Note

Double-Stranded RNA Interference of a Rice PI/GLO Paralog, OsMADS2, Uncovers Its Second-Whorl-Specific Function in Floral Organ Patterning

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ABSTRACT

Unlike many eudicot species, grasses have duplicated PI/GLO-like genes. Functional analysis of one of the rice PI/GLO paralogs, OsMADS2, is reported here. Our data demonstrate its essential role in lodicule development and implicate the second PI/GLO paralog, OsMADS4, to suffice for stamen specification. We provide the first evidence for differential contributions of grass PI/GLO paralogs in patterning second- and third-whorl floral organs.

SPECIFICATION of petals (second whorl) and stamens (third whorl) in eudicot flowers requires a pair of related genes. In Arabidopsis, these genes are APETALA3 (AP3) and PISTILLATA (PI) and in Antirrhinum majus they are DEFICIENS (DEF) and GLOBOSA (GLO). Mutations in either of these pairs of “B-function” genes have similar homeotic effects in the second and third whorls (LOHMANN and WEIGEL 2002). These genes work with “A-function” genes to confer second-whorl organ identity and with “C-function” genes to specify third-whorl identity. Both B- and C-function genes require yet another group of genes, SE PALLATA (SEP1, SEP2, and SEP3), for their activity (LOHMANN and WEIGEL 2002). B-function genes have been identified in the monocot cereal grasses, maize and rice, which have highly derived floral organs in the first and second whorls. The latter genes are similar to their eudicot counterparts in that they are expressed in lodicules (second whorl) and stamens (third whorl) and their limited mutational analysis (silky1 of maize and spa1 of rice) reveals second- and third-whorl organ identity changes (AMBROSE et al. 2000; KOY ZUKA et al. 2000; NANDI et al. 2000; MUNSTER et al. 2001; NAGASAWA et al. 2003). As in Arabidopsis and Antirrhinum, a single AP3/DEF ortholog exists in diverse monocots such as lily (Lilium regale), wheat (Triticum aestivum), maize, and rice (MUNSTER et al. 2001). In contrast, PI/GLO-like genes are duplicated in these monocot species (MUNSTER et al. 2001). A significant issue to be addressed is whether gene duplication of PI/GLO-like genes in grasses has any functional importance. To elucidate the specific function of OsMADS2, one of the rice PI/GLO-like genes, we have created knockdown phenotypes by exploiting double-stranded RNA-mediated interference, an efficient method to silence genes of interest (BAULCOMBE 2002). Thirteen independent transgenic lines were generated after transformation with the plasmid pUbi-dsRNAiOsMADS2 (Figure 1A). The floral phenotypes in these transgenic plants were analyzed.

Rice flowers, also called spikelets, are unique in their architecture. Male and female reproductive organs occupy positions identical to those in primitive flowers (IRISH 2001). The morphology of organs that surround the reproductive structures differs radically. The eudicot sepals and petals are replaced in rice florets by the lemma/palea and lodicules, respectively (IRISH 2001). Additionally, small bracts, termed outer glumes, sub-tend the spikelet. The gross morphology of flowers in pUbi-dsRNAiOsMADS2 transgenic plants resembles wild-type flowers, particularly with regard to the outer glumes, lemma, and palea (Figure 1, B and C). On the other hand, the second-whorl lodicules (Figure 2F) are approximately three times larger than the small and fleshy wild-type lodicules. The latter are usually wider at the base and narrower at the apex (Figure 2A). The larger transgenic lodicules are also wide at the base, but their significantly greater apical growth produces a structure mimicking the more peripheral organs (Figure 2F). These overgrown second-whorl structures have a green midvein, a feature typical of the outer glume and lemma/palea (Figure 1, B and C) but normally absent in lodicules.

The epidermal cell surfaces of sterile whorl organs in rice flowers are distinctive (PRASAD et al. 2001). We therefore carried out scanning electron microscopy...
projections and trichomes characterize the palea abaxial surface of the wild-type outer glume. Since rice has two sets of lodicules, we used pUbi-dsRNAiOsMADS2 transformed lodicules to establish their identity in pUbi-dsRNAiOsMADS2 expression cassettes. K and C represent K-box and C-terminal sequences, respectively. Embryogenic rice calli were transformed with this plasmid and transgenic plants were regenerated as described in Prasad et al. (2001). (B and C) Floral phenotypes of pUbi-dsRNAiOsMADS2 transgenic spikelets. Partially opened wild-type (B) and transgenic rice florets (C) showing outer glume (shaded arrows); le, lemma; pa, palea. Stamens are marked by an open arrowhead; open arrows point to the midvein in the glume and palea. Bar, 1 mm.

Figure 1.—(A) Schematic of transgene construct pUbi-dsRNAiOsMADS2. Transcripts generated can create double-stranded RNA molecules with a loop, LB, the left border; RB, right border; T-DNA repeats flank the HygR and dsRNAiOsMADS2 expression cassettes. K and C represent K-box and C-terminal sequences, respectively. Embryogenic rice calli were transformed with this plasmid and transgenic plants were regenerated as described in Prasad et al. (2001). (B and C) Floral phenotypes of pUbi-dsRNAiOsMADS2 transgenic spikelets. Partially opened wild-type (B) and transgenic rice florets (C) showing outer glume (shaded arrows); le, lemma; pa, palea. Stamens are marked by an open arrowhead; open arrows point to the midvein in the glume and palea. Bar, 1 mm.

(SEM) of the abaxial and adaxial cell surfaces of the transformed lodicules to establish their identity in pUbi-dsRNAiOsMADS2 transgenic plants. The epidermal cells of the wild-type outer glume are arranged in long smooth files (Figure 2, E and K). Cells with rounded projections and trichomes characterize the palea abaxial surface (Figure 2B), while the adaxial surface has smooth, wide, rectangular cells (Figure 2L). Although the epidermal cell morphology of palea is similar to that of the lemma, it can be distinguished from the lemma by a unique marginal tissue that is smooth and trichomeless (Figure 2, A, F, M, and N), rather like the cells of the outer glumes (Figure 2, E and K). Wild-type lodicules have an interlocking pavement-like arrangement of small, compact, rectangular cells (Figure 2, C, D, and O). Epidermal cells of the transgenic outer glume and palea are similar to wild type (compare Figure 2, B and E, with Figure 2, G and J). Strikingly, the enlarged lodicle-like organs of transgenic flowers have characteristics of the outer glume or the palea marginal tissue (Figure 2, H, I, and P–R). The proximal/basal region of these second-whorl organs is a mosaic of cells in elongated files together with cells with some lodicule features (Figure 2, I, P, and Q). The distal/apical portion of these enlarged structures consists of cells morphologically identical to the wild-type outer glume or the palea marginal tissue (compare Figure 2, H and R, with Figure 2, E, K, M, and N). Thus, knockdown of OsMADS2 perturbs the lodicule differentiation, creating cell types present in more peripheral floral organs. Although OsMADS2 is expressed throughout stamen primordia specification and differentiation (Kyozuka et al. 2000; Nandi et al. 2000), loss of its expression has no effect on stamen cellular differentiation (data not shown). The six stamens and single central carpel of these flowers are normal since the transgenic spikelets were fertile.

To determine early developmental effects of OsMADS2 knockdown on second-whorl organ patterning, spikelets undergoing organogenesis were taken up for SEM (Figure 3). The initiation of floral organ primordia in these transgenic spikelets is indistinguishable from that of the wild type (Figure 3, A vs. D). The lemma and palea primordia are the earliest to form and develop as hood-shaped organs, enclosing the inner structures of both wild-type and transgenic spikelets (Figure 3, B and E). Dissection of the lemma and palea exposes the developing lodicules and stamens. Differentiation of stamen primordia into anthers and filaments occurs comparably in wild-type and transgenic spikelets (Figure 3, C, F, and I). Wild-type lodicules initiate as a fleshy cup-shaped structure with a broad basal and a narrow apical end (Figure 3C). Lodicule differentiation in transgenic spikelets deviates from this early stage. While the transgenic lodicule is also cup shaped, it is flattened to a greater extent, thereby losing the typical thick fleshy characteristic seen in wild-type lodicules (Figure 3F). The epidermal cell surface features of these transgenic lodicules are also distinct from those in the wild type (compare Figure 3G with Figure 3H). Further, the apical end of transgenic lodicules continues to grow instead of terminating growth, as seen in comparably staged wild-type lodicules (compare Figure 3I with Figure 3C).

Since rice has two PI/GLO1-like genes, OsMADS2 and OsMADS4, that share substantial sequence similarity, especially in the MADS box (89% identical), we have examined the specificity of the OsMADS2 knockdown in our transgenic lines. Northern blot analysis on total RNA from wild-type and pUbi-dsRNAiOsMADS2 transgenic panicles was used to detect endogenous OsMADS2 transcripts and none were found in transgenic plants (Figure 4A). This suggests a complete transcriptional knockdown of OsMADS2. As expected, the 2.3-kb transcript from the transgene (DsOsMADS2: antisense, linker, and sense transcript) was detected (Figure 4A). This RNA is partially processed into small (~24-nucleotide) RNA molecules (our unpublished data), which likely trigger gene silencing. To determine OsMADS4 transcript levels in the transgenic panicles, RT-PCR was carried out using primers specific to this gene alone. No change in OsMADS4 transcript levels was found, a finding replicated in Northern blot analysis also (Figure 4B and data not shown).

Although stamens of eudicot and monocot flowers
Figure 2.—Comparison of epidermal cell morphologies in sterile whorl organs of a wild-type vs. pUBi-dsRNAiOsMADS2 transgenic floret by SEM. (A and F) Partially dissected wild-type and transgenic spikelets, respectively. The lemma has been removed from these florets. Open arrowhead points to the palea marginal tissue. B, C, D, and E show surfaces of wild-type palea, apical/distal lodicule, basal/proximal lodicule parts, and outer glume, respectively. (G) Cell surface of a transgenic palea. (H) Apical portion of a transgenic lodicule with features identical to the wild-type outer glume in E or the marginal tissue of the palea in M. (I) The central portion of a transgenic lodicule showing a mosaic of lodicule is indicated by open arrowheads and glume/palea marginal tissue cell types by open arrows. (J) Abaxial surface of a transgenic outer glume. (K and L) Adaxial surface of a wild-type outer glume and palea, respectively. (M and N) Abaxial and adaxial surfaces of the wild-type palea marginal tissue showing features as in the outer glume. (O) Adaxial surface of a wild-type lodicule. (P) Abaxial surface of the proximal part of a transgenic lodicule. Open arrowheads indicate islands of cells similar to the outer glume or the marginal tissue of the palea. Bars: A and F, 1 mm; B, G, and I, 50 μm; C–E, H, and J–R, 10 μm.

are homologous, the equivalence of lodicules to eudicot petals requires further investigation. Homologous organs can be expected to have a common descent, but monocot and eudicot petals are thought to have arisen independently (IRISH 2001). This indicates the likelihood of species-specific mechanisms for petal formation. In grasses, one such mechanism could originate from the duplication of a PI/GLO homolog. Here we provide evidence for functional relevance of such duplication in rice. Gene-specific knockdown of one of the rice PI/GLO-like genes followed by detailed phenotypic analysis of floral organ differentiation suggests a role only in lodicule development. The homeotic transformation of lodicule cell types to those present in more peripheral organs like the palea or glume, although obvious in these transgenic plants, is not complete. Perhaps B-function activity from SPW1 (OsMADS16) and OsMADS4 provides some partial lodicule identity. Alternatively, residual OsMADS2 activity, below our detection limits, persists to specify some lodicule characteristics. Transgenic rice plants expressing an antisense OsMADS4 cDNA are reported to be defective for both lodicule and stamen development (KANG et al. 1998). It is not known whether these cosuppression phenotypes originate from reduced levels of OsMADS4 alone or from the additional nonspecific cosuppression of OsMADS2 due to the general effects of antisense RNA on both members of this gene pair. From gross morphology, the defective lodicules of cosuppressed OsMADS4 transgenic plants were interpreted to be transformed lemma/palea-like organs (KANG et al. 1998). However, the study lacked cellular characterization of the transformed organs, and thus it is unclear whether the transformation to lemma/palea cell types was complete or whether any lodicule characteristics persisted. This is a relevant point since transformed second-whorl organs in spw1, a mutant in the sole rice AP3-like gene, do not have the full complement of all palea cell types. In spw1 flowers, lodicules acquire characteristics of only the marginal tissue of the palea, which are in fact similar to cell types of the outer glume (NAGASAWA et al. 2003 and our present study). In this respect, loss of either SPW1 or OsMADS2 creates similar transformation of second-whorl organs.

As predicted by the eudicot “ABC” model, rice B-function genes alone are not sufficient for lodicule development and they probably require another class of gene(s). Loss-of-function studies for candidate rice A-function genes are still awaited, as are partners and regulators of OsMADS2.

The second phenotype arising from cosuppression of OsMADS4 is conversion of stamens into carpel-like organs (KANG et al. 1998). In contrast, stamens are completely unaffected by loss of OsMADS2 RNA. Since Os-
MADS4 expression is unaltered in the latter transgenic plants, it must suffice for normal stamen development. An ancestral PI motif characterizes the C terminus of PI/GLO-like proteins in all flowering plants. Very recent emerging evidence demonstrates that this motif is essential for PI function (Lamb and Irish 2003). This motif is present in both the rice PI/GLO paralogs. We speculate that the amino acid sequence deviations within the core consensus found in OsMADS2 may underlie its functional divergence.

The only dicot B-function mutant that alters only second-whorl organ (petals) development is green petal (gp) of petunia. Although this petunia B-function gene is expressed in both second- and third-whorl organs, homeotic conversion occurs only in the second whorl of the gp mutant wherein petals are converted to sepal

(Kush et al. 1993; van der Krol et al. 1993). GP has been predicted to function redundantly with another petunia B class gene, PhBX, for stamen specification (Tsuchimoto et al. 2000). Here we provide the evidence that the rice B-function gene OsMADS2 that is specifically required for second-whorl (lodicule) development is dispensable for specifying the third whorl. Our data also shed light on the importance of PI/GLO-like gene duplication for functional diversification and grass floral organ patterning.

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LITERATURE CITED


Note


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