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The *LAX1* and *FRIZZY PANICLE 2* Genes Determine the Inflorescence Architecture of Rice by Controlling Rachis-Branch and Spikelet Development

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We have analyzed two mutants that exhibit altered panicle architecture in rice (*Oryza sativa* L.). In *lax1-2,* which is a new and stronger allele of the previously reported *lax* mutant, initiation and/or maintenance of rachis-branches, lateral spikelets, and terminal spikelets was severely prevented. *In situ* hybridization analysis using *OSH1,* a rice *knotted1* (*kn1*) ortholog, confirmed the absence of lateral meristems in *lax1-2* panicles. These defects indicate that the *LAX1* gene is required for the initiation/maintenance of axillary meristems in the rice panicle. In addition to its role in forming lateral meristems, the wild-type *LAX1* gene acts as a floral meristem identity gene which specifies the terminal spikelet meristem. A comparison of the defects in *lax1-1* and *lax1-2* plants suggested that the sensitivities to reduced *LAX1* activity were not uniform among different types of meristems. In the *fzp2* mutant panicle, the basic branching pattern of the panicle was indistinguishable from that of the wild type; however, specification of both terminal and lateral spikelet meristems was blocked, and sequential rounds of branching occurred at the point where the spikelet meristems are initiated in the wild-type panicle. This resulted in the generation of a panicle composed of excessive ramification of rachis-branches. The *lax1-1 fzp2* double mutants exhibited a novel, basically additive, phenotype, which suggests that *LAX1* and *FZP2* function in genetically independent pathways. **© 2001 Academic Press**

Key Words: **rice; shoot branching; inflorescence; meristem identity; meristem determinacy.**

INTRODUCTION

Plant form is established postembryonically by the continuous formation of lateral organs and axillary shoots. The progressive growth of axillary shoots during development creates a particular pattern of branched growth which is defined genetically and is thus characteristic to each species. The transition of the shoot apical meristem (SAM) from vegetative to reproductive development causes a change of the branching pattern that leads to the generation of a more complex structure, the inflorescence. Generally, the architecture of inflorescences depends mainly on a basic branching pattern and the positioning of flowers (Weberling, 1989; Coen and Nugent, 1994). Even though molecular genetic approaches in the past decade using *Arabidopsis* and *Antirrhinum* as model species have successfully identified a set of genes called floral meristem identity genes as key players in floral meristem initiation (Weigel and Meyerowitz, 1994; Yanofsky, 1995; Ng and Yanofsky, 2000), the genetic mechanisms determining the positioning of floral meristems are still unknown. Furthermore, studies on the inflorescence shoot branching at the molecular level have not been pursued.

Clear changes of phylotaxy after the transition to reproductive development are observed in most plant species. This indicates that the transition sets off a new regulatory mechanism to control inflorescence branching. Consistent with this, mutations that specifically affect either inflorescence branching or vegetative branching have been reported

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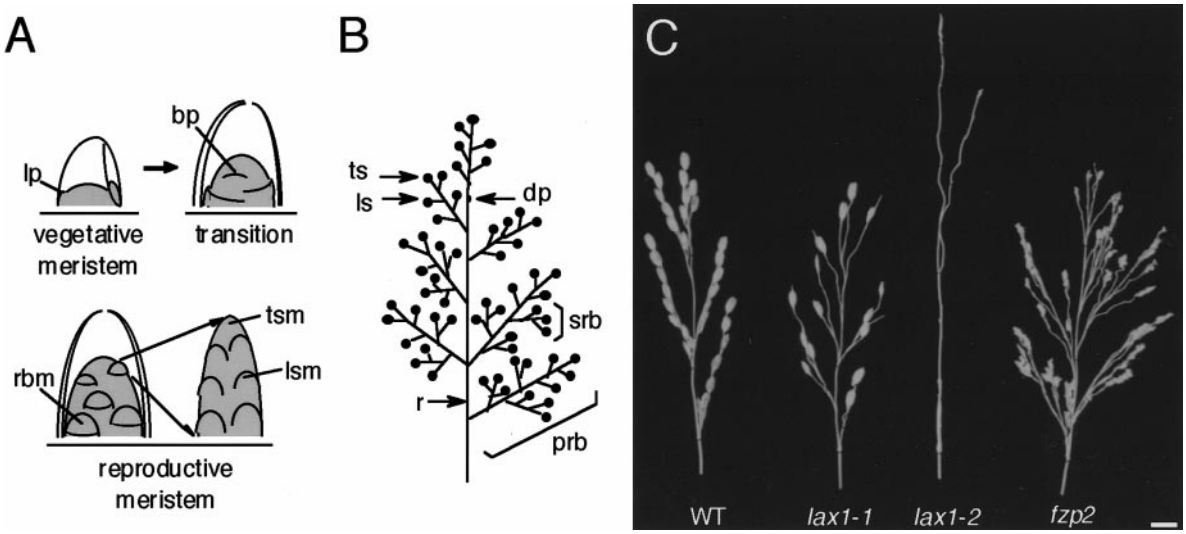

FIG. 1. Wild-type and mutant rice inflorescences used in this study. (A) Schematic diagram summarizing the development of rice meristems. Leaf primordia (lp) arise at angles of 180° during vegetative growth. In the transition to the reproductive phase, bract primordia (bp) develop at angles of 144°, from where rachis-branch meristems (rbm) arise. Bracts do not develop further, but rachis-branch meristems give rise to terminal (tsm) and lateral (lsm) spikelet meristems at 180°. (B) Structure of a mature rice inflorescence (panicle). A mature rice panicle is composed of primary rachis-branches (prb), secondary rachis-branches (srb), sometimes tertiary rachis-branches (not shown), and a rachis, the main stem of the panicle. The apical meristem of the inflorescence is degenerated after the production of the primary rachis-branches and left as a knob called a degenerate point (dp) in a mature panicle. The primary node is the position where the bract of the first primary rachis-branches formed. Lateral spikelets (ls) are formed on panicle branches and each branch ends in the terminal spikelets (ts). (C) Panicle morphology of mutants. From the left to the right, wild-type, *lax1-1*, *lax1-2*, and *fzp2* panicles. All lateral spikelets are absent, but fertile terminal spikelets are produced normally in *lax1-1*. Formation of rachis-branches and spikelets is extremely reduced in *lax1-2*. Primary rachis-branches are formed normally in the *fzp2;* however spikelets are not produced at all. Bar, 1 cm.

in a variety of species (Allen and Sussex, 1996; Napoli and Ruehle, 1996; Sheridon, 1998; Souer *et al.*, 1998). Moreover, it was recently demonstrated that the transition leads to a change in the activation pattern of the preformed axillary meristem from acropetal to basipetal in *Arabidopsis* (Grbic and Bleecker, 2000). Also, a gene which is specifically involved in axillary meristem initiation only in the vegetative phase is also known. In the *lateral suppressor* (*Ls*) mutant of tomato, the vegetative lateral shoot branching is totally absent, whereas the inflorescence shows a normal appearance. Conservation between the LS amino acid sequence and the gene family containing the VHIID domain indicated the role of *LS* gene in gibberellic acid (GA) signaling (Schumacher *et al.*, 1999).

On the other hand, to some extent, lateral branching in the vegetative and reproductive developmental phases is controlled by common mechanisms. The maize *TEOSINTE BRANCHED* (*TB1*) mutant plants, for example, produce excess branching in the vegetative as well as the reproductive developmental phases due to the loss of apical dominance, suggesting that *TB1* is involved in the suppression of lateral branch formation in both developmental phases (Doebley *et al.*, 1997). Expression of the *Shootmeristemless* (*STM*) of *Arabidopsis, knotted 1* (*kn1*) of maize, and their cognate orthologs in other species in all the axillary meristems suggests that the *KN1* family of homeotic genes is likely to be involved in shoot branching throughout development (Jackson *et al.*, 1994; Long *et al.*, 1996).

Genetic and molecular analyses of mutants that show altered growth patterns and morphology offer powerful tools for unveiling the complex processes of plant development. Because of their distinctive architecture, grasses are useful for studying the genetic control of inflorescence form as well as the evolution of plant morphology. Among the grass species, we chose rice (*Oryza sativa* L.) because of its advantages for molecular biological studies (Izawa and Shimamoto, 1996). In grass species, flowers are called florets and are aggregated into groups known as spikelets (Hoshikawa, 1989; Bell, 1991; McSteen *et al.*, 2000). The number of florets present in a single spikelet varies depending upon the species. For example, in rice, a spikelet contains a single floret, whereas a maize spikelet contains two florets. A rice inflorescence is termed a panicle since the rachis, the main axis of the rice inflorescence, is repeatedly branched. After the transition, the primary inflorescence meristem produces several rachis-branch primordia in the axil of bracts. Subsequently, the primary rachis-branches begin to form secondary rachis-branches or

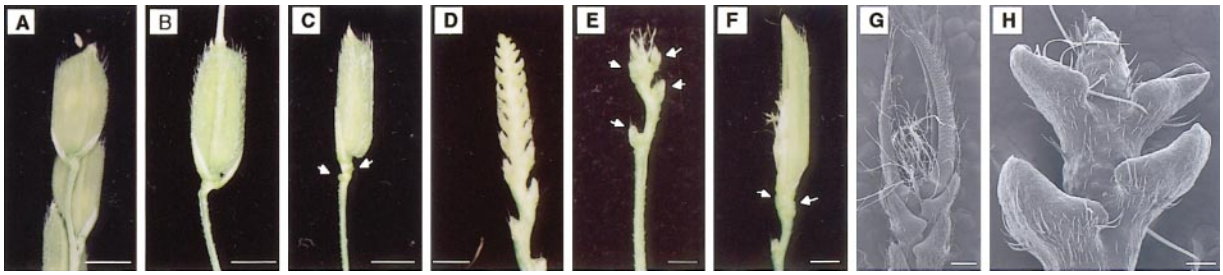

FIG. 2. Terminal spikelets in *lax1* mutants. (A) Wild-type spikelets. Each floret is enclosed by a pair of glumes. (B) *lax1-1*. (C) *lax1-1*/*lax1-2*. The extra bracts are observed (arrows). (D) The apical region of the *lax1-2* panicle showing the strong phenotype. Abnormally big extra bracts are produced without the formation of axillary lateral organs. (E and F) The apical region of the *lax1-2* panicle showing moderate (E) and weak (F) defects. Arrows in (C), (E), and (F) indicate the extra bracts. (G and H) SEM views of the terminal structure produced in the *lax1-2* panicle. Bar, 1 cm in (A) to (F), 1 mm in (G) and (H).

lateral spikelet primordia. Then, each rachis-branch ends in a fertile spikelet after it has formed a defined number of spikelets. Therefore, in addition to the primary inflorescence meristem, at least three different new types of reproductive meristems are initiated in rice panicle development; the rachis-branch meristem (RBM), the lateral spikelet meristem (LSM), and the terminal spikelet meristem (TSM). It is of interest to determine how the generation and development of these meristems are controlled. In rice, many mutants with altered panicle morphology are known, but they have not been satisfactorily analyzed (Kinoshita and Takahashi, 1991; Murai and Izawa, 1994; Kyozuka, 1999).

In this study, we describe two mutants that exhibit altered panicle branching. Our analyses of the two mutant loci indicated that the *LAX1* and *FZP2* genes play fundamental roles in the establishment of rice panicle architecture.

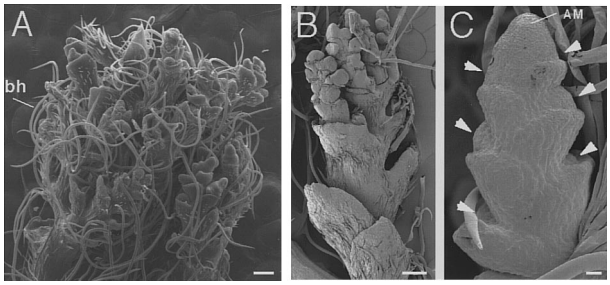

FIG. 3. SEM view of a young *fzp2* panicle. (A) Overview of *fzp2* panicle. The whole panicle is composed of a massive formation of branch meristems. No abnormality is found in the development of bract hair (bh). Bar, 100 μm. (B) Closer view of one branch. Continuous formation of axillary shoot meristems is observed. Bar, 50 μm. (C) A very young branch with lateral meristems arising from the axils of bracts (arrowheads). Phylotaxy of the meristems is apparently 180°.

MATERIALS AND METHODS

Mutagenesis

lax1-2 and *fzp2* mutants were found among 5540 γ-irradiated M2 plants (cv. Shiokari) grown in the field. The *lax1* mutation (Futsuhara *et al.*, 1979) was introduced to cv. Shiokari in eight successive crossings. In this paper, we refer to this original *lax1* allele as *lax1-1*.

SEM

Young panicles from wild types and mutants were dissected and fixed overnight at 4°C in 2.5% glutaraldehyde (2.5% GA in a 50 mM phosphate buffer, pH 7.0). Tissues were dehydrated through an ethanol series of 25 to 100% and dried. Specimens were coated and then analyzed on a scanning electron microscope (Hitachi S-4700, Hitachi, Japan) using an accelerating voltage of 15 kV.

Histology and in Situ Hybridization

Young panicles were fixed with FAA solution at 4°C overnight followed by dehydration steps and then embedded in paraffin (Paraplast+; Oxford Labware). The tissues were sliced into 10-μm sections, dried overnight onto 3-amino-propyltriethoxy silane-coated slides (ProbeOn Plus, Fisher Biotech Co.), and used for hemaxylin staining and *in situ* hybridization.

In situ hybridization was performed according to Kyozuka *et al.* (1998). The N-terminus and 5′ untranslated region of the *OSH1* (Matsuoka *et al.*, 1993) were used as a template to make digoxigenin-labeled RNA probes. Hybridization was performed overnight at 55°C. After hybridization, sections were washed in 4× SSPE followed by one washing with RNase solution (20 mg/L) at 37°C for 30 min and four washings (30 min each) in 0.5× SSPE solution at 65°C.

RESULTS

Development of the Wild-Type Rice Panicle

Here, we briefly summarize the normal development of the rice panicle (Fig. 1A). After the transition to reproduc-

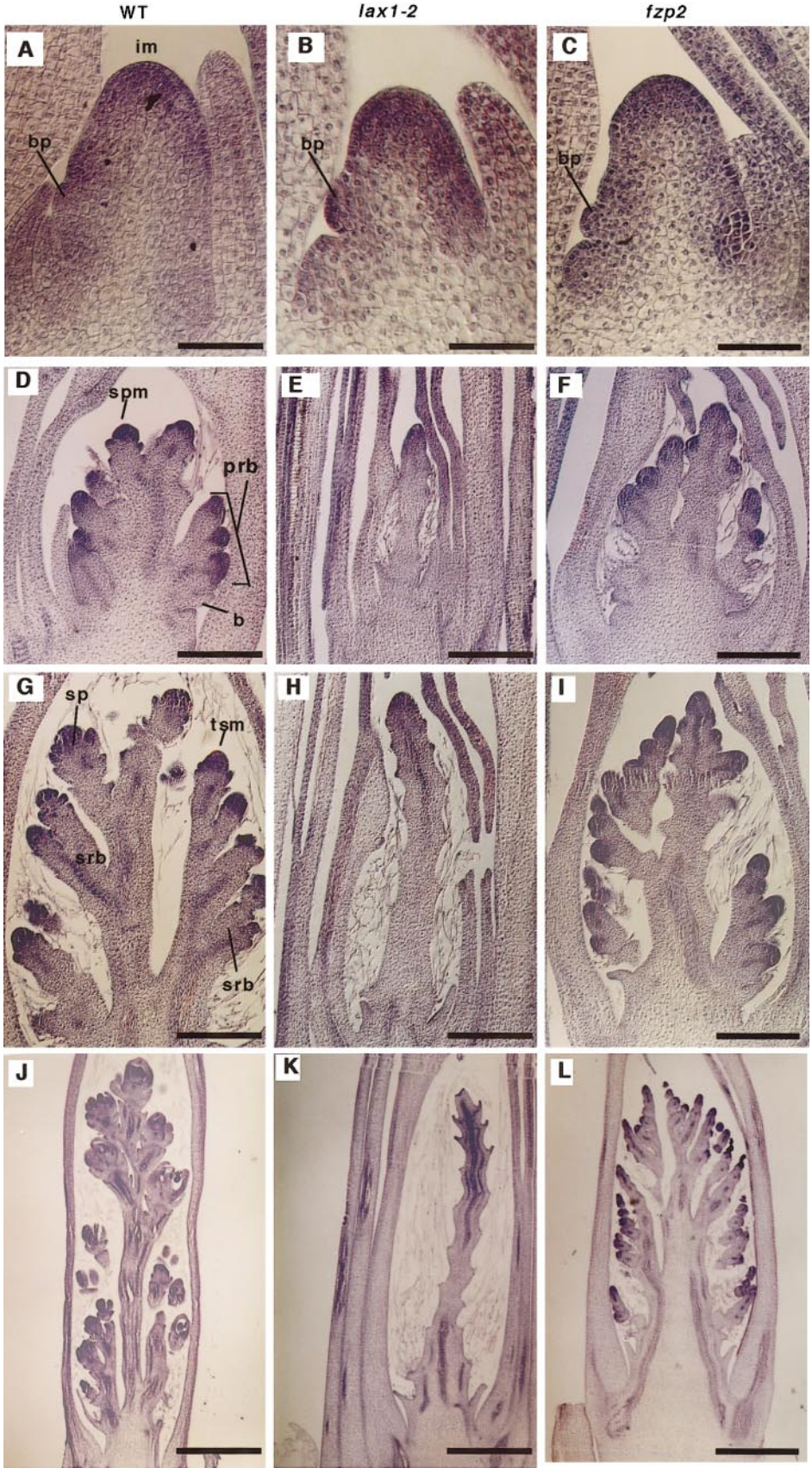

TABLE 1
Phenotype of the *lax1-2* Mutation

	n	Culm length (cm)[a]	No. of tillers	No. of primary branches	Length of panicle[b]	No. of nodes[c]
WT	26	44.2 ± 4.9	5.1 ± 1.4	6.2 ± 1.0	10.6 ± 0.7	11.0 ± 0.9
lax1-2	17	43.3 ± 2.1	5.8 ± 1.9	2.6 ± 0.9	15.0 ± 1.0	19.0 ± 3.0

[a] Length from the ground to the panicle node.
[b] Length between the panicle node and the degenerated point.
[c] Number of nodes on the main axis.

tive development, the inflorescence meristem produces several bract primordia at an angle of 144°. Primary rachis-branches arise as axillary meristems in the axil of these bracts. After a fixed number of primary rachis-branches are initiated, the primary inflorescence meristem aborts and is left as a scar called a degenerate point. The rachis-branch meristem generates secondary rachis-branches or lateral spikelets at an angle of 180° and subsequently converts to a terminal spikelet meristem. The mature wild-type rice panicle consisting of rachis, rachis-branches, lateral spikelets, and terminal spikelets is illustrated in Fig. 1B.

Rice Mutants Showing Defects in Panicle Morphology

We analyzed three mutants representing two genetic loci, *lax1-1*, *lax1-2*, and *fzp2*, all exhibiting abnormalities in panicle morphology (Fig. 1C). As reported previously, all lateral spikelets were absent in the *lax1-1* mutant, whereas each branch ends in a fertile spikelet (Futsuhara *et al.*, 1979). In *lax1-2* mutants, abnormalities in panicle morphology were much severer than in *lax1-1*. The number of rachis-branches was dramatically reduced, and no normal spikelets were initiated. Although the phenotype observed in *lax1-2* mutant panicles was much stronger than that of *lax1-1* and the previously reported *lax1* mutants, there were some similarities. The formation of lateral spikelets was completely suppressed while terminal spikelets were less affected in both *lax1-1* and *lax1-2*. Therefore, we performed complementation analysis between *lax1-1* and *lax1-2* by crossing pollen from a *lax1-1* mutant to *lax1-2*/+ plants, since the *lax1-2* mutant plants are completely sterile. The resultant F1 plants segregated seven wild-type and eight mutant phenotypes, suggesting that *lax1-1* and *lax1-2* have defects at the same locus. Genotypes of the *lax1* locus in these F1 plants were further confirmed in the F2 generation, in which wild-type plants and *lax1-1* plants segregated from F1 plants showing the wild-type phenotype. In contrast, *lax1-1*, *lax1-2*, and their intermediates segregated in the progeny of self-fertilized *lax1-1/lax1-2* plants (data not shown). Because *lax1-2* is a more severe mutant allele of the *lax1* locus, we mainly used the *lax1-2* allele in later analyses.

Table 1 shows a comparison of panicle features between wild-type and *lax1-2* plants. The number of primary rachis-branches was reduced to less than half that of the wild-type panicle in *lax1-2*. In contrast to this reduction, the number of nodes produced on each rachis-branch was significantly increased. As a result, the length of the panicle was increased. We could not detect any morphological differences between wild-type and *lax1-2* plants during their vegetative phase. The number of tillers produced before heading, the heading time, and the plant height were also comparable in wild type and *lax1-2*.

Phenotypic alterations of the *fzp2* panicle resembled the previously reported *frizzy panicle* (*fzp*) mutant (Mackill *et*

FIG. 4. Development of wild-type and mutant panicles. (A, D, G, and J) Wild-type panicle. (B, E, H, and K) *lax1-2* panicle. (C, F, I, and L) *fzp2* panicle. (A, B, and C) Primary inflorescence meristem (im) soon after the onset of reproductive development. Bract primordia (bp) can be seen in wild-type, *lax1-2*, and *fzp2* inflorescences. Differences were not apparent between wild-type, *lax1-2*, and *fzp2* inflorescence meristems at this stage. Bar, 100 μm. (D, E, and F) Late rachis-branch initiation stage panicle. Primary rachis-branches (prb) are formed and the development of spikelet meristems (spm) has initiated in the wild-type panicle. In contrast, neither rachis-branch formation or spikelet initiation is observed in the *lax1-2* panicle. The basic architecture of the panicle is similar in wild type and the *fzp2;* however, no sign of spikelet initiation is observed in the *fzp2* panicle. b, bract of rachis-branch. Bar, 200 μm. (G, H, and I), Spikelet initiation stage panicle. Development of spikelets (sp) proceeds basipetally in the wild-type panicle. Direct transformation of a rachis-branch meristem to a terminal spikelet meristem (tsm) is evident in (G) since no residual rachis-branch meristem is present around the terminal spikelet meristem. In the *lax1-2*, the panicle continues to elongate without forming lateral meristems. Continuous generation of lateral rachis-branch meristems is evident in the *fzp2* panicle. srb, secondary rachis-branch. Bar, 200 μm. (J, K, and L) Late spikelet initiation stage panicle. Development of spikelet organs has completed in the wild-type panicle. In contrast, no spikelet is seen in *lax1-2* and *fzp2* panicles. Bar, 400 μm.

TABLE 2
Phenotype of the *fzp2* Mutation

	n	Culm length (cm)[a]	No. of tillers	No. of primary branches	Length of panicle[b]	No. of nodes[c]
WT	33	45.4 ± 3.0	5.5 ± 1.8	6.1 ± 0.7	11.1 ± 1.0	7.6 ± 0.6
fzp2	13	43.8 ± 4.1	6.0 ± 2.1	5.2 ± 0.7	11.9 ± 1.1	6.2 ± 0.4

[a] Length from the ground to the panicle node.
[b] Length between the panicle node and the degenerated point.
[c] Number of nodes on the main axis.

al., 1991), although their genetic interaction is at present unknown. In the *fzp* and *fzp2* mutants, the basic architecture of the panicle branching was normal; however, spikelets did not initiate at all. Instead, the meristems continued to produce new meristems, which resulted in the generation of a panicle composed of excessive ramification of rachis-branches. As shown in Table 2, the number of primary rachis-branches or the number of nodes per primary rachis-branch did not differ significantly between wild-type and *fzp2* panicles.

Indeterminate Growth of the Terminal Spikelet Meristem in lax1-2

The formation of the terminal spikelets was rarely affected in *lax1-1* (Fig. 2B). In contrast, as shown in Fig. 2, a range of abnormalities was observed in the terminal spikelets of the *lax1-2* panicle. In the most distinctive case, the rachis-branch meristem produced abnormal bracts indeterminately at the angle of 180° without generating axillary meristems (Fig. 2D). These bracts were abnormally big and noticeable, while the bracts of spikelet and panicle branches were not apparent in the mature wild-type panicle (Figs. 2A and 2H). Incomplete spikelets were sometimes generated at the apex, suggesting that partial transformation of the rachis-branch meristem to a spikelet meristem had taken place even in *lax1-2* (Figs. 2C, 2E, and 2F). At low frequency, floral organs were formed in the *lax1-2* panicle; however, they were always sterile (Fig. 2F). Extra bracts were still observed when the floral structure was formed at the tip of rachis-branches in *lax1-2* and *lax1-1/lax1-2* (Figs. 2C, 2E, and 2F, arrows) but not in *lax1-1* (Fig. 2B). We found that the frequency of terminal spikelet formation was higher in later arisen tillers than in the main stem, which reached the heading stage faster. This may suggest that rice plants acquire more floral characteristics with time.

Indeterminate Branching of the Meristems in the fzp2 Panicle

An immature *fzp2* panicle was observed by SEM (Fig. 3). In the *fzp2* panicle, lateral meristems that would normally give rise to individual spikelets in the wild type behaved like meristems of rachis-branches. The *fzp2* "spikelet" meristems produced a certain number of bracts with axillary buds (Figs. 3B and 3C). The axillary meristems grew and reiterated the program of the *fzp2* "spikelet" meristem. As a result, meristems were repeatedly produced, and the whole structure of the *fzp2* panicle became a mass of proliferating meristems (Fig. 3A). Interestingly, each apical meristem initiated only several bracts and axillary meristems and soon ceased its activity, suggesting that the apical meristems had a determinate nature. Considering that rachis-branch meristems are produced at an angle of 144° but lateral spikelets are produced at an angle of 180° in wild-type panicles, the meristems produced in *fzp2* possessed a floral fate since the phylotaxy of the production of bracts and axillary meristems was 180° (Fig. 3C).

Development of the Panicle in lax1-2 and fzp2 Mutant Plants

For a better understanding of the defects in *lax1* and *fzp2*, the development of panicles in the two mutants was observed by sectioning (Fig. 4). Primary inflorescence meristems shortly after the transition are shown in Figs. 4A, 4B, and 4C. Differences were not apparent between wild-type, *lax1-2*, and *fzp2* inflorescence meristems at this stage. The absence of rachis-branch initiation in *lax1-2* became evident by the late rachis-branch initiation stage (Fig. 4E). The rachis-branch meristem of the *lax1-2* continued to produce bract-like knobs which did not subtend axillary meristems but increased the length of the panicle (Figs. 4H and 4K).

The basic architecture of the panicle appeared to be similar between the wild-type and the *fzp2* except that the differentiation of glumes in the terminal spikelets was evident in the wild-type panicle by the late rachis-branch initiation stage (Figs. 4D and 4F). In the later stage, when all spikelet primordia had initiated in the wild-type panicle, the differences became clearer (Figs. 4G, 4I, 4J, and 4L). The apical meristems in *fzp2* continued the initiation of new rachis-branch primordial instead of generating spikelet organs. SAMs of the newly formed rachis-branches reiterated the same branching patterns and initiated the next order branches. Bract hair developed in both *lax1-2* and *fzp2* as in the wild-type panicle.

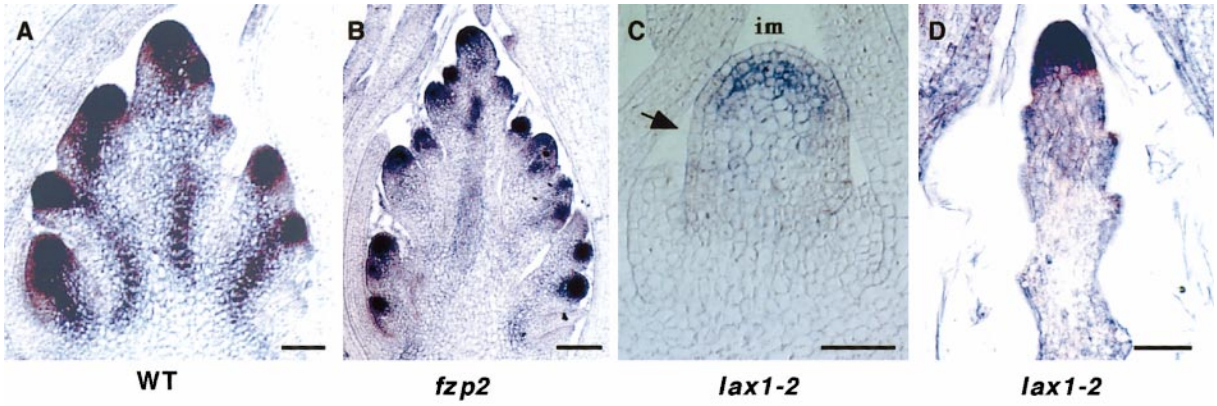

FIG. 5. *OSH1* expression in longitudinal sections of a young panicle. (A) Wild-type panicle at early rachis-branch initiation stage. *OSH1* mRNA accumulates in all the apical meristems and vascular regions. (B) *fzp2* mutant panicle at the late rachis-branch initiation stage. Accumulation of the *OSH1* mRNA is detected in the shoot meristems and vascular regions. (C) Inflorescence meristem (im) of *lax1-2* immediately after the transition to reproductive phase. Down-regulation of *OSH1* expression is observed in the region where a bract primordium will develop (arrow). (D) Upper region of the *lax1-2* panicle. *OSH1* accumulates only at the top of the rachis, and no signal is observed at the axils of the bract-like knobs, confirming the absence of lateral meristems. Bar in (A, B, C, and D), 100 μm.

Expression of OSH1, a Rice Ortholog of KN1, in lax1-2 and fzp2 Mutants

We used *OSH1*, a *kn1* ortholog of rice, as a molecular marker for the initiation/maintenance of rachis-branch meristems. In the development of the wild-type rice panicle, *OSH1* is expressed in the inflorescence meristems, and its expression is down-regulated in incipient rachis-branches and the rachis-branch primordia (Sentoku *et al.*, 1999). In the growing rice panicle, *OSH1* RNA is detected in the apical meristems (Fig. 5A). When spikelet meristems start to initiate, *OSH1* mRNA accumulates in the spikelet meristems (data not shown). In *fzp2* panicle, *OSH1* mRNA was observed in all meristems (Fig. 5B). In contrast, *OSH1* mRNA accumulated only in the apical region of the *lax1-2* panicle and not in the axils of abnormal bracts, although weak expressions were sometimes observed in the tip of bract-like structures (Figs. 5C and 5D). The absence of *OSH1* mRNA accumulation confirmed that lateral meristems were not initiated in the *lax1-2* panicle.

Phenotype of the lax1 fzp2 Double Mutant

In order to examine the genetic interaction between *LAX1* and *FZP2* genes, double-mutant plants were produced by crossing *lax1-1* pollen to *fzp2*/+ carpels. Resultant F1 plants were self-pollinated, and the phenotype of the F2 generation was examined. From the F1 population, a line which segregated *lax1-1*, *fzp2*, and plants exhibiting a novel phenotype was obtained. The ratio of segregation was 12:2:4:2 in wt:*lax1-1*:*fzp2*: novel plants. Plants with the last phenotype were again segregated in the selfed progeny of F2 plants that showed the *lax1-1* phenotype, confirming that the plants exhibiting the novel phenotype were *lax1-1 fzp2* double mutants. As shown in Fig. 6A, double-mutant panicles were distinct from either *lax1-1* or *fzp2*. The formation of lateral spikelets was prevented, as in *lax1-1*, but no terminal spikelets were produced at all. Primary rachis-branches also elongated in a zigzag morphology not seen in *lax1-1* that was caused by a deviation in the growth axis at each point where a rachis-branch or a spikelet should form (Fig. 6B). SEM analysis of young double-mutant panicles revealed that new meristems arose in the axis of lateral bracts; however, different from *fzp2* meristems, they did not elongate forming further lateral branches (Fig. 6C). The elongation ceased with the consumption of the meristem into differentiated tissue resembling that of the *lax1-1* (Fig. 6D). In conjunction with these observations, the phenotype of the *lax1-1 fzp2* double-mutant plant suggested that the two genes may function in genetically different pathways.

DISCUSSION

LAX1 Is Required for the Initiation/Maintenance of Lateral Meristems in the Rice Panicle

Three types of shoot apical meristems with different identities, RBM, LSM, and TSM, are newly generated in rice after the transition from vegetative to reproductive development. Absence of lateral spikelets in *lax1-1* allele clearly indicates that *LAX1* function is required for the formation of lateral spikelet meristems. Furthermore, isolation of *lax1-2*, a new and stronger *lax1* allele, revealed that the *LAX1* gene is necessary for the formation of rachis-branch meristems and specification of terminal spikelet meris-

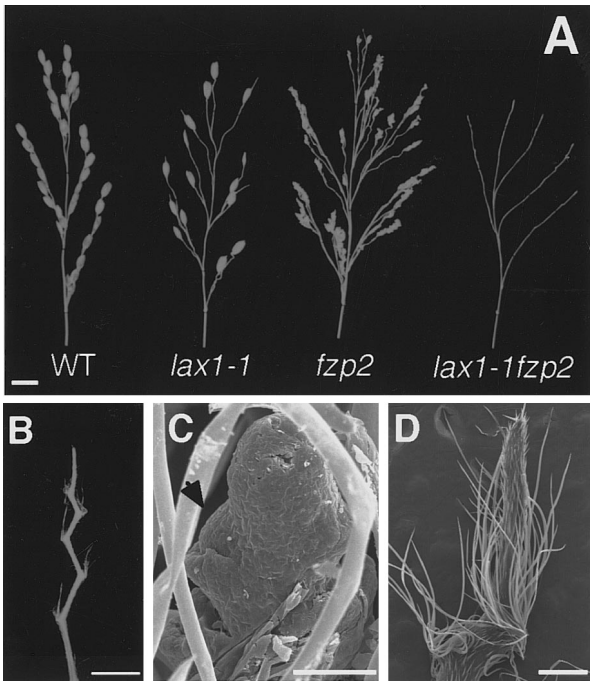

FIG. 6. Phenotype of the *lax1-1 fzp2* double mutant. (A) A comparison of the panicle morphology between wild-type, *lax1-1*, *fzp2*, and the double-mutant plants. Bar, 1 cm. (B) Close-up view of a rachis-branch in the double-mutant panicle before heading. (C) A SEM view of the rachis-branch of the double mutant at the early stage of panicle development. Formation of axillary meristem is observed at this stage (arrow). (D) A SEM view of the apex of the branch in the double mutant at the same stage as shown in (B).

tems. Thus, the function of the *LAX1* gene is required for normal development of all the three meristems in a panicle (Fig. 7).

The fate of the branch meristem apices in rice inflorescence has been controversial. In a generally accepted view, the rachis-branch meristems and the lateral spikelet meristems are interpreted as axillary meristems, whereas the terminal spikelet meristem is thought to be transformed from the rachis-branch meristems. On the other hand, some suggest that the rachis-branch meristem remains indeterminate and all the spikelets, including the one formed at the top of each branch, are thus produced laterally (Clifford, 1987). Based on the following observations, we favor the former interpretation. In *lax1-1* mutants, although lateral spikelets are not produced, terminal spikelets are normally formed, indicating that the initiation of the terminal and lateral spikelets may be controlled by different genetic programs. In addition, the different defects observed in terminal spikelets and lateral spikelets in the *lax1-2* also support this notion. Finally, transversal sections throughout panicle development also showed that each rachis-branch meristem is directly converted into a spikelet (Fig. 4G). Furthermore, we did not find a residual rachis-branch meristem after the formation of the terminal spikelet meristem that would have remained if the terminal spikelet was initiated laterally.

Comparison of the defects between *lax1-1* and *lax1-2* alleles showed that the dependence of the meristem initiation/maintenance on *LAX1* function varied among the meristems. Production of lateral spikelets was completely suppressed even in a weak allele of *lax1* mutants, *lax1-1*. In contrast, defects in the specification of terminal spikelets were evident only in *lax1-2*. The number of rachis-branches was only slightly reduced in *lax1-1*, but it was decreased to none in *lax1-2*. Although the exact mechanism of genetic separation of gene functions observed in the phenotypes of *lax1-1* and *lax1-2* is unknown, two models might explain it. First, the *LAX1* gene might have two separable functions. One is necessary for lateral spikelet initiation/maintenance, while the second function is required for rachis-branch formation and transformation of the rachis-branch meristems into terminal spikelet meristems. With regard to the lateral spikelet formation, we did not detect any differences between *lax1-1*, *lax1-1*/*lax1-2*, and *lax1-2* plants. This suggests that the function required for lateral spikelet formation might be separable from the rest of the

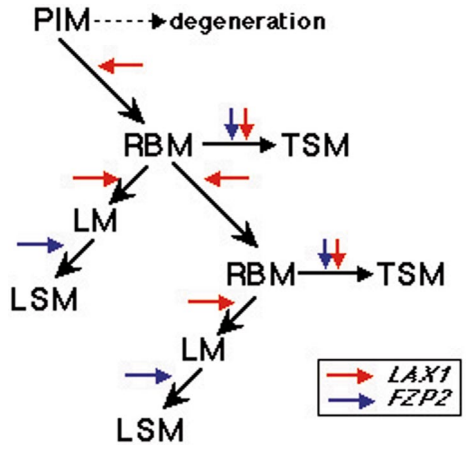

FIG. 7. Model explaining the action of *LAX1* and *FZP2* during rice panicle development. As a first step in the rice panicle development, rachis-branch meristems (RBM) are generated from the primary inflorescence meristem (PIM). Then, each rachis-branch meristem produces lateral meristems (LM) which are subsequently specified as lateral spikelet meristems (LSM). *LAX1* is required for the formation of rachis-branch meristems and the generation of lateral meristems from rachis-branch meristems. Lateral meristems on rachis-branches are specified as spikelet meristems by the action of *FZP2* gene. Finally, rachis-branch meristems are converted to terminal spikelet meristems (TSM). The *FZP2* gene is necessary for the conversion of the apical meristems of rachis-branches to terminal spikelet meristems. Interestingly, *LAX1* is also required to confer a determinacy to the rachis-branch meristems.

LAX1 function and both *lax1-1* and *lax1-2* alleles lack this portion of *LAX1* gene function. Alternatively, a simple quantitative difference of the *LAX1* gene product in the two mutant alleles may have caused the phenotypic differences. The observation that *lax1-1*/*lax1-2 trans*-heterozygote exhibited an intermediate level of defect with respect to terminal spikelet formation and generation of rachis-branches supports this quantitative model.

The LAX1 Gene Is Required to Suppress the Indeterminate Growth of Rachis Branch Meristems

In this study, we observed the indeterminate growth of the rachis-branch meristems of the *lax1-2* mutant as a recessive, loss-of-function phenotype. Thus, we presume that the wild-type *LAX1* gene may prevent the indeterminate growth of the SAM by inducing the transformation of the rachis-branch meristem into a terminal spikelet meristem. In this respect, the *LAX1* gene could be defined also as a meristem identity gene required for specifying meristem determinacy.

Several genes required for the suppression of the indeterminate growth of the apical meristems have been isolated. In *Arabidopsis* and *Antirrhinum*, which have indeterminate inflorescences, this feature is controlled by the *TFL1* and *CEN* genes, respectively (Shannon and Meeks-Wagner, 1991; Alvarez *et al.*, 1992; Bradley *et al.*, 1996). *TFL1* and *CEN* encode homologous proteins, suggesting that the basic mechanisms controlling inflorescence determinacy are conserved between the two species (Bradley *et al.*, 1996, 1997; Ohshima *et al.*, 1997). Although the functions of *TFL1*/*CEN* homologs in rice are yet to be reported, the phenotype of *lax1-2* described in this study might suggest the existence of a mechanism controlling meristem determinacy that is independent of *TFL1*/*CEN*. The determinacy of a floral meristem is controlled by *AGAMOUS* (*AG*) and its orthologs in diverse plant species including maize and rice (Yanofsky *et al.*, 1990; Mena *et al.*, 1996; Kang *et al.*, 1998). In the *indeterminate spikelet* (*ids*) mutant of maize, the spikelet meristem acquired indeterminacy, leading to the production of additional florets in a single spikelet instead of the two florets observed in a normal maize spikelet (Chuck *et al.*, 1998). Thus, the determinacy of spikelet meristem is controlled by the *IDS* gene in maize. Here, we showed that the *LAX1* gene dictates the determinacy of rachis-branch meristems in the rice panicle. In view of the above observations, it seems quite likely that distinct sets of genes are utilized to establish the determinacy/indeterminacy of different types of meristems in grass inflorescences.

Similar Mutations in Maize

Several mutant loci exhibiting inflorescence defects similar to those of the *lax1* or *fzp2* mutants have been described in maize (Sheridan, 1988; McSteen *et al.*, 2000). In the *bif2* mutant, the tassel has few branches and spikelets are unpaired, suggesting that initiation of tassel branch meristems and spikelet meristems is blocked in *bif2*. Severe reduction of the number of branches in tassel and ear shoots occurs in other maize mutants, such as *ba1* and *ba2*. Among these loci, *bif2* and *ba2* are mapped on the long arm of chromosome 3, which has synteny with chromosome 1 of rice, to where the *LAX1* gene is mapped. This correspondence raised the possibility that *LAX1* might be an ortholog of the *BIF2* or the *BA2* gene. Mutants showing *fzp* or *fzp2*-like phenotypes are also known in maize. In the *ramosa* mutant, indeterminate branches are produced in place of spikelet pairs. Similarly, the transition from spikelets to florets is blocked and indeterminate growth of spikelets is often observed in the ears of the *branched silkeless* (*bd*) mutant (Colombo *et al.*, 1998). Isolation of these genes and comparative studies between rice and maize will provide valuable information for understanding the development of grass inflorescences.

FZP2 Is a Meristem Identity Gene Required for Spikelet Identity

In *fzp2* mutant plants, spikelets are led to the indeterminate generation of meristems. Thus, *FZP2* may be considered a spikelet meristem identity gene. The *fzp2* phenotype can be interpreted as the transformation of the floral meristems to inflorescence shoots as shown in *Arabidopsis lfy* and *Antirrhinum floricaula* (*flo*) mutants (Weigel *et al.*, 1992; Coen *et al.*, 1990). However, we detected no difference in the coding sequences of the *RFL* gene, the *FLO*/*LFY* homolog of rice (Kyozuka *et al.*, 1998), between the wild type and the *fzp2* mutants. Thus, it is unlikely that the *RFL* is the causal gene of the *fzp2* mutant. Future isolation of the *FZP2* gene may elucidate whether the mechanisms controlling the onset of floral meristems have been conserved in these divergent species.

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