Grass plants develop unique floral patterns that determine grain production. However, the molecular mechanism underlying the specification of floral organ identities and meristem determinacy, including the interaction among floral homeotic genes, remains largely unknown in grasses. Here, we report the interactions of rice (Oryza sativa) floral homeotic genes, OsMADS3 (a C-class gene), OsMADS13 (a D-class gene), and DROOPING LEAF (DL), in specifying floral organ identities and floral meristem determinacy. The interaction among these genes was revealed through the analysis of double mutants.\textit{osmads13-3 osmads3-4} displayed a loss of floral meristem determinacy and generated abundant carpelloid structures containing severe defective ovules in the flower center, which were not detectable in the single mutant. In addition, in situ hybridization and yeast two-hybrid analyses revealed that OsMADS3 and DL did not regulate each other’s transcription or interact at the protein level. This indicates that OsMADS3 plays a synergistic role with OsMADS13 in both ovule development and floral meristem termination. Strikingly, \textit{osmads3-4 dl-sup6} displayed a severe loss of floral meristem determinacy and produced supernumerary whorls of lodicule-like organs at the forth whorl, suggesting that OsMADS3 and DL synergistically terminate the floral meristem. Furthermore, the defects of \textit{osmads13-3 dl-sup6} flowers appeared identical to those of \textit{dl-sup6}, and the \textit{OsMADS13} expression was undetectable in \textit{dl-sup6} flowers. These observations suggest that DL and OsMADS13 may function in the same pathway specifying the identity of carpel/ovule and floral meristem. Collectively, we propose a model to illustrate the role of OsMADS3, DL, and OsMADS13 in the specification of flower organ identity and meristem determinancy in rice.


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thought to be partially applicable in explaining grass floral development, grasses have diversified genetic components in specifying the identity of floral organs and meristem (Thompson and Hake, 2009). For example, loss-of-function mutants of the orthologs of Arabidopsis AP3 in maize (Silky1) and in rice (SUPERWOMEN1 [SPW1] or OsMADS16) display homeotic transformations of stamens to carpels and of lodicules to lemma- or palea-like structures, suggesting the conservation of class B genes between grasses and Arabidopsis (Ambrose et al., 2000; Nagasawa et al., 2003; Whipple et al., 2007). Grasses have duplicated and subfunctionalized C-class genes (Kramer et al., 2004; Zahn et al., 2006). For example, rice has two AG homologs, OsMADS3 and OsMADS58 (Kramer et al., 2004). OsMADS3 plays key roles in both stamen identity specification and late anther development, while OsMADS58 is crucial for specifying floral meristem determinacy and carpel architecture (Yamaguchi et al., 2006; Hu et al., 2011). Similarly, there is a pair of AG homologs in maize: Zea agamous1 (zag1) and Zea mays mads2 (zmm2). The zag1 gene has been shown to determine floral meristem determinacy, while the biological function of zmm2 remains unclear (Mena et al., 1996).

In rice, OsMADS13 is a D-class gene that is orthologous to Arabidopsis SEEDSTICK (STK) and to FLORAL BINDING PROTEIN7 (FBP7) and FBP11 in petunia (Petunia hybrida). Coexpression of FBP7 and FBP11 causes the conversion of ovules into carpelloid organs (Colombo et al., 1995). The osmads13 mutants are associated with homeotic transformation of ovules into carpelloid structures and indeterminate flowers (Dreni et al., 2007; Yamaki et al., 2011). This is in contrast to the mutation of the Arabidopsis STK gene, which does not display altered ovule identity (Pinyopich et al., 2003). In Arabidopsis, AG, STK, SHATTERPROOF1 (SHP1), and SHP2 are grouped in the monophyletic AG-like clade and have been shown to be involved in ovule identity specification. STK is the only D-lineage gene and is expressed in the ovule. stk single mutants develop a slightly abnormal ovule with a defect in funiculus development, while the stk shp1 shp2 triple mutant demonstrates the conversion of ovules into leaf-like or carpel-like organs (Favaro et al., 2003; Pinyopich et al., 2003). Furthermore, STK, SHP1, SHP2, and AG were shown to form multimeric complexes in yeast in the presence of SEP MADS box factors, and the defect of ovule development in sep1/SEP1 sep2 SEp3 is similar to that in the shp1 shp2 stk triple mutant (Favaro et al., 2002), suggesting the role of Arabidopsis SEP genes participating in ovule identity specification. In addition, AG was shown to be involved in specifying ovule identity by affecting the expression of SHP1 and SHP2 (Brambilla et al., 2007).

The rice DROOPING LEAF (DL) gene, which is orthologous to Arabidopsis CRABS CLAW (CRC), encodes a YABBY domain protein and plays a crucial role in specifying the carpel identity and floral meristem determinacy (Yamaguchi et al., 2004). Severe dl mutants display complete homeotic transformation of carpels into stamens, while mutations of CRC cause abnormal carpel development (Alvarez and Smyth, 1999; Yamaguchi et al., 2004). Moreover, DL/CRC interacts antagonistically with class B genes (Alvarez and Smyth, 1999; Yamaguchi et al., 2004), suggesting that DL and CRC play a conserved and diversified role in controlling carpel identity in rice and Arabidopsis, respectively.

Grasses have diversified SEP-like genes, with at least five SEP-like members (OsMADS1, OsMADS5, OsMADS7, OsMADS8, and OsMADS34) in rice (Malcomber and Kellogg, 2005; Zahn et al., 2005; Arora et al., 2007). OsMADS1 (also called LEAFY HULL STERILE1) has been characterized as a SEPALLATA (SEP)-like gene in rice, which is required for specifying the lemma/palea identity and the meristem of inner floral organs (Jeon et al., 2000; Prasad et al., 2001, 2005; Malcomber and Kellogg, 2004; Agrawal et al., 2005; Chen et al., 2006b). Knockdown of both OsMADS7 and OsMADS8 results in late flowering, homeotic transformations of lodicules, stamens, and carpels into palea/lemma-like organs, and a loss of floral determinacy. Simultaneous reduction of the expression of four rice SEP-like genes, OsMADS1, OsMADS5, OsMADS7, and OsMADS8, causes homeotic transformation of all floral organs except the lemma into leaf-like organs (Cui et al., 2010). OsMADS34 (also called PANICLE PHYTOMER2) plays a key role in controlling the development of inflorescences and spikelets in rice (Gao et al., 2010; Kobayashi et al., 2010). Moreover, investigations of the double mutant osmads34 osmads1 indicates that OsMADS34 and OsMADS1 redundantly specify the identities of floral organs, including the lemma/palea, lodicules, stamens, and carpel (Gao et al., 2010). All these data suggest the conserved and diversified functions of rice SEP-like genes in specifying flower organ identity. More recently AGAMOUS-LIKE6 (AGL6) genes in monocots and dicots have been also shown to play key roles in specifying floral organ and meristem identity (Hsu et al., 2003; Fan et al., 2007; Ohmori et al., 2009; Reinheimer and Kellogg, 2009; Rijkemma et al., 2009; Thompson et al., 2009; Li et al., 2010; Viene et al., 2010). AGL6-like genes are ancient and widely distributed in gymnosperms and angiosperms and form a sister clade to SEP-like genes (Purugganan et al., 1995; Theissen et al., 2000; Becker and Theissen, 2003; Zahn et al., 2005). Mutations in AGL6 homologous genes in grasses result in defective floral organ identity and meristem determinacy (Ohmori et al., 2009; Thompson et al., 2009; Li et al., 2010).

Although several genes are reported to play roles in specifying flower development in rice, their genetic interactions remain largely unknown. In this study, we characterized the genetic interaction of OsMADS3, DL, and OsMADS13 in specifying floral organs and floral meristem determinacy and provided new insights into the molecular mechanisms that regulate flower development in rice.
RESULTS

Identification of New Alleles of OsMADS3, OsMADS3, and DL

To identify rice mutants with floral defects, we screened a population of rice mutants for defective flowers in the japonica subspecies 9522 background treated by ^60Co γ-ray (280 Gy; Chen et al., 2006a). One mutant line displaying complete female sterility was identified. Genetic analysis and map-based cloning indicated that this mutant has a one-base deletion in the fifth exon in OsMADS13 (Os012g10540; Supplemental Fig. S1A), causing a frameshift at amino acid 132 and the formation of a premature stop codon. OsMADS13 expression was specifically reduced in pistils of the mutant (Supplemental Fig. S1B). As the first two mutants of OsMADS13 (osmads13-1 and osmads13-2) have been reported (Dreni et al., 2007; Yamaki et al., 2011) and a genetic analysis indicated that our mutant is allelic to the reported osmads13-1, we named this mutant osmads13-3. This mutation is not associated with obvious alteration of the outer three whorl organs, although some osmads13-3 flowers (31%) displayed three or four stigmas (n = 121; Fig. 1, A and Q) instead of two stigmas in wild-type flowers. Like the osmads13-1 mutant, osmads13-3 showed complete female sterility with aborted ovule development (Fig. 1, B and Q) and carpelloid structures (Supplemental Fig. S1, F and G). In addition, the ectopic expression of DL was observed in the carpelloid structures of osmads13-3 (Fig. 2, A–F), suggesting that these ectopic structures have the carpel identity.

Subsequently, we identified a new null mutant of DL, called dl-sup6, which was allelic with the reported dl-1 mutant (Nagasawa et al., 2003; Yamaguchi et al., 2004). Sequence analysis showed the insertion of one DNA fragment at the second intron of the DL gene (Supplemental Fig. S2A), which abolished the expression of DL in the mutant (Supplemental Fig. S2B). Because of five previously identified strong dl alleles (dl-sup1 to dl-sup5; Nagasawa et al., 2003; Yamaguchi et al., 2004), we named this mutant dl-sup6. Like the severe dl mutants, dl-sup6 displayed a phenotype of drooping leaves (Supplemental Fig. S2C) with ectopic stamens at the position of the carpel (Supplemental Figs. S2, E–H, and 4Q). Some flowers displayed a loss of floral meristem determinacy (Supplemental Fig. S2, F and J). In some cases, ectopic lodicule-like structures or fused anthers were observed in dl-sup6 (Supplemental Fig. S2, E–G). Scanning electronic microscopy (SEM) observation revealed that dl-sup6 flowers developed normally at stage Sp6 (Supplemental Fig. S2I). The staging of flowers refers to a previous report (Ikeda et al., 2004). At stage Sp7 or Sp8, dl-sup6 flowers generated ectopic stamen primordia (Supplemental Fig. S2J). In addition, dl-sup6 lemmas displayed alternating numbers of vascular tissues, with three, four, or five vascular bundles (Supplemental Fig. S2L), while the wild-type lemma had the characteristic five vascular bundles (Yuan et al., 2009), suggesting an important role of DL in specifying lemma identity.

In addition, we recently characterized a new weak allele of OsMADS3 called osmads3-4, which is allelic to osmads3-1 (Hu et al., 2011). In osmads3-4, a two-base deletion was observed in the fifth exon of OsMADS3, leading to premature translational termination at amino acid 137 within the K domain. osmads3-4 flowers developed ectopic lodicule-like structures in whorl 2 and lodicule-like or lodicule-anther mosaic organs in whorl 3 (Fig. 1, C, J, and R). Unlike severe allele osmads3-3 (Yamaguchi et al., 2006), most osmads3-4 flowers displayed normal pistil development in the forth whorl (Fig. 1D).

OsMADS3 and OsMADS13 Synergistically Specify Ovule Identity and Floral Meristem Determinacy

To investigate the genetic interaction between OsMADS3 and OsMADS3 in determining rice flower development, we constructed the double mutant osmads13-3 osmads3-4. osmads13-3 osmads3-4 flowers displayed similar developmental defects in the second and third whorls to osmads3-4 (Fig. 1, E and S). Surprisingly, osmads13-3 osmads3-4 flowers displayed indeterminate floral development with supernumerary whorls of carpelloid structures without detectable ovule morphology in the flower center (Fig. 1, E–H), which was not observed with the corresponding single mutant. SEM observation showed that osmads13-3 osmads3-4 floral meristem was similar to that of osmads3-4 at stage Sp6 during the formation of stamen primordia (Fig. 1, J and K). At early stage Sp8, when the wild-type flower displays one carpel primordium in the fourth whorl and the floral meristem terminates (Fig. 1L), osmads13-3 osmads3-4 generated both primary and secondary carpel primordia and the floral meristem still persisted (Fig. 1, M and N), suggesting that floral stem cells are not terminated in a timely manner in the double mutant (Fig. 1S). In support of this, the expression of OSH1, a maker gene of rice floral meristem (Yamaki et al., 2005), was detectable in the indeterminate floral meristem of osmads13-3 osmads3-4 at stage Sp8, while the floral meristem in the wild-type flowers had been consumed at the same stage (Fig. 1, O and P). These observations suggest that OsMADS13 and OsMADS3 play synergistic roles in ovule development and determinacy of the floral meristem.

To further elucidate the mechanism of OsMADS13 and OsMADS3 in floral development, a yeast two-hybrid experiment was performed, and we observed no interaction of these two proteins, as judged by the growth condition in selective culture medium (Supplemental Fig. S3). RNA in situ hybridization analysis indicated that the OsMADS13 expression pattern was not obviously reduced in osmads3-4 at stage Sp8, when the ovule forms (Fig. 3, A–C), and the OsMADS3 mRNA signal was not obviously changed in osmads13-3 (Fig. 3G). Thus, OsMADS13 and OsMADS3 do not seem to influence each other at the transcriptional level.
To further characterize the potential interaction between OsMADS3 and DL in controlling rice flower development, the double mutant osmads3-4 dl-sup6 was constructed. Morphological observations indicated that osmads3-4 dl-sup6 flowers had an altered vascular pattern in the lemma, resembling that of dl-sup6 (data not shown), suggesting that DL controls lemma identity independent of OsMADS3. This is consistent with the lack of expression of OsMADS3 in whorl 1 (Yamaguchi et al., 2006). The floral organs in the second and third whorls of osmads3-4 dl-sup6 appeared similar to those of osmads3-4 (Fig. 4, A, B, Q, and R, compared with Fig. 1). Furthermore, osmads3-4 dl-sup6 developed ectopic floral organ primordia that were similar to those of osmads3-4 at stage Sp6 (Fig. 4F, compared with Fig. 1G), suggesting that OsMADS3 functions in lodicule and stamen develop-
ment independent of DL. This is in agreement with the fact that DL is not expressed in lodicules and stamens (Fig. 2; Yamaguchi et al., 2004). Strikingly, osmads3-4 dl-sup6 flowers generated supernumerary whorls of undifferentiated lodicule-like organs in the position of the pistil, which seemed to be arranged in bilateral symmetry along the elongated axis (Fig. 4, C–F). In addition, the floral meristem was observed on the top of the axis (Fig. 4E). This phenotype implies a severe loss of floral meristem determinacy, which was further confirmed by the in situ hybridization of OSH1 mRNA (Fig. 4H). SEM observation showed that at the early stage Sp8, the osmads3-4 dl-sup6 flower violated the normal development process and formed an indeterminate floral meristem in the flower center (Fig. 4G). Transverse section analysis indicated that these underdeveloped tissues were morphologically close to those of lodicules, with the characteristic pattern of vascular bundles (Fig. 4, I–K). Also, this indication was confirmed by the SEM observation that the morphology of epidermal cells of these underdeveloped tissues appeared similar to that of lodicules (Fig. 4, L and M). Meanwhile, the mRNA of the rice B-class gene SPW1 (OsMADS16), which accumulates in wild-type lodicules and stamens (Fig. 4N; Nagasawa et al., 2003), was detectable in these undifferentiated organs (Fig. 4O). This was combined with the presence of transcripts of the putative class A gene OsMADS15 (also called Degenerative Palea [DEP]; Wang et al., 2010a) in the undifferentiated tissues within the flower center of osmads3-4 dl-sup6 (Fig. 4P). In addition, the normal expression pattern of DL was detectable in osmads13-3 (Fig. 2G) and OsMADS3 expression was detected in ectopic stamens of dl-sup6 (Fig. 3H), suggesting that OsMADS3 and DL do not affect the expression of each other at the transcriptional level. These results suggest that OsMADS3 and DL may define the floral meristem in parallel during rice flower development.

**Analysis of the Interaction between OsMADS13 and DL**

To determine the relationship between OsMADS13 and DL, we constructed the osmads13-3 dl-sup6 double mutant, and osmads13-3 dl-sup6 displayed flower defects similar to those of dl-sup6 (Fig. 5; Supplemental Fig. S2). Moreover, in situ analysis showed that OsMADS13 transcripts were not obviously detected in dl-sup6 flowers (Fig. 3D). In contrast, DL expression was ectopically observed in the indeterminate organ within the carpel in one osmads3-13-3 flower. The signal is indicated by the arrow. G, Normal expression of DL in osmads3-4 at stage Sp7. H, Ectopic expression of DL in osmads3-4 osmads13-3 at stage Sp8. ca, Carpel; fm, floral meristem; le, lemma; lo, lodicule; pa, palea; st, stamen. Bars = 50 μm in A and 100 μm in B to H.
OsMADS3 transcripts were observed in the ab-
osmads13-3 normal ovule in stage Sp8. Bars = sup6 at stage Sp8. ca, Capel; es, ectopic stamen; fm, floral meristem; lo, lodicules; st, stamen. E, The expression of OsMADS3 in the wild-type stamen primordia at stage Sp6. F, At stage Sp8, there is detectable expression of OsMADS3 in the wild-type ovule. G, OsMADS3 transcripts were observed in the ab-

**DISCUSSION**

**Rice Has a Conserved and Diversified Mechanism Controlling Ovule Identity**

Ovule development is of importance in the plant life cycle. The ovule is the source of the megagametophyte and the precursors of seeds, consisting of the nucleus, integument(s), and funiculus (Reiser and Fischer, 1993; Colombo et al., 2008). Previous studies in petunia, Arabidopsis, and rice revealed that the MADS box genes belonging to the AG clade are necessary for specifying ovule identity.

In rice, the AG clade contains four MADS box members: two C-lineage genes, OsMADS3 and OsMADS58, and two D-lineage genes, OsMADS13 and OsMADS21 (Kramer et al., 2004; Zahn et al., 2006). The expression of OsMADS13 is restricted in the ovule, which is very similar to that of STK, FBP7, and FBP11. In contrast, OsMADS21 is mainly expressed in developing seeds (Lee et al., 2003; Dreni et al., 2007) and was thought to play a minor role in controlling ovule development (Dreni et al., 2007). Grass species including maize, wheat (Triticum aestivum), barley, and rice have duplicated C-class genes (Mena et al., 1996; Kramer et al., 2004; Yamaguchi et al., 2006). To date, there is no evidence indicating that class C genes are required for carpel identity in grasses (Thompson and Hake, 2009). In rice, analyses of mutations of OsMADS3 and knockdown of OsMADS58 suggested that the two C-class genes have subfunctionalized and redundant functions in rice flower development (Yamaguchi et al., 2006; Hu et al., 2011; M.M. Kater, personal communication; Fig. 6). osmads3-3 is a strong allele of OsMADS3, displaying homeotic transformation of nearly all stamens in whorl 3 into lodicule-like organs, suggesting a major role of OsMADS3 in stamen specification (Yamaguchi et al., 2006). The intermediate mutant osmads3-4 displays defective postmeiotic anther development with an abnormal accumulation of reactive oxygen species. OsMADS3 was also shown to directly regulate the expression of MT-1-4b, which encodes a type 1 small Cys-rich and metal-binding protein with superoxide anion- and hydroxyl radical-scavenging activity, suggesting that OsMADS3 is a key transcriptional regulator in rice male reproductive development, at least in part by regulating reactive oxygen species homeostasis through MT-1-4b (Hu et al., 2011). Previously, OsMADS58 was shown to play a key role in regulating floral meristem determinacy and normal carpel morphogenesis by the analysis of OsMADS58 RNA-silenced lines (Yamaguchi et al., 2006). However, a T-DNA insertion knockout mutant of OsMADS58 was recently identified and showed no obvious floral defects (M.M. Kater, personal communication). The osmads3-4 osmads58 double mutant displayed more severe defects of inner floral organs and meristem determinacy, suggesting that OsMADS58 and OsAMDS3 redundantly regulate inner floral organ identity and flower determinacy (M.M. Kater, personal communication). Therefore, it will be interesting to investigate the genetic interaction of OsMADS58 with OsMADS13 and DL in the future.

Similarly, two duplicated AG homologs (zag1 and zmm2) are present in the maize genome, and mutations in zag1 cause loss of floral meristem determinacy in the ear, without obvious alteration of floral organ identity (Mena et al., 1996). Currently, no mutants of zmm2 have
been identified, but the expression pattern of *zm2* is in agreement with that of class C function (Mena et al., 1996). Here, our genetic analysis of the double mutant *osmads13-3 osmads3-4* indicated that *OsMADS3* plays a critical role in ovule formation and floral meristem determinacy redundantly with *OsMADS13* (Fig. 6). These data also support that the C-class and D-class genes probably retain their functions even though they underwent multiple subfunctionalization events and several neofunctionalizations after duplication within the *AG* clade (Rijpkema et al., 2010).

In rice, the YABBY domain gene *DL* was shown to be crucial for carpel specification (Nagasawa et al., 2003; Yamaguchi et al., 2004), which is different from the well-known ABC genes. In addition, the role of *DL* is distinct from the closely related YABBY gene CRC of Arabidopsis, which plays a mild role in carpel development (Alvarez and Smyth, 1999; Bowman and Smyth, 1999; Yamaguchi et al., 2004). Analysis of *osmads3-4 dl-sup6* flowers indicated that *DL* and *OsMADS3* play a redundant role in terminating floral meristem, but they may function in a distinct pathway (Fig. 6). The ectopic expression of *SPW1* in the supernumerary whorls of indeterminate organs of the double mutant flower may be explained by the antagonistic role of *DL* in reacting to class B genes in the flower center (Yamaguchi et al., 2004; this study). The ectopic expression of the putative class A gene *OsMADS15* in the floral center may be caused by the mutation of *OsMADS3*. In Arabidopsis and *Antirrhinum*, A- and

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**Figure 4.** Flower phenotype of *dl-sup6 osmads3-4*. A, One *dl-sup6* flower showing ectopic stamens in the center. B, One *dl-sup6 osmads3-4* flower showing the phenotypes in the second and third whorls similar to *osmads3-4*. C, Closeup of a *dl-sup6 osmads3-4* flower showing mosaic organs and indeterminate organs in the center. D, SEM observation of one *dl-sup6 osmads3-4* flower showing the supernumerary whorls of indeterminate undifferentiated organs in the floral center. E, Closeup of D. F, SEM observation of one *dl-sup6 osmads3-4* flower at stage Sp6. G, SEM observation of one *dl-sup6 osmads3-4* flower after stage Sp7 showing ectopic organs and indeterminate meristem. H, Expression pattern of *OSH1* in the *dl-sup6 osmads3-4* flower at stage Sp8. I and J, Transverse sections of one *dl-sup6 osmads3-4* flower showing the ectopic organs in the flower center. K, Transverse section of one wild-type lodicule. L and M, SEM analysis of epidermal cells of *osmads3-4 dl-sup6* ectopic organs and wild-type lodicules, respectively. N, Expression of *SPW1* in wild-type lodicules and stamens. O, Transcripts of *SPW1* detectable in lodicule-like organs of one *dl-sup6 osmads3-4* flower center. P, *OsMADS15* is expressed in lodicule-like organs in one *dl-sup6 osmads3-4* flower center. Q and R, Floral diagrams of *dl-sup6* (Q) and *osmads3-4 dl-sup6* (R). est, Ectopic stamen; fm, floral meristem; l-a, lodicule-anther mosaic organs; ll, lodicule-like structure; lo, lodicules; mtp, marginal tissue of the palea; st, stamen. Bars = 1 mm in A, B, and D; 500 µm in C; 100 µm in E, H to K, and N to P; 50 µm in F; and 20 µm in L and M.
C-class genes were shown to be antagonistic to each other (Coen and Meyerowitz, 1991). Given the conserved role of the C gene in plant flower development, in combination with the ectopic formation of lodicule-like organs in some $dl$-sup6 flowers, we hypothesize that OsMADS3 and DL likely inhibit the expression of putative class A genes such as OsMADS15 in inner flower organs (Fig. 6).

The rice genome contains four putative A-class genes encoding AP1/FRUITFULL-like proteins: OsMADS14, OsMADS15, OsMADS18, and OsMADS20 (Fornara et al., 2004; Kater et al., 2006; Preston and Kellogg, 2006). However, few class A mutants have been identified in addition to those in Arabidopsis, and the roles of class A genes in floral organ identity are not as clear as was hypothesized by the ABCDE model (Preston and Kellogg, 2006). Unfortunately, besides the dep mutant, no other single or double knockout mutant lines for these rice genes have been reported. The dep mutant containing a single nucleotide G-to-C substitution at position 94 of the first exon of OsMADS15 displayed shrunken paleas and slightly elongated lemmas and glumes (Wang et al., 2010a), which are different from the mutant phenotype of class A genes AP1 and AP2 in Arabidopsis, with the conversion of sepals into leaf- or bract-like structures and petals into stamen-like organs or loss of sepals (Mandel et al., 1992; Jofuku et al., 1994). Therefore, whether DEP functions as an Arabidopsis A-class gene in rice flower development remains to be investigated. AP2 transcription factors in maize and rice have been shown to regulate shoot apical meristem determinacy. In maize, indeterminate spikelet 1 (ids1) and the paralog of ids1, sid1, are required for floral meristem determinacy, and ids1 sid1 double mutants have no floral meristem, which was replaced by the formation of many bract-like organs, terminating in an ovule-like structure (Chuck et al., 2008). Similarly, mutations in the ids1-like gene SUPERNUMERARY BRACT in rice result in a delayed transition of spikelet meristem to floral meristem, with additional bract-like organs (Lee et al., 2007).

Furthermore, in this study, our finding suggests that OsMADS13 and DL specify carpel/ovule and floral meristem identity in the same pathway. Besides the observation that $osmads13-3$ $dl$-sup6 displayed flower defects similar to that of $dl$-sup6, no obvious OsMADS13 expression was detectable in $dl$-sup6 flowers, and DL transcripts were ectopically detected in osmads13-3 flowers, suggesting that DL may directly or indirectly regulate OsMADS13 expression. In other words, loss of OsMADS13 expression in $dl$-sup6 may result from the altered carpel/ovule identity in $dl$-sup6, or DL regulates carpel/ovule and meristem identity by controlling OsMADS13 expression. Furthermore, the ectopic ex-

Figure 5. Flower phenotypes of osmads13-3 $dl$-sup6. A, One osmads13-3 $dl$-sup6 flower with a weak phenotype, in which several ectopic stamens formed. B, One osmads13-3 $dl$-sup6 flower with a severe phenotype.

Figure 6. Proposed model to illustrate the genetic interaction between OsMADS3, OsMADS13, and DL in rice flower development. A, Interactions between rice floral organ homeotic genes of A-function genes (such as OsMADS15), SPW1, OsMADS3, OsMADS13, and DL. Different colors represent the expression patterns of genes in lodicules, stamens, the carpel, and the ovule. OsMADS3 possibly represses the expression of A-function genes such as OsMADS15 in the inner floral organs; DL may antagonize the expression of SPW1 and OsMADS15. While OsMADS13 may indirectly limit the expression of DL in the ovule, DL may directly or indirectly positively regulate OsMADS13 expression. The broken arrow indicates the possibly indirect or direct regulation of the OsMADS13 expression by DL. B, Functions of OsMADS3, DL, and OsMADS13 in specifying floral organ identities and floral meristem termination. Green lines and red arrows indicate the functions of repression and promotion, respectively. OsMADS3 regulates the number of lodicules in whorl 2 by suppressing lodicule development, particularly near the palea (Yamaguchi et al., 2006), represses the formation of lodicules and determines the stamen identity in whorl 3, and specifies ovule identity in the floral center. DL represses the formation of stamens and specifies the carpel identity in the flower center, while OsMADS13 represses carpel formation and determines ovule identity. OsMADS13 may terminate floral meristem terminal in parallel with OsMADS3, and DL may regulate the floral meristem determinacy in the same pathway of OsMADS13. OsMADS3 and DL can redundantly terminate the floral meristem.
pression of DL in osmads13-3 is likely caused by the altered identities of ovule and meristem, and OsMADS13 may indirectly restrict the expression of DL in the ovule (Fig. 6).

**Regulation of Rice Floral Meristem Termination**

Floral organs are formed by a floral meristem, a pool of pluripotent and dividing cells (Prunet et al., 2009). The regulation of the floral meristem seems to be widely conserved among angiosperms (Ferrario et al., 2004; Prunet et al., 2009). In Arabidopsis, AG is a master regulator terminating the floral meristem by turning WUSCHEL (WUS) off (Sieburth et al., 1998; Sun et al., 2009). In addition to homeotic transformations of stamens into petals, strong ag alleles (ag-1-ag-3) showed a complete loss of floral meristem determinacy, and the carpel was replaced by a new flower (Bowman et al., 1989, 1991; Yanofsky et al., 1990). The genomes of both eudicot and monocot species, including Antirrhinum, rice, maize, and barley, contain duplicated and sub-functionalized AG homologs (Zahn et al., 2006). Recent analysis of the osmads3-4 osmads58 double mutant suggests that two rice C-class genes, OsMADS3 and OsMADS58, redundantly regulate floral meristem determinacy (M.M. Kater, personal communication). In Antirrhinum, the class C MADS box gene PLENA (PLE) specifies reproductive organ identity and floral meristem termination, and the phenotype of ple mutants is similar to ag mutants, with homeotic conversion of reproductive organs to perianth organs (with the exception of nested flowers appearing inside whorl 4 instead of whorl 3 in strong ag mutants) and a loss of floral determinacy. In contrast, the mutation of FAR-INELLI (FAR), the close paralog of PLE, displayed normal flower development only with partial male sterility (Bradley et al., 1993; Davies et al., 1999). Moreover, the B-class MADS box genes DEF and GLO, which are not normally expressed in the fourth whorl, appeared to be ectopically expressed in ple far double mutants, suggesting a distinct role of the C class in Antirrhinum genes from that in Arabidopsis in redundantly and negatively regulating the B-function MADS box genes.

It is known that AG regulates the floral meristem by indirectly repressing the expression of WUS (Lenhard et al., 2001). Recently, KNÜCKLES (KNU) encoding a C2H2 zinc-finger protein was shown to serve as the mediator in this feedback loop (Sun et al., 2009). AG directly regulates the expression of KNU, which can negatively regulate WUS expression (Sun et al., 2009). It remains unclear whether there is a similar mechanism in grasses. In this work, our genetic analyses elucidate the role of OsMADS3, OsMADS13, and DL in floral meristem determinacy (Fig. 6). There are 13 WOX (for WUSCHEL-related homeobox gene family) members in the rice genome, and OsWUS was found to be closely related to the Arabidopsis WUS gene (Nardmann and Werr, 2006; Dai et al., 2007; Nardmann et al., 2007; Zhang et al., 2010). But the biological function of OsWUS remains unclear. Nardmann and Werr (2006) isolated two WUS homologs (ZmWUS1 and ZmWUS2) in maize and rice OsWUS and found that they were not expressed in the organizing center of the vegetative shoot apical meristem, as was the WUS gene in Arabidopsis.

Similar to the role of eudicot SEP-like genes in floral meristem determinacy, grass SEP- and AGL6-like genes are capable of regulating carpel/ovule development and floral meristem determinacy (Jeon et al., 2000; Prasad et al., 2001, 2005; Agrawal et al., 2005; Chen et al., 2006b; Ohmori et al., 2009; Reinheimer and Kellogg, 2009; Thompson et al., 2009; Cui et al., 2010; Gao et al., 2010; Kobayashi et al., 2010; Li et al., 2010). However, how these genes regulate floral organ identity and meristem determinacy in grasses remains less understood. It is likely that SEP-like and/or AGL6-like proteins act as mediators that constitute multimeric complexes with MADS domain proteins from different clades to regulate flower development in grasses (Immink et al., 2009; Seok et al., 2010; Wang et al., 2010b). In maize, double mutants of the AGL6-like gene bearded-ear (bde) and the class C gene zag1 display a severe ear phenotype with the conversion of floral meristems to branch-like meristems, which is not detectable in either single mutant, suggesting that bde and zag1 redundantly specify floral meristem identity (Thompson et al., 2009). Moreover, BDE and ZAG1 can physically interact, suggesting that these two proteins act in complexes to control floral development in the maize ear (Thompson et al., 2009). OsMADS7 (also called OsMADS45) and OsMADS8 (also called OsMADS24) were shown to have a similar interaction profile to those of Arabidopsis SEP proteins (Kater et al., 2006; Cui et al., 2010). They can interact with the AG-like protein OsMADS13, which is similar to STK. OsMADS7 and OsMADS8 also interact with Arabidopsis STK and petunia FBP7 (Favaro et al., 2002, 2003).

In summary, this study reveals the genetic interaction of the floral homeotic genes OsMADS3, OsMADS13, and DL and describes an unknown model to illustrate the role of OsMADS3, DL, and OsMADS13 in the specification of flower organ identity and meristem determinacy in rice.

**MATERIALS AND METHODS**

**Plant Materials**

The mutants osmads13-3 and dl-sup6 were identified from an M2 population of Oryza sativa subspecies japonica ‘9522’ mutagenized with radiation of 60Co γ-ray (Chen et al., 2006a). The strong allele of OsMADS13 (osmads13-1) and the weak allele (dl-2) were kindly provided by Prof. Martin M. Kater (Università degli Studi di Milano) and Prof. Hiro-Yuki Hirano (University of Tokyo), respectively. Prior to the analysis, osmads13-3, osmads3-4, and dl-sup6 were all crossed with wild-type 9522 three times. Double mutant plants were isolated by phenotype observation and verified by genotyping with primers 3TPF/3TPR and 13TPF/13TPR for osmads3-4 and osmads13-3, respectively (Supplemental Table S1). Mutant and wild-type rice plants were planted in paddy fields under normal conditions in Shanghai or in a greenhouse at Shanghai Jiao Tong University.
Histological Analysis and Microscopy Observation

Materials were fixed and dehydrated as described by Li et al. (2006). For histological analysis, tissues were substituted by xylene and embedded in Paraplast plus. Then, materials were sectioned to 8 μm thick, stained with toluidine blue, and photographed using a Nikon E600 microscope and a Nikon DXM1200 digital camera. SEM observation was performed with JSM-6300LV (JEOL) as described previously (Li et al. 2006). The dividing of the ovule stages refers to a previous report (Lopez-Dee et al., 1999).

In Situ Hybridization

Treatment of samples was as described previously (Li et al., 2006). For the construction of specific probes for OsMADS13, SPW1/OsMADS16, and DL gene-specific fragments of OsMADS13 cDNA (367–958 bp), OsMADS16 cDNA (211–686 bp), and DL cDNA (121–639 bp) were amplified by reverse transcription (RT)-PCR using primers 13FF/13FP, 16FF/16PPR, and DLPPF/DLPPR, respectively (Supplemental Table S1) and cloned into pbLue-script II KS+ phagemid vector (Stratagene). The probe construct of cDNA (211–686 bp), and

Yeast Two-Hybrid Analysis

The MATCHMAKER GAL4 Two-Hybrid System (Clontech) was used to detect the interaction between OsMADS5 and OsMADS13. cDNA fragments encoding the ICD domain of OsMADS5 and OsMADS13 were amplified by RT-PCR with primers 3YF/3YR and 13YF/13YR, respectively (Supplemental Table S1), and the cDNA fragment encoding the ICD1 domain of OsMADS6 was amplified by RT-PCR with primers 6YF/6YR (Supplemental Table S1). Then, these cDNA fragments were cloned into pGBK7 and pGAD7T to fuse with the BD (bait domain) and AD (activation domain) of GAL4, respectively. Recombinant vectors were named AD-13, BD-13, AD-3, BD-3, AD-6, and BD-6 respectively. Self-activation was assayed on selective synthetic dropout medium plates (∼Leu−/His+/3-amino-1,2,4-triazole-3-AT or ∼Trp−/His+/3-AT). Then, combinations of AD-3/BD-3 and BD-3/AD-13 were transformed into yeast strain AH109 simultaneously according to the protocol. The transformants cotransformed with plasmids encoding OsMADS6 and OsMADS13 were used as a positive control (Favaro et al., 2002), and the transformants containing plasmids pGAD7T and pGBK7T were used as a negative control. The interaction was judged by the growth condition on selective medium (2 His/+3-AT) according to the protocol from the company.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Schematic representation of osmads13 mutants and abnormal ovule development of osmads13-3.

Supplemental Figure S2. Schematic representation of dl-sup mutants and the phenotype of dl-supp.

Supplemental Figure S3. OsMADS5 does not interact with OsMADS13 in yeast cells.

Supplemental Table S1. Primers used in this research.

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LITERATURE CITED

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

Alvarez J, Smyth DR (1999) CRABS CLAW and SPATULA, two Arabidopsis genes that control carpel development in parallel with AGAMOUS. Development 126: 2377–2386

Ambrose BA, Lerner DR, Ciceri P, Padilla CM, Yanosky MF, Schmidt RJ (2000) Molecular and genetic analyses of the silky1 gene reveal conservation of floral organ specification between eudicots and monocots. Mol Cell 3: 569–579

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

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

Brecker A, Theissen G (2005) The major clades of MADS-box genes and their role in the development and evolution of flowering plants. Mol Phylogenet Evol 29: 464–489

Bowman JL, Dews GN, Meyerowitz EM (1991) Expression of the Arabidopsis floral homeotic gene AGAMOUS is restricted to specific cell types late in flower development. Plant Cell 3: 749–758

Bowman JL, Smyth DR (1999) CRABS CLAW, a gene that regulates carpel and nectary development in Arabidopsis, encodes a novel protein with zinc finger and helix-loop-helix domains. Development 126: 2387–2396

Bowman JL, Smyth DR, Meyerowitz EM (1989) Genes directing flower development in Arabidopsis. Plant Cell 1: 37–52

Bradley D, Carpenter R, Sommer H, Hartley N, Coen E (1993) Complementary floral homeotic phenotypes result from opposite orientations of a transposon on the plemis locus of Antirrhinum. Cell 72: 85–95

Brambilla V, Battaglia R, Colombo M, Masiero S, Bencivenga S, Kater MM, Colombo L (2007) Genetic and molecular interactions between BELL1 and MADS box factors support ovule development in Arabidopsis. Plant Cell 19: 2544–2556

Chen L, Chu H, Yuan Z, Zhang DB (2006a) Isolation and genetic analysis for rice mutants treated with 60 Co γ-rays. J Xiamen Univ (Nat Sci) 45: 82–85

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

Chu HW, Qian Q, Liang WQ, Yin CS, Tan HX, Yao X, Yuan Z, Yang J, Huang H, Luo D, et al. (2006) The Floral Organ Number4 gene encoding a putative ortholog of Arabidopsis CLAVATATA regulates apical meristem size in rice. Plant Physiol 142: 1029–1052

Chuck G, Meeley R, Hake S (2008) Floral meristem initiation and meristem cell fate are regulated by the maize AP2 genes ids1 and ids1. Development 135: 3013–3019

Clifford H (1987) Spikelet and Floral Morphology. Smithsonian Institution Press, Washington, DC

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

Colombo L, Battaglia R, Kater MM (2008) Arabidopsis ovule development and its evolutionary conservation. Trends Plant Sci 13: 444–450

Colombo L, Franken J, Koetje E, van Went J, Dons HJ, Angenent GC, van Tunen AJ (1995) The petunia MADS box gene FBI1 determines ovule identity. Plant Cell 7: 1859–1868

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

Dai M, Hu Y, Zhao Y, Liu H, Zhou DX (2007) A WUSCHEL-LIKE HOME-OXO gene represses a YABBY gene expression required for rice leaf development. Plant Physiol 144: 380–390

Davies B, Motte P, Keck E, Saedler H, Sommer H, Schwarz-Sommer Z (1999) PLENA and FARINELLI: redundancy and regulatory interactions between two Antirrhinum MADS-box factors controlling flower development. EMBO J 18: 4023–4034

Ditta G, Pinophich A, Robles P, Pelaz S, Yanosky MF (2004) The SEP4 gene of Arabidopsis thaliana functions in floral organ and meristem identity. Curr Biol 14: 1935–1940
Dreni L, Jacchia S, Fornara E, Fornari M, Ouwerverk PB, An G, Colombo L, Kater MM (2007) The D-lineage MADS-box gene OsMADS13 controls ovule identity in rice. Plant J 52: 690–699

Fan J, Li W, Dong X, Guo W, Shu H (2007) Ecotropic expression of a hyacinth AGL6 homolog caused earlier flowering and homeotic conversion in Arabidopsis. Sci China C Life Sci 50: 676–689

Favaro R, Immink RG, Ferioli V, Bernasconi B, Byzova M, Angenent GC, Kater M, Colombo L (2002) Ovule-specific MADS-box proteins have conserved protein-protein interactions in monocot and dicot plants. Mol Genet Genomics 268: 152–159

Favaro R, Pinyopich A, Battaglia R, Kooller M, Borghi L, Ditta G, Yanofsky MF, Kater MM, Colombo L (2003) MADS-box protein complexes control carpel and ovule development in Arabidopsis. Plant Cell 15: 2603–2617

Ferrasio S, Immink RG, Angenent GC (2004) Conservation and diversity in flower lard. Curr Opin Plant Biol 7: 84–91

Fornara F, Parenicová L, Falasca G, Pelucchi N, Masiero S, CiannaS M, Lopez-Dee Z, Altamura MM, Colombo L, Kater MM (2004) Functional characterization of OsMADS18, a member of the APL/QUA subfamily of MADS box genes. Plant Physiol 135: 2207–2219

Gao XC, Liang WQ, Yin CS, Ji SM, Wang H, Xu X, Guo CC, Kong HZ, Xue HP, Zhang DB (2010) The SEPALATA-like gene OsMADS34 is required for rice inflorescence and spikelet development. Plant Physiol 153: 728–740

Grass Phyllology Working Group (2001) Phyllology and subclamatic classification of the grasses (Poaceae). Ann Mo Bot Gard 88: 373–457

Hsu HF, Huang CH, Chou LT, Yang CH (2003) Ecotropic expression of an orchid (Oncidium ‘Romeo Kasze’) AGL6-like gene promotes flowering by activating flowering time genes in Arabidopsis thaliana. Plant Cell Physiol 44: 783–794

Hu LF, Liang WQ, Yin CS, Cui X, Zong J, Wang X, Hu JP, Zhang DB (2011) Rice MADS3 regulates ROS homeostasis during late anther development. Plant Cell 23: 515–533

Ikeda K, Nakasawa N, Nagato Y (2004) Developmental course of inflorescence and spikelet in rice. Breed Sci 54: 147–156

Immink RG, Tonaco IA, de Folter S, Shchennikova A, van Dijk AD, Busscher-Hu LF, Liang WQ, Yin CS, Cui X, Zong J, Wang X, Hu JP, Zhang DB (2006) The shoot stem cell niche in angiosperms: expression patterns of WUS orthologues in rice and maize imply major modifications in the course of mono- and dicot evolution. Mol Biol Evol 23: 2492–2504

Johann J, Zimmermann D, Durantini D, Kranz E, Werr W (2007) WOX gene phylogeny in Poaceae: a comparative approach addressing leaf and embryo development. Mol Biol Evol 24: 2474–2484

Liu C, Thong Z, Yu H (2009) Coming into bloom: the specification of floral meristems. Development 136: 3379–3391

Lopez-Dee ZP, Wittich P, Enrico Pe M, Rigola I, Del Buono I, Gorla MS, Kater MM, Colombo L (1996) OsMADS13, a novel rice MADS-box gene expressed during ovule development. Dev Genet 25: 237–244

Malcomber ST, Kellogg EA (2004) Heterogeneous expression patterns and separate roles of the SEPALATA LEAFY HULL STERILE1 genes in grasses. Plant Cell 16: 1692–1706

McLendon ST, Kellogg EA (2005) SEPALATA gene diversification: brave new whorls. Trends Plant Sci 10: 427–435

Mena M, Ambrose BA, Meeley RB, Briggs SP, Yanofsky MF, Schmidt RJ (1996) Diversification of C-function activity in maize flower development. Science 274: 1537–1540

Nagasa A, MiyiShi M, Sano I, Satoh H, Hirano H, Sakai H, Nagato Y (2002) SUPERWOMAN1 and DROOPING LEAF genes control floral organ identity in rice. Development 130: 705–718

Ohmori S, Kimizu M, Sugita M, Miyao A, Hirochika H, Uchida E, Nagato Y, Yoshida H (2009) MOSAIC FLORAL ORGAN1, an AGL6-like MADS box gene, regulates floral organ identity and meristem fate in rice. Plant Cell 21: 3008–3025

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

Pelaz S, Gustafson-Brown C, Kohalmi SE, Crosby WL, Yanofsky MF (2001a) APETAL1 and SEPALATA interact to promote flower development. Plant J 26: 385–394

Pelaz S, Tapia-López R, Alvarez-Buylla ER, Yanofsky MF (2001b) Conversion of leaves into petals in Arabidopsis. Curr Biol 11: 182–184

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

Prakash S, Parameswaran S, Vijayraghavan U (2005) OsMADS1, a rice MADS-box factor, controls differentiation of specific cell types in the lemma and palea and is an early-acting regulator of inner floral organs. Plant Physiol 134: 93–1028

Prakash S, Srimar P, Kumar CS, Kushalappa K, Vijayraghavan U (2001) Ecotopic expression of rice OsMADS1 reveals a role in specifying the lemma and palea, grass floral organs analogous to sepals. Dev Genes Evol 211: 281–290

Preston JC, Kellogg EA (2006) Reconstructing the evolutionary history of paralogous APETAL1/FRUITFULL-like genes in grasses (Poaceae). Genetics 174: 421–437

Prunet N, Morel P, Negriu T, Trehin C (2009) Time to stop: flower meristem termination. Plant Physiol 150: 1764–1772

Proppingan MD, Rounsley SD, Schmidt RJ, Yanofsky MF (1995) Molecular evolution of flower development: diversification of the plant MADS-box regulatory gene family. Genetics 140: 345–358

Reinheimer R, Kellogg EA (2009) Evolution of AGL6-like MADS box genes in grasses (Poaceae): ovule expression is ancient and palea expression is new. Plant Cell 21: 2951–2960

Reiser L, Fischer RL (1993) The ovule and the embryo sac. Plant Cell 5: 200–203

Rudall PJ, Stuppy W, Jennifer C, Kellogg EA, Briggs BG (2005) Evolution of
of reproductive structures in grasses (Poaceae) inferred by sister-group comparison with their putative closest living relatives, Ecdiecioletaceae. Am J Bot 92: 1432–1443

Seok HY, Park HY, Park JI, Lee YM, Lee SY, An G, Moon YH (2010) Rice ternary MADS protein complexes containing class B MADS heterodimer. Biochem Biophys Res Commun 401: 598–604

Sieburth LE, Drews GN, Meyerowitz EM (1998) Non-autonomy of AGAMOUS function in flower development: use of a Cre/loxP method for mosaic analysis in Arabidopsis. Development 125: 4303–4312

Sun B, Xu Y, Ng KH, Ito T (2009) A timing mechanism for stem cell maintenance and differentiation in the Arabidopsis floral meristem. Genes Dev 23: 1791–1804

Theissen G, Becker A, Di Rosa A, Kanno A, Kim JT, Münster T, Winter KU, Saedler H (2000) A short history of MADS-box genes in plants. Plant Mol Biol 42: 115–149

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

Thompson BE, Hake S (2009) Translational biology: from Arabidopsis flowers to grass inflorescence architecture. Plant Physiol 149: 38–45

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

Wang K, Tang D, Hong L, Xu W, Huang J, Li M, Gu M, Xue YB, Cheng ZK (2010a) DEP and AFO regulate reproductive habit in rice. PLoS Genet 6: e1000818

Wang YQ, Melzer R, Theissen G (2010b) Molecular interactions of orthologues of floral homeotic proteins from the gymnosperm Gnetum Gnetum gnemon provide a clue to the evolutionary origin of ‘floral quartets.’ Plant J 64: 177–190

Whipple CJ, Zanis MJ, Kellogg EA, Schmidt RJ (2007) Conservation of B class gene expression in the second whorl of a basal grass and outgroups links the origin of lodicules and petals. Proc Natl Acad Sci USA 104: 1081–1086

Yamaguchi T, Lee DY, Miyao A, Hirochika H, An G, Hirano HY (2006) Functional diversification of the two C-class MADS box genes OSMADS53 and OSMADS58 in Oryza sativa. Plant Cell 18: 15–28

Yamaguchi T, Nagasawa N, Kawasaki S, Matsuoka M, Nagato Y, Hirano HY (2004) The YABBY gene DROOPING LEAF regulates carpel specification and midrib development in Oryza sativa. Plant Cell 16: 500–509

Yamaki S, Nagato Y, Kurata N, Nonomura KI (2011) Ovule is a lateral organ finally differentiated from the terminating floral meristem in rice. Dev Biol 351: 208–216

Yamaki S, Satoh H, Nagato Y (2005) Gypsy embryo specifies ovule curvature by regulating ovule/integument development in rice. Planta 222: 408–417

Yano T, Kanno A, Kim JT, Münster T, Winter KU, Saedler H (2000) A short history of MADS-box genes in plants. Plant Mol Biol 42: 115–149

Zahn LM, Kong HZ, Leebens-Mack JH, Kim S, Soltis PS, Landherr LL, Solitis DE, Depamphilis CW, Ma H (2005) The evolution of the SEPALLATA subfamily of MADS-box genes: a preangiosperm origin with multiple duplications throughout angiosperm history. Genetics 169: 2209–2223

Zahn LM, Leebens-Mack JH, Arrington JM, Hu Y, Landherr LL, dePamphilis CW, Becker A, Theissen G, Ma H (2006) Conservation and divergence in the AGAMOUS subfamily of MADS-box genes: evidence of independent sub- and neofunctionalization events. Evol Dev 8: 30–45

Zhang D, Wilson ZA (2009) Stamen specification and anther development in rice. Chin Sci Bull 54: 2342–2353

Zhang X, Zong J, Liu J, Yin YJ, Zhang DB (2010) Genome-wide analysis of WOX gene family in rice, sorghum, maize, Arabidopsis and poplar. J Integr Plant Biol 52: 1016–1026