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

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25 **Highlight:** MADS-box proteins are critical transcription factors required for floral
26 development, but their functions in monocots are still relatively uncharacterised. Here we
27 review how changes in MADS-box proteins throughout evolution have created a diverse
28 range of monocot flowers and identify key targets for crop improvement and breeding.

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31 **Key words:** *Arabidopsis*, barley, floral development; inflorescence, lily, MADS-box;
32 monocots; rice, transcription factors, wheat

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34

35 **Abstract**

36 Many monocot plants have high social and economic value. These include grasses such as

37 rice (*Oryza sativa*), wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), which produce

38 soft commodities for many food and beverage industries, and ornamental flowers like lily

39 (*Lilium longiflorum*) and orchid (*Oncidium Gower Ramsey*), which represent an important

40 component of international flower markets. There is constant pressure to improve the

41 development and diversity of these species with a significant emphasis on flower

42 development, and this is particularly relevant considering the impact of changing

43 environments on reproduction and thus yield. MADS-box proteins are a family of

44 transcription factors that contain a conserved 56 amino acid MADS-box motif. In plants,

45 attention has been devoted to characterisation of this family due to their roles in inflorescence

46 and flower development, which holds promise for the modification of floral architecture for

47 plant breeding. This has been explored in diverse angiosperms, but particularly the dicot

48 model *Arabidopsis thaliana*. The focus of this review is on the less-well characterised roles of

49 the MADS-box proteins in monocot flower development and how changes in MADS-box

50 proteins throughout evolution may have contributed to creating a diverse range of flowers.

51 Examining these changes within the monocots can identify the importance of certain genes

52 and pinpoint those which might be useful in future crop improvement and breeding strategies.

53

54
55 **Introduction**

56

57 The grass family, Poaceae, diverged from other Poales around 55-70 million years ago

58 (Bommert *et al.*, 2005). The inflorescence morphology of grasses is one of the major

59 determinants of yield and is thus a key breeding target (Bommert *et al.*, 2005). Identifying

60 genes and proteins that are involved in flower development and their behaviour in high-

61 yielding varieties and varieties that are resistant to biotic and abiotic stresses, may help to

62 identify pathways that can be targeted for the improvement of important crops.

63

64 Much of our knowledge of flower structure, morphology and genetics has been gained

65 through study of the model dicotyledonous plants *Arabidopsis thaliana* and *Antirrhinum*

66 *majus*. *Arabidopsis* flowers contain 4 concentric whorls of organs including 4 sepals, 4 petals,

67 6 stamen and 2 fused carpels. In general, flowers in the grasses share a similar structure, but

68 exhibit some key differences. The rice spikelet comprises a single fertile floret that contains

lemma and palea in whorl 1, two lodicules in whorl 2, six stamens in whorl 3 and a pistil in whorl 4 (Figure 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 (Bell, 1991; Clifford, 1987). Their morphology is very similar except for three vascular strand in the lemma compared to two in palea (Ambrose *et al.*, 2000), and a higher density of trichomes and more stomata in the lemma compared to 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 (Ambrose *et al.*, 2000; Bowman, 1997; Kyozuka *et al.*, 2000; Prasad *et al.*, 2001; Xu *et al.*, 2017). Their function is to protect the florets and kernels from pathogens 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 (Figure 1B), while other grasses have long, branched 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 (Figure 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).

103   Orchids are also members of the monocotyledons, in the family Orchidaceae, but are distinct

104   from the true grasses. Orchid flowers have a zygomorphic structure, which is very different

105   from any of the grass floret structures and within the orchid family there is also great diversity

106   (Pan *et al.*, 2014). *Oncidium Gower Ramsey*, the variety that has been frequently used for

107   floral characterisation, has three types of perianth organs. In the first whorl three small sepals

108   can be identified, while in the second whorl, two petals and the very distinctive lip, or

109   labellum, are found (Figure 1D); because the sepals and petals are not significantly different

110   in some plant species, they are often called tepals. The labellum is particularly interesting

111   from an evolutionary perspective since it represents a unique floral structure that may indicate

112   a shift in protein function and interactions in the highly conserved MADS-box family

113   (Mondragon-Palomino and Theissen, 2008). It is essential for the interaction with pollinators

114   and different models have been proposed to describe the protein interactions leading to

115   labellum development (Mondragon-Palomino and Theissen, 2008).

116

117   Lily (*Lilium longiflorum*) from the monocot family Liliaceae produce flowers that have three

118   sepals in the first whorl, three petals in the second whorl, six stamens in the third whorl and

119   three fused carpels in the fourth whorl (Figure 1E). In *Lilium longiflorum*, most parts of the

120   sepals and petals are still connected to each other giving the lily flowers their distinct trumpet

121   form and distinguishing them from other lily species. Similar to orchids, the sepals and petals

122   are almost identical, which earned them the general name tepals (Tzeng and Yang, 2001).

123   Orchid flowers probably originated from a flower with lily-like actinomorphic perianth with

124   undifferentiated whorls of tepals (Mondragon-Palomino and Theissen, 2008).

125

126   **The MADS-box protein family**

127

128   The MADS-box acronym is derived from MCM1 (yeast), AG (*Arabidopsis*), DEFICIENS

129   (Antirrhinum) and SRF (mammals), the first four proteins discovered in the transcription

130   factor family (Lawton-Rauh *et al.*, 2000; Shore and Sharrocks, 1995). The MADS-box

131   proteins are involved in diverse developmental processes in flowering plants, cardiac muscle

132   development in animals and pheromone response in yeast (Becker and Theissen, 2003;

133   Pelucchi *et al.*, 2002; Schwarz-Sommer *et al.*, 1990).

134

135   In plants, the MADS-box genes have been proposed to be the driving force behind much

136   floral diversity (Theissen and Saedler, 2001; Yamaguchi and Hirano, 2006). Therefore, better

137 insight into their expression and function, and their conservation in different species is
138 important to inform breeding strategies targeting alterations in floral architecture. The
139 MADS-box domain is highly conserved across different species in dicots and monocots,
140 which makes the functional diversity of the proteins extremely interesting. In this review the
141 expression patterns and functions of MADS-box genes relative to flower development in six
142 different monocot species including barley, wheat, maize (*Zea mays*), rice, orchid and lily
143 have been compared. The cereals barley, wheat, maize and rice are mainly cultivated for food
144 purposes, while orchid and lily have economic value as ornamental plants and flowers.

145

146 **MADS-box protein structure is conserved between diverse plant species**

147
148 The MADS-box genes have been divided in two groups: Type I and Type II (Becker and
149 Theissen, 2003). Type I genes seem to have a faster evolutionary rate than Type II genes. The
150 number of duplications of Type I genes is higher, however, even in the shorter time frame
151 (Gramzow and Theissen, 2013). In plants the Type II MADS-box genes are called MIKC-
152 type genes, an acronym of the 4 different domains that have been identified in all genes of this
153 type (Becker and Theissen, 2003).

154

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

166

167 Dependent on the structure of the intervening (I) domain and K-box, the MIKC-type MADS-
168 box proteins can be further subdivided into two categories: the MIKC^c-type and the MIKC*-
169 type proteins. The I-domain in the MIKC^c-type proteins is only encoded by 1 exon, while that

170 in the MIKC*-type proteins is longer, with 4 or 5 exons (Becker and Theissen, 2003; Zhao *et*
171 *al.*, 2006a).

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

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

192 The floral organ identity MADS-box genes of the MIKC^c-type have been divided into five
193 different classes based on their homeotic function: class A, B, C, D and E genes (Bowman *et*
194 *al.*, 1989, 1991; Coen and Meyerowitz, 1991; Theißen, 2001; Weigel and Meyerowitz, 1994).
195 The A- and E-class protein complexes specify sepals in the first whorl. Complexes of A-, B-
196 and E-class proteins specify petals in the second whorl (Honma and Goto, 2001). B-, C- and
197 E-class complexes specify stamens in the third whorl and C- and E-class protein complexes
198 specify carpels in the fourth whorl (Coen and Meyerowitz, 1991; Honma and Goto, 2001). D-
199 class proteins specify ovules together with E-class genes (Figure 3) (Angenent and Colombo,
200 1996; Becker and Theissen, 2003; Colombo *et al.*, 1995; Li *et al.*, 2011; Theissen and
201 Saedler, 2001; Wang *et al.*, 2015a). Another group of genes, phylogenetically related to the
202 B-class genes was identified and was named the B$_{sister}$ or Bs genes (Becker *et al.*, 2002).

203 Genes in this class are mainly expressed in female reproductive organs, especially in the
204 ovules (Becker *et al.*, 2002; Becker and Theissen, 2003; Munster *et al.*, 2001). All of these
205 genes also fall into separate clades, named after the first proteins identified (Figure 4). The
206 genes in the SQUA-clade all determine either inflorescence or floral meristem identity and
207 some have additional A-type functions, while genes in the DEF/GLO clade have class B
208 functions (Theißen *et al.*, 1996). The AG-clade consists of an AG- and an AGL11 (or STK)-
209 lineage and the class E genes are all part of the SEP/AGL2-clade.

210

211 **The ABCDE model in monocots**

212

213 MADS-box genes involved in flower development have been studied in a wide variety of
214 species. In monocots, most research has been undertaken in rice, wheat and maize.
215 Comparing the expression patterns and functions of MADS-box floral genes in different
216 monocot species provides information on the differences in their morphology and how
217 evolution may have affected different floral structures and floral diversity among these
218 species. While rice, wheat and barley have a similar floral pattern, the flowers in orchid and
219 lily are very different. The emergence of unique organs like the labellum in orchid and the
220 differentiation between male tassels and female ears in maize are also interesting to be
221 elucidated. Comparing the expression and function of the ABCDE MADS-box genes within
222 these monocot species provides an interesting opportunity to elucidate more about their role
223 in shaping these different floral structures.

224

225 **A-class genes**

226

227 In *Arabidopsis* and *Antirrhinum*, the A-class genes *AP1* and *SQUA* are responsible for the
228 transition from vegetative to reproductive growth, determination of floral organ identity and
229 the regulation of fruit maturation (Fornara *et al.*, 2004). Their orthologues in monocots have
230 some level of conservation, but there is some divergence in sequence, expression pattern and
231 function (Zhang and Yuan, 2014). In the core eudicots there are two different gene clades
232 within the class A genes: euAP1 and euFUL, which have arisen from a duplication event that
233 coincided with the origin of this angiosperm group (Litt and Irish, 2003; Shan *et al.*, 2007). In
234 non-core eudicots and monocots, only sequences that are similar to euFUL genes have been
235 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 (Figure 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: RRNa-LaLT/NLa and CFAT/A. These motifs contain an acidic transcription activation domain and a farnesylation signal (Chen *et al.*, 2008; Fornara *et al.*, 2004; Litt and Irish, 2003). Both of these motifs have not 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 (Figure 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* 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 (Figure 5B) (Moon *et al.*, 1999b; 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.*, 2000b; 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 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 to 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 (Fornara *et al.*, 2003; Masiero *et al.*, 2002; Pelucchi *et al.*, 2002). *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-2-Hybrid and 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 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.*, 2003b), 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 (Figure 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) (Li *et al.*, 2014; Zhao *et al.*, 2011). *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 MPSS analysis, in situ hybridization and promotor:GUS 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 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* (Figure 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 (Sasani *et al.*, 2009; Tranquilli and Dubcovsky, 2000; Yan *et al.*, 2003).

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 (Figure 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, 2013; Murai *et al.*, 2003). *WFUL3* seems to have a function in floral meristem development together with *WFUL2*, while *WFUL2* has a specialised 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 (Figure 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). Aside 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 (Figure 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 to 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 completely different expression patterns to 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, which 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 this is an ancestral function (Becker and Theissen, 2003; Munster *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 (Figure 6B) (Becker and Theissen, 2003; Winter *et al.*, 2002a; Zahn *et al.*, 2005b).

AP3-like genes

In higher eudicots, an euAP3 motif is found in the AP3-like proteins, but absent in non-core eudicots and non-eudicots. Instead a highly conserved paleoAP3 motif (YGxHDLRLA) is observed in their sequences (Figure 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

405 Yang, 2001). Only one DEF-like gene has been found in most monocots, so it is presumed

406 that no gene duplication event happened here, except for orchids, where the gene duplication

407 seems to have occurred in the DEF-clade instead of the GLO-clade (Table 1) (Chen *et al.*,

408 2012). The paleoAP3 motif seems to have significant sequence diversification in the GLO-

409 like lineage after duplication, where it has been termed a PI-like motif (Figure 6A) (Kramer *et*

410 *al.*, 1998; Moon *et al.*, 1999a). The observation of these different motifs in the monocot B-

411 class MADS-box genes shows that AP3 homologues were highly conserved in most monocots

412 during evolution and that they are more closely related to the lower eudicots than to the higher

413 eudicots (Tzeng and Yang, 2001).

414

415 In rice, *OsMADS16* is a member of DEF-clade and expression is detected in lodicule and

416 stamen primordia from initiation onwards, as revealed by RNA blot analysis and in situ

417 hybridization (Figure 6B) (Fornara *et al.*, 2003; Moon *et al.*, 1999a; Nagasawa *et al.*, 2003).

418 DEF- and GLO-like proteins, like AP3 and PI in *Arabidopsis*, form obligate heterodimers,

419 which might have originated after the gymnosperm-angiosperm split but before the monocot-

420 eudicot split (Davies *et al.*, 1996; Goto and Meyerowitz, 1994; Winter *et al.*, 2002b). The

421 interaction between proteins of the GLO- and the DEF-clade is conserved, as shown by the

422 interaction of OsMADS16 with OsMADS4 and OsMADS2 by yeast-two-hybrid analysis

423 (Moon *et al.*, 1999a; Yao *et al.*, 2008). They form a heterodimer and may auto-regulate their

424 own expression (Yadav *et al.*, 2007), similar to AP3 and PI in *Arabidopsis* (Krizek and

425 Meyerowitz, 1996). The function of *OsMADS16* seems to be well conserved between rice and

426 *Arabidopsis* (Yamaguchi and Hirano, 2006). A loss-of-function mutant of *OsMADS16*,

427 known as *spw1 (superwoman1)*, shows the homoetic transformation of stamens into carpels

428 and lodicules into palea-like organs (Nagasawa *et al.*, 2003). Similarly, *SILKY1*, the *AP3*

429 orthologue in maize, is required for the normal development of lodicules and stamens.

430 *SILKY1* is expressed in the centre of the floral meristem after the lemma and palea primordia

431 have initiated as well as in lodicules and stamens throughout their development (Ambrose *et*

432 *al.*, 2000). A loss-of-function mutation of *SILKY1* results in homeotic transformations of

433 stamens to carpels and lodicules to lemma- or palea-like organs (Ambrose *et al.*, 2000).

434 OsMADS16 also seems to interact with OsMADS3 (C-class), OsMADS15 (A-class),

435 OsMADS8 (E-class) and OsMADS6 (AGL6-like) (Lee *et al.*, 2003a).

436 In wheat, two homeologous genes of *WAP3* (*TaMADS#51* and *TaMADS#82*) on

437 chromosomes 7B and 7D respectively were identified as AP3-like B-class genes (Table 1)

438 (Hama *et al.*, 2004). *WAP3/TaMADS#51* expression is only detected in young spikes at the

439 floral organ development stage, while *WAP3/TaMADS#82* expression was lower in young
440 spikes, but higher in spikes at heading stage (Figure 6B) (Hama *et al.*, 2004).

441

442 The DEF-like genes in orchid are subdivided into four different clades (Mondragon-Palomino
443 and Theissen, 2008). *OMADS3* (clade 2)*,* one AP3-like gene in orchid, does not contain the
444 C-terminal motif, which differs from the other B-class genes found so far (Figure 6) (Hsu and
445 Yang, 2002). The conserved K box sequence (QYQRM), however, is present (Hsu and Yang,
446 2002; Tsai and Chen, 2006). Its expression can be detected in all four floral organs as well as
447 in vegetative leaves as shown by a combination of RT-PCR and Northern analysis (Hsu and
448 Yang, 2002) which is different from other B-class genes that show specific expression in
449 flowers (Figure 6B). Yeast-two-hybrid analysis showed that OMADS3 is able to form strong
450 homodimers (Hsu and Yang, 2002; Tsai and Chen, 2006). Three other DEF-like genes are
451 found in orchid; *OMADS12* (clade 4), *OMADS5* (clade 1) with expression in sepals and petals
452 and *OMADS9* (clade 3) which is highly expressed in petals and absent in vegetative tissues ;
453 (Figure 6B) (Chang *et al.*, 2010; Hsu *et al.*, 2015). OMADS5 and OMADS9 may play a
454 different role in the formation of the sepal, petal and labellum (Chang *et al.*, 2010). The
455 difference for petal and lip formation may be due to the expression of *OMADS5* in the petal
456 and its absence in the lip. OMADS5 may have a negative role in regulating labellum
457 formation (Chang *et al.*, 2010) which was further supported by the reduced expression of
458 *OMADS5* in lip-like sepals and lip-like petals of peloric orchid mutants of *O. Gower Ramsey*
459 (Chang *et al.*, 2010). OMADS5 and OMADS9 are able to form homodimers and heterodimers
460 with each other and with OMADS3 (Chang *et al.*, 2010). *OMADS12* is weakly expressed in
461 stamen, but strongly expressed in the carpel (Hsu *et al.*, 2015). Its expression is completely
462 absent in the sepal, petal and labellum (Hsu *et al.*, 2015). This indicates that clade 4 in *O.*
463 *Gower Ramsey* does not appear to affect perianth differentiation (Hsu *et al.*, 2015).

464

465 In lily, the *LMADS1* gene is the functional counterpart of *AP3* in *Arabidopsis* (Table 1)
466 (Tzeng and Yang, 2001) with conserved function in regulating petal and stamen development.
467 *LMADS1* is expressed in all four floral whorls, but the protein is only detected in petals and
468 stamens, as revealed by Western blot analysis, suggesting post-transcriptional regulation
469 (Tzeng and Yang, 2001). *LMADS1* transcripts were also strongly detected in late-developing
470 carpels (Tzeng and Yang, 2001). Yeast-two-hybrid analysis showed that LMADS1 can form
471 strong homodimers, similar to OMADS3 (Hsu and Yang, 2002; Tsai and Chen, 2006; Tzeng
472 *et al.*, 2004; Tzeng and Yang, 2001). The highly conserved paleoAP3 motif (YGSHDLRLA)

473   was found at the C-terminus of LMADS1 (Figure 6A). Within the K box, the highly

474   conserved sequence (QYEKM) was also identified (Tzeng and Yang, 2001).

475

476   Briefly, wheat has two *AP3* homologues showing different expression patterns, possibly

477   indicating divergent functions. A series of duplication events in orchid are proposed to form

478   4 different clades of AP3-like B-class genes with functional diversification which may

479   contribute to the development of the unique orchid floral structure, the labellum. Unlike the

480   A-class genes, lily AP3-like genes now show more similarity with the AP3-like genes in

481   grasses and *Arabidopsis* than with those in orchid.

482

483   ***PI-like genes***

484

485   Several GLO-like genes have been identified in rice, barley, wheat, maize and lily (Chang *et*

486   *al.*, 2010; Chen *et al.*, 2012; Chung *et al.*, 1995; Hama *et al.*, 2004; Munster *et al.*, 2001);

487   proteins of the GLO-like lineage have a conserved PI-motif in their C-terminal domain

488   (Figure 6).

489

490   In rice the PI-like genes *OsMADS2* and *OsMADS4* are mainly expressed in lodicules, stamens

491   and carpels (Figure 6B) (Chung *et al.*, 1995; Fornara *et al.*, 2003; Kyozuka *et al.*, 2000). The

492   function of *OsMADS2* is similar to that of PI in *Arabidopsis*, based upon RNAi analysis

493   (Kang and An, 2005; Prasad and Vijayraghavan, 2003; Yadav *et al.*, 2007; Yao *et al.*, 2008).

494   RNAi knock-down lines of *OsMADS2* showed continued growth of the distal region of

495   second whorl organs forming an elongated bract-like structure, but no apparent changes in

496   stamen shape (Yadav *et al.*, 2007; Yao *et al.*, 2008; Yoshida *et al.*, 2007). *OsMADS2* is

497   transiently expressed early in all floral tissues and later strongly expressed in early stamen

498   primordia as shown by in situ hybridization (Kyozuka *et al.*, 2000; Yadav *et al.*, 2007).

499   Similar expression levels are detected in developing lodicules and stamens, but are later

500   substantially reduced in differentiating stamens (Kyozuka *et al.*, 2000; Yadav *et al.*, 2007).

501   *OsMADS4* transcription activation occurs very early and uniformly during spikelet meristem

502   initiation (Chung *et al.*, 1995; Yadav *et al.*, 2007). During floret organ development high

503   levels of *OsMADS4* expression occur in stamen and carpel with reduced expression in

504   differentiating lodicules (Yadav *et al.*, 2007). RNAi lines of *OsMADS4* showed no phenotypic

505   alterations, indicating that OsMADS4 and OsMADS2 might be acting redundantly in stamen

506   specification (Yao *et al.*, 2008; Yoshida *et al.*, 2007). Supporting this, in the double knock-

down mutants of *OsMADS2* and *OsMADS4* the stamens were transformed into carpel-like organs (Yao *et al.*, 2008; Yoshida *et al.*, 2007). 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 (Yao *et al.*, 2008; Yoshida *et al.*, 2007).

The *PI* orthologs *ZMM18*, *ZMM29* and *ZMM16* in maize show an expression pattern similar to that of *OsMADS2* and *OsMADS4* (Figure 6B) (Fornara *et al.*, 2003). *ZMM16* is the orthologue of *OsMADS2*, while *ZMM18* and *ZMM29* are orthologous to *OsMADS4* (Table 1) (Munster *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 stamen and lodicules (Whipple *et al.*, 2004). *ZMM16* was also weakly detected in vegetative organs (Munster *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 (Munster *et al.*, 2001). The gene pair *ZMM18* and *ZMM29* appear to have originated by a gene duplication event (Munster *et al.*, 2001). Using an electrophoretic mobility shift assay (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, Figure 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 (Figure 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

540 formation, whereas the presence of OMADS3/8/9 and the absence of OMADS5 are likely to

541 be required for labellum formation (Chang *et al.*, 2010).

542

543 *LMADS8* and *LMADS9* were identified as the *PI* orthologs in *Lilium longiflorum* (Table 1)

544 (Chen *et al.*, 2012). qRT-PCR analysis revealed that *LMADS8* is highly expressed in the first

545 and second whorl tepals in young and mature flowers, but is absent in vegetative leaves, roots

546 and stem (Chen *et al.*, 2012). The expression pattern of *LMADS9* is very similar to that of

547 *LMADS8* (Figure 6B). As seen in *Arabidopsis* AP3 and PI, and OsMADS4 and OsMADS16

548 in rice, LMADS8 and LMADS9 are able to form heterodimers with the AP3-like LMADS1

549 proteins, and can also form homodimers and heterodimers with each other as shown by yeast-

550 two-hybrid analysis (Chen *et al.*, 2012). The function of LMADS8 and LMADS9 seems to be

551 involved in tepal formation and to a minor extent in early stamen formation (Chen *et al.*,

552 2012). Interestingly, *LMADS9* is a truncated version of *LMADS8*, missing the PI-motif in the

553 C-terminal region (Figure 6A) (Chen *et al.*, 2012). Ectopic expression of *LMADS8* and

554 *LMADS9* in *Arabidopsis* partially converts sepals into petal-like organs (Chen *et al.*, 2012).

555 Overexpression of *LMADS8* in the *pi* mutant of *Arabidopsis* completely rescued the

556 phenotype, while overexpression of *LMADS9* only partially rescued the phenotype (Chen *et*

557 *al.*, 2012).

558

559 Overall, the PI-like B-class genes in the grasses seem to have a conserved expression pattern

560 and function. Only one PI-like gene is found in orchid, with a different protein-protein

561 interaction pattern and function, indicating that the B-class genes are essential for the unique

562 floral structure of orchids (Chang *et al.*, 2010). Even though LMADS9 does not have the

563 defining PI-motif at its C-terminus, it does not seem to have lost its interaction possibilities

564 and, possibly may have retained its function (Chen *et al.*, 2012).

565

566

567 **The Bsister-genes are phylogenetically closely related to the B-class genes but have**

568 **different functions**

569

570 Close relatives of B-class genes have been identified in various species including rice, maize,

571 barley and wheat and have been termed the B_{sister} (B_s) genes. They are mainly expressed in

572 female reproductive organs, especially ovules. The two lineages were most likely generated

573 by gene duplication (Becker and Theissen, 2003; Munster *et al.*, 2001). Compared with the B-

574 class genes, B_{sister} genes share a shorter I domain, a sub-terminal PI-motif-derived sequence

575 and in some cases a paleoAP3 motif in the C-terminal region (Figure 7A) (Becker *et al.*,

576 2002). In *Arabidopsis*, two B_{sister} genes have been identified, *ABS* and *GOA* (Becker *et al.*,

577 2002; Mizzotti *et al.*, 2012; Nesi *et al.*, 2002). *ABS* is expressed in the endothelial layer of the

578 inner integuments of mature ovules and is necessary for inner integument differentiation (Nesi

579 *et al.*, 2002). *GOA* has a broad expression pattern in ovule primordia and in ovules, which

580 later is restricted to the outer integuments (Prasad *et al.*, 2010). It has functions in ovule outer

581 integument development and the regulation of fruit longitudinal growth (Prasad *et al.*, 2010;

582 Yang *et al.*, 2012).

583

584 The Bsister genes form three subclades in monocots: OsMADS29, OsMADS30 and

585 OsMADS31 (Yang *et al.*, 2012), which are named after the three B_{sister} genes found in the rice

586 genome (Table 1). Expression analysis showed that *OsMADS29* expression is restricted to

587 developing seeds, while *OsMADS30* is expressed throughout all organs in the plant (Figure

588 7B) (Yang *et al.*, 2012). Suppressed expression of *OsMADS29* by an antisense construct

589 results in reduced and delayed cell degradation of the nucellar projection, abnormal

590 endosperm development and altered seed morphology (Yin and Xue, 2012), indicating that

591 *OsMADS29* is important for the degradation of the nucellar projection and the nucellus.

592 Yeast-two-hybrid analysis showed that OsMADS29 interacts with all five E-class MADS-box

593 genes and both AGL6-like MADS-box genes (Nayar *et al.*, 2014). It also interacts with A-

594 class OsMADS14 and OsMADS18, C-class OsMADS3 and B_{sister} protein OsMADS31 and

595 forms homodimers (Nayar *et al.*, 2014). *OsMADS30* lacks the characteristic B_{sister} motifs

596 (Becker *et al.*, 2002; Yang *et al.*, 2012) and has a different C-terminal due to the insertion of a

597 mobile element (OsME), which has altered function and expression profile (Figure 7A)

598 (Schilling *et al.*, 2015). In maize, *ZMM17* has been identified as a B_{sister} gene; *ZMM17* is

599 expressed in all organ primordia of the female spikelet, but later restricted to the ovule and the

600 developing silk as determined by northern hybridization analysis (Becker *et al.*, 2002; Yang *et

601 al.*, 2012). *WBsis* was classified as a B_{sister} gene and part of *OsMADS29*-like clade in wheat

602 because of the high sequence similarity with *OsMADS29* and *OsMADS31* (Yamada et al.,

603 2009). *WBsis* is expressed in the endothelial layer of the inner integument of the ovule,

604 similar to *ABS* in *Arabidopsis*, weak expression is also detected in the nucellus and the outer

605 integument (Mizzotti *et al.*, 2012; Yamada *et al.*, 2009; Yang *et al.*, 2012).

606

607 All B$_{sister}$ genes discussed here show a similar expression pattern, except *OsMADS30* which

608 also has a diverged function. No B$_{sister}$ genes have been thoroughly investigated in barley,

609 orchid and lily.

610

611 **C- and D-class genes**

612

613 C-class genes in eudicots specify the plant reproductive organs alone (carpels) or together

614 with the B-class genes (stamens) (Fornara *et al.*, 2003). They also seem to be involved in the

615 negative regulation of A-class MADS-box genes (Gustafson-Brown *et al.*, 1994; Wang *et al.*,

616 2015b). Upon the discovery of the function of the MADS-box genes *FBP7* and *FBP11* in

617 *Petunia* in regulating ovule organ identity, the ABC model was extended to incorporate a D

618 function (Angenent *et al.*, 1995; Colombo *et al.*, 1995). D-gene function is involved in the

619 determination of the identity of the central meristem, the progenitor tissue of the placenta and

620 the ovules (Angenent and Colombo, 1996). Both C- and D-class genes belong to the AG-like

621 subfamily and have arisen through a gene duplication event close to base of the angiosperm

622 emergence (Becker and Theissen, 2003).

623

624 C- and D-class proteins can be distinguished by the structure of the N-terminal part of the K-

625 box. In D-lineage, a glutamine at position 105 is conserved, while this residue is not found in

626 C-lineage (Figures 7 and 8) (Dreni *et al.*, 2007; Kramer *et al.*, 2004). Most D-lineage proteins

627 also have a non-polar hydrophobic residue at position 106, whereas C-lineage proteins have a

628 polar residue at that position (Dreni *et al.*, 2007). Monocot D-lineage proteins have a specific

629 single amino acid insertion at position 90 and at position 113 there is a histidine residue. Both

630 of these are not present in C-lineage proteins (Dreni *et al.*, 2007). Furthermore there is a

631 conserved AG motif I and AG motif II in the C-terminal region of AG-like proteins, which

632 can be found in C- and D-class proteins (Kramer *et al.*, 2004). A nine-amino acid motif

633 downstream of the AG motif II is specific for D-class proteins (Hsu *et al.*, 2010) (Figures 8

634 and 9).

635

636 In rice, two duplicated C-class genes *OsMADS3* and *OsMADS58* have partially

637 subfunctionalized (Table 1) (Kang *et al.*, 1995; Yamaguchi *et al.*, 2006). *OsMADS3* shows

638 high sequence similarity and expression with *Arabidopsis AG* (C-class gene). In situ

639 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 (Dreni *et al.*, 2011; Yamaguchi *et al.*, 2006). 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 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) (Hirabayashi and Murai, 2009; Meguro *et al.*, 2003; Murai, 2013; Shitsukawa *et al.*, 2007; Zhao *et al.*, 2006a). Although they share high level sequence similarity to rice *OsMADS58* and *OsMADS3* respectively, they have different expression patterns and functions (Murai, 2013; Wei *et al.*, 2011). 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 heading stage seen by Northern blot analysis, suggesting 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 (Figure 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).

673 The maize orthologues of rice *OsMADS3* are *ZMM2* and *ZMM23*, and *OsMADS58* is *ZAG1*

674 (Table 1) (Li *et al.*, 2014; Münster *et al.*, 2002; Schmidt *et al.*, 1993; Theißen *et al.*, 1995).

675 *ZAG1* is expressed early in stamen and carpel primordia as shown by RNA blot analysis and

676 in situ hybridization (Schmidt *et al.*, 1993). *ZMM2* is mainly expressed in the anthers (Figure

677 8B) (Li *et al.*, 2014; Mena *et al.*, 1996). Analysis of loss-of-function mutants showed that

678 *ZAG1* determines the floral meristem, while *ZMM2* participates in regulating the formation of

679 stamens and carpels (Mena *et al.*, 1996; Wei *et al.*, 2011). The orchid genes, *OMADS4* and

680 *OMADS2* are both placed in the AG-clade, with *OMADS4* having a C-class function and

681 *OMADS2* a D-class function (Table 1) (Hsu *et al.*, 2010). qRT-PCR analysis showed that

682 *OMADS4* is expressed in stamens, the stigmatic cavity and ovule (Figure 8B) (Hsu *et al.*,

683 2010), which is similar to that of *AG* in *Arabidopsis* (Yanofsky et al., 1990). Yeast-two-

684 hybrid analysis showed that OMADS4 and OMADS2 can form homodimers and

685 heterodimers with each other (Hsu *et al.*, 2010). *LMADS10*, the C-class gene in Lily, is

686 expressed in stamens and carpels (Hsu *et al.*, 2010). This is very similar to the expression

687 pattern in *Oncidium Gower Ramsey* (Figure 8B). Ectopic expression of *LMADS10* in

688 *Arabidopsis* caused early flowering and produced small, curly leaves and floral organ

689 conversions like carpelloid sepals (Hsu *et al.*, 2010). Overexpression of *OMADS4* in

690 *Arabidopsis* only showed a moderate early flowering phenotype with no homeotic floral

691 organ changes (Hsu *et al.*, 2010).

692

693 Rice has two duplicated D-lineage genes: *OsMADS13* and *OsMADS21* (Table 1) (Dreni *et al.*,

694 2007; Kramer *et al.*, 2004). *OsMADS13* is expressed in the ovule primordium and the inner

695 cell layer of the carpel wall. Its expression persists during development of the ovule, mainly

696 in the integuments (Lopez-Dee *et al.*, 1999). In a *Tos17* insertion mutant of *OsMADS13*,

697 ovule primordia developed into carpelloid structures that grew out of the carpel, giving rise to

698 ectopic styles and stigmas (Dreni *et al.* (2007); Yamaki *et al.* (2011). The *osmads3-3*

699 *osmads13* double mutant showed a complete loss of floral meristem determinacy inside the

700 fourth whorl, while the *osmads13 osmads58* double mutant showed a similar but milder

701 phenotype (Dreni *et al.*, 2011; Li *et al.*, 2011). OsMADS13 interacts with the E-class MADS-

702 box proteins, OsMADS7 and OsMADS8, and is involved in ovule specification and floral

703 meristem determinacy (Dreni *et al.*, 2007; Fornara *et al.*, 2003; Yamaguchi and Hirano,

704 2006). RT-PCR and in situ hybridization showed that *OsMADS21* is expressed at low levels

705 in the inner two whorls of the flower and ovules, its expression overlaps with that of

706 *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 (Figure 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 upregulation of *OsMADS21* resulted in partial complementation of *osmads13* phenotype, but ovule development was not completely restored (Dreni *et al.*, 2007; Dreni *et al.*, 2011). These results suggest that *OsMADS21* has lost its function in determining ovule identity, presumably because of its redundancy with *OsMADS13* (Dreni *et al.*, 2007; Fornara *et al.*, 2003; Yamaguchi and Hirano, 2006).

The closest relative to the *Arabidopsis* D-function gene *STK* in wheat is *WSTK*, also known as TaAG-3 (Table 1) (Paolacci *et al.*, 2007; Zhao *et al.*, 2006a). Yeast-two-hybrid analysis has shown that WSTK forms a complex with the E-class protein WSEP (Murai, 2013; Shitsukawa *et al.*, 2007; Yamada *et al.*, 2009). 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 (Yamada *et al.*, 2009; Zhao *et al.*, 2006a). The closest relative to *OsMADS*21 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 (Li *et al.*, 2014; Schmidt *et al.*, 1993; Theiβen *et al.*, 1995). *ZAG2* is a floral specific gene, but 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 (Figure 9B) (Hsu *et al.*, 2010). Ectopic expression of *OMADS2* shows the same phenotype as *LMADS10*, except there are no floral organ conversions (Hsu *et al.*, 2010). *LMADS2* was identified as the D-class protein in *Lilium 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).

741

742   The gene duplication event of C-class genes is also seen in some grasses, for instance, in

743   maize, leading to three different C-class genes and possible subfunctionalization (Dreni and

744   Kater, 2014). In contrast, only one C-class gene and one D-class gene have currently been

745   found in *O. Gower Ramsey* and *L. longiflorum*, but their expression patterns are highly

746   conserved compared with those of *Arabidopsis* and rice.

747   **E-class genes**

748

749   E-class genes belong to *AGL2*-subfamily and specify flower organ identity by forming higher-

750   order protein complexes with the class A, B or C proteins respectively (Becker and Theissen,

751   2003; Pelaz *et al.*, 2000; Theißen, 2001). This ability to form tetrameric complexes also

752   contributes to the development of floral quartets to control sepal, petal, stamen and carpel

753   formation or their equivalents in grasses (Becker and Theissen, 2003; Fornara *et al.*, 2003;

754   Theissen and Saedler, 2001). In *Arabidopsis*, *SEP1/2/3/4* have been identified as E-class

755   genes (Huang *et al.*, 1995; Ma *et al.*, 1991; Mandel and Yanofsky, 1998). *SEP1*, *SEP2* and

756   *SEP4* are expressed in all four whorls of the flower, with SEP4 showing higher expression in

757   the central dome (Ditta *et al.*, 2004; Flanagan and Ma, 1994; Savidge *et al.*, 1995). *SEP3* is

758   only expressed in the inner three whorls (Mandel and Yanofsky, 1998).

759

760   *AGL2*-like genes were deduced to have undergone a gene duplication event before the origin

761   of the extant angiosperms, and after the divergence between extant gymnosperms and

762   angiosperms, creating the SEP3- and LOFSEP-lineages (Malcomber and Kellogg, 2005;

763   Zahn *et al.*, 2005a). Furthermore, SEP3- and LOFSEP-lineages may have undergone more

764   gene duplication events in the grasses, leading to three LOFSEP lineages: OsMADS1-,

765   OsMADS5- and OsMADS34-clades and two SEP3-lineages: OsMADS7- and OsMADS8-

766   clade (Malcomber and Kellogg, 2005; Zahn *et al.*, 2005a). In addition, two motifs (SEPI and

767   SEPII) that consist of hydrophobic and polar residues were observed in AGL2-like proteins

768   (Vandenbussche *et al.*, 2003; Zahn *et al.*, 2005a). Clade-specific changes in these motifs can

769   be seen, for instance, the OsMADS5-clade in grasses have lost the final 12-15 amino acids

770   within the SEPII motif, possibly caused by a recent gene duplication followed by a frameshift

771   mutation (Vandenbussche *et al.*, 2003; Zahn *et al.*, 2005a).

772

773   *LOFSEP-lineage*

OsMADS1-clade

OsMADS1, one well-characterised 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.*, 2000a; 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 (Figure 10B) (Chung *et al.*, 1994; Kobayashi *et al.*, 2010; Prasad *et al.*, 2001). Overexpression of *OsMADS1* caused stunted panicles, irregular positioned branches and spikelets and the rudimentary glumes were transformed into palea/lemma-like structures (Prasad *et al.*, 2005; Prasad *et al.*, 2001). Different mutants of *OsMADS1* have been investigated. Jeon *et al.* (2000a) reported that *lhs-1 (leafy hull sterile1),* which contains two missense mutations in *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 (Cui *et al.*, 2010; Hu *et al.*, 2015; Lim *et al.*, 2000; Moon *et al.*, 1999b). Two maize homologs of *OsMADS1*, *ZMM8* and *ZMM14* are thought to determine the alternative identity of the upper vs the lower floret within each spikelet primordium (Becker and Theissen, 2003; Cacharrón *et al.*, 1999). Their expression was only detectable in the upper floret, but not in the lower floret of the developing spike, shown by in situ hybridization (Figure 10B) (Cacharrón *et al.*, 1995; Cacharrón *et al.*, 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

808 detected in the spikelet axis at the most proximal position (Shitsukawa *et al.*, 2007). Later,
809 their expression was observed in the glume, lemma and palea until maturity of the floral
810 organs (Shitsukawa *et al.*, 2007). Shitsukawa *et al.* (2007) showed that expression of *WLHS1-*
811 *B* is much lower than that of *WLHS1-A* and *–D*. WLHS1-B and WLHS1-D interact with B-
812 class WAP3 and WPI2 and all E-class genes, with the exception of WLHS1-A (Shitsukawa *et*
813 *al.*, 2007). It has been suggested that the lack of interaction with WLHS1-A is due to the loss
814 of the K box in WLHS1-A (Davies *et al.*, 1996; Shitsukawa *et al.*, 2007). Overexpression of
815 *WLHS1* homeologues in *Arabidopsis* showed no phenotype for *WLHS1-A* and early flowering
816 and late production of terminal flowers for *WLHS1-B* and *–D* (Shitsukawa *et al.*, 2007).

817

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

828

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

834

835 *OsMADS34-clade*
836 Unlike other *SEP*-like genes involved in controlling flower development, *OsMADS34*
837 (*PANICLE PHYTOMER2* [*PAP2*]), one *LOFSEP* gene, is required for rice inflorescence and
838 spikelet development (Gao *et al.*, 2010; Kobayashi *et al.*, 2010; Lin *et al.*, 2014). *osmads34-1*
839 showed altered inflorescence shape with increased primary branch number and decreased
840 secondary branch number. In addition, *osmads34-1* showed fewer spikelets and changed
841 spikelet morphology, containing elongated sterile lemmas with lemma/palea-like features

842   (Gao *et al.*, 2010)(Gao et al., 2010). Recently *OsMADS34/PAP2* was shown to be involved in

843   the transition from vegetative to reproductive development via specifying inflorescence

844   meristem identity together with three *AP1/FUL*-like genes *OsMADS14*, *OsMADS15* and

845   *OsMADS18* (Kobayashi et al., 2012). These findings clearly show that OsMADS34 is a

846   positive regulator of inflorescence meristem identity and spikelet meristem identity as well as

847   a suppressor of elongation of the glumes (Kobayashi *et al.*, 2010; Kobayashi *et al.*, 2012).

848

849   In maize and wheat, the function of *OsMADS34* homologs have not been elucidated, and only

850   expression data is reported. Two maize homologues of *OsMADS34*, *ZMM24* and *ZMM31* are

851   expressed in early developing tassels and ears, and *ZMM24* shows high expression throughout

852   ear development (Danilevskaya *et al.*, 2008). *TaSEP-5* was identified as the orthologue of

853   *OsMADS34* in wheat and its three homeologues are located on chromosomes 5A, 5B and 5D

854   with high expression level at the early spike developmental stages, which decreases, but

855   increases again in spikes at the booting and heading stages (Paolacci *et al.*, 2007). Notably,

856   *TaSEP-5* is highly expressed in the glumes, lemma and palea (Paolacci *et al.*, 2007).

857

858   *Orchid and lily*

859   To date there is no direct genetic evidence showing the function of the *OsMADS1*-like gene

860   *OMADS11* in orchid. *OMADS11* is highly expressed in the sepal, petal, lip, carpel, anther cap

861   and stigmatic cavity and has no expression signal in vegetative leaves and stamens as was

862   shown by RT-PCR. Ectopic expression of *OMADS11* in *Arabidopsis* showed early flowering

863   phenotypes and smaller, curled leaves (Chang *et al.*, 2009). In lily, *LMADS3* and *LMADS4*

864   were identified as E-class genes (Table 1) (Tzeng *et al.*, 2003). *LMADS4* is a *SEP1/2*

865   orthologue, which is expressed in the inflorescence meristem, floral buds of different

866   developmental stages and in all four whorls of the flower (Chang *et al.*, 2009; Tzeng *et al.*,

867   2003). *LMADS4* is also expressed in the vegetative leaf and in the inflorescence stem (Tzeng

868   *et al.*, 2003). *Arabidopsis* plants with ectopic expression of *LMADS4* were indistinguishable

869   from the wild type plants (Tzeng *et al.*, 2003).

870

871   **SEP3-lineage**

872

873   *OsMADS7-clade*

874   *OsMADS7* has redundant function in specifying rice flower development with *OsMADS8*, as

875   suggested by the observation that *OsMADS7* and *OsMADS8* share almost identical expression

876   patterns (Kang *et al.*, 1997; Pelucchi *et al.*, 2002). *OsMADS7* and *OsMADS8* are expressed

877   early in the floral meristem where the lodicule and stamen primordia develop (Kang *et al.*,

878   1997; Pelucchi *et al.*, 2002). Subsequently they are expressed in lodicules, developing stamen

879   and carpel primordia throughout floret development (Figure 10B) (Kang *et al.*, 1997; Pelucchi

880   *et al.*, 2002). Overexpression and knockdown of *OsMADS7* shows similar phenotypes to that

881   of *OsMADS8* (Cui *et al.*, 2010; Jeon *et al.*, 2000b; Kang *et al.*, 1997). Knock-down of both

882   *OsMADS7* and *OsMADS8* resulted in late flowering and homeotic transformation of lodicules,

883   stamens and carpels into palea/lemma-like structures, while knockdown of *OsMADS7* or

884   *OsMADS8* using RNAi only showed mild phenotypes (Cui *et al.*, 2010). In vitro and in vivo

885   assays showed that OsMADS7 interacts with OsMADS8 and OsMADS1 and can form

886   homodimers (Cui *et al.*, 2010).

887

888   *ZMM6* in maize is weakly expressed in all organs of the upper and lower floret during the

889   inflorescence development and strongly expressed in the endosperm transfer cell region and

890   the embryo during maize kernel development (Figure 10B) (Cacharrón *et al.*, 1995;

891   Cacharrón *et al.*, 1999; Lid *et al.*, 2004). Loss-of-function of *ZMM6* with a *Mutator*-insertion

892   showed no obvious developmental defects in the kernel (Lid *et al.*, 2004).

893   In barley, *HvBM7* (also known as *BM9*) expression has been found in anthers, but not in the

894   lemma or palea and later also in lodicules and the carpel (Figure 10B) (Schmitz *et al.*, 2000).

895   The wheat SEP-like protein WSEP has three homeologues in the wheat genome on

896   chromosomes 7A, 7B and 7D (Paolacci *et al.*, 2007; Shitsukawa *et al.*, 2007). Just before

897   initiation of the lodicule, stamen and carpel formation, *WSEP* expression was detected in

898   whorls 2, 3 and 4 (Shitsukawa *et al.*, 2007). In all subsequent stages, expression was also

899   detected in the palea of the floret (Figure 10B). qRT-PCR showed that there is no difference

900   in expression between the three homeologues (Shitsukawa *et al.*, 2007). Overexpression of

901   *WSEP* in *Arabidopsis* showed early flowering and four to five curled leaves phenotypes for all

902   three homeologues (Shitsukawa *et al.*, 2007). The strong expression of *WSEP* not only during

903   floral organ differentiation, but also after floral organ determination, suggests that *WSEP*

904   genes are involved in both floral organ differentiation but also in their subsequent

905   development (Chang *et al.*, 2009; Murai, 2013; Shitsukawa *et al.*, 2007). WSEP interacts with

906   the A-class WAP1, the B-class WAP3 and WPI2, the C-class WAG1 and WAG2, the D-class

907   WSTK and all E-class genes, except WLHS1-A (Shitsukawa *et al.*, 2007).

908

909   *OsMADS8-clade*

910   The expression pattern of the *OsMADS8* homologue in maize *ZMM27* is similar to that of

911   *ZMM6*, showing weak expression during development of the inflorescence and strong

912   expression during maize kernel development (Lid *et al.*, 2004). Further, loss of function of

913   *ZMM27* in a *Mutator*-insertional mutant did not induce obvious defects and neither did the

914   double mutant with *ZMM6 (Lid et al., 2004). TaMADS1* was identified as the *OsMADS*8

915   orthologue in wheat, with the three homeologues located on chromosomes 5A, 5B and 5D

916   (Paolacci *et al.*, 2007). Northern blot analysis and in situ hybridization showed that they are

917   uniformly expressed in the spikelet primordia and later confined to the carpels and stamens

918   (Zhao *et al.*, 2006b). Overexpression of *TaMADS1* in *Arabidopsis* showed mild to severe

919   phenotypes with early flowering and abnormal floral organs (Zhao *et al.*, 2006b).

920

921   *Orchid and lily*

922   Expression of the *OsMADS7*-like gene in orchid, *OMADS6*, is abundant in the sepal, petal,

923   labellum, carpel, anther cap and stigmatic cavity, and weak in the stamen, as shown by RT-

924   PCR (Figure 10B) (Chang *et al.*, 2009). Overexpression of *OMADS*6 in *Arabidopsis* resulted

925   in early flowering, two to four small curled leaves, terminal flowers composed of two to three

926   flowers and homeotic conversions of sepals into carpel-like structures and petals into stamen-

927   like structures (Chang *et al.*, 2009). In lily, *LMADS3* is a *SEP3* orthologue, which shows

928   almost identical expression to that of the *OsMADS*1-like gene in lily, *LMADS4* (Tzeng *et al.*,

929   2003*)*. Northern blot analysis showed that *LMADS3* is expressed in the inflorescence

930   meristem and later in all four floral organs, but absent in vegetative leaves (Tzeng *et al.*,

931   2003). Overexpression of *LMADS3* in *Arabidopsis* resulted in early flowering, two to three

932   small curled rosette leaves and two curled cauline leaves (Tzeng *et al.*, 2003). Inflorescence

933   determinacy was lost, as was production of terminal flowers at the end of the inflorescence

934   that had two to three carpels.

935

936   **AGL6-like genes**

937

938   The *AGL6* subfamily is thought to be sister to the E-class AGL2-like genes (Becker and

939   Theissen, 2003). Rijpkema et al (2009) proposed adding *AGL6*-like genes to the E-class of the

940   ABCDE model. *Arabidopsis* has two AGL6-like genes: *AGL6* and *AGL13*, both of which

941   have various divergent functions in the plant, although no loss-of-function mutants have been

942   described so far (Dreni and Zhang, 2016). AGL6 in *Arabidopsis* can interact with some type I

943 MADS proteins, which is unusual for MIKC^c-type MADS proteins (Dreni and Zhang, 2016).
944 AGL6-like proteins have a C-terminus with two short, but highly conserved regions named
945 AGL6-I and AGL6-II motifs (Ohmori *et al.*, 2009).

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

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

964 Rice has two *AGL6*-like genes: *OsMADS6* and *OsMADS17*, which have different expression
965 patterns (Ohmori *et al.*, 2009; Reinheimer and Kellogg, 2009). RT-PCR and in situ
966 hybridization showed that *OsMADS6* is expressed in the floral meristem at early stages and
967 later in the emerging palea primordium (Li *et al.*, 2010). It is also detected in developing
968 palea, lodicules, ovule integuments, carpels and weakly in lemma (Figure 11B) (Dreni and
969 Zhang, 2016; Li *et al.*, 2010). Mutants of *OsMADS6* (also called *mfo1*), showed disturbed
970 palea and lodicule identities and had extra carpels or spikelets (Ohmori *et al.*, 2009). *mfo1*
971 *lhs1* double mutant resulted in a severe phenotype including the loss of spikelet meristem
972 determinacy, suggesting that together with OsMADS1, OsMADS6 determines floral organ
973 and meristem identities (Li *et al.*, 2010; Ohmori *et al.*, 2009). This also suggests that
974 OsMADS6 has a very similar function to the E-class genes, which regulate the development
975 of all four whorls and floral meristem determinacy (Li *et al.*, 2010). OsMADS6 can also form
976 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* (Lee *et al.*, 2003a; Moon *et al.*, 1999b; Seok *et al.*, 2010). OsMADS6 also interacts with the D-class protein OsMADS13 and 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 primordia and is also detected in the anther wall (Figure 11B) (Reinheimer and Kellogg, 2009). Suppression of *OsMADS17* by RNAi did not result in any morphological abnormalities (Ohmori *et al.*, 2009). In *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 (Figure 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 the E-class gene *OMADS6* and to 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

1010 sepals into carpel-like structures and petals into staminoid structures were also observed (Hsu
1011 *et al.*, 2003).

1012

1013 AGL6-like genes seem to be involved in diverse processes in all four whorls, with conserved
1014 expression and function in most of the species. In orchid there seems to be a specialised
1015 function for these genes in the labellum formation.

1016

1017 **Conclusions and perspectives**

1018

1019 MADS-box ABCDE genes are crucial for floral development and their evolutionary changes
1020 with gene duplication, sub-functionalization and neo-functionalization led to novel
1021 morphological forms in plants. Understanding the function of these MADS-box genes can
1022 provide information on how different floral structures originated and identify targets for
1023 future crop improvement.

1024 In grasses, the A-class genes underwent more gene duplications and acquired functions in
1025 specifying the grass-specific flower organs such as the palea and lodicule. Clearly the whole
1026 picture of A-class genes in grasses still remains to be elucidated.

1027 As in other species, the function of B-class genes is relatively conserved in most grasses even
1028 though there may has been gene duplication and sub-functionalization. Exceptionally, in
1029 orchids, two separate duplication events have led to some remarkable changes in floral
1030 structure. *OMADS3* in orchid lost the C-terminal motifs of MADS-box proteins and has
1031 expression signal in the vegetative leaves (Hsu and Yang, 2002; Tsai and Chen, 2006). It is
1032 speculated that *LMADS1* in lily may represent an ancestral form of the B function gene, which
1033 retains the ability to form homodimers and regulates petal and stamen development (Tzeng
1034 and Yang, 2001). Notably, the *OsMADS30* B_{sister} gene has gone through neo-
1035 functionalization, giving it a function in vegetative development instead of ovule and seed
1036 development (Schilling *et al.*, 2015). Until now, little is known about the Bsister genes in
1037 most of the species described.

1038 Despite gene duplication events the C- and D-class genes seem to have retained most of their
1039 function and expression patterns in monocots. Sub-functionalization has lead to genes
1040 working redundantly and the rice D-class gene *OsMADS21*, has lost its ability to determine
1041 ovule development because of redundancy with *OsMADS13* (Dreni *et al.*, 2007; Fornara *et
1042 al.*, 2003; Prasad *et al.*, 2005; Yamaguchi and Hirano, 2006). Its higher expression in

1043 developing kernels might suggest OsMADS21 has gone through neo-functionalization and
1044 has a function after fertilization (Arora *et al.*, 2007).

1045 The E-class genes are more difficult to compare than the other classes of genes from the
1046 ABCDE model as they have diversified with the function in inflorescence and spikelet
1047 development during evolution. The expression of *OsMADS1* homologs in grasses varies from
1048 species to species with the developmental pattern of florets in the spikelet. *OsMADS1*-like
1049 genes may have been involved in morphological diversification of inflorescences during the
1050 evolution of grass species (Yamaguchi and Hirano, 2006).

1051 Expression of *AGL6*-like genes in the palea is conserved in all spikelet-bearing grasses. This
1052 could indicate that AGL6-like genes might play an conserved role in palea development
1053 (Reinheimer and Kellogg, 2009). It has been proposed that AGL6-like genes may have played
1054 an important role in the evolution of unique flower features, such as the labellum in orchids
1055 (Dreni and Zhang, 2016).

1056

1057 Characterisation of these genes, their structure, their expression pattern and their function will
1058 give greater insight into their role in flower development. Importantly, phylogenetic analysis
1059 can sometimes be misleading, and data from functional analysis experiments are needed to
1060 confirm whether genes belong in specific clades and still retain a function in flower
1061 development. In line with this, neo-functionalization likely plays a relatively important and
1062 unexplored role in monocot floral diversity.The identification of orthologues is currently
1063 heavily reliant on sequence similarities, but due to the many gene duplication events that have
1064 shaped the MADS-box family, some MADS-box genes in monocots have gained new roles,
1065 or lost their ancestral function. It must also be noted that most of these sequences are
1066 extracted from reference genomes, and therefore a much greater level of diversity may be
1067 present in the pangenome that is not represented here. Since flower development is one of the
1068 major determinants for yield in important crops, improving our understanding about the genes
1069 and networks involved in flower development is an essential tool to help towards devising
1070 new strategies for crop improvement.

1071

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

Figure Legends

Table 1 The ABCDE genes in *Arabidopsis* and monocot species. Listed are the genes in model organism *Arabidopsis* and the orthologs 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.

Figure 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 3 stamens. (C) Maize has two separate inflorescences, a male (tassel) and a female (ear) one. 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, 6 stamens in the third whorl and a carpel in the fourth whorl.

Figure 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 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 to 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.

Figure 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 in 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).

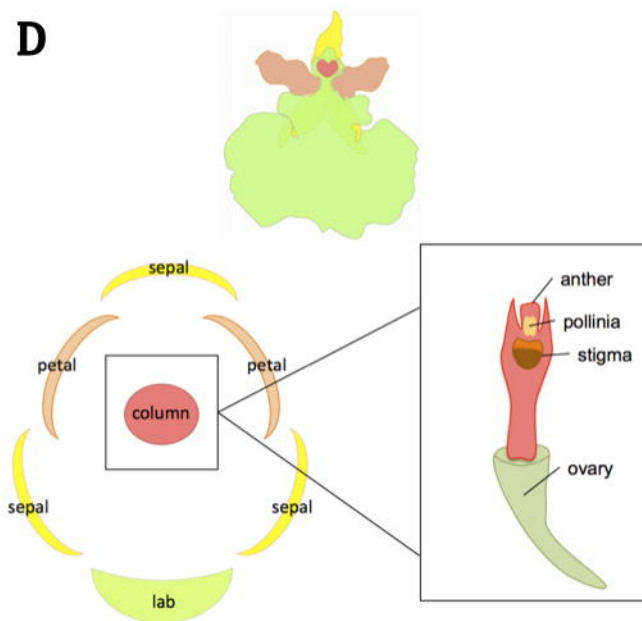**Figure 4 Phylogenetic analysis of ABCDE MADS-box genes from** *Arabidopsis, Amborella trichopoda, Populus trichocarpa***, rice, maize, wheat, barley, orchid and lily.** Phylogenetic tree obtained with RAxML tree building through Geneious version 8.0 by Biomatters. Available from http://www.geneious.com. 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 the supplemental figures S1-7.

Figure 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 for 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 multiple genes expressed in this tissues, while orange indicates only one gene expressed in this tissue. White square indicate there is no expression and grey squares indicate no data is available about the expression in these tissues.

Figure 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 in 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 homologs. 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 multiple genes expressed in this tissues, while orange indicates only one gene expressed in this tissue. White square

indicate there is no expression and grey squares indicate no data is available about the expression in these tissues.

Figure 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 seems 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 Figure 8). OsMADS30 contains neither of the two motifs. (B) The expression pattern of B$_{sister}$ genes that have been investigated show conservation in the female reproductive organs. Red squares indicate multiple genes expressed in this tissues, while orange indicates only one gene expressed in this tissue. White square indicate there is no expression and grey squares indicate no data is available about the expression in these tissues.

Figure 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 homologs. Expression of C-class genes seems to be conserved in all species. (B) The expression pattern of C-class genes are conserved across all species that have been investigated to date. Red squares indicate multiple genes expressed in this tissues, while orange indicates only one gene expressed in this tissue. White square indicate there is no expression and grey squares indicate no data is available about the expression in these tissues.

Figure 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 doesn't 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 multiple genes expressed in this tissues, while orange indicates only one gene expressed in this tissue. White square indicate there is no expression and grey squares indicate no data is available about the expression in these tissues.

Figure 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 multiple genes expressed in this tissues, while orange indicates only one gene expressed in this tissue. White square indicate there is no expression and grey squares indicate no data is available about the expression in these tissues.

Figure 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 multiple genes expressed in this tissues, while orange indicates only one gene expressed in this tissue. White square indicate there is no expression and grey squares indicate no data is available about the expression in these tissues.

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

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

Supplemental Figure S3 Sequence alignment of B$_{sister}$-class proteins in *Arabidopsis, Amborella trichopoda, Populus trichocarpa*, **rice, maize, wheat, barley, orchid and lily.**

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

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

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

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

A

lemma — palea

le
1
lo 2 lo
ca 3
4
sta
pa 1

B

lemma — palea

le
1
lo 2 lo
ca 3
4
sta
pa 1

C

uf

uf lf

glu
le
lo lo
ca

pa

glu

glu
le
lo lo
sta
pa

pa
sta
lo lo
le
glu

D

sepal

petal petal

column

sepal sepal

lab

anther
pollinia
stigma

ovary

E

tepal
sta

ca

| | 1 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 200 | 211 |

ZMM2 — MADS-box, Interv..., K-box, C-terminal

WAG2 — MADS-box, Interv..., K-box, C-terminal

OsMADS3 — MADS-box, Interv..., K-box, C-terminal

OMADS4 — MADS-box, Interv..., K-box, C-terminal

LMADS10 — MADS-box, Interv..., K-box, C-terminal

HvBM3 — MADS-box, Interv..., K-box, C-terminal

AG — MADS-box, Interv..., K-box, C-terminal

A

B

	Class A					
Tissue \ **Species**	**Rice**	**Maize**	**Barley**	**Wheat**	**Orchid**	**Lily**
Inflorescence meristem				WFUL1, -2, -3		LMADS7
Spikelet meristem				WFUL1, -2, -3		
Floral meristem	OsMADS14, -15, -18			WFUL1, -2, -3		
Sepal/Lemma-Palea	OsMADS14, -15, -18	ZMM4, -15, ZAP1	HvBM14, -15, -18	WFUL1, -2, -3		
Petal/Lodicules	OsMADS14, -15	ZMM4, -15, ZAP1	HvBM14, -15, -18	WFUL1, -2, -3		
Labellum					OMADS10	
Stamen	OsMADS14, -18		HvBM14, -15, -18	WFUL1, -3	OMADS10	
Carpel	OsMADS14, -18		HvBM14, -15, -18	WFUL1, -3	OMADS10	LMADS5, -6
Vegetative leaves	OsMADS18	ZMM4, -15	HvBM18	WFUL1	OMADS10	LMADS5, -6
Vegetative stem		ZMM4, -15	HvBM18			LMADS5, -6, -7
Roots	OsMADS18	ZMM4, -15	HvBM18			

A

| | 180 | 190 | 200 | 210 | 220 | 230 | 240 | 250 253 |

(Sequence alignment showing taxa: AmPI, PI, PtPI1, PtPI2, ZMM18, ZMM29, HvBM2, WPI2, OsMADS2, ZMM16, OsMADS4, HvPI, WPI1, OMADS8, LMADS8, LMADS9, PtD, AmAP3, SILKY1, OsMADS16, WAP3-a, HvBM16, WAP3-b, WAP3-c, LMADS1, OMADS9, OMADS3, OMADS5, AP3, PtAP3)

B

Tissue　　　　　Species	Rice	Maize	Barley	Wheat	Orchid	Lily
Inflorescence meristem						
Spikelet meristem				WAP3/TaMADS#51		
Floral meristem		ZMM16, -18, -29		WAP3/TaMADS#51		
Sepal/Lemma-Palea					OMADS3, -5, -8	LMADS1, -8, -9
Petal/Lodicules	OsMADS2, -4, -16	SILKY1, ZMM16, -18, -29		WPI1, WPI2	OMADS3, -5, -8, -9	LMADS1, -8, -9
Labellum					OMADS3, -8, -9	
Stamen	OsMADS2, -4, -16	SILKY1, ZMM16, -18, -29		WPI1, WPI2	OMADS3, -8, -12	LMADS1
Carpel	OsMADS2, -4				OMADS3, -8, -12	LMADS1
Vegetative leaves					OMADS3, -8	
Vegetative stem						
Roots					OMADS8	

Class B

A

		240	250	260	270	280	290	299
GOA
WBsis30b
WBsis30c
WBsis30a
HvBM30
ZmBS2
OsMADS30
ZmBS3
OsMADS31
HvBM31
WBsis31b
WBsis31a
AmBsis
ZMM17
WBsis29c
HvBM29
WBsis29b
WBsis29a
PtBsis3
ABS
PtBsis2
PtBsis1

B

Class B_sister

Tissue \ Species	Rice	Maize	Barley	Wheat	Orchid	Lily
Inflorescence meristem						
Spikelet meristem						
Floral meristem						
Sepal/Lemma-Palea	OsMADS30					
Petal/Lodicules	OsMADS30					
Labellum						
Stamen	OsMADS30					
Carpel	OsMADS30	ZMM17		Wbsis		
Vegetative leaves	OsMADS30					
Vegetative stem	OsMADS30					
Roots	OsMADS30					
Seed	OsMADS29					

A

	270	280	290	300	310	320	326

AG
PtAG1
PtAG2
AmAG
ZMM2
ZMM23
OsMADS3
WAG2A
HvAG1
WAG2B
LMADS10
OMADS4
OsMADS58
ZAG1
HvAG2
WAG1

B

Tissue \ Species	Rice	Maize	Barley	Wheat	Orchid	Lily
Inflorescence meristem						
Spikelet meristem						
Floral meristem	OsMADS3, -58	ZAG1				
Sepal/Lemma-Palea						
Petal/Lodicules						
Labellum						
Stamen	OsMADS3, -58	ZMM2, -23		WAG1, -2	OMADS4	LMADS10
Carpel	OsMADS3, -58			WAG1, -2	OMADS4	LMADS10
Vegetative leaves						
Vegetative stem						
Roots						
Seed						

Class C

A

	80	90	100	110	120	130	140	150
ZAG2	SSSGPPLLEHNAQ	QFYQQESAKLRNQIQ	KLQNTNRHLVGDSVGNLSLKELKQLESRLEKGISKIRARKSELLAAEIS
ZMM1	SSSGPPLLEHNAQ	QFYQQESVKLRNQIQ	KLQNTNRHLVGDSVGNLSLKELKQLESRLEKGISKIRARKSELLAAEIN
OsMADS13	STSGAPLIEVNAQ	QYYQQESAKLRHQIQ	KLQNTNKHLVGDNVSNLSLKELKQLESKLEKGISKIRARKNELLASEIN
HvBM13	STSGAPLIEINAQ	QYYQQETAKMRHQIQ	KLQNTNKHLVGDSVGNLSLKELKQLESRLEKGISKIRARKNELLSSEIN
TaAG-3B	STSGVPLIEVNAQ	QYYQQESAARLRHQIQ	KLQSTNKHLVGDSVGNLSLKELKQLESRLEKGISKIRARKNELLSFEIN
WSTK	STSGVPLIEVNAQ	QYYQQESAKLRHQIQ	KLQSTNKHLVGDSVGNLSLKELKQLESRLEKGISKIRARKNELLSSEIN
TaAG-3A	STSGVPLIEVNAQ	QYYQQESAKLRHQIQ	KLQSTNKHLVGDSVGNLSLKELKQLESRLEKGISKIRARKNELLSSEIN
OsMADS21	SGSA-PVIDVNSH	QYFQQESAKMRHQIQ	KLQNANRHLIGESIGNMTAKELKSLENRLEKGISRIRSKKHELLFSEIE
HvBM21	SGSA-PAIDVNSQ	QYFQQESAKLRQQIG	NLQNANRHLMGDSVGNLTVKELKTLENRLDKSIRIRSKKHELLSAEIE
TaAG-4A	SGSA-PAIDVNSQ	QYFQQESAKLRHQIQ	SLQNANRNLMGESVGNLTLKELKSLENRLDKGISRIRAKKHELLFAEIE
TaAG-4B	SGSA-PAIDVNSQ	QYFQQESAKLRHQIQ	SLQNANRNLMGESVGNLTLKELKSLENRLDKGIGRIRAKKHELLFAEIE
STK	TNTS-TVQEINA-	AYYQQESAKLRQIQ	TIQNSNRNLMGDSLSLSLSVKELKQVENRLEKAISRIRSKKHELLLVBIE
PtAGL11-1	SNAS-SITEINA-	QYYQQESAKLRQIQ	KLQNSNRNLMGESVGNLSLKELKQLENRLEKGISRIRSKKHELLLAEIE
PtAGL11-2	SNTA-SITEINA-	QYYQQESAKLRQIQ	KLQNSNRNLMGESVGMIRIRIRSKKHELLLAEIE
OMADS2	SNSG-ALVEVNSQ	QYYQQESAKMRHQIQ	KLNNSSRHLMGELSSINLKELKQLENRLERGITRVRSKKHELLAEIE
AmAGL11	SSAT-SISEANS-	QYYQQEATKLRQQIQ	KLQNANRHMGDGLSADTIKELKQLEHRLERGLTRIRSKKHELLFABIE
LMADS2	SSNSNSLIQVNSQ	QYFQQESAKLRHQIQ	KITNANRHLVGEALSLTVKELKQLENRLERGLTRIRSKKHELLFABIE

B

Class D

Tissue \\ Species	Rice	Maize	Barley	Wheat	Orchid	Lily
Inflorescence meristem						
Spikelet meristem						
Floral meristem		*ZAG2*				
Sepal/Lemma-Palea						
Petal/Lodicules						
Labellum						
Stamen	*OsMADS21*			*WSTK, TaAG-4*		
Carpel	*OsMADS13, -21*	*ZMM1*		*WSTK, TaAG-4*	*OMADS2*	*LMADS2*
Vegetative leaves						
Vegetative stem						
Roots						
Seed	*OsMADS21*					

A

B

Class E

Tissue / Species	Rice	Maize	Barley	Wheat	Orchid	Lily
Inflorescence meristem				WLHS1, TaSEP-5		LMADS3, -4
Spikelet meristem		ZMM6, -14, -27, -24, -31		WSEP, TaSEP-5, TaMADS1		
Floral meristem	OsMADS1, -7, -8		HvBM1	TaSEP-5	OMADS11	
Sepal/Lemma-Palea	OsMADS1, -34	ZMM24	HvBM1	WLHS1, WSEP, TaSEP-6, -5	OMADS6, -11	LMADS3, -4
Petal/Lodicules	OsMADS7, -8	ZMM24	HvBM1, -7	WSEP, TaSEP-6	OMADS6, -11	LMADS3, -4
Labellum					OMADS6, -11	
Stamen	OsMADS7, -8, -34		HvBM7	WSEP, TaSEP-6, TaMADS1		LMADS3, -4
Carpel	OsMADS1, -7, -8	ZMM14, -24	HvBM1, -7	WSEP, TaSEP-6, TaMADS1	OMADS6, -11	LMADS3, -4
Vegetative leaves						LMADS3, -4
Vegetative stem						LMADS3, -4
Roots						
Seed		ZMM6, -27				

A Protein sequence alignment showing AGL6, OMADS1, PtAGL6, OsMADS17, OMADS7, AmAGL6, ZAG3, ZAG5, OsMADS6, TaAGL6-1C, TaAGL6-1B, HvBM6, TaAGL6-1A with position markers 220, 230, 240, 250, 260, 270, 280.

B

Tissue \ Species	Rice	Maize	Barley	Wheat	Orchid	Lily
Inflorescence meristem						
Spikelet meristem						
Floral meristem	OsMADS6, -17	ZAG3				
Sepal/Lemma-Palea	OsMADS6	ZAG3			OMADS7	
Petal/Lodicules	OsMADS6	ZAG3			OMADS7	
Labellum					OMADS1, -7	
Stamen	OsMADS17	ZAG3			OMADS7	
Carpel	OsMADS6	ZAG3			OMADS1, -7	
Vegetative leaves						
Vegetative stem						
Roots						
Seed						

Class AGL6