Rice MADS6 Interacts with the Floral Homeotic Genes SUPERWOMAN1, MADS3, MADS58, MADS13, and DROOPING LEAF in Specifying Floral Organ Identities and Meristem Fate

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AGAMOUS-LIKE6 (AGL6) genes play essential roles in flower development, but whether and how they work with floral organ identity genes remain less understood. Here, we describe interactions of the rice (Oryza sativa) AGL6 gene MADS6 with other rice floral homeotic genes in flower development. Genetic analyses revealed that MADS6 specifies the identity of the three inner whorls and floral meristem determinacy redundantly with SUPERWOMAN1/MADS16 (B-gene) or MADS3 (C-gene). MADS6 was shown to define carpel/ovule development and floral determinacy by interacting with MADS13 (D-gene) and control the palea and floral meristem identities together with the YABBY gene DROOPING LEAF. Expression analyses revealed that the transcript levels of six B-, C-, and E-class genes were reduced in mads6-1 at the early flower developmental stage, suggesting that MADS6 is a key regulator of early flower development. Moreover, MADS6 can directly bind to a putative regulatory motif on MADS58 (C-gene), and mads6-1 mads58 displayed phenotypes similar to that of mads6-1. These results suggest that MADS6 is a key player in specifying flower development via interacting with other floral homeotic genes in rice, thus providing new insights into the mechanism by which flower development is controlled.

INTRODUCTION

The flowers of angiosperms display a huge diversity in structure (Theissen and Melzer, 2007). The ABCDE model proposed that the combinatorial action of class A, B, C, and E floral homeotic genes determines floral organ identity in eudicot model species, such as Arabidopsis thaliana, Petunia hybrida, and Antirrhinum majus. In particular, A- and E-function genes specify the identity of sepals in the first outer whorl; A-, B-, and E-function genes together determine the identity of petals in the second whorl; B-, C-, and E-function genes coordinately define stamen identity in the third whorl; and C- and E-function genes act together to specify carpels in the fourth whorl (Coen and Meyerowitz, 1991; Pelaz et al., 2000; Theissen, 2001; Theissen and Saedler, 2001; Ditta et al., 2004).

As one of the largest families in higher plants, the grass family (Poaceae) contains many economically important crops, such as rice (Oryza sativa), barley (Hordeum vulgare), and maize (Zea mays) (Clayton and Renvoize, 1986; Linder and Rudall, 2005). These crops evolved floral organization and morphologies distinct from those of eudicots and even other monocots (Grass Phylogeny Working Group, 2001; Rudall et al., 2005; Whipple et al., 2007). Spikelet, the structural unit of grass flowers, has a varied number of bract-like organs and florets. In rice, a spikelet consists of two pairs of sterile glumes (i.e., rudimentary glumes and empty glumes) and one floret that contains a lemma and a palea in whorl 1, two lodicules in whorl 2, six stamens in whorl 3, and a pistil in whorl 4 (Yuan et al., 2009; Zhang and Wilson, 2009). However, the molecular basis underlying grass flower development remains poorly understood (Clifford, 1987; Whipple et al., 2007).

Recently, emerging evidence suggests that the ABCDE genetic model is partially applicable to grasses such as rice and maize (Kyoizuka et al., 2000; Kater et al., 2006; Preston and Kellogg, 2006; Prusinkiewicz et al., 2007; Reinheimer and Kellogg, 2009; Thompson and Hake, 2009; Zhang and Wilson, 2009). For instance, mutations in maize Silky1 and rice SUPERWOMAN1 (SPW1) or MADS16, both of which are orthologs of the Arabidopsis B-function gene, AP3, cause homeotic transformations of stamens to carpels and lodicules to lemma- or palea-like structures (Ambrose et al., 2000; Nagasawa et al., 2003; Whipple et al., 2004), suggesting the conserved role of the B-class genes...
MADS6 Regulates Rice Flower Development

from grasses to Arabidopsis. In grasses, there are duplicated and subfunctionalized C-class genes (Kramer et al., 2004; Zahn et al., 2006). For example, rice contains two AG homologs, MADS3 and MADS58 (Kramer et al., 2004). MADS3 plays a key role in stamen and ovule identity specification, late anther development, and floral meristem determinacy (Yamaguchi et al., 2006; Hu et al., 2011; Li et al., 2011). Using an RNA interference approach, MADS58 was shown to be required for defining floral meristem determinacy and carpel architecture (Yamaguchi et al., 2006). Similarly, maize has three AG homologs: zag1 (zea agamous1), zmm2 (Zea mays mads2), and zmm23 (Münster et al., 2002). The zag1 gene is required for floral meristem determinacy, but the biological functions of zmm2 and zmm23 have not been elucidated (Mena et al., 1996). Rice contains two D-class genes, MADS13 and MADS21, which are orthologous to the Arabidopsis SEEDSTICK (STK) and petunia FLORAL BINDING PROTEIN7 (FBP7) and FBP11 genes (Colombo et al., 1995). MADS13 was shown to be involved in ovule identity specification and floral meristem termination (Dreni et al., 2007; Li et al., 2011). However, mutants of the Arabidopsis STK gene do not display altered ovule identity (Pinyopich et al., 2003).

Grasses have diversified E-class (SEPALLATA [SEP]) genes. Rice has at least five SEP-like genes: MADS1/LEAFY HULL STERILE1 (LHS1), MADS5, MADS7, MADS8, and MADS34 (Malcomber and Kellogg, 2004, 2005; Zahn et al., 2005; Arora et al., 2007). LHS1 specifies the identity of lemma and palea and the meristem of inner floral organs (Jean et al., 2000; Agraywal et al., 2005; Prasad et al., 2005; Chen et al., 2006a). Transgenic plants with reduced expression of both MADS7 and MADS8 exhibit 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 (i.e., LHS1, MADS5, MADS7, and MADS8) causes homeotic transformation of all floral organs except the lemma into leaf-like organs (Cui et al., 2010). MADS34 (PANICLE PHYTOMER2) controls the development of inflorescences and spikelets (Gao et al., 2010; Kobayashi et al., 2010). Analysis of mads34 mads1 indicates that MADS34 and LHS1 redundantly specify the identities of all floral organs, including lemma/palea, lodicules, stamens, and carpel (Gao et al., 2010).

Sequence and phylogenetic analyses indicated that AGAMOUS-LIKE6 (AGL6)-like and SEP-like genes have high sequence similarities, forming sister clades on the phylogenetic tree (Theissen et al., 2000; Becker and Theissen, 2003; Zahn et al., 2005). SEP-like genes are only found in angiosperms, while AGL6 genes are ancient and widely distributed in gymnosperms and angiosperms. Recently, AGL6-like genes in monocots and eudicots were shown to play essential roles in flower development (Schauer et al., 2007; Ohmori et al., 2009; Reinheimer and Kellogg, 2009; Rijkema et al., 2009; Li et al., 2010). The Arabidopsis genome contains two AGL6 genes, AGL6 and AGL13 (Vandenbussche et al., 2003a), suggesting possible functional redundancy between the two genes. Mutation or knockdown of AGL6 or AGL13 does not result in an abnormal flower phenotype (Schauer et al., 2007; Koo et al., 2010; Yoo et al., 2011). Loss-of-function mutants of the only Petunia AGL6 gene, PhAGL6, show no morphological abnormalities of floral organs, but Ph AGL6 functions redundantly with the SEP genes FBP2 and FBP5 in petal and anther development, and its protein physically interacts with FBP2 (Vandenbussche et al., 2003b; Rijkema et al., 2009).

The AGL6-like genes from grasses form two paralogous clades: the MADS17 clade containing only the rice MADS17 gene, and the MADS6 clade, which includes rice MADS6 (also called MOSAIC FLORAL ORGANS1 [MFO1]) and maize ZAG3 and ZAG5 (Ohmori et al., 2009; Reinheimer and Kellogg, 2009; Li et al., 2010). Grass AGL6-like genes were shown to be expressed in paleas, lodicules, ovules, and floral meristems, and each of these expression domains may represent a distinct function of the gene product (Reinheimer and Kellogg, 2009). In rice, the expression level of MADS6 is high in floral meristem at early stages and in the palea and inner floral organ primordia (lodicule, stamen, and pistil) at later stages (Ohmori et al., 2009; Reinheimer and Kellogg, 2009). MADS6 transcripts were detected in the floral meristem at the early stage and in the lemma, palea, lodicule, pistil, and (weakly) in empty glumes and stamens at late stages, with its protein product functioning redundantly with MADS6 in flower development (Ohmori et al., 2009; Reinheimer and Kellogg, 2009). Our previous studies revealed that the palea of mads6-1 flowers develops five to six vascular bundles, which resembles the identity of a wild-type lemma, suggesting the role of MADS6 in specifying the identity of palea. In addition, mads6-1 flowers are retarded in development at the early stage, exhibit homeotic conversion of lodicules and stamens into glume-like and mosaic structures, have defective carpels and ovules, and contain indeterminate meristem at later flower developmental stages. Furthermore, we showed that the MADS6 gene is able to specify floral state by determining floral organ and meristem identities together with LHS1/MADS1 because mads1-1 mads6-1 double mutants display severe floral defects, such as no inner floral organs or glume-like structures within flowers and strongly indeterminate floral meristem, phenotypes not observed in the single mutants (Li et al., 2010). A mutation of the maize AGL6 gene zea agamous3 (zag3) results in extra mosaic or fused floral organs in the upper floral meristem and additional floral meristems in the lower floral meristem. zag3 and the maize homolog of AG, zagi, can genetically and physically interact in promoting floral meristem identity (Thompson et al., 2009). These findings suggest that AGL6 genes have SEP-like functions in flower development.

Despite the findings that AGL6-like genes have a role in defining floral organ and meristem identities, whether and how they interact with other floral homeotic genes in these processes remain largely unknown. Here, we report that MADS6 interacts with several known flower homeotic genes in specifying flower development and determining floral meristem fate in rice. We show that MADS6 not only interacts with B-, D-, and E-class proteins but also regulates the expression of these genes, thus providing novel insights into the mechanism by which AGL6-like genes exert their functions in plant flower development.

RESULTS

Transcriptome Analysis of mads6-1 Flowers

To further elucidate the regulatory role of MADS6, we compared genome-wide mRNA levels in wild-type and mads6-1 flowers
at stage Sp6, when stamen primordia are formed, using microarray analyses with an Agilent 4 × 4 4K oligonucleotide DNA chip. Stage Sp6 flowers were collected according to spikelet length and flower morphology defined by Ikeda et al. (2004), and three independent biological replicates were performed to assess its reproducibility. Data were analyzed by the Empirical Bayes method (Smyth, 2004). Initial filtering of candidate genes was performed using a false discovery rate cutoff of 0.5%, followed by a secondary selection using at least twofold changes in gene expression as the cutoff. Fifty-nine genes were found to have at least twofold changes in expression in mads6-1 flowers compared with the wild type. Among them, 26 were upregulated and 33 were downregulated (see Supplemental Table 1 online).

The expression of six MADS box genes, including the B-class genes MADS4 and SPW1, C-class genes MADS3 and MADS58, and E-class genes MADS7 and MADS8, was downregulated in the mutant (Table 1). In particular, MADS8 and MADS7 were downregulated ~22.5-fold (for one MADS8 probe of chip) and 11.6-fold, respectively. The microarray data were further confirmed by serial quantitative RT-PCR (qRT-PCR) analysis, which showed that the mRNA levels of these six genes were reduced from stage 4 to stage 6 and came back up at stage 8 (Figure 1).

To clarify the functional relationship between MADS6 and the floral homeotic genes whose expressions were significantly downregulated in mads6-1, we conducted detailed genetic analyses using double mutants between mads6-1 and spw-1, mads3-4, mads58, mads13-3, and dl-sup6. In addition to phenotypic analysis, we also performed situ analysis to determine the regulatory relationship between MADS6 and SPW1, MADS3, MADS58, MADS13, and DL at the transcription level.

Interaction between MADS6 and SPW1

SPW1 is a B-class gene required for determining the identity of lodicules and stamens. In spw1-1 flowers, lodicules are transformed into glume-like structures and stamens into carpel-like organs (Nagasawa et al., 2003) (Figures 2A, 2D, 2P, and 2R). Unlike mads6-1 or mads6-1 spw-1, MADS6 and SPW1 synergistically specify lodicule identity. Similar to spw1-1, mads6-1 spw1-1 flowers displayed the conversion of stamens into carpel-like structures, each of which contains one to four stigmas (Figures 2F and 2S). Consistent with this phenotype, the expression of the carpel marker gene DL was found in the ectopic organs (see Supplemental Figures 1K to 1M online). These results suggest that SPW1 plays a more important role in stamen identity. In the double mutant, the average number of carpel-like organs per flower was 5.86 (n = 43) compared with 2.09 in spw1-1 and 4.24 in mads6-1 (see Supplemental Table 2 online). The average number of carpel-like organs per flower was 5.07 (n = 43) in the double mutant, while it was 7.00 for spw1-1 (n = 43) and 1.10 for mads6-1 (n = 43) compared with 2.09 in spw1-1 and 4.24 in mads6-1 (see Supplemental Table 2 online). More interestingly, about half of the flowers (22 in 43) in the double mutant developed new inflorescence-like organs at the position of wild-type lodicules (Figures 2G and 2H), a phenotype that was not observed in single mutant flowers, implying that MADS6 and SPW1 can repress the inflorescence primordia in the second whorl in a redundant manner. These observations suggested that both MADS6 and SPW1 function in specifying the identity of floral organs in the three inner whorls and the determinacy of floral meristem. Furthermore, the functions of these two proteins are redundant in some while independent from each other in other aspects of flower development.

To analyze phenotypes of the double mutant in more depth, we performed scanning electron microscopy. No structural differences between the flowers of spw1-1 and the wild type were detected at stage Sp6, when stamen primordia initiate (Figure 2I). However, similar to that of mads6-1 (Li et al., 2010), the inner flower organ primordia of the double mutant displayed retarded growth (Figure 2K). At stage Sp8, spw1-1 displayed ectopic

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**Table 1. Microarray Data Showing the Downregulated Expression of Six MADS Box Genes at Stage Sp6 in mads6-1 Flower**

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Fold Change</th>
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<tbody>
<tr>
<td>MADS16</td>
<td>-8.4</td>
</tr>
<tr>
<td>MADS4</td>
<td>-3.7</td>
</tr>
<tr>
<td>MADS3</td>
<td>-7.7</td>
</tr>
<tr>
<td>MADS58</td>
<td>-22.5*</td>
</tr>
<tr>
<td>MADS58</td>
<td>-6.1*</td>
</tr>
<tr>
<td>MADS7</td>
<td>-11.6</td>
</tr>
<tr>
<td>MADS8</td>
<td>-6.3</td>
</tr>
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*Different probes for MADS8.
carpel primordia (Figure 2J), and the double mutant flowers developed into carpel- and glume-like structures (Figures 2L and 2M). Also, additional inflorescence primordia were detectable in some mads6-1 spw1-1 flowers (Figures 2N and 2O). Results of the scanning electron microscopy experiment were consistent with the genetic analysis, further substantiating the role MADS6 and SPW1 in regulating flower development.

RNA in situ hybridization analysis of the mads6-1 mutant revealed that at stage Sp5, when lodicule primordia are formed, the expression level of SPW1 in the lodicule primordium was slightly weaker than in the wild type (Figures 3A and 3E) (Nagasawa et al., 2003). At stage Sp6, the expression of SPW1 was detected in lodicule and stamen primordia of mads6-1 (Figures 3B and 3F). At stages Sp7 and Sp8, the expression of SPW1 was observed in lodicule and stamen primordia in mads6-1 similar with its expression pattern in the wild type (Figures 3C, 3D, 3G, and 3H). In spw1-1, the expression of MADS6 was detected in paleas, lodicules, carpels, and the receptacle similar to its expression profile in the wild-type plants (Figures 3I to 3L) (Ohmori et al., 2009; Reinheimer and Kellogg, 2009; Li et al., 2010). These in situ analysis results are consistent with the microarray and qRT-PCR experiments, supporting the notion that MADS6 is able to activate the expression of SPW1 at the early flower developmental stage but does not obviously affect its expression at late stages.

**MADS6 and DL Redundantly Regulate Floral Meristem Identity**

DL, which belongs to the YABBY gene family, determines carpel identity and regulates vascular pattern of the lemma (Nagasawa et al., 2003; Yamaguchi et al., 2004; Li et al., 2011). In the construction of double mutants, we used the null allele dl-sup6, which is allelic to the previously reported dl-2 mutant (Nagasawa et al., 2003; Yamaguchi et al., 2004; Li et al., 2011). dl-sup6 has drooping leaves, ectopic stamens in the fourth
to MADS6 lodicules and stamens, whereas palea, which is similar to that of the wild-type palea, was found in 58% of the flowers (Li et al., 2011).

Determinacy in some of the flowers (Figures 4A, 4B, and 4J) (Li et al., 2010). By contrast, MADS6 expression was observed in the dl-sup6 floral primordia at stage Sp4, and in palea and carpel primordia as well as the floral meristem at stages Sp7 and Sp8, just like in the wild type (see Supplemental Figures 1H to 1J online). Together, these results suggested that, whereas MADS6 may repress the expression of DL, DL does not have an obvious effect on MADS6 expression.

**MADS6 and MADS13 Redundantly Regulate Carpel/Ovule Identity and Floral Determinacy**

MADS13 is a D-class gene that functions in specifying ovule identity and floral meristem determinacy (Dreni et al., 2007). We recently identified a strong MADS13 allele, mads13-3, which showed carpelloid structures, indeterminate floral organs, and complete female sterility caused by aborted ovule development (Figures 5A, 5B, and 5K) (Li et al., 2011).

Flowers of the mads6-1 dl-sup6 double mutant could be divided into two types based on palea morphology. Type I was found in 58% of the flowers (n = 56). They displayed widened palea, which is similar to that of mads6-1 (Figure 4C). Type II flowers (42%; n = 56) had two palea-like organs (Figures 4D and 4K), which were not observed in the single mutants. Examination of transverse sections showed that these two palea-like organs had the characteristic marginal tissue of the lemma but contained three vascular bundles similar to that of wild-type palea (Figures 4I and 4K). Consistent with this finding, two palea-like primordia were observed at stage Sp6 on the palea side in type II flowers (Figure 4G). These results revealed that MADS6 specifies the palea identity together with MADS13.

The phenotypes of the mads6-1 dl-sup6 floral organs in whorls 2 and 3 appeared similar to that of mads6-1 (Figure 4E), suggesting that MADS6 is involved in defining the identities of lodicules and stamens, whereas DL does not play a role in it. This finding is consistent with the lack of DL expression in whorls 2 and 3 (see Supplemental Figure 1 online). Interestingly, all flowers in mads6-1 dl-sup6 displayed an inflorescence-like structure in whorl 4 (n = 62) (Figures 4E, 4F, 4H, and 4K), yet this phenotype was rarely observed in mads6-1 (Li et al., 2010). These results suggested that MADS6 and DL act synergistically in terminating floral meristem development.

RNA in situ hybridization analysis detected DL expression in the wild-type lemma and carpel, not in the palea (Yamaguchi et al., 2004) (see Supplemental Figures 1A to 1C online). However, ectopic expression of DL was observed in the altered palea organ and ectopic carpels or abnormal ovules in mads6-1 (see Supplemental Figures 1D to 1G online) (Ohmori et al., 2009; Li et al., 2010). By contrast, MADS6 expression was observed in the dl-sup6 floral primordia at stage Sp4, and in palea and carpel primordia as well as the floral meristem at stages Sp7 and Sp8, just like in the wild type (see Supplemental Figures 1H to 1J online). Together, these results suggested that, whereas MADS6 may repress the expression of DL, DL does not have an obvious effect on MADS6 expression.
MADS6 and MADS13 have partial functional redundancy in specifying carpel/ovule identity and terminating floral stem cell activity.

In situ hybridization analysis detected the expression of MADS13 in wild-type ovules at stage Sp8 (see Supplemental Figures 2A and 2B online), which is consistent with a previous report (Dreni et al., 2007). In mads6-1, MADS13 expression was detected in ovule primordia at early Sp8 stage, similar to its expression in the wild type (see Supplemental Figure 2C online), and in ectopic ovules at late Sp8 stage (see Supplemental Figures 2D and 2E online). In mads13-3, the expression of MADS6 was observed in the floral meristem and primordia of the palea, lodicules, and the carpel at stages Sp7 to Sp8 (see Supplemental Figures 2F to 2H online), which is similar to its expression pattern in the wild type. These results suggested that MADS6 and MADS13 do not obviously regulate the expression of each other at the transcriptional level during early stages of ovule specification.

**MADS6 Positively Regulates the Expression of MADS7 and MADS8**

MADS7 and MADS8 are the two closest rice homologs of Arabidopsis SEP3 (Malcomber and Kellogg, 2005; Zahn et al., 2005; Arora et al., 2007). Like SEP3, MADS7 and MADS8 are expressed in the three inner whorls (Cui et al., 2010). MADS6 was shown to interact with MADS7 and MADS8 in a yeast two-hybrid analysis (Moon et al., 1999). Moreover, Cui et al. (2010) revealed
that plants in which both MADS7 and MADS8 were silenced displayed strong defects in flower development, where stamens were transformed into lodicules or glume-like organs and flowers appeared to lose determinacy by generating higher order carpels, suggesting functional redundancy between MADS7 and MADS8 in specifying flower development.

In situ hybridization detected the expression of MADS7 in the primodia of lodicules, stamens, and carpels in the wild type (Figures 6A to 6C), which agrees with results from the previous report (Cui et al., 2010). In addition, the expression of MADS8 was detected in the floral meristem (Figure 6D) and whorls 2, 3, and 4 (Figures 6E and 6F) in the wild type. In mads6-1, however, the expression of MADS7 and MADS8 was markedly reduced in floral meristem in the early stage of flower development (Figures 6G to 6L), which is consistent with the microarray data and qRT-PCR analysis (Table 1, Figure 1). Together, these results supported the notion that MADS6 positively regulates the expression of MADS7 and MADS8 during rice flower development.

**MADS6 Positively Regulates the Expression of MADS3 and MADS8**

In mads6-1 flowers, the initiation of stamen development is much delayed and stamens are partially converted into lodicule-like or lodicule-anther mosaic organs during early flower development (Ohmori et al., 2009; Li et al., 2010). MADS3, a C-class gene in rice, has been shown to play a key role in controlling the development of lodicules and stamens (Yamaguchi et al., 2006; Hu et al., 2011). More recently we showed that MADS3 also defines carpel development and floral determinacy redundantly with MADS13 (Li et al., 2011). In addition, two intermediate

![Figure 5. Flower Phenotypes of mads6-1 mads13-3.](image-url)
alleles, mads3-2 and mads3-4, displayed mild homeotic transformation from stamens into lodicule-like or lodicule-anther mosaic organs (Yamaguchi et al., 2006; Hu et al., 2011) (Figures 7A, 7F, and 7J).

The morphology of palea in the mads6-1 mads3-4 double mutant appeared similar to mads6-1, suggesting that MADS6’s function in regulating palea identity is independent of MADS3. However, more severe defects were observed in whorls 2 to 4. In the double mutant, the average number of ectopic lodicule- or glume-like organs increased to 7.25 (n = 91), compared with 5.34 (n = 79) in mads6-1, and the number of stamens in the double mutant decreased to 2.45 (n = 91) from 6.00 in the wild type (Figures 7B, 7E, 7I, and 7K; see Supplemental Table 3 online). Defects in the double mutant also seemed to be intensified in whorl 4, where elongated floral axis or an inflorescence-like structure was observed in the floral center, and the average number of carpels was 2.2 (n = 91) (Figures 7C and 7D; see Supplemental Table 3 online). Scanning electron microscopy observation indicated that mads6-1 mads3-4 had delayed inner floral organ development at stage Sp6 (Figure 7G), which is similar to that of mads6-1. At stage Sp8, the mads6-1 mads3-4 double mutant displayed lodicule- or glume-like organs compared with the wild type or mads3-4 (Figure 7H). These results suggested that MADS6 and MADS3 synergistically determine the identity of the three inner floral organs and floral determinacy.

In situ analysis detected the expression of MADS3 at stage Sp5 in the wild type at the position where the stamen primordium was to be formed (Figure 8A). At stages Sp7 and Sp8, the mRNA of MADS3 was detectable in whorls 3 and 4 of the wild-type flowers (Figures 8B and 8C) (Yamaguchi et al., 2006). However, in mads6-1, the expression of MADS3 seemed much weaker and delayed (Figures 8D to 8F). Similarly, reduced expression of MADS8 was observed in mads6-1 at the early stage (Figures 8G to 8J). This result is consistent with the microarray data and qRT-PCR analysis, suggesting that MADS6 may promote the expression of MADS3 and MADS8 during early flower development. Conversely, we observed no obvious change in MADS6 expression between mads3-4 and the wild type, with the exception of some signals in ectopic lodicule-like primordia (Figures 8K to 8N), suggesting that MADS3 does not affect the expression of MADS6 at the transcriptional level to significant degrees.

The mads58 allele contains a dSpm element insertion in the second intron of MADS58, which leads to ∼35-fold reduction in the expression of MADS58 but no obvious defects in flower development (Figure 9A) (Dreni et al., 2011). The defects of floral organs in the outer three whorls were almost identical in mads6-1 mads58 and mads6-1, except that the number of stigmas and abnormal carpels/ovules was higher in the double mutant (Figures 9B to 9G). The average stigma number per flower was 6.56 (n = 50) in mads6-1 mads58 and 2.77 (n = 44) in mads6-1. About half of the mads6-1 mads58 flowers displayed defective ovule development, each showing two or three carpel/ovule-like structures. The average number of carpel/ovule per flower was 1.5 (n = 50) in mads6-1 mads58 and 1.1 in mads6-1 (n = 44) under the same growth condition (Figures 9E and 9F).

MADS box proteins can regulate gene expression by binding as homo- or heterodimers to sequences containing a consensus.
core element called the CArG box (Riechmann et al., 1996). For instance, the second intron of the Arabidopsis C-class gene AG contains one CArG-box, M1 [CC(A/T)6G], which is bound by SEP3 and acts as an enhancer sequence required for the proper expression of AG (Deyholos and Sieburth, 2000; Kaufmann et al., 2009) (Figure 10A). To further investigate the regulatory role of MADS6 during rice flower development, we searched the homeotic genes investigated in this study for putative CArG box sequences using the plant CARE tool (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/; Lescot et al., 2002). We found five CArG motifs in the second intron of MADS58 and grouped them into two types: M2 [CC(A/T)8G] and M3 [CC(A/T)7G]. The second intron of MADS3 was also found to contain five CArG motifs, which could be grouped into M2, M3, and M4 [CC(A/T)4 (A/T/C/G)2G] types (Figure 10A).

To test whether MADS6 is able to bind to the CArG motifs in the second introns of MADS3 and MADS58, we performed chromatin immunoprecipitation (ChIP)-qPCR analysis. First, we developed rabbit polyclonal antibodies against a bacterial-expressed recombinant protein that contains 86 amino acids (amino acid 165 to amino acid 250) from the N terminus of MADS6 (see Methods). Specificity of the antibody was validated using immunoblot analysis, by which we detected, using a protein extract from wild-type flowers, a band of 30 kD that is close to the expected size (see Supplemental Figure 3 online). ChIP-qPCR analysis using the affinity-purified MADS6 antibody showed specific enrichment of the M3-containing region of MADS58 but not the other CArG motifs in MADS3 and MADS58 (Figure 10B; see Methods and Supplemental Table 4 online). These results suggested that MADS6 may directly regulate the expression of...
MADS6 is a Key Regulator in Specifying Floral Organ Identities

Flowering plants (angiosperms) evolved a tremendous diversity of floral structures (Theissen and Melzer, 2007). In this study, we investigated the genetic interaction of MADS6 with floral homeotic genes SPW1 (B-class), MADS3 and MADS58 (C-class), MADS13 (D-class), and DL and demonstrated that interactions of MADS6 with these floral homeotic genes play essential roles in rice flower development. Expression analyses indicated that the expression of B-, C-, and E-class genes in mads6-1 was reduced at early flower developmental stages (Figure 1, Table 1). This may explain the delayed development of floral organ primordia in the mutant. However, repression of these six MADS box genes at the transcript level was less obvious at late flower developmental stages. Ohmori et al. (2009) reported the upregulation of MADS14, MADS15, MADS3, MADS58, and LHS1 in mfo1-1 elongated lodicules and MADS14, MADS15, and MADS3 in the mfo1-1 carpel during late flower developmental stages, suggesting the developmental stage–dependent control of these floral homeotic genes in rice. In this study, we did not detect obvious changes in the expression pattern of MADS6 in spw1-1, mads3-4, and mads13-3. Based on these results, we propose that MADS6 may act as an upstream regulator that activates the expression of B- (MADS4 and SPW1), C- (MADS3 and MADS58), and E-class (MADS7 and MADS8) genes during early flower development (Figure 11A). Alternatively, the reduced expression of B-, C-, and E-class genes may be a consequence of delayed development of flower organ primordia in mads6-1.

Phylogenetic and functional analyses revealed that AGL6 family members are closely related to SEP-like genes (Becker and Theissen, 2003). Arabidopsis SEP and AGL6 genes were shown to activate the expression of B- and C-class genes (Liu et al., 2009a; Koo et al., 2010). Moreover, ChIP sequencing analysis revealed direct association of SEP3 with the regulatory regions of MADS box genes, where peaks of association were found to be located in the promoters of AP1, AP3, SEP1, and SEP2 and the second intron of AG (Kauffmann et al., 2009). In this study, ChIP-qPCR assays revealed direct association of MADS6 with a predicted regulatory motif in the second intron of MADS58 (Figure 10). In addition, dramatically reduced expression of MADS58 was observed in mads6-1 during early flower development (Figures 1 and 8; Table 1), suggesting that MADS6 functions upstream of the C-class gene MADS58 and may directly activate MADS58 expression (Figure 11A).

Similar to SEP proteins (Immink et al., 2009), MADS6 have been shown to form complexes with MADS4, SPW1, MADS13, MADS7, MADS8, MADS14, and MADS15 (Moon et al., 1999; Favaro et al., 2002; Seok et al., 2010) (Figure 11), suggesting that MADS58 through binding to the CArG element in the second intron.

DISCUSSION

MADS6 Regulates Rice Flower Development
AGL6-like proteins act as integrators that form multimeric complexes with MADS domain proteins from different clades in rice (Figure 11). We hypothesize that these MADS6-containing protein complexes may also be involved in the transcriptional control of floral homeotic genes in rice. This is exemplified by the findings that in Arabidopsis AP3 and PI form functional heterodimers, which can bind DNA in vitro (Riechmann et al., 1996) and regulate their own transcription in vivo (Goto and Meyerowitz, 1994; Krizek and Meyerowitz, 1996). Further determination of whether such auto/cross-regulatory loops also exist during flower development in grasses will help explain how MADS6 acts both upstream from and in concert with these floral MADS box genes.

The ABCDE Model and Control of Palea Identity in Grasses

Compared with eudicot plants, rice has both conserved and unique genes that act in the regulation of flower development (Figure 11). As a result, the ABCDE genetic model is only partially applicable to the mechanism of flower development in grasses (Thompson and Hake, 2009; Li et al., 2011). For example, B- and C-class function seems to be conserved in grasses, Arabidopsis, and other eudicot species, whereas the function of AGL6 genes, equivalents of the Arabidopsis class E or SEP genes, is partially conserved from eudicots to grasses (Figure 11).

In grasses, AGL6-like genes acquired a new expression domain in the palea, suggesting that their proteins may be key regulators of palea identity (Ohmori et al., 2009; Reinheimer and Kellogg, 2009; Li et al., 2010). It has been hypothesized that during evolution of grass flowers, the palea was a congenital fusion of two perianth parts along with the lemma, which would represent a modified trimerous calyx (Francis, 1920; Zanis, 2007). Congenital fusion (also called phylogenetic fusion and zonal growth) refers to a compound structure that is developed as a homogeneous unit but is potentially derived from separate origins (Cusick, 1966; Verbeke, 1992). According to comparative morphological analysis, the development of compound organs resulting from fusions between individual primordia is thought to play a key role in floral morphogenesis during evolution (Verbeke, 1992). However, the mechanism underlying congenital fusion remains poorly understood.

Our results in this study suggest that both MADS6 and DL are required for the proper establishment of palea identity and morphology. In the wild type, DL was shown to have zygomorphic expression in the lemma, but in mads6 mutants, its expression becomes detectable in the palea as well. In addition, mads6 mutants display loss of the palea identity (Ohmori et al., 2009; Li et al., 2010). DL was recently shown to play a critical role in specifying lemma identity (Figure 11), and dl-sup6 lemmas had altered developmental identity with an increased number of vascular tissues but no obvious change of palea morphology (Li et al., 2011). Intriguingly, some flowers of the mads6-1 dl-sup6 double mutants displayed complete loss of palea identity determination, leading to two separate organs. The complete loss of palea identity may be explained by the idea that congenital fusion...
of the palea relies on proper establishment of the palea identity. This interpretation is analogous to what was described as the dedoublement phenomenon in Arabidopsis stamens, where a single stamen primordium in the medial position is split in two, producing two pairs of medial stamens (Ronse Decraene and Smets, 1993; Bowman and Smyth, 1998). However, ap3 or pi mutants show the transformation of these organs into carpels, resulting in just single organs in these positions (Bowman et al., 1991; Goto and Meyerowitz, 1994; Krizek and Meyerowitz, 1996). Similarly, this interpretation was used to explain the stamen number reduction in Lepidium (Brassicaceae) (i.e., stamens are reduced either by apparent loss of primordia [in the two lateral positions] or by fusion of two primordia into one [in the two medial positions]) (Endress, 1992; Bowman and Smyth, 1998). Here, we propose that MADS6 may specify the palea identity through repressing the expression of DL in the palea (Figure 11). Consistent with this view, investigations from model dicot plants suggest that the congenital fusion often acts downstream of organ identity specification such that a loss of organ identity alters proper fusion (Alvarez and Smyth, 1999; Vandenbussche et al., 2004; Prunet et al., 2009). In Arabidopsis, analysis of double mutants of crabs claw (crc) and spatula (spt) with homeotic mutants indicated that A and B organ identity genes are capable of negatively regulating the function of CRC and SPT in carpel development (Alvarez and Smyth, 1999). CRC was shown to suppress the radial growth but trigger the longitudinal growth of the developing gynoecium, and SPT is able to promote the development of the carpel margins and the derived tissues (Alvarez and Smyth, 1999).

Previously, we showed that the rice CYCLOIDEA-like homolog RETARDED PALEA1 (REPI) is a palea-specific gene that regulates palea identity and initiation by regulating cell proliferation and expansion, but it does not affect lemma development (Yuan et al., 2009). The rep1 mutants display altered palea identity, such as an increased number of vascular bundles in the palea, which resembles a lemma-like organ (Yuan et al., 2009). Whether and how MADS6 interacts with REP1 in specifying palea identity requires further elucidation.

We have shown that in addition to specifying palea identity, MADS6 also regulates lodicule development by interacting with SPW1, defines the stamen, carpel, and meristem identities with MADS3 and MADS58, and specifies carpel/ovule development and floral meristem determinacy together with MADS13 (Figure 11). Furthermore, MADS6 regulates flower development redundantly with E-class genes, such as LHS1, MADS7, and MADS8. Although no obvious protein–protein interaction or transcriptional regulation between MADS6 and LHS1 was found (Li et al., 2010), MADS6 may promote the expression of MADS7 and MADS8 and physically interact with the products of these two genes (Moon et al., 1999) (Figure 11). Similarly, Arabidopsis AGL6 was shown to interact with several MADS proteins, such as SEP1, SEP3, SHATTERPROOF2, AP1, and FUL (de Folter et al., 2005).

Control of Floral Meristem Determinacy

Floral organs originate from the floral meristem, a pool of pluripotent stem cells (Liu et al., 2009b; Prunet et al., 2009). Mechanisms for the maintenance of floral meristem determinacy seem to be widely conserved among angiosperms (Ferrario et al., 2004; Prunet et al., 2009). Generally, floral meristem activity is abolished after the formation of a fixed pattern of floral organs. It has been shown that Arabidopsis AG is a key regulator in terminating floral meristem by turning WUSCHEL (WUS) off (Lenhard, et al., 2001; Lohmann et al., 2001). Whether grasses use a similar mechanism remains unknown. In the rice genome, there are 13 putative WOX (WUSCHEL-related homeobox gene family) genes; Os WUS was found to be closely related in sequence to the Arabidopsis WUS gene (Nardmann and Werr, 2006; Dai et al., 2007; Zhang et al., 2010b). But the biological function of Os WUS remains unclear. Unlike the WUS gene in Arabidopsis, Os WUS is not expressed in the organizing center of
et al., 2006). In rice, analyses of et al., 1996; Kramer et al., 2004; Yamaguchi et al., 2006; Zahn 2006). Grass species have duplicated C-class genes (Mena et al., 1999). MADS4 and physically interacting with the MADS4-SPW1 complex (Seok et al., 2010). MADS6 regulates the stamen, carpel, and meristem identities by activating the expression of the MADS3 and MADS58 at early stages and specifies carpel/ovule development and controls floral meristem termination by interacting with MADS13 (Moon et al., 1999). MADS4 or MADS13 interacts with MADS7 and MADS8, respectively (Kater et al., 2006). In addition, MADS6 determines flower development redundantly with LHS1, MADS7, and MADS8 (Ohmori et al., 2009; Li et al., 2010). It also promotes the expression of MADS7 and MADS8 and physically interacts with MADS7 and MADS8 (Moon et al., 1999). Furthermore, MADS6, MADS3, MADS58, DL, and MADS13 redundantly specify floral meristem determinacy (Yamaguchi et al., 2004, 2006; Dreni et al., 2011; Li et al., 2011).

(B) ABCDE model in rice flower development. The putative A-class gene DEP defines palea identity (Wang et al., 2010; Li et al., 2011). The E-function genes LHS1, MADS6, and MADS34 specify lemma/palea identities. The A-class gene DEP, B-class genes SPW1, MAD52, and MAD54, in combination with the E-class genes LHS1, MAD5, MAD57, MADS8, and MAD54, specify the lodicule identity. The B-class genes, the C-class genes MAD53 and MAD55, together with the E-class genes determine the stamen identity. DL specifies carpel identity. The D-class gene MAD51 and E-class genes determine carpel/ovule identity. Additionally, DL antagonistically regulates the expression of the B-class genes, and the C-class gene MAD53 represses the expression of the putative A-class gene DEP (MADS15) (Li et al., 2011).

c, ca, carpel; le, lemma; lo, lodicule; ov, ovule; pa, palea; st, stamen.
[See online article for color version of this figure.]

the vegetative shoot apical meristem (Nardmann and Werr, 2006). Grass species have duplicated C-class genes (Mena et al., 1996; Kramer et al., 2004; Yamaguchi et al., 2006; Zahn et al., 2006). In rice, analyses of mad53 and mad58 mutants suggested that MAD53 plays a major role in specifying stamen identity and late anther development (Yamaguchi et al., 2006; Hu et al., 2011), and MAD58 plays a redundant role with MAD53 in specifying floral meristem determinacy and carpel identity (Dreni et al., 2011). More recently, our double mutant analysis revealed that MAD53 and MAD513 redundantly regulate carpel/ovule development and floral meristem determinacy, suggesting that the C-class and D-class genes in rice retain their conserved function even though they had multiple subfunctionalization and/or neofunctionalization events after duplication within AG clade (Li et al., 2011) (Figure 11).

In this study, we have shown that MADS6 regulates floral meristem determinacy together with C-class genes. Mutations in MADS6 cause greatly reduced expression of the AG homologs MAD53 and MAD58, and mad56-1 mad54-3 displayed severe defects in floral meristem determinacy, such as the presence of inflorescence-like structures (Figure 7). Although mad56-1 mad58 and mad56-1 displayed similar defects in the outer three-whorl floral organs, the double mutant flowers had an increased number of stigma and carpel/carpel-like structures compared with mad56-1 (Figure 9), indicating additional defects in floral determinacy. In maize, BDE and ZAG1 were shown to physically interact (Thompson et al., 2009), yet such an interaction was not found between MAD56 and MAD53/MAD58 (Moon et al., 1999). This may suggest that rice MAD56 has conserved and divergent mechanisms from its counterpart in maize in forming protein complexes to specify floral development. Alternatively, MAD56 may have weak interaction with MAD53/58, which was not detected by previous attempts (Moon et al., 1999).

Our data suggest that in addition to C-class genes, MAD56 has other partners in determining floral meristem identity. MAD56 functions redundantly with SPW1, MAD513, and DL, respectively, in specifying floral meristem identity. Some flowers in mad56-1 spw1-1 exhibited new inflorescence-like organs in lieu of lodicules, suggesting that MAD56 and SPW1 play an important role in floral development, particularly in repressing meristem activity after the establishment of lodicule identity. Their genetic interaction may be explained by their protein–protein interaction observed by Seok et al. (2010). In addition to its role in promoting the expression of MAD54 and SPW1 during early flower development, MAD56 was also shown to physically interact with MAD54 and SPW1, suggesting that MAD56-MAD54-SPW1 complex formation may be essential for flower development (Seok et al., 2010) (Figure 11). Additionally, analysis of mad56-1 mad13-3 suggests the interaction of MAD56 with MAD513 in specifying floral meristem identity. MAD56 can interact with MAD513 at the protein level (Favaro et al., 2002), but they do not seem to regulate each other’s expression as revealed in this study (Figure 11).

Rice DL is different from the well-known ABC genes (Nagasawa et al., 2003; Yamaguchi et al., 2004). Moreover, the role of DL is distinct from the closely related Arabidopsis YABBY gene CRC, which only plays a partial role in carpel identity (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). Our recent analysis of mad3-4 dl-sup6 flowers indicated that DL and MAD53 act redundantly in specifying carpel identity and terminating floral meristem, but they may function in distinct pathways (Li et al., 2011) (Figure 11). Furthermore, previous investigation indicated that DL plays an antagonistic role with class B genes (Yamaguchi et al., 2004; Li et al., 2011). The mad56-1 dl-sup6 double mutant flowers displayed inflorescence-like structures in whorl 4, suggesting that MAD56 and DL may synergistically terminate the floral meristem (Figure 11).
MADS6 is able to specify floral meristem by interacting with E-class genes such as LHS1/MADS1, MADS7, and MADS8 (Figure 11). One possible mechanism is that MADS6 could positively regulate the expression of MADS7 and MADS8. mads1 mads6 double mutants had severe indeterminate floral meristem (Ohmori et al., 2009; Li et al., 2010), while our microarray (this study) and in situ analyses (Li et al., 2010) did not reveal obvious expression changes in LHS1/MADS1 in mads6-1. Although Moon et al. (1999) reported the protein–protein interaction between MADS6 and LHS1/MADS1 in yeast cells, we did not observe this interaction (Li et al., 2010), possibly due to weak protein interaction.

In summary, using double mutant analyses in combination with in situ and ChIP-qPCR experiments, we reveal interactions of MADS6 with floral homeotic genes SPW1, MADS3, MADS8, MADS13, and DL in regulating rice flower development and show that MADS6 positively regulates the expression of the B-, C-, and E-class genes during early flower development. A model (Figure 11) is proposed to illustrate the role of floral homeotic genes in the specification of flower organ identity and meristem determinacy in rice.

METHODS

Plant Materials
The mads6-1, mads13-3, mads3-4, and dl-sup6 mutants were identified previously (Chen et al., 2006b; Hu et al., 2011; Li et al., 2010, 2011). spw1-1 and mads58 were kindly provided by Hajime Sakai, Yasuo Nagato, and Venkatesan Sundaresan. Double mutants were isolated by phenotype observation and verified by genotyping. Methods for genotyping dl-sup6, mads13-3, and mads3-4 were described previously (Li et al., 2011). Primer pairs 6TPF/6TPR, 16TPF/16TPR, and 58TPF/58TPR were used for genotyping mads6-1, spw1-1, and mads58, respectively (see Supplemental Table 4 online). Mutant and wild-type rice (Oryza sativa) plants were planted in the paddie field or greenhouse in Shanghai Jiao Tong University, China.

Histological Analysis and Microscopy Observation
Flower materials were collected at various stages of development according to spikelet size and morphology descriptions defined by Ikeda et al. (2004), fixed in FAA (10% formalin, 50% ethanol, and 5% acetic acid), and dehydrated in a series of graded ethanol (Chu et al., 2006; Li et al., 2006). For histological analysis, tissues were then infiltrated with xylene and embedded in paraplast plus. Then, materials were sectioned to 8 μm thick and stained with toluidine blue (Bio Basic) and photographed using a Nikon E600 microscope and a Nikon DXM1200 digital camera. Electron microscopy scanning was performed with a JSM-6360LV (Jeol) as described previously (Li et al., 2006).

In Situ Hybridization
Samples were treated as described previously (Li et al., 2006). Constructs of gene-specific probes for MADS13, SPW1, DL, and MADS6 were described previously (Li et al., 2010, 2011). Constructs of MADS3 and MADS58 probes were generated based on descriptions by Yamaguchi et al. (2006) and probes of MADS7 and MADS8 were made as described by Cui et al. (2010). Digoxigenin-labeled antisense and sense probes were transcribed in vitro as described by Li et al. (2006). Images were obtained using an Olympus Nikon E600 microscope.

Microarray Analysis
Microarray experiments were performed as described by Hu et al. (2011). Total RNAs were isolated from three replicates of stage Sp6 flowers from the wild type and mads6-1. Developing flower primordia were collected based on microscopy analysis and spikelet length described by Ikeda et al. (2004).

qRT-PCR
Total RNA was isolated from wild-type and mads6-1 flowers at stage Sp4/Sp5, Sp6, early Sp8, and late Sp8. qRT-PCR conditions were the same as those described by Zhang et al. (2010a). Primers for MADS3 and MADS4 were described by Chen et al. (2006a), and primers for SPW1, MADS7, MADS8, and MADS58 were described by Cui et al. (2010). All samples were run with three replicates. Data acquisition and analyses were performed using the Roche Light Cycler software. Sample amounts used were normalized using ACTIN expression.

Preparation of the MADS6 Polyclonal Antibody
The MADS6-specific fragment (493 to 753 bp) was amplified by PCR using the primer pair 6APF/6APR (see Supplemental Table 4 online). PCR products were cloned into pET-32a (Novagen) to produce p32-P-MADS6. The 6XHis-MADS6 fused protein was expressed in Escherichia coli using p32-P-MADS6. Proteins were purified according to the manufacturer’s instructions, and the antibody was prepared as described by Huang et al. (2000) using the specific MADS6 fragment. Nuclear extracts from the wild-type flowers at stage Sp7 were used for immunoblot analysis to test the specificity of the MADS6 antibody, following the protocol used for the ChIP experiments (Zhang et al., 2010b), except that the tissue was not fixed. The glutathione S-transferase–tagged full-length MADS6 protein was used as the positive control for immunoblot analysis.

ChIP-qPCR and Fold Enrichment Analysis
Rice spikelets at stage Sp7 were treated and sonicated with an Ultrasonic Crasher Noise Isolating Chamber (Scientz). The procedure for ChIP of the MADS6-DNA complexes in wild-type flowers was modified from Haring et al. (2007). For each PCR reaction, 0.5 μl of recovered DNA from immune precipitation (IP) or mock was used as template, three biological replicates were included, and each reaction was repeated three times. Primers used for qPCR analyses are labeled in Figure 10 and listed in Supplemental Table 4 online, and reactions were performed on a Rotor-Gene RG3000A detection system (Corbett Research) using SYBR Green I. The normalized mean cycle threshold (Ct) of each gene was calculated and used to determine fold change according to the method described by Rotor-Gene version 6.0 (Build 38) software and Zhang et al. (2010a). The difference between the Ct of the antibody enrichment and no antibody control (mock) was calculated to obtain the relative enrichment of the fragments containing the putative CArG motifs. Quantification involved normalization of the Ct of each IP or the control sample to obtain a nonspecific adjustment ∆Ct (ΔCt IP – ΔCt mock), followed by calculation of relative enrichment of each fragment using the equation 2^[-ΔCt IP – ΔCt mock].

Accession Numbers
Sequence data from this article for the cDNAs of MADS6, SPW1, MADS3, MADS8, DL, MADS13, MADS7, and MADS8 can be found in the GenBank/EMBL data libraries under accession numbers AK069103, AK069317, AK108568, AK111723, AK242416, AK070425, AK100263, and AK072867, respectively. Locus identifications in the Rice Genome
Annotation Project Database are as follows: MADS6 (Os02g45770), SPW1 (Os06g49840), MADS3 (Os01g10504), MADS58 (Os05g11414), DL (Os03g11600), MADS13 (Os12g10540), MADS7 (Os08g41950), and MADS8 (Os09g32948). Microarray data accession number in NCBI is GSE29349.

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

**Supplemental Figure 1.** Spatial and Temporal Expression Pattern of DL and MADS6 as Revealed by In Situ Hybridization.

**Supplemental Figure 2.** Spatial and Temporal Expression Pattern of MADS13 and MADS6.

**Supplemental Figure 3.** Specificity Analysis of MADS6 Polyclonal Antibodies.

**Supplemental Table 1.** Up- or Downregulated (at Least Twofold Change in Expression, P Value < 0.05) Non-MADS Box Genes in mads6-1 Identified by Bayes Analysis (≤0.5% FDR).

**Supplemental Table 2.** Number of Floral Organs in Wild-Type and mads6-1, spw1-1, and mads6-1 spw1-1 Flowers.

**Supplemental Table 3.** Number of Floral Organs in Wild-Type and mads6-1, mads3-4, and mads6-1 mads3-4 Flowers.

**Supplemental Table 4.** Primers Used in This Study.

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AUTHOR CONTRIBUTIONS

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Rice *MADS6* Interacts with the Floral Homeotic Genes *SUPERWOMAN1, MADS3, MADS58, MADS13*, and *DROOPING LEAF* in Specifying Floral Organ Identities and Meristem Fate

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