expression profiles, it has been proposed that *AGL6* genes might have been important for the evolution of the unique flower features of orchids. In Arabidopsis, data suggest that they may also function during the vegetative phase, in flowering time regulation, and in shaping the inflorescence architecture. In addition, a number of *AGL6* subfamily genes from monocots, eudicots, and gymnosperms have been overexpressed in Arabidopsis and tobacco, suggesting similar functions (reviewed by Rijpkema *et al.*, 2009; Viaene *et al.*, 2010).

If *AGL6* genes play just the same roles as *AGL2*, it would be expected that these two ancestral clades are not both essential for the plant and one or the other should frequently have been lost in different angiosperm lineages. In constrast, there seems to be a strong positive selection pressure on *AGL6* in known flowering plant genomes, with the possible exception of flax, suggesting that their functions cannot be identical to those of the *AGL2* subfamily genes. Furthermore, the *AGL2* subfamily expanded in several conserved clades during the evolution of angiosperms, whereas the expansion of the *AGL6* subfamily was more limited. However, these questions are yet to be addressed thoroughly. Several studies pointed to a possible correlation of the *AGL6* genes with the development of male and female sporogenous tissues, and also with the first stages of male and female gametophyte development. Moreover, at least in Arabidopsis, AGL6 is a possible interactor of some type I MADS proteins (de Folter *et al.*, 2005), which is unusual for MIKC^C-type MADS proteins. Indeed, many type I MADS proteins are known players in female gametophyte, and embryo and endosperm development (reviewed by Masiero *et al.*, 2011).

The strong expression of Arabidopsis *AGL6* in the ovule endothelium suggests an unknown role in the development of this important cell layer. It will be interesting to test whether

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AGL6 forms a MADS complex with SEEDSTICK (STK) and ARABIDOPSIS B_{SISTER} (ABS), which are essential for endothelium development (Nesi *et al.*, 2002; Mizzotti *et al.*, 2012). For the less conserved *AGL6*-like clade of core eudicots, which are frequently expressed in vegetative tissues, no function has been yet assigned. Thus, the functional significance and evolutionary origins of *AGL6* genes still await clarification.

Due to the similarity and close phylogenetic relationship of AGL6 and AGL2 proteins, it has been speculated for a long time that AGL6 might mediate the formation of floral identity quartets in gymnosperms, as they are the only possible players of the E function in these plants, where all the *AGL6* sister clades are missing. However, the notion that gymnosperms and angiosperms possess essentially a similar set of $MIKC^C$ TF subfamilies does not automatically imply that MADS tetramers must form in the same way in all seed plants. Notably, the gene duplication event that led to the formation of the *DEF* and *GLO* subfamilies within B class genes occurred in the angiosperm lineage after its separation from the gymnosperms lineage (Zahn *et al.*, 2005*b*), and the establishment of a DEF/GLO obligate heterodimer occurred even later in the most evolved angiosperm taxa (Winter *et al.*, 2002; Melzer *et al.*, 2014). Moreover, studies conducted in *G. gnemon* MADS proteins supported their ability to form DNA-bound tetramers made only of B and C subunits, or only C (but not only B) subunits, which does not require the E proteins acting like glue (Melzer *et al.*, 2010; Wang *et al.*, 2010). On the other hand, whether these complexes observed *in vitro* are also able to trigger proper transcription of their target genes without the need for the putative AGL6 and AGL2 transcriptional activation motifs, is not yet clear (Wang *et al.*, 2010). Regarding these aspects, the AGL6 members are still far less understood than other MIKC TFs and deserve more studies in the future.

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Alignment of eudicot AGL6-like, eudicot euAGL6 (orange), and grass ZAG3 clade proteins.

Figure S2. Phylogeny of *AGL6* subfamily genes in seed plants, constructed by the parsimony method.

Figure S3. Phylogeny of *AGL6* subfamily genes in seed plants, constructed by the NJ method.

Figure S4. Multifasta protein whole sequence alignment.

Figure S5. Multifasta codon alignment used to infer the phylogeny.

Table S1. Gene accession numbers.

Acknowledgements

We thank Junyan Li for editing the paper, and Professor Wen-Chieh Tsai (Institute of Tropical Plant Sciences, National Cheng Kung University, Tainan, Taiwan) for providing full *AGL6* subfamily sequences from *P. equestris.* Our research on rice E function genes is supported by the National Natural Science Foundation of China (NSFC) (31230051), the NSFC Research Fund for International Young Scientists (31550110198), the Programme of Introducing Talents of Discipline to Universities (111 Project, B14016), and the China Postdoctoral Science Foundation (2014M560328). LD is supported by a Marie Skłodowska-Curie Global Fellowship (Horizon 2020 call H2020-MSCA-IF-2014, project 661678 - RiceStyle). We apologize to the authors of papers that we could not discuss in this manuscript, due to space constraints.

References

Agrawal GK, Abe K, Yamazaki M, Miyao A, Hirochika H. 2005. Conservation of the E-function for floral organ identity in rice revealed by the analysis of tissue culture-induced loss-of-function mutants of the OsMADS1 gene. Plant Molecular Biology **59,** 125–135.

Airoldi CA, Davies B. 2012. Gene duplication and the evolution of plant MADS-box transcription factors. Journal of Genetics and Genomics **39,** 157–165.

Amborella Genome Project. 2013. The Amborella genome and the evolution of flowering plants. Science **342,** 1241089.

Angenent GC, Franken J, Busscher M, van Dijken A, van Went JL, Dons HJ, van Tunen AJ. 1995. A novel class of MADS box genes is involved in ovule development in petunia. The Plant Cell **7,** 1569–1582.

Arora R, Agarwal P, Ray S, Singh AK, Singh VP, Tyagi AK, Kapoor S. 2007. MADS-box gene family in rice: genome-wide identification, organization and expression profiling during reproductive development and stress. BMC Genomics **8,** 242.

Becker A, Theissen G. 2003. The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Molecular Phylogenetics and Evolution **29,** 464–489.

Bell CD, Soltis DE, Soltis PS. 2005. The age of the angiosperms: a molecular timescale without a clock. Evolution **59,** 1245–1258.

Bowers JE, Chapman BA, Rong J, Paterson AH. 2003. Unraveling angiosperms genome evolution by phylogenetic analysis of chromosomal duplications events. Nature **422,** 433–438.

Cai J, Liu X, Vanneste K, *et al*. 2014. The genome sequence of the orchid Phalaenopsis equestris. Nature Genetics **47,** 65–72.

Carlsbecker A, Tandre K, Johanson U, Englund M, Engström P. 2004. The MADS-box gene DAL1 is a potential mediator of the juvenile-to-adult transition in Norway spruce (Picea abies). The Plant Journal **40,** 546–557.

Chen ZX, Wu JG, Ding WN, Chen HM, Wu P, Shi CH. 2006. Morphogenesis and molecular basis on naked seed rice, a novel homeotic mutation of OsMADS1 regulating transcript level of AP3 homologue in rice. Planta **223,** 882–890.

Ciaffi M, Paolacci AR, Tanzarella OA, Porceddu E. 2011. Molecular aspects of flower development in grasses. Sexual Plant Reproduction **24,** 247–282.

Coen ES, Meyerowitz EM. 1991. The war of the whorls: genetic interactions controlling flower development. Nature **353,** 31–37.

Colombo L, Franken J, Koetje E, van Went J, Dons HJ, Angenent GC, van Tunen AJ. 1995. The petunia MADS box gene FBP11 determines ovule identity. The Plant Cell **7,** 1859–1868.

Conrad LJ, Khanday I, Johnson C, Guiderdoni E, An G, Vijayraghavan U, Sundaresan V. 2014. The polycomb group gene EMF2B is essential for maintenance of floral meristem determinacy in rice. The Plant Journal **80,** 883–94.

Cui R, Han J, Zhao S, Su K, Wu F, Du X, Xu Q, Chong K, Theissen G, Meng Z. 2010. Functional conservation and diversification of class E floral homeotic genes in rice (Oryza sativa). The Plant Journal **61,** 767–781.

de Folter S, Immink RGH, Kieffer M, *et al*. 2005. Comprehensive interaction map of the Arabidopsis MADS Box transcription factors. The Plant Cell **17,** 1424–1433.

Ditta G, Pinyopich A, Robles P, Pelaz S, Yanofsky MF. 2004. The SEP4 gene of Arabidopsis thaliana functions in floral organ and meristem identity. Current Biology **14,** 1935–1940.

Dreni L, Kater MM. 2014. MADS reloaded: evolution of the AGAMOUS subfamily genes. New Phytologist **201,** 717–732.

Dreni L, Pilatone A, Yun D, Erreni S, Pajoro A, Caporali E, Zhang D, Kater MM. 2011. Functional analysis of all AGAMOUS subfamily

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members in rice reveals their roles in reproductive organ identity determination and meristem determinacy. The Plant Cell **23,** 2850–2863.

Duan Y, Xing Z, Diao Z, *et al*. 2012. Characterization of Osmads6-5, a null allele, reveals that OsMADS6 is a critical regulator for early flower development in rice (Oryza sativa L.). Plant Molecular Biology **80,** 429–442.

Edger PP, Pires JC. 2009. Gene and genome duplications: the impact of dosage-sensitivity on the fate of nuclear genes. Chromosome Research **17,** 699–717.

Favaro R, Immink RGH, Ferioli V, Bernasconi B, Byzova M, Angenent GC, Kater M, Colombo L. 2002. Ovule-specific MADS-box proteins have conserved protein–protein interactions in monocot and dicot plants. Molecular Genetics and Genomics **268,** 152–159.

Favaro R, Pinyopich A, Battaglia R, Kooiker M, Borghi L, Ditta G, Yanofsky M, Kater M, Colombo L. 2003. MADS-box protein complexes control carpel and ovule development in Arabidopsis. The Plant Cell **15,** 2603–2611.

Ferrario S. 2003. The MADS box gene FBP2 is required for SEPALLATA function in Petunia. The Plant Cell **15,** 914–925.

Gramzow L, Theissen G. 2010. A hitchhiker's guide to the MADS world of plants. Genome Biology **11,** 214.

Gramzow L, Weilandt L, Theißen G. 2014. MADS goes genomic in conifers: towards determining the ancestral set of MADS-box genes in seed plants. Annals of Botany **114,** 1407–29.

Guimarães LA, Diva DM, Masiero S, Resentini F, Gomes ACMM, Silveira ÉD, Florentino LH, Rodrigues JCM, Colombo L, Vera VT. 2013. BbrizAGL6 is differentially expressed during embryo sac formation of apomictic and sexual Brachiaria brizantha plants. Plant Molecular Biology Reporter **31,** 1397–1406.

Hecht V, Foucher F, Ferrándiz C, *et al*. 2005. Conservation of Arabidopsis flowering genes in model legumes. Plant Physiology **137,** 1420–34.

Heijmans K, Ament K, Rijpkema AS, Zethof J, Wolters-Arts M, Gerats T, Vandenbussche M. 2012. Redefining C and D in the Petunia ABC. The Plant Cell **24,** 2305–2317.

Henschel K, Kofuji R, Hasebe M, Saedler H, Münster T, Theissen G. 2002. Two ancient classes of MIKC-type MADS-box genes are present in the moss Physcomitrella patens. Molecular Biology and Evolution **19,** 801–814.

Honma T, Goto K. 2001. Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature **409,** 525–529.

Hsu WH, Yeh TJ, Huang KY, Li JY, Chen HY, Yang CH. 2014. AGAMOUS-LIKE13, a putative ancestor for the E functional genes, specifies male and female gametophyte morphogenesis. The Plant Journal **77,** 1–15.

Hu Y, Liang W, Yin C, *et al*. 2015. Interactions of OsMADS1 with floral homeotic genes in rice flower development. Molecular Plant **8,** 1366–1384.

Huang X, Effgen S, Meyer RC, Theres K, Koornneef M. 2012. Epistatic natural allelic variation reveals a function of AGAMOUS-LIKE6 in axillary bud formation in Arabidopsis. The Plant Cell **24,** 2364–2379.

Ikeda K, Sunohara H, Nagato Y. 2004. Developmental course of inflorescence and spikelet in rice. Breeding Science **54,** 147–156.

Ito T, Wellmer F, Yu H, Das P, Ito N, Alves-Ferreira M, Riechmann JL, Meyerowitz EM. 2004. The homeotic protein AGAMOUS controls microsporogenesis by regulation of SPOROCYTELESS. Nature **430,** 356–360.

Itoh JI, Nonomura KI, Ikeda K, Yamaki S, Inukai Y, Yamagishi H, Kitano H, Nagato Y. 2005. Rice plant development: from zygote to spikelet. Plant and Cell Physiology **46,** 23–47.

Jeon JS, Jang S, Lee S, *et al*. 2000. leafy hull sterile1 is a homeotic mutation in a rice MADS box gene affecting rice flower development. The Plant Cell **12,** 871–84.

Jetha K, Theißen G, Melzer R. 2014. Arabidopsis SEPALLATA proteins differ in cooperative DNA-binding during the formation of floral quartet-like complexes. Nucleic Acids Research **42,** 10927–10942.

Jiao Y, Li J, Tang H, Paterson AH. 2014. Integrated syntenic and phylogenomic analyses reveal an ancient genome duplication in monocots. The Plant Cell **26,** 2792–2802.

Kater MM, Dreni L, Colombo L. 2006. Functional conservation of MADS-box factors controlling floral organ identity in rice and Arabidopsis. Journal of Experimental Botany **57,** 3433–3444.

Koo SC, Bracko O, Park MS, *et al*. 2010. Control of lateral organ development and flowering time by the Arabidopsis thaliana MADS-box gene AGAMOUS-LIKE6. The Plant Journal **62,** 807–816.

Kramer EM, Dorit RL, Irish VF. 1998. Molecular evolution of genes controlling petal and stamen development: duplication and divergence within the APETALA3 and PISTILLATA MADS-box gene lineages. Genetics **149,** 765–783.

Kramer EM, Jaramillo MA, Di Stilio VS. 2004. Patterns of gene duplication and functional evolution during the diversification of the AGAMOUS subfamily of MADS box genes in angiosperms. Genetics **166,** 1011–1023.

Lee DS, Chen LJ, Li CY, *et al*. 2013. The Bsister MADS gene FST determines ovule patterning and development of the zygotic embryo and endosperm. PLoS One **8,** e58748.

Li H, Liang W, Hu Y, Zhu L, Yin C, Xu J, Dreni L, Kater MM, Zhang D. 2011*a*. Rice MADS6 interacts with the floral homeotic genes SUPERWOMAN1, MADS3, MADS58, MADS13, and DROOPING LEAF in specifying floral organ identities and meristem fate. The Plant Cell **23,** 2536–2552.

Li H, Liang W, Jia R, Yin C, Zong J, Kong H, Zhang D. 2010. The AGL6-like gene OsMADS6 regulates floral organ and meristem identities in rice. Cell Research **20,** 299–313.

Li H, Liang W, Yin C, Zhu L, Zhang D. 2011*b*. Genetic interaction of OsMADS3, DROOPING LEAF, and OsMADS13 in specifying rice floral organ identities and meristem determinacy. Plant Physiology **156,** 263–274.

Litt A, Irish VF. 2003. Duplication and diversification in the APETALA1/FRUITFULL floral homeotic gene lineage: implications for the evolution of floral development. Genetics **165,** 821–833.

Lovisetto A, Guzzo F, Busatto N, Casadoro G. 2013. Gymnosperm B-sister genes may be involved in ovule/seed development and, in some species, in the growth of fleshy fruit-like structures. Annals of Botany **112,** 535–544.

Ma H, Yanofsky MF, Meyerowitz EM. 1991. AGL1–AGL6, an Arabidopsis gene family with similarity to floral homeotic and transcription factor genes. Genes and Development **5,** 484–495.

Malcomber ST, Kellogg EA. 2005. SEPALLATA gene diversification: brave new whorls. Trends in Plant Science **10,** 427–435.

Masiero S, Colombo L, Grini PE, Schnittger A, Kater MM. 2011. The emerging importance of type I MADS box transcription factors for plant reproduction. The Plant Cell **23,** 865–872.

Melzer R, Harter A, Rumpler F, Kim S, Soltis PS, Soltis DE, Theissen G. 2014. DEF- and GLO-like proteins may have lost most of their interaction partners during angiosperm evolution. Annals of Botany **114,** 1431–1443.

Melzer R, Theissen G. 2009. Reconstitution of 'floral quartets' in vitro involving class B and class E floral homeotic proteins. Nucleic Acids Research **37,** 2723–2736.

Melzer R, Verelst W, Theissen G. 2009. The class E floral homeotic protein SEPALLATA3 is sufficient to loop DNA in 'floral quartet'-like complexes in vitro. Nucleic Acids Research **37,** 144–157.

Melzer R, Wang YQ, Theissen G. 2010. The naked and the dead: the ABCs of gymnosperm reproduction and the origin of the angiosperm flower. Seminars in Cell and Developmental Biology **21,** 118–28.

Mena M, Mandel MA, Lerner DR, Yanofsky MF, Schmidt RJ. 1995. A characterization of the MADS-box gene family in maize. The Plant Journal **8,** 845–54.

Mizzotti C, Mendes MA, Caporali E, Schnittger A, Kater MM, Battaglia R, Colombo L. 2012. The MADS box genes SEEDSTICK and ARABIDOPSIS Bsister play a maternal role in fertilization and seed development. The Plant Journal **70,** 409–20.

Moon YH, Kang HG, Jung JY, Jeon JS, Sung SK, An G. 1999. Determination of the motif responsible for interaction between the rice APETALA1/AGAMOUS-LIKE9 family proteins using a yeast two-hybrid system. Plant Physiology **120,** 1193–1204.

Moore MJ, Bell CD, Soltis PS, Soltis DE. 2007. Using plastid genome-scale data to resolve enigmatic relationships among basal angiosperms. Proceedings of the National Academy of Sciences, USA **104,** 19363–19368.

Mouradov A, Glassick T V., Hamdorf BA, Murphy LC, Marla SS, Yang Y, Teasdale RD. 1998. Family of MADS-Box genes expressed

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early in male and female reproductive structures of monterey pine. Plant Physiology **117,** 55–62.

Nagasawa N, Miyoshi M, Sano Y, Satoh H, Hirano H, Sakai H, Nagato Y. 2003. SUPERWOMAN1 and DROOPING LEAF genes control floral organ identity in rice. Development **130,** 705–718.

Nayar S, Kapoor M, Kapoor S. 2014. Post-translational regulation of rice MADS29 function: homodimerization or binary interactions with other seed-expressed MADS proteins modulate its translocation into the nucleus. Journal of Experimental Botany **65,** 5339–5350.

Nayar S, Sharma R, Tyagi AK, Kapoor S. 2013. Functional delineation of rice MADS29 reveals its role in embryo and endosperm development by affecting hormone homeostasis. Journal of Experimental Botany **64,** 4239–4253.

Nesi N, Debeaujon I, Jond C, Stewart AJ, Jenkins GI, Caboche M, Lepiniec L. 2002. The TRANSPARENT TESTA16 locus encodes the ARABIDOPSIS BSISTER MADS domain protein and is required for proper development and pigmentation of the seed coat. The Plant Cell **14,** 2463–2479.

Ohmori S, Kimizu M, Sugita M, Miyao A, Hirochika H, Uchida E, Nagato Y, Yoshida H. 2009. MOSAIC FLORAL ORGANS1, an AGL6-like MADS box gene, regulates floral organ identity and meristem fate in rice. The Plant Cell **21,** 3008–3025.

Paterson AH, Bowers JE, Chapman BA. 2004. Ancient polyploidization predating divergence of the cereals, and its consequences for comparative genomics. Proceedings of the National Academy of Sciences, USA **101,** 9903–9908.

Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF. 2000. B and C floral organ identity functions require SEPALLATA MADS-box genes. Nature **405,** 200–203.

Pellegrini L, Tan S, Richmond TJ. 1995. Structure of serum response factor core bound to DNA. Nature **376,** 490–498.

Pelucchi N, Fornara F, Favalli C, Masiero S, Lago C, Pè EM, Colombo L, Kater MM. 2002. Comparative analysis of rice MADS-box genes expressed during flower development. Sexual Plant Reproduction **15,** 113–122.

Peng Z, Lu Y, Li L, *et al*. 2013. The draft genome of the fast-growing non-timber forest species moso bamboo (Phyllostachys heterocycla). Nature Genetics **45,** 456–461, 461e1–2.

Pinyopich A, Ditta GS, Savidge B, Liljegren SJ, Baumann E, Wisman E, Yanofsky MF. 2003. Assessing the redundancy of MADS-box genes during carpel and ovule development. Nature **424,** 85–88.

Prasad K, Ambrose BA. 2010. Shaping up the fruit: control of fruit size by an Arabidopsis B-sister MADS-box gene. Plant Signaling and Behavior **5,** 899–902.

Prasad K, P. S, Kumar S, Kushalappa K, Vijayraghavan U. 2001. Ectopic expression of rice OsMADS1 reveals a role in specifying the lemma and palea, grass floral organs analogous to sepals. Development Genes and Evolution **211,** 281–290.

Prasad K, Parameswaran S, Vijayraghavan U. 2005. OsMADS1, a rice MADS-box factor, controls differentiation of specific cell types in the lemma and palea and is an early-acting regulator of inner floral organs. The Plant Journal **43,** 915–928.

Prasad K, Vijayraghavan U. 2003. Double-stranded RNA interference of a rice PI/GLO paralog, OsMADS2, uncovers its second-whorl-specific function in floral organ patterning. Genetics **165,** 2301–2305.

Prasad K, Zhang X, Tobón E, Ambrose BA. 2010. The Arabidopsis B-sister MADS-box protein, GORDITA, represses fruit growth and contributes to integument development. The Plant Journal **62,** 203–214.

Reinheimer R, Kellogg EA. 2009. Evolution of AGL6-like MADS box genes in grasses (Poaceae): ovule expression is ancient and palea expression is new. The Plant Cell **21,** 2591–2605.

Rijpkema AS, Zethof J, Gerats T, Vandenbussche M. 2009. The petunia AGL6 gene has a SEPALLATA-like function in floral patterning. The Plant Journal **60,** 1–9.

Rounsley SD, Ditta GS, Yanofsky MF. 1995. Diverse roles for MADS box genes in Arabidopsis development. The Plant Cell **7,** 1259–1269.

Ruelens P, de Maagd RA, Proost S, Theißen G, Geuten K, Kaufmann K. 2013. FLOWERING LOCUS C in monocots and the tandem origin of angiosperm-specific MADS-box genes. Nature Communications **4,** 2280.

Sang X, Li Y, Luo Z, *et al*. 2012. CHIMERIC FLORAL ORGANS1, encoding a monocot-specific MADS box protein, regulates floral organ identity in rice. Plant Physiology **160,** 788–807.

Schauer SE, Schlüter PM, Baskar R, Gheyselinck J, Bolaños A, Curtis MD, Grossniklaus U. 2009. Intronic regulatory elements determine the divergent expression patterns of *AGAMOUS-LIKE6* subfamily members in Arabidopsis. The Plant Journal **59,** 987–1000.

Schneider H, Schuettpelz E, Pryer KM, Cranfill R, Magallón S, Lupia R. 2004. Ferns diversified in the shadow of angiosperms. Nature **428,** 553–557.

Seok HY, Park HY, Park JI, Lee YM, Lee SY, An G, Moon YH. 2010. Rice ternary MADS protein complexes containing class B MADS heterodimer. Biochemical and Biophysical Research Communications **401,** 598–604.

Shan H, Zhang N, Liu C, Xu G, Zhang J, Chen Z, Kong H. 2007. Patterns of gene duplication and functional diversification during the evolution of the AP1/SQUA subfamily of plant MADS-box genes. Molecular Phylogenetics and Evolution **44,** 26–41.

Smaczniak C, Immink RGH, Muiño JM, *et al*. 2012. Characterization of MADS-domain transcription factor complexes in Arabidopsis flower development. Proceedings of the National Academy of Sciences, USA **109,** 1560–1565.

Su CL, Chen WC, Lee AY, Chen CY, Chang YCA, Chao YT, Shih MC. 2013. A modified ABCDE model of flowering in orchids based on gene expression profiling studies of the moth orchid Phalaenopsis aphrodite. PLoS One **8,** e80462.

Tandre K, Albert VA, Sundås A, Engström P. 1995. Conifer homologues to genes that control floral development in angiosperms. Plant Molecular Biology **27,** 69–78.

Tang X, Zhang ZY, Zhang WJ, Zhao XM, Li X, Zhang D, Liu QQ, Tang WH. 2010. Global gene profiling of laser-captured pollen mother cells indicates molecular pathways and gene subfamilies involved in rice meiosis. Plant Physiology **154,** 1855–70.

Tapia-López R, García-Ponce B, Dubrovsky JG, Garay-Arroyo A, Pérez-Ruíz RV, Kim S-H, Acevedo F, Pelaz S, Alvarez-Buylla ER. 2008. An AGAMOUS-related MADS-box gene, XAL1 (AGL12), regulates root meristem cell proliferation and flowering transition in Arabidopsis. Plant Physiology **146,** 1182–1192.

Theissen G. 2001. Development of floral organ identity: stories from the MADS house. Current Opinion in Plant Biology **4,** 75–85.

Theissen G, Saedler H. 2001. Plant biology. Floral quartets. Nature **409,** 469–471.

Thiel T, Graner A, Waugh R, Grosse I, Close TJ, Stein N. 2009. Evidence and evolutionary analysis of ancient whole-genome duplication in barley predating the divergence from rice. BMC Evolutionary Biology **9,** 209.

Thompson BE, Bartling L, Whipple C, Hall DH, Sakai H, Schmidt R, Hake S. 2009. bearded-ear encodes a MADS box transcription factor critical for maize floral development. The Plant Cell **21,** 2578–2590.

Tsuchimoto S, Mayama T, van der Krol A, Ohtsubo E. 2000. The whorl-specific action of a petunia class B floral homeotic gene. Genes to Cells **5,** 89–99.

Vandenbussche M, Zethof J, Souer E, Koes R, Tornielli GB, Pezzotti M, Ferrario S, Angenent GC, Gerats T. 2003. Toward the analysis of the petunia MADS box gene family by reverse and forward transposon insertion mutagenesis approaches: B, C, and D floral organ identity functions require SEPALLATA-like MADS box genes in petunia. The Plant Cell **15,** 2680–2693.

Vekemans D, Proost S, Vanneste K, Coenen H, Viaene T, Ruelens P, Maere S, Van de Peer Y, Geuten K. 2012. Gamma paleohexaploidy in the stem lineage of core eudicots: significance for MADS-box gene and species diversification. Molecular Biology and Evolution **29,** 3793–3806.

Viaene T, Vekemans D, Becker A, Melzer S, Geuten K. 2010. Expression divergence of the AGL6 MADS domain transcription factor lineage after a core eudicot duplication suggests functional diversification. BMC Plant Biology **10,** 148.

Vision TJ, Brown DG, Tanksley SD. 2000. The origins of genomic duplications in Arabidopsis. Science **290,** 2114–2117.

Wang YQ, Melzer R, Theissen G. 2010. Molecular interactions of orthologues of floral homeotic proteins from the gymnosperm Gnetum

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gnemon provide a clue to the evolutionary origin of 'floral quartets'. The Plant Journal **64,** 177–190.

Wang H, Zhang L, Cai Q, *et al*. 2015. OsMADS32 interacts with PI-like proteins and regulates rice flower development. Journal of Integrative Plant Biology **57,** 504–513.

Wang X, Shi X, Hao B, Ge S, Luo J. 2005. Duplication and DNA segmental loss in the rice genome: implications for diploidization. New Phytologist **165,** 937–946.

Whipple CJ, Zanis MJ, Kellogg EA, Schmidt RJ. 2007. Conservation of B class gene expression in the second whorl of a basal grass and outgroups links the origin of lodicules and petals. Proceedings of the National Academy of Sciences, USA **104,** 1081–1086.

Winter KU, Becker A, Münster T, Kim JT, Saedler H, Theissen G. 1999. MADS-box genes reveal that gnetophytes are more closely related to conifers than to flowering plants. Proceedings of the National Academy of Sciences, USA **96,** 7342–7347.

Winter KU, Weiser C, Kaufmann K, Bohne A, Kirchner C, Kanno A, Saedler H, Theissen G. 2002. Evolution of class B floral homeotic proteins: obligate heterodimerization originated from homodimerization. Molecular Biology and Evolution **19,** 587–596.

Wong CE, Singh MB, Bhalla PL. 2013. Novel members of the AGAMOUS LIKE 6 subfamily of MIKCC-type MADS-box genes in soybean. BMC Plant Biology **13,** 105.

Yadav SR, Khanday I, Majhi BB, Veluthambi K, Vijayraghavan U. 2011. Auxin-responsive OsMGH3, a common downstream target of OsMADS1 and OsMADS6, controls rice floret fertility. Plant and Cell Physiology **52,** 2123–2135.

Yang X, Wu F, Lin X, Du X, Chong K, Gramzow L, Schilling S, Becker A, Theißen G, Meng Z. 2012. Live and let die—the B(sister) MADS-box gene OsMADS29 controls the degeneration of cells in maternal tissues during seed development of rice (Oryza sativa). PLoS One **7,** e51435.

Yao SG, Ohmori S, Kimizu M, Yoshida H. 2008. Unequal genetic redundancy of rice PISTILLATA orthologs, OsMADS2 and OsMADS4, in lodicule and stamen development. Plant and Cell Physiology **49,** 853–857.

Yin LL, Xue HW. 2012. The MADS29 transcription factor regulates the degradation of the nucellus and the nucellar projection during rice seed development. The Plant Cell **24,** 1049–1065.

Yoo SK, Hong SM, Lee JS, Ahn JH. 2011*a*. A genetic screen for leaf movement mutants identifies a potential role for AGAMOUS-LIKE 6 (AGL6) in circadian-clock control. Molecules and Cells **31,** 281–287.

Yoo SK, Wu X, Lee JS, Ahn JH. 2011*b*. AGAMOUS-LIKE 6 is a floral promoter that negatively regulates the FLC/MAF clade genes and positively regulates FT in Arabidopsis. The Plant Journal **65,** 62–76.

Yun D, Liang W, Dreni L, Yin C, Zhou Z, Kater MM, Zhang D. 2013. OsMADS16 genetically interacts with OsMADS3 and OsMADS58 in specifying floral patterning in rice. Molecular Plant **6,** 743–756.

Zahn LM, Kong H, Leebens-Mack JH, Kim S, Soltis PS, Landherr LL, Soltis DE, Depamphilis CW, Ma H. 2005*a*. The evolution of the SEPALLATA subfamily of MADS-box genes: a preangiosperm origin with multiple duplications throughout angiosperm history. Genetics **169,** 2209–23.

Zahn LM, Leebens-Mack J, DePamphilis CW, Ma H, Theissen G. 2005*b*. To B or not to B a flower: the role of DEFICIENS and GLOBOSA orthologs in the evolution of the angiosperms. Journal of Heredity **96,** 225–240.

Zhang D, Yuan Z. 2014. Molecular control of grass inflorescence development. Annual Review of Plant Biology **65,** 553–78.

Zhang D, Yuan Z, An G, Dreni L, Hu J, Kater MM. 2013. Panicle development. In: Zhang Q, Wing RA, eds. Genetics and genomics of rice, plant genetics and genomics: crops and models 5 . New York: Springer Science+Business Media, 279–295.

Zhang J, Nallamilli BR, Mujahid H, Peng Z. 2010. OsMADS6 plays an essential role in endosperm nutrient accumulation and is subject to epigenetic regulation in rice (Oryza sativa). The Plant Journal **64,** 604–617.

Zhang J, Tang W, Huang Y, Niu X, Zhao Y, Han Y, Liu Y. 2015. Down-regulation of a LBD-like gene, OsIG1, leads to occurrence of unusual double ovules and developmental abnormalities of various floral organs and megagametophyte in rice. Journal of Experimental Botany **66,** 99–112.

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