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The *YABBY* Gene *DROOPING LEAF* Regulates Carpel Specification and Midrib Development in *Oryza sativa* [W]

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In this article, we report that carpel specification in the *Oryza sativa* (rice) flower is regulated by the floral homeotic gene *DROOPING LEAF* (*DL*) that is distinct from the well-known ABC genes. Severe loss-of-function mutations of *DL* cause complete homeotic transformation of carpels into stamens. Molecular cloning reveals that *DL* is a member of the *YABBY* gene family and is closely related to the *CRABS CLAW* (*CRC*) gene of *Arabidopsis thaliana*. *DL* is expressed in the presumptive region (carpel anlagen), where carpel primordia would initiate, and in carpel primordia. These results suggest that carpel specification is regulated by *DL* in rice flower development. Whereas *CRC* plays only a partial role in carpel identity, *DL* may have been recruited to have the more essential function of specifying carpels during the evolution of rice. We also show that *DL* interacts antagonistically with class B genes and controls floral meristem determinacy. In addition, severe and weak *dl* alleles fail to form a midrib in the leaf. The phenotypic analysis of *dl* mutants, together with analyses of the spatial expression patterns and ectopic expression of *DL*, demonstrate that *DL* regulates midrib formation by promoting cell proliferation in the central region of the rice leaf.

INTRODUCTION

The specification of each type of floral organ is a key process in flower development. Molecular and genetic studies in two model eudicots, *Arabidopsis thaliana* (Arabidopsis) and *Antirrhinum majus* (snapdragon), have established the ABC model for the determination of floral organ identities (Bowman et al., 1991; Coen and Meyerowitz, 1991). This model proposes that each class of floral homeotic gene, termed A, B, and C, works in two adjacent whorls, and combinatorial activities of these genes specify four types of floral organ: sepals, petals, stamens, and carpels.

AGAMOUS (*AG*) plays a central role in specifying carpel identity in the Arabidopsis flower (Yanofsky et al., 1990; Bowman et al., 1991; Bowman et al., 1999). Loss-of-function mutations in *AG* result in homeotic transformation of stamens into petals in whorl 3 and of carpels into reiterating *ag* flowers in whorl 4. Thus, *AG* has functions that specify stamen and carpel identity, repress the activity of class A genes in the inner two whorls, and control floral meristem determinacy. It has been suggested, however, that carpel identity also is regulated in an *AG*-independent pathway because mutants lacking *AG* function, such as the *ap2 ag* double mutant, retain carpeloid properties (Bowman et al., 1991). Further genetic studies have revealed that *CRABS CLAW* (*CRC*) and *SPATULA* (*SPT*) act redundantly in this pathway to specify carpels and are negatively regulated by class A and class B genes (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). *CRC* is a member of the *YABBY* family of genes that encode plant-specific transcription factors with a zinc-finger domain and a helix-loop-helix domain (named the *YABBY* domain). In addition to *CRC* and *SPT*, the *SHATTERPROOF* MADS box genes partially contribute to carpel development (Pinyopich et al., 2003).

Although much progress has been made in our understanding of the molecular mechanisms of flower development in Arabidopsis, molecular developmental studies are incomplete in monocots. In the Poaceae (grass) family, flower developmental studies have focused on *Zea mays* (maize) and *Oryza sativa* (rice) (Schmidt and Ambrose, 1998; Goto et al., 2001) because of the availability of mutants, molecular tools, and information from genome analysis. In addition, grasses bear unique flowers that are distinct from those of dicots. The grass flowers have stamens and carpels like dicot flowers but lack obvious sepals and petals. Lodicules, which are specific to grass flowers and correspond to petals in dicots, are formed in the outer whorl of stamens. These three floral organs are subtended by palea and lemma. The grass carpels constitute a simple structure composed of stigmas, styles, and an ovary, in which a single ovule develops. The carpels do not differentiate to form transmitting tissues and septa as they do in Arabidopsis.

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Mutations in the *silky1* (*si1*) gene in maize and in the *SUPERWOMAN1* (*SPW1*) gene in rice cause homeotic transformation of stamens and lodicules into carpels and palea/lemma-like structures, respectively. (Ambrose et al., 2000; Nagasawa et al., 2003). These phenotypes are similar to those of class B mutants in Arabidopsis and *A. majus*. Isolation of the causative genes of these mutations revealed that both *si1* and *SPW1* encode MADS box genes that are orthologous to Arabidopsis *APETALA3* ([*AP3*]; Jack et al., 1992) and are expressed in the same domains in developing flowers as *AP3*. These results suggest that the function of class B genes and the mechanisms that specify petals/lodicules and stamens are conserved between dicots and monocots. By contrast, a loss-of-function mutation in *ZAG1*, an ortholog of *AG*, is not associated with any homeotic change in the maize flower, whereas the *zag1* flower, like the *ag* flower in Arabidopsis, lacks floral determinacy (Mena et al., 1996). The lack of homeotic changes in *zag1* is hypothesized to be attributable to the fact that this mutation is masked by the redundant functions of *ZMM2*, a paralog of *ZAG1*. Thus, the genetic mechanism that specifies carpel identity in grass species remains unclear.

Leaf morphologies also diverge in eudicots and grass species. Grass leaves have parallel venation, whereas most dicot leaves have reticulate venation. Rice leaves have three types of vein arranged in parallel: the central vein and the lateral large and small veins. The central vein comprises a strong structure, called the midrib, which serves to keep the leaves upright. Although some reports have described mutants lacking a midrib (Rao et al., 1988; Fladung et al., 1991), the genes controlling midrib formation have not been described in detail.

We found previously that severe mutations in the *DROOPING LEAF* (*DL*) locus in rice cause homeotic mutation of carpels into stamens in flowers and failure of midrib formation in leaves (Nagasawa et al., 2003). In this report, we describe isolation of the *DL* gene and its function in flower development and midrib formation. Our results show that *DL* is a member of the *YABBY* gene family and encodes a putative transcription factor that contains zinc-finger and helix-loop-helix domains. Phenotype and expression analyses indicate that *DL* is necessary for carpel specification and is negatively regulated by class B genes in rice. Among the Arabidopsis *YABBY* genes, *DL* is most similar to *CRC*, which has a partial role in carpel identity. Our results suggest that genes in the *DL*/*CRC* subfamily act in carpel development in both monocots and dicots, but their contribution to carpel specification may have been more crucial in grass flower development during the evolution of grasses. Finally, we show that *DL* regulates midrib formation by specifying cell fate to promote cell proliferation specifically in the central region of the leaf.

RESULTS

DL Regulates Carpel Identity and Floral Meristem Determinacy in the Rice Flower

Rice flowers have two lodicules, six stamens, and one pistil, which is presumably formed from three carpels, and these floral organs are subtended by palea and lemma (Figure 1A). In this article, we refer to the regions where lodicules, stamens, and a pistil develop in rice as whorl 2, whorl 3, and whorl 4, respectively, in line with the definition of regions in Arabidopsis.

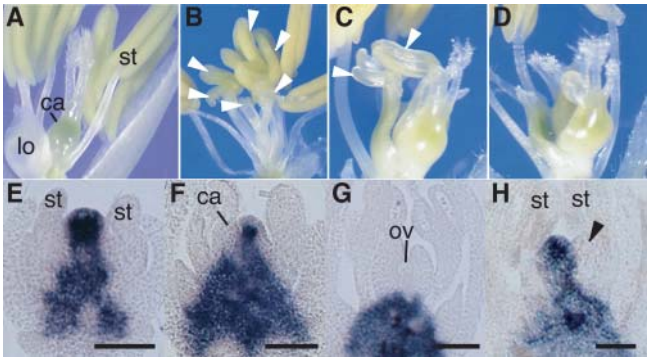

Figure 1. Flower Phenotypes and Expression Patterns of *OSH1*.

(A) Wild-type flower.
(B) Complete transformation of carpels into stamens in the *dl-sup1* flower.
(C) Partial transformation of carpels in the intermediate *dl-3* flower.
(D) Multiple carpels produced in the *dl-3* flower.
(E) to **(G)** Spatial expression patterns of *OSH1* in the wild-type flower.
(H) Spatial expression patterns of *OSH1* in *dl-sup1*.
Arrowheads indicate ectopic stamens. ca, carpel; lo, lodicule; ov, ovule; st, stamen. Bars = 20 μm.

Here, we isolated and analyzed five new *dl* alleles in addition to four previously described *dl* alleles (Nagasawa et al., 2003). Three severe alleles, *dl-sup3*, *dl-sup4*, and *dl-sup5*, showed homeotic transformation of carpels into stamens (Figure 1B) and a variation in the number of ectopic stamens from two to seven. No vestigial carpel-like or ovule-like tissues were observed. An intermediate allele, *dl-3*, showed pleiotropic abnormalities, such as partial transformation of carpels (Figure 1C), the production of multiple carpels (Figure 1D), the formation of cell clusters, and an increase in the number of styles (data not shown). No phenotypic change was observed in other floral organs in any of the alleles, suggesting that mutations of *DL* affect only the innermost whorl. These results indicate that *DL* is necessary for the specification of carpel identity.

The indeterminate formation of ectopic stamens in the severe *dl* alleles and the production of multiple carpels and clusters of undifferentiated cells in the intermediate *dl* allele suggested that *DL* might regulate floral meristem determinacy. To test this hypothesis, we examined the spatial expression pattern of the class I knox gene *OSH1*, which is a molecular marker of meristematic indeterminate cells in rice (Sato et al., 1996). In the wild-type flower, *OSH1* was expressed in the floral meristem and receptacle in the early stages of development (Figure 1E). The domain expressing *OSH1* in the meristem became smaller as carpel primordia arose (Figure 1F) and completely disappeared when carpels developed in the fourth whorl (Figure 1G). By contrast, *OSH1* expression in the *dl-sup1* flower was maintained in the central region even after ectopic stamens were produced (Figure 1H). These results indicate that *DL* is required for regulating floral meristem determinacy in addition to specifying carpel identity.

DL Is Required for Midrib Formation in Leaves

The *dl* mutations cause defects in midrib formation in leaves, resulting in drooping leaf phenotypes (Nagasawa et al., 2003). Here, we examined the defects in midrib structure in these mutants in detail. The midrib in wild-type rice leaves had two structural characteristics. First, it had two large locules, called clear cells (Figure 2A), which are thought to be formed by programmed cell death after the accumulation of a large number of cells in the central region of the leaf (see below) and which constituted hollow thin cylinders aligned from proximal to distal in the leaves. Second, it formed a small vascular bundle at the adaxial side opposite the central vascular bundle (Figures 2A and 2C). In the *dl* mutants, the central vein lacked both clear cells and the adaxial small vascular bundle (Figure 2E). In addition, the structure of the central vein in the *dl* mutants resembled that of the lateral large veins (Figures 2D and 2E).

To determine why the midrib was missing, we analyzed the developmental patterns of leaves in the early stages. In the wild type, the central vascular bundle was initiated at the end of plastchron1 (P1). During P3 and P4, the cells in the central region of the leaf proliferated along the adaxial-abaxial axis, and a large number of cells accumulated adaxial to the central vascular bundle (Figure 2F). The midrib structure was differentiated from the large number of proliferated cells in the central region after the P5 stage. By contrast, in *dl-sup1* mutants, the specific cell proliferation in the central region did not occur while the central vascular bundle was being formed (Figure 2G). The number of cell files along the adaxial-abaxial axis in the center of the *dl-sup1* leaf was reduced to approximately one-third of the number in the wild-type leaf. A failure to accumulate enough cells in the central region seems to be the primary cause of leaves lacking midribs. Taken together, these observations suggest that *DL* regulates midrib formation by inducing cell proliferation in the central region of the leaf.

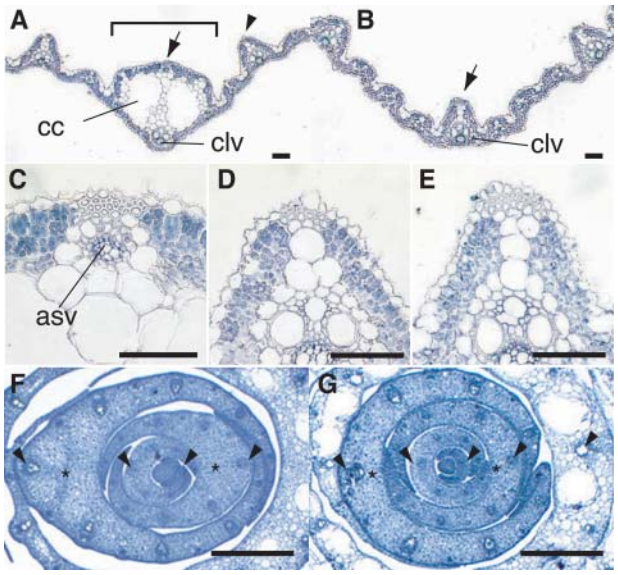

Figure 2. Leaf Phenotypes.

(A) and **(B)** Cross-section of the leaf blade of the wild type **(A)** and *dl-sup1* **(B)**. The bracket indicates the midrib structure.

(C) to **(E)** Enlarged views of the inner structure at the adaxial sides of the wild-type midrib **(C)**, indicated by the arrow in **(A)**; the wild-type lateral vein **(D)**, indicated by the arrowhead in **(A)**; and the *dl-sup1* central large vein **(E)**, indicated by the arrow in **(B)**.

(F) and **(G)** Cross-section of the shoot apex of the wild type **(F)** and *dl-sup1* **(G)**. Arrowheads indicate the central vascular bundles, and asterisks indicate the central regions of the leaves that differ in the wild type and *dl-sup1*.

asv, adaxial small vascular bundle; cc, clear cells; clv, central large vascular bundle. Bars = 100 μm.

Isolation of *DL*

To determine the molecular functions of *DL*, we first tried to isolate the *DL* gene by a map-based cloning strategy (see supplemental data online). The *dl* locus was mapped to restriction fragment length polymorphism (RFLP) markers on the short arm of rice chromosome 3. A BAC contig encompassing ~300 kb was constructed by screening using the closest marker C316 and subsequent chromosome walking. Next, we narrowed down the putative *DL* region using the mutant line fm28, which is supposed to have chromosomal deletion involving the *dl* and *leafy hull sterile* loci (Yoshimura et al., 1997), which are tightly linked together. The deleted region in fm28 was mapped by DNA gel blot analysis using DNA fragments arbitrarily isolated from a BAC clone (2E1213), and the deletion was found to be <40 kb. A λ contig covering the whole deletion then was constructed.

While these experiments were in progress, a new *dl* allele (*dl-sup3*) was isolated from R2 progenies that were derived from rice plants regenerated through tissue culture. Because the retrotransposon *TOS17* is known to be mobilized and to cause somaclonal mutations during tissue culture (Hirochika et al., 1996), we examined whether the mutation in *dl-sup3* was caused by the insertion of *TOS17*. DNA gel blot analysis using a probe for *TOS17* indicated that one of the bands cosegregated with the drooping leaf phenotype (data not shown). Subsequent DNA gel blot analysis using a probe for the DNA sequence flanking the *TOS17* in this band showed that the sequence cosegregated with the *dl* phenotype and genotype (Figure 3A). The *TOS17* insert was found to be located in a region of ~40 kb, which corresponded to the region found to contain the *dl* locus in our map-based approach. Thus, the results of our two approaches for isolating the *DL* gene were in agreement.

Sequence analysis of both the genomic DNA and the cDNA revealed that the putative *DL* gene covered ~10 kb and comprised seven exons and six introns (Figure 3B). To verify whether this gene was *DL*, a construct containing the genomic sequence of the candidate gene was transformed into the *dl-1* plant. Leaves in the transgenic plants stood upright, and the midrib was formed as in the wild type (see supplemental data online). The slight phenotypic alterations in the pistil observed in this intermediate allele also were rescued by the transgene (data not shown).

Next, we determined the DNA sequences of this candidate gene for each of the *dl* mutant alleles. In all severe alleles, the mutations detected were associated with serious defects at the molecular level: nucleotide substitution occurs at an RNA

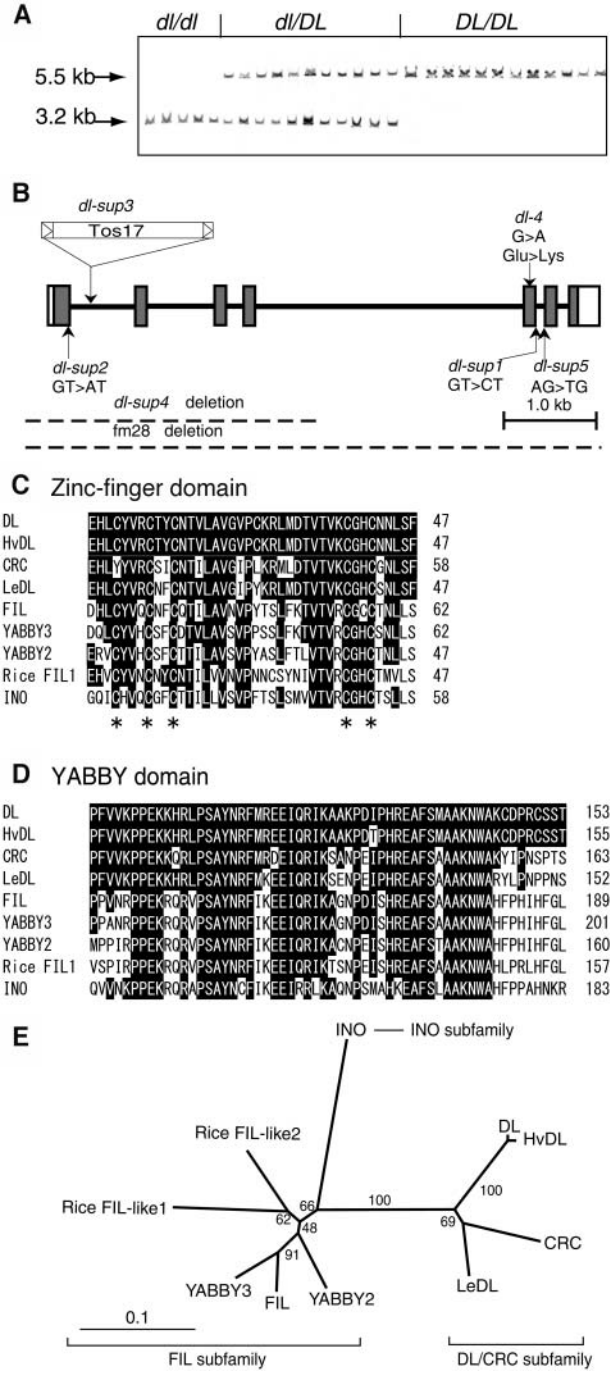

Figure 3. Gene Isolation and Structural Features of *DL*.

(A) Cosegregation of the *TOS17* insertion with *DL* genotypes. The genotypes, which were determined by the drooping leaf phenotype of the next generation (R_3), are shown above the lanes. The 5.5-kb *XbaI* band corresponds to the wild-type allele, whereas the 3.2-kb band is produced by the insertion of *TOS17*, which contains an internal *XbaI* site.

(B) Genomic structure of *DL* and mutations in the six *dl* alleles. Boxes indicate exons and thick lines indicate introns. The coding regions are shown by shaded boxes.

splicing site in *dl-sup1*, *dl-sup2*, and *dl-sup5*; the DNA sequence, including exon 1 to exon 4, is deleted in *dl-sup4*; and *TOS17* is inserted into the first intron of *dl-sup* (Figure 3B). In the weak *dl-4* allele, a single base change causes an amino acid replacement from Glu (118) to Lys in the *YABBY* domain (Figure 3B; see also supplemental data online).

Taken together, these results clearly indicate that the candidate gene that we isolated is derived from the *dl* locus.

DL Is a Member of the *YABBY* Gene Family and Is Closely Related to Arabidopsis *CRC*

The longest *DL* cDNA isolated was 1037 bp and encodes a putative protein of 194 amino acids (see supplemental data online). Database analysis revealed that *DL* is a member of the *YABBY* gene family, which is specific to plant genomes. In Arabidopsis, this family includes developmental genes such as *CRC* (Bowman and Smyth, 1999), *FILAMENTOUS FLOWER* (*FIL*) (Sawa et al., 1999), and *INNER NO OUTER* (*INO*) (Villanueva et al., 1999). Like other *YABBY* genes, *DL* encodes a protein containing two distinct domains: a zinc-finger domain in the N-terminal region and a C-terminal *YABBY* domain, which may form a helix-loop-helix structure and shares low similarity with the HMG box (Bowman and Smyth, 1999).

The amino acid identities between DL and other YABBY proteins (except HvDL, which belongs to the same grass family as rice) were found to vary from 48.6 to 87.5% (mean 65.0%) in the zinc-finger domain and from 62.7 to 83.6% (mean 74.6%) in the *YABBY* domains (Figures 3C and 3D). Although the overall homology in the zinc-finger domain was lower than that in the *YABBY* domain, five Cys residues were found to be conserved among nine proteins examined, except for the first Cys in CRC (Figure 3C). No obvious homology was observed in the central region or outside of the two domains.

A phylogenic tree constructed from a comparison of the two domains indicated that the *YABBY* genes are divided into three subfamilies: the *DL*/*CRC* subfamily, including *DL* and Arabidopsis *CRC*; the *FIL* subfamily, including almost all *YABBY* genes in Arabidopsis and rice (only one EST of rice is shown); and the *INO* subfamily, including only *INO* (Figure 3E). Some amino acid sequences, such as SF in the C-terminal region of the zinc-finger domain and PFVVK in the N-terminal region of the *YABBY* domain, were found to be specific to proteins in the *DL*/*CRC*

(C) Zinc-finger domain. The five conserved Cys residues are indicated with asterisks.

(D) YABBY domain. The regions containing both domains have been extended slightly beyond the original definition (Bowman and Smyth, 1999) through the accumulation of more *YABBY* sequences. Amino acids identical to those of *DL* are indicated with black boxes.

(E) Phylogeny of the *YABBY* gene family. The tree was constructed by the neighbor-joining method (Saitou and Nei, 1987). Numbers denote bootstrap values. Amino acid sequences of HvDL (*Hordeum vulgare* [barley] *DL* homolog) and LeDL (*Lycopersicon esculentum* [tomato] *DL* homolog) were deduced from the following EST sequences: HvDL (BG369278, AL509850) and LeDL (AI485831, AI483816). Rice FIL-like proteins have been described by Sawa et al. (1999).

subfamily. Thus, the phylogenic analysis and the amino acid comparison together indicate that *CRC* is the *YABBY* gene in Arabidopsis that is most similar to *DL* in rice.

Temporal and Spatial Expression Patterns of *DL*

We analyzed the spatial and temporal expression patterns of *DL* during flower and leaf development by in situ hybridization. In the wild-type flower, a bulge corresponding to the carpel primordia first arose at the flower meristem near the lemma (Figure 4A), and then the primordia developed from the flank of the meristem toward the opposite side, enclosing the meristem (Figures 4B and 4C). The meristem remained morphologically undifferentiated and later developed into the ovule. Thus, in rice flower development, the central region of the flower meristem is not consumed by carpel primordia. This contrasts with flower development in Arabidopsis, in which carpel primordia develop at the expense of the floral meristem.

DL transcripts were not detected from the early stages of flower development to the stage when the stamen primordia arose (Figure 4D). *DL* expression was detected first in a few cells in the flower meristem at the lemma side (Figure 4E) and then in cells at the flank of the meristem (Figure 4F). These *DL* expression domains corresponded to the presumptive region (carpel anlagen), where carpel primordia would initiate. Shortly after this expression, carpel primordia began to form. At this stage, *DL* was expressed specifically and uniformly in carpel primordia (Figures 4G to 4I). No expression was detected in the flower meristem (Figures 4E and 4F) or the ovule primordium (Figures 4G to 4I). By contrast, expression of *DL* was not observed in *dl-sup1* mutants throughout flower development (data not shown). Thus, the expression domains of *DL* are restricted to the carpel anlagen and the carpel primordia, and these expression patterns of *DL* are consistent with its specific role in carpel development.

During leaf development, *DL* transcripts were detected first in the central region of the P1 primordia (Figures 5A and 5B). As the wild-type leaf grows, the central region becomes thicker through cell proliferation, and at this stage, *DL* expression was restricted to several cell arrays in the central region (Figure 5C). During the P1 to P3 stages, the *DL* expression domain spanned from the adaxial to the abaxial epidermal tissues. At the P4 stage, *DL* expression disappeared from the abaxial mesophyll, which was almost mature, but was maintained in the central region abaxial to the central vascular bundle (Figure 5C). The expression of *DL* was hardly detectable in the P5 leaves (Figure 5C). The disappearance of *DL* transcripts was confirmed by longitudinal sections, which contained all parts of the P5 leaves (data not shown). *DL* expression also was detected in the central region of the leaf primordia in the embryo (Figure 5D) and in the region that corresponds to the midrib in the lemma (Figures 4E and 4G). Thus, the expression of *DL* is consistent with its specific role in midrib development.

DL Expression Is Negatively Regulated by *SPW1*

Loss-of-function mutations of *SPW1* cause homeotic conversion of lodicules and stamens into palea-like organs and carpels,

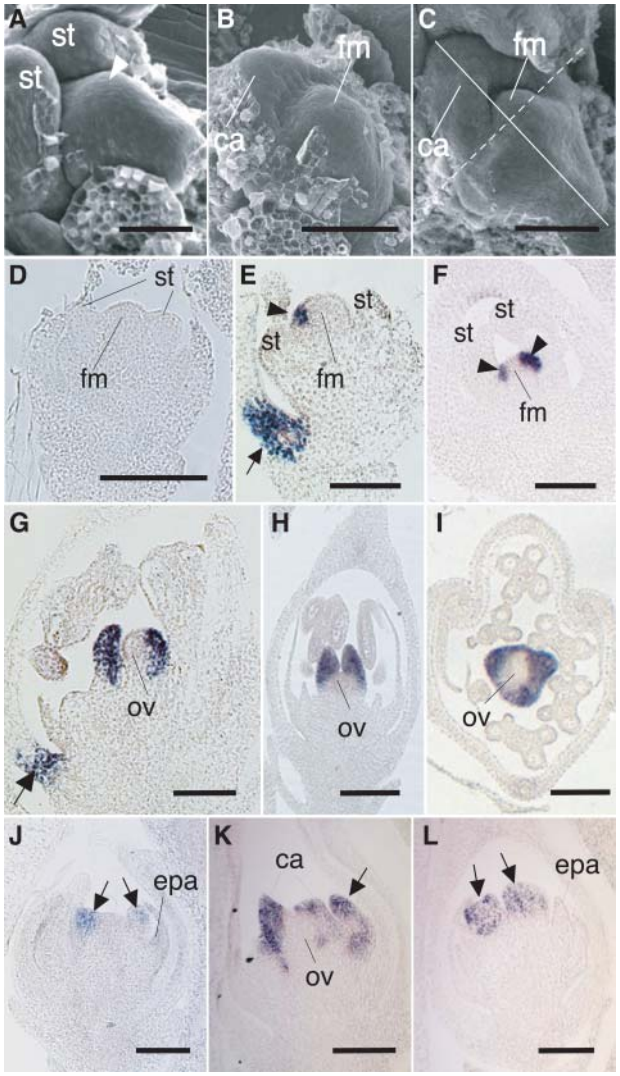

Figure 4. Scanning Electron Micrographs of Carpel Development and in Situ Localization of *DL* Transcripts.

(A) Scanning electron micrograph of the wild-type floral meristem just before carpel initiation.

(B) and **(C)** Scanning electron micrographs of carpel primordia and the flower meristem in the wild-type flower.

(D) to **(I)** Localization of *DL* transcripts in the wild-type flower (**[D]** to **[H]**) longitudinal sections; **[I]** transverse section). Sectioned planes are indicated in **(C)** by a solid line (for **[E]** and **[G]**) and by a dashed line (for **[D]**, **[F]**, and **[H]**).

(J) to **(L)** Localization of *DL* transcripts in the *spw1* flower. The flower stage shown in **(J)** is the same as that shown in **(D)**.

Arrowheads in **(A)**, **(E)**, and **(F)** indicate the carpel anlagen. Arrows in **(E)** and **(G)** are *DL* signals in the region that correspond to the midrib in the lemma. Arrows in **(J)** to **(L)** indicate ectopic carpels in whorl 3. ca, carpel; epa, ectopic palea-like organ; fm, floral meristem; ov, ovule; st, stamen. Bars = 20 μm.

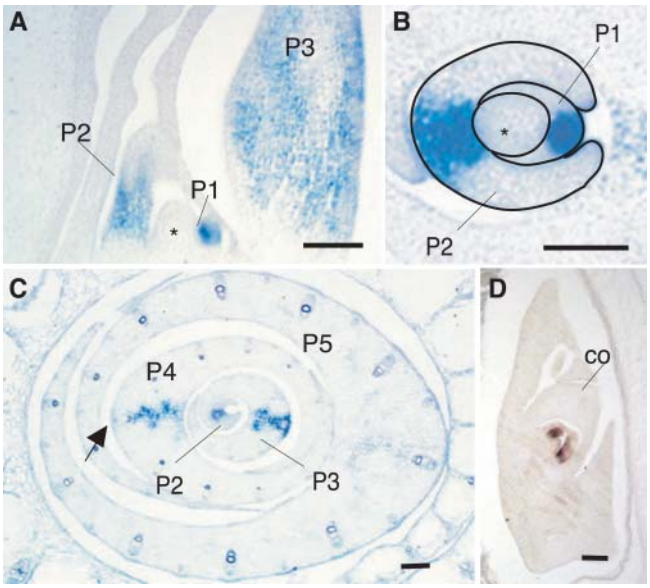

Figure 5. Localization of *DL* Transcripts in Developing Wild-Type Leaves and an Embryo.

(A) to **(C)** DL expression in leaves. Longitudinal **(A)** and transverse (**[B]** and **[C]**) section of a vegetative shoot apex. P1 and P2 leaf primordia and the shoot apical meristem are outlined in **(B)**. The section of **(C)** was taken 100 μm above that shown in **(B)**.
(D) *DL* expression in an embryo 10 d after pollination, when two leaves had initiated.
Plastochron numbers are indicated as P1 to P5. Asterisks indicate the shoot apical meristem. The arrow shows the abaxial mesophyll. co, coleoptyle. Bars = 20 μm.

respectively (Nagasawa et al., 2003). To investigate how *DL* genetically interacts with *SPW1*, we examined the *DL* expression pattern in the *spw1-1* flower. *DL* transcripts first were detected ectopically in the primordia in whorl 3, which developed ectopic carpels (Figure 4J). The timing of this expression was earlier than that observed in the wild type (cf. Figure 4D). Expression of *DL* continued during the development of both the ectopic carpels and the original carpels (Figures 4K and 4L). These observations indicate that *DL* is involved in the specification of the ectopic carpels in the *spw1* mutant and that the expression of *DL* in whorl 3 may be regulated negatively by *SPW1* in the wild type. No expression was observed in the ectopic palea-like organs in whorl 2 in the *spw1* mutant (Figures 4J and 4L).

Ectopic Expression of *DL*

To gain further insight into the functions of *DL*, we produced transgenic rice plants that overexpressed *DL* under the control of the constitutive rice actin promoter. *ACTIN:DL* plants showed aberrant seedling phenotypes and died after producing four to eight leaves. Leaf blades in *ACTIN:DL* plants curled toward the inside, forming a cylinder-like structure. New leaves were prevented from emerging from these cylindrical leaves (Figure 6D). Transverse sections showed that the leaf blades were thickened, and midrib-like structures were formed in the lateral regions as well as in the central region in *ACTIN:DL* lines (Figure 6E). Clear cells were ectopically formed in the lateral regions, and the small adaxial vascular bundle that is specific to the midrib in the wild-type leaf also developed at the adaxial side opposite the large vascular bundle in the *ACTIN:DL* leaf. The leaf blades became gradually thinner toward the marginal regions. Analysis of earlier developmental stages of the leaves showed that cells proliferated along the adaxial-abaxial axis not only in the central regions but also in the lateral regions in *ACTIN:DL* lines (Figure 6F). Taken together, these observations support the idea that *DL* regulates midrib formation by promoting cell proliferation in the central region of wild-type leaves.

No alterations in adaxial-abaxial identity were observed in the arrangement of the leaf inner structure (Figures 6G and 6H). Bulliform cells, which are responsible for the leaf rolling that occurs under drought conditions in rice, were deposited in the adaxial side of the leaf blade in *ACTIN:DL* lines as they are in the wild type. The sclerenchyma was formed abaxial to the larger vascular bundle in *ACTIN:DL* lines as it is in the wild type.

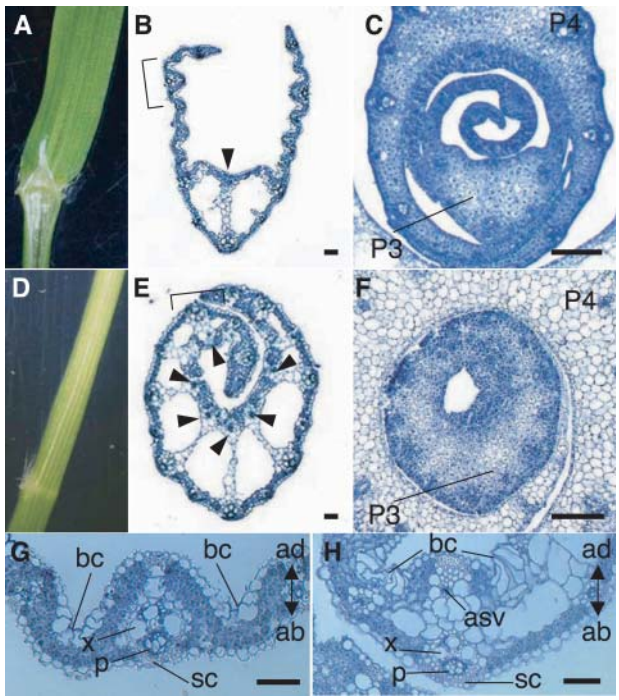

Figure 6. Phenotypes of *ACTIN:DL* Plants.

(A) and **(D)** Morphology of leaf blades.
(B) and **(E)** Transverse sections of the leaf blades of seedlings.
(C) and **(F)** Transverse sections of developing (P3 to P4) leaf blades.
(G) and **(H)** Enlarged view of the regions shown by the brackets in **(B)** and **(E)**, respectively.
(A), **(B)**, **(C)**, and **(G)** Wild-type.
(D), **(E)**, **(F)**, and **(H)** *ACTIN:DL*.
Arrowheads indicate adaxial small vascular bundles. ab, abaxial; ad, adaxial; asv, adaxial small vascular bundle; bc, bulliform cells; p, phloem; sc, sclerenchyma; x, xylem. Bars = 200 μm.

Similarly, the arrangement of xylem and phloem in *ACTIN:DL* lines was the same as that in the wild type. These observations suggest that *DL* is not sufficient for the control of dorsoventrality.

DISCUSSION

We have isolated the rice *DL* gene by two alternative strategies: map-based cloning and transposon tagging. *DL* encodes a putative transcriptional regulator that contains zinc-finger and YABBY domains and is most similar to *CRC* among the Arabidopsis *YABBY* genes. Analyses of phenotypes and special expression patterns strongly suggest that *DL* is involved in carpel specification in the flower and midrib development in the leaf.

DL Functions in Flower Development

A severe or intermediate mutation in *DL* causes complete or partial homeotic conversion of carpels into stamens without affecting the identities of other floral organs, suggesting that *DL* functions only in whorl 4. The expression of *DL* is detected first in the presumptive region, where carpels initiate, and then specifically expressed in carpel primordia. Thus, the spatial and temporal patterns of *DL* expression in carpel development are correlated with floral phenotypes. *DL* also is expressed in ectopic carpel primordia in whorl 3 of the *spw1* flower, in which stamens are homeotically transformed into carpels, suggesting that *DL* is involved in the development of these ectopic carpels. In fact, neither the original carpels nor the ectopic carpels observed in *spw1* were initiated in *dl-sup1 spw1-1* double mutants (Nagasawa et al., 2003). These results clearly indicate that *DL* is a floral homeotic gene that regulates carpel specification in rice.

The flowers in severe loss-of-function *dl* mutants seemingly resemble those in *superman* (*sup*) mutants of Arabidopsis (Bowman et al., 1992; Sakai et al., 1995) because the number of stamens is increased in both types of plant. A detailed comparison of the mutant phenotypes suggests, however, that the functions of *DL* and *SUP* differ (Nagasawa et al., 2003). The spatial expression patterns of *DL* also indicate that the functions of the two genes are distinct. First, *DL* is specifically expressed in carpel anlagen and carpel primordia, whereas *SUP* is expressed in the inner part of whorl 3 (Sakai et al., 1995). Second, *DL* is ectopically expressed in whorl 3 of the *spw1* flower, whereas the spatial expression pattern of *SUP* is not altered in the *ap3* flower, although its expression level is decreased (Sakai et al., 2000). Neither carpels nor stamens are developed in the *dl-sup1 spw1-1* double mutant (Nagasawa et al., 2003), whereas the *ap3 sup* double mutant shows a phenotype similar to that of the *ap3* single mutant (Sakai et al., 2000). Arabidopsis *SUP* is thought to be involved in maintaining the boundary between whorl 3 and whorl 4, and defects in this gene cause extra stamens to form in whorl 3 at the expense of carpel development (Sakai et al., 1995). By contrast, the severe phenotypes of *dl* mutants may be explained by homeotic transformation of carpels into stamens, together with loss of floral meristem determinacy.

Homeotic transformation of stamens into carpels in *spw1* mutants suggests that *DL* is regulated negatively by *SPW1* in whorl 3 in the wild type (Nagasawa et al., 2003). Here, we have clearly proved this hypothesis by showing that *DL* is expressed in the ectopic carpel primordia in whorl 3 of the *spw1* flower. Conversely, homeotic transformation of carpels into stamens, coupled with the spatial expression pattern of *SPW1* in severe *dl* mutants, indicates that *SPW1* is negatively controlled by *DL* in whorl 4 in the wild type (Nagasawa et al., 2003). Thus, *DL* and *SPW1* antagonistically regulate each other. Further molecular studies may be required to address the question of whether this mutual negative regulation is direct or indirect. Although *DL* transcripts were detected in whorl 3, they were not detected in whorl 2 in the *spw1* flower, suggesting that *DL* is repressed by class A genes or requires the function of class C genes.

It is of great interest to know how class C genes are involved in carpel development in rice. In maize, a loss-of-function mutation of *ZAG1* does not affect floral organ identity on its own (Mena et al., 1996) but causes loss of carpel identity when coupled with a mutation in *si1* (Ambrose et al., 2000). Moreover, it has been suggested that the *ZAG1* paralog *ZMM2* acts redundantly in carpel identity (Mena et al., 1996). In rice, antisense suppression of the *AG* ortholog *OsMADS3* produces flowers with abnormal carpels but does not cause clear homeotic change in carpels (Kang et al., 1998). By contrast, two other functions of class C genes—namely, the negative regulation of class A genes and stamen specification in conjunction with class B genes—have been demonstrated by the results from antisense suppression and ectopic expression of *OsMADS3* (Kang et al., 1998; Kyozuka and Shimamoto, 2002). We have found that rice has another *AG* ortholog in addition to *OsMADS3* (T. Yamaguchi and H.-Y. Hirano, unpublished data). Therefore, to reveal the contribution of class C genes to carpel specification in rice, an approach based on the knockout of both class C genes will be required.

We also have shown that *DL* regulates determinacy of the floral meristem. This was predicted from the phenotypes of the *dl* mutants and confirmed by the expression pattern of *OSH1*. Studies of *zag1* mutants have demonstrated that this class C gene in the grass family, like *AG* in Arabidopsis, is involved in the control of floral determinacy as is the *indeterminate floral apex1* gene (Mena et al., 1996; Laudencia-Chingcuanco and Hake, 2002). The regulation of floral meristem determinacy and the interaction between *DL* and these genes will be interesting subjects for future studies of flower development in rice. It also is of great interest to note that some genes, such as *DL* and *AG*, are involved in the regulation of both carpel identity and floral meristem determinacy. Therefore, the two functions may be closely associated with each other.

DL Function in Midrib Formation

In developing leaves, before the formation of a midrib structure, the central region adaxial to the central vascular bundle is thickened by cell proliferation. This proliferation provides enough cells to form a midrib structure. In *dl* mutants, this specific cell proliferation seems not to occur because the number of cell files in the center of the leaf is reduced and no obvious thickening of the central region is observed. In the wild type, *DL* starts to be

strongly expressed in the central region of the leaf before thickening and continues to be expressed during thickening. Therefore, the function of *DL* may be to specify cell fate to promote the specific proliferation of cells in the center of the leaf.

Our results from the overexpression of *DL* strongly support this hypothesis. In *ACTIN:DL* plants, the lateral regions of the leaf also became thick from vigorous cell proliferation, resulting in the formation of midrib-like structures in these regions. Thus, we conclude that the primary function of *DL* in leaf development is to specify the midrib by promoting cell proliferation specifically along the adaxial-abaxial axis. The leaf thickness was reduced toward the peripheral region and midrib-like structures did not form in the leaf margins in *ACTIN:DL* plants, suggesting that the function of *DL* is regulated negatively by unknown factors in the peripheral and marginal regions. Furthermore, as *DL* is expressed and functions to form the midrib only in the central region of the leaf in the wild type, it may be associated with the central lateral properties of the leaf.

Evolutionary Implications of *DL* Function

Phylogenic analysis revealed that *DL* is most similar to *CRC* among Arabidopsis *YABBY* genes. We have isolated seven additional rice *YABBY* genes, which are classified into the *FIL* or *INO* subfamilies (K. Harada, A. Takamura, T. Yamaguchi, and H.-Y. Hirano, unpublished data). We could not identify any other *YABBY* genes in the sequence database of the rice genome, for which sequencing has been almost completed. Because all of the Arabidopsis *YABBY* genes have been described (Bowman and Smyth, 1999; Sawa et al., 1999; Siegfried et al., 1999; Villanueva et al., 1999), these analyses confirm that *DL* and *CRC* are orthologs.

Our studies indicate that *DL* has three functions in rice flower development: specification of carpel identity, control of floral meristem determinacy, and antagonistic regulation with class B genes. In Arabidopsis, genetic and molecular analyses have revealed that *CRC* is involved in an *AG*-independent pathway of carpel specification and in control of floral determinacy (Alvarez and Smyth, 1999). *CRC* also is negatively regulated by class B genes (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). In addition, the formation of ectopic stamen interior to the carpels in *crc*/*crc ag*/+ plants suggests that *CRC* represses class B genes (Alvarez and Smyth, 1999). From these observations, we conclude that the functions of the *DL*/*CRC* subfamily are fundamentally conserved between rice and Arabidopsis.

Unlike severe loss-of-function mutations of *DL*, however, loss-of-function mutations of *CRC* do not result in homeotic transformation of carpels (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). *DL* may have been recruited to acquire critical functions to specify carpel identity during grass evolution (see below). Alternatively, genes in the *DL*/*CRC* subfamily of *YABBY* genes may contribute differently to carpel specification, depending on the plant species. It is possible that the diverse contribution of *DL*/*CRC* may be associated with differences in carpel architecture. The Arabidopsis carpel, especially the ovary, has a complex structure consisting of various differentiated tissues, such as septa, abaxial repla, transmitting tissues, and placenta (Bowman et al., 1999), whereas the rice pistil is simple because its ovary does not contain these differentiated tissues and encloses a single ovule. It is plausible that the *crc* mutation does not cause serious defects in carpel development in Arabidopsis because many genes coordinately specify the complex differentiation of the carpel (Alvarez and Smyth, 1999; Bowman and Smyth, 1999; Eshed et al., 1999; Kuusk et al., 2002; Pinyopich et al., 2003). By contrast, loss of the function of *DL* may be critical in rice because it is thought that only a few genes regulate carpel development. In either case, we conclude that *DL* has a predominant role in carpel specification in rice.

Defects in both carpel identity and midrib formation have been reported in mutants of two other grass species, *Pennisetum americanum* (pearl millet) and *Panicum aestivum* (Rao et al., 1988; Fladung et al., 1991). Because the phenotypes of these mutants are quite similar to those of the rice *dl* mutants, these mutations may be caused by *DL* orthologs in each plant. This suggests that genes in the *DL*/*CRC* subfamily may have been recruited to play an essential role in carpel specification and midrib formation during grass evolution. As *crc* mutants do not show any phenotypic changes in the leaf and *CRC* is not expressed in the leaf (Alvarez and Smyth, 1999; Bowman and Smyth, 1999), the *DL* function that controls midrib formation may have been acquired in the monocot lineage or during grass evolution.

Our results have shown that *DL* is a member of the *YABBY* gene family. In Arabidopsis, the function of *YABBY* genes is thought to be to promote abaxial cell fate in the lateral organs (Eshed et al., 1999; Sawa et al., 1999; Siegfried et al., 1999; Bowman, 2000), except for *CRC*, which plays a role in nectary development in addition to its role in regulating abaxial cell fate in carpels (Alvarez and Smyth, 1999; Bowman and Smyth, 1999). It is unlikely, however, that *DL* is involved in abaxial specification in rice. The phenotypes of *dl* mutants were not associated with abaxial identity, and no expression of *DL* was observed on the abaxial side in either the carpel or the leaf. Overexpression of *DL* did not cause structural alterations associated with dorsoventrality of the leaf. The *YABBY* genes seem to have evolved recently during plant evolution because database analysis indicates that no *YABBY*-like genes have been detected in animal and fungal genomes. Therefore, it is plausible that the *YABBY* genes have acquired their diversified functions during the evolution of monocots and dicots. Furthermore, because *DL*/*CRC* and *INO* regulate development of the carpel and outer integument, respectively (Alvarez and Smyth, 1999; Bowman and Smyth, 1999; Villanueva et al. 1999), both of which are characteristic of angiosperms, elucidation of the *YABBY* gene function in various angiosperms, including primitive species, may aid our understanding of the evolution of body plans in angiosperms and the mechanisms by which they are determined.

METHODS

Materials

The rice strains used in this study were *O. sativa* spp *japonica*. Taichung 65 (T65) was used for the wild type in cDNA isolation, histological observation, and in situ hybridization. Mutants *dl-1*, *dl-2, dl-sup1*, and *dl-sup2* were described by Nagasawa et al. (2003). *dl-sup3*, *dl-sup4*, and

dl-sup5 were isolated from the R_2 population of plants (Nipponbare) regenerated by the method of Hirochika et al. (1996). *dl-3* and *dl-4* were obtained from an M_2 population of Kinmaze that had been chemically mutagenized with *N*-methyl-*N*-nitrosourea. A chromosomal deletion line, fm28, was isolated from the M_2 population (Nihon-masari), which was produced by γ-ray irradiation.

Isolation of *DL*

The *dl* locus has been mapped to the short arm of rice chromosome 3 (Yoshimura et al., 1997). The *dl* mutant (Japonica) was crossed with Kasalath (Indica), and F_2 plants with the drooping leaf phenotype were selected and used for the following experiments. The *dl* locus was mapped among RFLP markers, and the marker C316 was found to be closest to this locus (no recombinant was found in 304 chromosomes). Next, we constructed a BAC contig by screening of a BAC library (Nakamura et al., 1997) with C316 and subsequent chromosomal walking. This BAC contig covered ~300 kb. Sequences at both ends of the BAC contig showed recombination with the *dl* locus (R176, one recombinant; L1L, four recombinants), indicating that the *dl* locus is contained in this contig. Next, we used a mutant line, fm28, which probably has a chromosomal deletion that includes *dl*. The deleted region in fm28 was mapped by DNA gel blot analysis using DNA fragments arbitrarily isolated from a BAC clone (2E1213), and the size of the deletion was found to be <40 kb. The DNA (15 to 18 kb) derived from the BAC clone 2E1213 was subcloned into λDASH (Stratagene, La Jolla, CA), and a λ contig covering the deleted region was constructed.

A new mutant with drooping leaf was identified in the R_2 population of regenerated rice plants through tissue culture. Genetic complementation analysis showed that this new mutant was allelic to other *dl* mutants, such as *dl-1*. The genotypes of 28 R_2 plants, determined by the phenotype of the next generation, were *DL*/*DL* (12 plants), *DL*/*dl* (11 plants), and *dl*/*dl* (5 plants). DNA gel blot analysis using the retrotransposon *TOS17* as a probe detected a 3.2-kb band in all plants with the *dl*/*dl* and *DL*/*dl* genotypes but was not detected in plants with *DL*/*DL*. DNA fragments specific to the *dl*/*dl* and *DL*/*dl* genotypes were isolated from a gel, and a DNA fragment (~500 bp) flanking *TOS17* was amplified by thermal asymmetric interlaced PCR. This fragment was used as a probe for subsequent DNA gel blot analysis.

Isolation of *DL* cDNA and DNA Sequencing

A cDNA library was constructed from poly(A)$^+$ RNA isolated from inflorescences containing developing flowers at early stages using λZAPII (Stratagene). The cDNA library was screened with a 4.0-kb DNA fragment flanking *TOS17*. After a second screening, five positive clones were obtained out of 5×10^5 clones. All five clones are identical, and the longest cDNA was 1019 bp. The λDNA (D14) including the *DL* genomic DNA was subcloned into pBluescript SK+ (Stratagene). The nucleotide sequence was determined with a DYE primer cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA) after making nested deletion series using a double-stranded nested deletion kit (Pharmacia, Piscataway, NJ). For identification of mutations in the *dl* mutants, DNA fragments including several exons were amplified with primers containing sequences complementary to universal primers (21M13 and RV) at the outside introns, and nucleotide sequences were determined by the DYE primer method.

Complementation Test and Overexpression of *DL*

The EcoRI fragment (thick line in Figure 1 in the supplemental data online) of λD14 was isolated, cloned into a binary vector, and used for complementation analysis. For overexpression, *DL* cDNA was inserted in the binary vector containing the cassette of the rice actin (*Act1*) promoter and the nos terminator (Sentoku et al., 2000). *Agrobacterium tumefaciens*–mediated transformation was performed using calli derived from scutellum according to Hiei et al. (1994).

In Situ Hybridization

A part of the *DL* cDNA (100 to 447) was amplified and cloned into a T-dvector and used for the synthesis of an RNA probe to avoid cross-hybridization among highly conserved sequences corresponding to the YABBY domain. In situ hybridization with digoxigenin-labeled RNA was conducted as described by Kouchi and Hata (1993). Tissues were fixed with 4% (w/v) paraformaldehyde and 0.25% glutaraldehyde in 0.1 M sodium phosphate buffer and embedded in Paraplast Plus (Oxford Labware, St. Louis, MO). Microtome sections (7 to 10 μm thick) were applied to glass slides treated with Vectabond (Vector Laboratories, Burlingame, CA).

Sequence data of the cDNA and genomic DNA of *DL* have been deposited with the EMBL/GenBank data libraries under accession numbers AB106553 and AB106554, respectively.

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REFERENCES

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

Ambrose, B.A., Lerner, D.R., Ciceri, P., Padilla, C.M., Yanofsky, M.F., and Schmidt, R.J. (2000). Molecular and genetic analyses of the *silky1* gene reveal conservation in floral organ specification between eudicots and monocots. Mol. Cell **5,** 569–579.

Bowman, J.L. (2000). The *YABBY* gene family and abaxial cell fate. Curr. Opin. Plant Biol. **3,** 17–22.

Bowman, J.L., Baum, S.F., Eshed, Y., Putterill, J., and Alvarez, J. (1999). Molecular genetics of gynoecium development in *Arabidopsis*. Curr. Top. Dev. Biol. **45,** 155–205.

Bowman, J.L., Sakai, H., Jack, T., Weigel, D., Mayer, U., and Meyerowitz, E.M. (1992). *SUPERMAN*, a regulator of floral homeotic genes in *Arabidopsis*. Development **114,** 599–615.

Bowman, J.L., and Smyth, D.R. (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, J.L., Smyth, D.R., and Meyerowitz, E.M. (1991). Genetic interactions among floral homeotic genes of *Arabidopsis*. Development **112,** 1–20.

Coen, E.S., and Meyerowitz, E.M. (1991). The war of the whorls: Genetic interactions controlling flower development. Nature **353,** 31–37.

Eshed, Y., Baum, S.F., and Bowman, J.L. (1999). Distinct mechanisms promote polarity establishment in carpels of *Arabidopsis*. Cell **9,** 199–209.

Fladung, M., Bossinger, G., Roeb, G.W., and Salamini, F. (1991). Correlated alterations in leaf and flower morphology and rate of leaf photosynthesis in a *midribless* (*mbl*) mutant of *Panicum maximum* Jacq. Planta **184,** 356–361.

Goto, K., Kyozuka, J., and Bowman, J.L. (2001). Turning floral organs into leaves, leaves into floral organs. Curr. Opin. Genet. Dev. **11,** 449–456.

Hiei, Y., Ohta, S., Komari, T., and Kumashiro, T. (1994). Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. Plant J. **6,** 271–282.

Hirochika, H., Sugimoto, K., Otsuki, Y., Tsugawa, H., and Kanda, M. (1996). Retrotransposons of rice involved in mutations induced by tissue culture. Proc. Natl. Acad. Sci. USA **93,** 7783–7788.

Jack, T., Brockman, L.L., and Meyerowitz, E.M. (1992). The homeotic gene APETALA3 of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. Cell **68,** 683–697.

Kang, H.-G., Jeon, J.-S., Lee, S., and An, G. (1998). Identification of class B and class C floral organ identity genes from rice plants. Plant Mol. Biol. **38,** 1021–1029.

Kouchi, H., and Hata, S. (1993). Isolation and characterization of novel nodulin cDNAs representing genes expressed at early stages of soybean nodule development. Mol. Gen. Genet. **238,** 106–119.

Kuusk, S., Sohlberg, J.J., Long, J.A., Fridborg, I., and Sundberg, E. (2002). *STY1* and *STY2* promote the formation of apical tissues during *Arabidopsis* gynoecium development. Development **129,** 4707–4717.

Kyozuka, J., and Shimamoto, K. (2002). Ectopic expression of *OsMADS3*, a rice ortholog of *AGAMOUS*, caused a homeotic transformation of lodicules to stamens in transgenic rice plants. Plant Cell Physiol. **43,** 130–135.

Laudencia-Chingcuanco, D., and Hake, S. (2002). The *indeterminate floral apex1* gene regulates meristem determinacy and identity in the maize inflorescence. Development **129,** 2629–2638.

Mena, M., Ambrose, B.A., Meeley, R.B., Briggs, S.P., Yanofsky, M.F., and Schmidt, R.J. (1996). Diversification of C-function activity in maize flower development. Science **274,** 1537–1540.

Nagasawa, N., Miyoshi, M., Sano, Y., Satoh, H., Hirano, H.-Y., Sakai, H., and Nagato, Y. (2003). *SUPERWOMAN 1* and *DROOPING LEAF* genes control floral organ identity in rice. Development **130,** 705–718.

Nakamura, S., Asakawa, S., Ohmido, N., Fukui, K., Shimizu, N., and Kawasaki, S. (1997). Construction of an 800-kb contig in the near-centromeric region of the rice blast resistance gene Pi-ta-2 using a highly representative rice BAC library. Mol. Gen. Genet. **254,** 611–620.

Pinyopich, A., Ditta, G.S., Savidge, B., Liljegren, S.J., Baumann, E., Wisman, E., and Yanofsky, M.F. (2003). Assessing the redundancy of MADS-box genes during carpel and ovule development. Nature **424,** 85–88.

Rao, S.A., Mengesha, M.H., and Reddy, C.R. (1988). Characteristics, inheritance, and allelic relationships of *midribless* mutants in pearl millet. J. Hered. **79,** 18–20.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4,** 406–425.

Sakai, H., Krizek, B.A., Jacobsen, S.E., and Meyerowitz, E.M. (2000). Regulation of *SUP* expression identifies multiple regulators involved in arabidopsis floral meristem development. Plant Cell **12,** 1607–1618.

Sakai, H., Medrano, L.J., and Meyerowitz, E.M. (1995). Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. Nature **378,** 199–203.

Sato, Y., Hong, S.K., Tagiri, A., Kitano, H., Yamamoto, N., Nagato, Y., and Matsuoka, M. (1996). A rice homeobox gene, *OSH1*, is expressed before organ differentiation in a specific region during early embryogenesis. Proc. Natl. Acad. Sci. USA **93,** 8117–8122.

Sawa, S., Watanabe, K., Goto, K., Kanaya, E., Morita, E.H., and Okada, K. (1999). *FILAMENTOUS FLOWER*, a meristem and organ identity gene of Arabidopsis, encodes a protein with a zinc finger and HMG-related domains. Genes Dev. **13,** 1079–1088.

Schmidt, R.J., and Ambrose, B.A. (1998). The blooming of grass flower development. Curr. Opin. Plant Biol. **1,** 60–67.

Sentoku, N., Sato, Y., and Matsuoka, M. (2000). Overexpression of rice *OSH* genes induces ectopic shoots on leaf sheaths of transgenic rice plants. Dev. Biol. **220,** 358–364.

Siegfried, K.R., Eshed, Y., Baum, S.F., Otsuga, D., Drews, G.N., and Bowman, J.L. (1999). Members of the *YABBY* gene family specify abaxial cell fate in *Arabidopsis*. Development **126,** 4117–4128.

Villanueva, J.M., Broadhvest, J., Hauser, B.A., Meister, R.J., Schneitz, K., and Gasser, C.S. (1999). *INNER NO OUTER* regulates abaxial- adaxial patterning in *Arabidopsis* ovules. Genes Dev. **13,** 3160–3169.

Yanofsky, M.F., Ma, H., Bowman, J.L., Drews, G.N., Feldmann, K.A., and Meyerowitz, E.M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. Nature **346,** 35–39.

Yoshimura, A., Ideta, O., and Iwata, N. (1997). Linkage map of phenotype and RFLP markers in rice. Plant Mol. Biol. **35,** 49–60.