

# LAX PANICLE2 of Rice Encodes a Novel Nuclear Protein and Regulates the Formation of Axillary Meristems <sup>W</sup>

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**Aerial architecture in higher plants is dependent on the activity of the shoot apical meristem (SAM) and axillary meristems (AMs). The SAM produces a main shoot and leaf primordia, while AMs are generated at the axils of leaf primordia and give rise to branches and flowers. Therefore, the formation of AMs is a critical step in the construction of plant architecture. Here, we characterized the rice (*Oryza sativa*) *lax panicle2* (*lax2*) mutant, which has altered AM formation. *LAX2* regulates the branching of the aboveground parts of a rice plant throughout plant development, except for the primary branch in the panicle. The *lax2* mutant is similar to *lax panicle1* (*lax1*) in that it lacks an AM in most of the lateral branching of the panicle and has a reduced number of AMs at the vegetative stage. The *lax1 lax2* double mutant synergistically enhances the reduced-branching phenotype, indicating the presence of multiple pathways for branching. *LAX2* encodes a nuclear protein that contains a plant-specific conserved domain and physically interacts with *LAX1*. We propose that *LAX2* is a novel factor that acts together with *LAX1* in rice to regulate the process of AM formation.**

## INTRODUCTION

The principal body plan of a plant is established during embryogenesis by the generation of an apical-basal axis. This bipolar organization is defined by a shoot apical meristem (SAM) and a root apical meristem (McSteen and Leyser, 2005). By contrast, the complexity of the adult plant architecture is generated by lateral growth, which is determined by the activity of post-embryonically produced secondary meristems, called axillary meristems (AMs). In rice (*Oryza sativa*), an AM produced at the axil of

the leaf generates a new shoot branch called the tiller, whereas an AM produced in the inflorescence generates a higher order of inflorescence branch, called the rachis branch, which bears a grass-specific structural unit of the inflorescence—the spikelet (Hoshikawa, 1989). More precisely, upon transition to the reproductive phase from the vegetative phase, the SAM of each tiller is transformed into an inflorescence meristem and forms an inflorescence called a panicle. During panicle development, the inflorescence meristem initiates primary branch meristems, each of which produces a primary panicle branch (PB). The PB also produces a certain number of secondary branch meristems, which produce secondary panicle branches (SBs) or differentiate into spikelets, depending on the time and position of their occurrence. Finally, the primary branch meristem and secondary branch meristem differentiate into a terminal spikelet. Thus, the pattern of AM formation is of special significance in crop production in various grass species because it regulates the number of shoot branches, inflorescence branches, and spikelets.

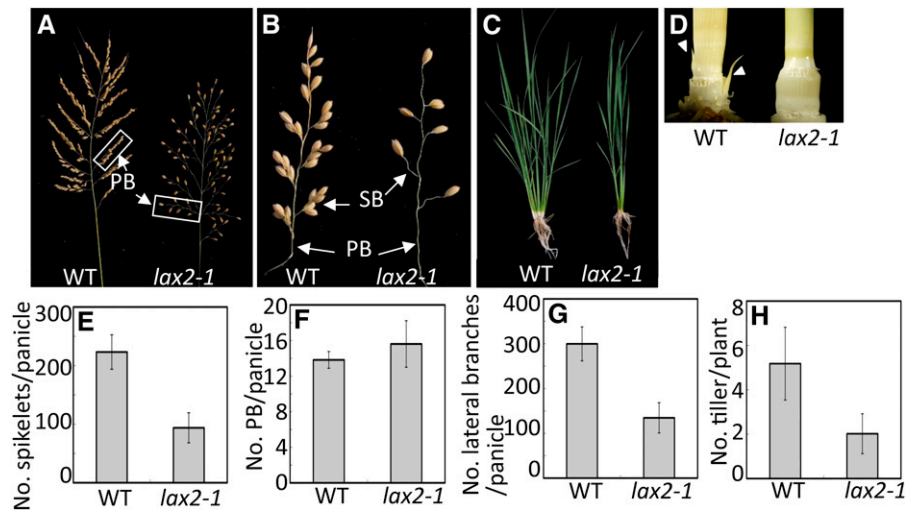
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**Figure 1.** Plant Morphology of the *lax2-1* Mutant.

(A) Mature panicles. The *lax2-1* mutant has a sparse appearance due to the production of fewer branches and spikelets. WT, wild type.

(B) Enlarged view of boxes in (A).

(C) Whole plants at the vegetative growth stage. *lax2-1* mutant has fewer tillers than the wild type.

(D) Culms of the wild type and *lax2-1* after removal of the surrounding leaves. White arrowheads indicate the prophyll, which encloses the AM.

(E) Quantification of the number of spikelets in a panicle.

(F) Quantification of the number of PBs per panicle.

(G) Quantification of the number of lateral branches, which is the sum of the number of PBs, SBs, and spikelets in a panicle.

(H) Quantification of the number of tillers per plant.

Error bars in (E) to (H) represent SD. The sample size for (E) to (H) is  $n = 9$ .

The pattern of lateral branching is affected by both the generation and growth of lateral shoots. Generation and outgrowth of lateral shoots are regulated by the combined action of hormones, such as auxin, cytokinin, and strigolactone, and environmental cues. Furthermore, a number of regulatory genes expressed in AM-specific and AM-nonspecific manners affect branching (McSteen, 2009).

Mutants in AM-nonspecific genes, including *Arabidopsis thaliana revoluta* and *cup-shaped cotyledon3*, show phenotypes not only in branching but also in various developmental aspects (SAM formation, leaf polarity, and vascular patterning), possibly derived from defects in general meristematic functions (Talbert et al., 1995; Otsuga et al., 2001; Hibara et al., 2006; Raman et al., 2008).

There is another type of mutant that exhibits AM-specific defects. In tomato (*Solanum lycopersicum*), *lateral suppressor (ls)* and *blind (bl)* mutants show defects in lateral branching (Schumacher et al., 1999; Schmitz et al., 2002). In *Arabidopsis*, *lateral suppressor (las)* and *regulator of axillary meristem (rax)* mutants are defective in AM formation (Greb et al., 2003; Müller et al., 2006). In maize (*Zea mays*), *barren stalk1 (ba1)* and *barren inflorescence2 (bif2)* mutants exhibit severe suppression of all types of AM (McSteen and Hake, 2001; Ritter et al., 2002; Gallavotti et al., 2004).

In rice, several mutants show AM-specific defects, including defects in panicle development. The mutants *monoculm1/small panicle (moc1/spa)*, *lax panicle1 (lax1)*, and *frizzy panicle (fzp)* have been shown to affect the patterning of AM (Komatsu et al., 2001, 2003; Li et al., 2003; Li et al., 2009). The genes underlying

these mutations have been isolated, and most of these genes encode transcriptional regulators with expression patterns that evoke their involvement in the patterning of AMs. Thus, the transcriptional network operates during the initiation or maintenance of the AM, although the direct or indirect connections between these factors have not been precisely determined.

Rice *MOC1*, which encodes a transcriptional regulator of the GRAS family, is homologous to tomato *LS* and *Arabidopsis LAS*. Tomato *BL* and *Arabidopsis RAX* are orthologous genes encoding R2R3-type MYB family transcription factors. Rice *LAX* and maize *BA1* are also orthologous; each encodes a transcription factor that contains a basic helix-loop-helix (bHLH) domain. The rice *FZP* gene, which encodes an ethylene-responsive element binding factor, is an ortholog of maize *BD1*. In many cases, transcriptional factors identified from AM-defective mutants are conserved in different plant species, suggesting that their functions in AM patterning are also conserved.

AM patterning can also be regulated at a level other than the transcriptional level. Rice *SHORT PANICLE1* encodes a putative transporter that belongs to the peptide transporter family, while *ERECT PANICLE2* encodes a novel plant-specific protein with unknown biochemical function that localizes to the endoplasmic reticulum (Li et al., 2009; Zhu et al., 2010). Maize *BIF2* encodes a Ser/Thr protein kinase that is orthologous to PINOID. PINOID phosphorylates and regulates PIN-FORMED1 (PIN1) localization to the plasma membrane and mediates directional auxin transport (Michniewicz et al., 2007). Interestingly, BIF2 and BA1 are both required for AM patterning and have direct interaction with

each other (Skirpan et al., 2008). These observations suggest the possibility that BIF2 regulates AM formation through posttranslational modifications of BA1. Another example of posttranslational regulation is the rice LAX1 protein. LAX1 is expressed in two to three layers of cells in the boundary between the SAM and the region of new meristem formation (Oikawa and Kyojuka, 2009). Interestingly, LAX1 movement between cells is required for the full functioning of LAX1.

Because the process of AM formation is regulated in multiple layers, elucidation of the genetic and biochemical relationship among the factors involved in this process is necessary to understand AM formation. Of particular interest is the analysis of mutants that show AM-specific defects and the clarification of genetic and biochemical interactions among the factors involved in AM formation. Here, we describe a novel gene, LAX2, that is involved in the maintenance of AM in rice. LAX2 regulates the branching of the aboveground parts of rice plants throughout plant development, except for the PB of the panicle. The *lax2* mutant is similar to the *lax1* mutant in that it lacks AMs for most of the lateral branching of the panicle (SBs and spikelets) and has reduced tillers at the vegetative stage. The synergistic enhancement of the branching phenotype of the *lax1 lax2* double mutant suggests that there are multiple pathways for branching. We show that LAX2 encodes a nuclear protein with a plant-specific conserved domain and that LAX2 physically interacts with LAX1 through a region containing this conserved domain. Thus, we propose that LAX2 is a novel genetic component that is required for the process of AM formation and acts together with LAX1 in rice.

## RESULTS

### *lax2* Affects Both Vegetative and Reproductive Branching

*lax2* mutations were isolated while screening for mutants with abnormal panicle development. We found three independent mutant alleles, all of which exhibited a sparse-panicle phenotype. These alleles, recovered from ethyl methanesulfonate, tissue culture, and  $\gamma$ -ray irradiation mutagenesis populations, were designated as *lax2-1*, *lax2-2*, and *lax2-3*, respectively.

The panicle of wild-type rice is composed of PBs, SBs, and spikelets (the grass-specific structural unit of the inflorescence). There are  $\sim 12$  PBs in a panicle; each PB bears several SBs and spikelets, and SBs also bear several spikelets. This multiple

order of branching is the basis for the characteristic appearance of the rice panicle. To characterize the nature of the sparse appearance of the *lax2-1* panicle, we counted the number of inflorescence branches in the wild type and mutant. The *lax2-1* mutant panicle produced fewer spikelets compared with the wild-type panicle (Figures 1A and 1E). Quantitative analysis of the *lax2-1* mutant panicle revealed that the number of PBs was not affected in the mutant (Figure 1F). However, the number of total lateral branches, which was the sum of the number of PBs, SBs, and spikelets in a panicle, was reduced in *lax2-1* (Figure 1G). This indicates that the sparse panicle appearance and the reduction in spikelet number in the *lax2-1* mutant are attributable both to reduced higher order branching, such as the number of SBs, and to a reduced number of spikelets borne directly on branches (Figure 1B). Because a spikelet other than a terminal spikelet is produced as a lateral branch from an inflorescence meristem, *lax2-1* mutants seem to have a defect in producing all the lateral branches other than PBs in panicles.

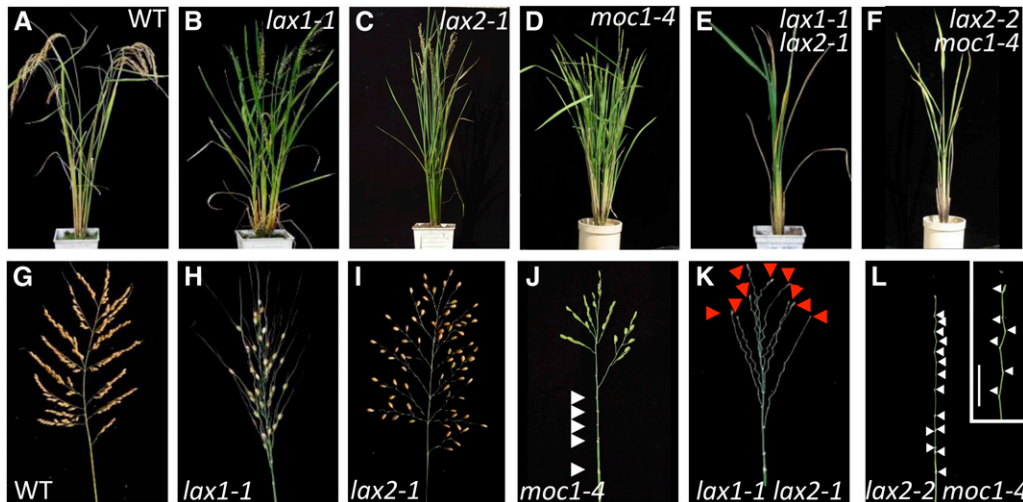
*lax2-1* mutants also had defects in vegetative development. In *lax2-1* mutants, the number of tillers produced during vegetative growth was reduced (Figures 1C and 1H). Because there was no trace of tiller buds at the base of the leaves in *lax2-1* mutants (Figure 1D), the reduction in the number of tillers is caused by a defect in AM formation and not by enhanced apical dominance or growth arrest of tiller buds. As a result of the reduction in the number of tillers in *lax2-1*, mature *lax2-1* plants had fewer panicles than wild-type plants. Thus, the *lax2-1* mutant failed to develop lateral branches during both vegetative and reproductive development, although the number of PBs in the panicle was not affected.

The other *lax2* mutant alleles, *lax2-2* and *lax2-3*, had similar branching defects, as observed in *lax2-1* mutants in panicles, except that the number of PBs in *lax2-2* and *lax2-3* was slightly increased (Table 1). Since *lax2* mutations reduce branching at most of the developmental stages, the increase in the number of PBs in these mutants could be due to an indirect effect to compensate for the reduction of the higher order branching and spikelets. The number of tillers in *lax2-2* and *lax2-3* was reduced but the degree of the reduction in *lax2-3* was not as evident as *lax2-1* or *lax2-2* (Table 1). Considering that all three *lax2* alleles carry mutations that are presumably null (discussed below) and all three *lax2* mutants originated from different genetic backgrounds (see Methods), the vegetative branching phenotype might be affected by genetic background.

**Table 1.** Phenotypic Characterization of *lax2* Mutants and Their Respective Parental Wild Types in Rice

Genotype	<i>n</i>	No. of Tillers per Plant	No. of Primary Branches per Panicle	No. of Secondary Branches per Panicle	No. of Spikelets per Panicle
Wild type (Zhonghua 11)	9	5.2 $\pm$ 1.6	13.8 $\pm$ 0.9	43.8 $\pm$ 5.9	223.2 $\pm$ 29.4
<i>lax2-1</i>	9	2.1 $\pm$ 0.9***	15.6 $\pm$ 2.6	18.3 $\pm$ 4.6***	93.8 $\pm$ 25.6***
Wild type (Nipponbare)	60	19.6 $\pm$ 3.6	8.9 $\pm$ 0.6	10.0 $\pm$ 2.0	76.8 $\pm$ 8.8
<i>lax2-2</i>	60	17.8 $\pm$ 2.8***	9.9 $\pm$ 0.9***	0.0 $\pm$ 0.0***	36.3 $\pm$ 4.0***
Wild type (Norin 8)	60	18.7 $\pm$ 4.3	9.4 $\pm$ 0.7	13.6 $\pm$ 2.2	92.5 $\pm$ 8.8
<i>lax2-3</i>	60	17.8 $\pm$ 3.2*	10.7 $\pm$ 0.8***	0.1 $\pm$ 0.2***	54.0 $\pm$ 4.3***

Average values  $\pm$  SD are shown. Asterisks (\*\*\*) and (\*) indicate that the differences between the mutants and their corresponding parental wild types are statistically significant at  $P < 0.001$  and  $< 0.05$ , respectively, according to the Student's *t* test.



**Figure 2.** Morphology of Double Mutants of *lax2* with *lax1* or *moc1*.

(A) to (F) Mature plants. WT, wild type.

(G) to (L) Panicle.

(A) and (G) The wild type.

(B) and (H) *lax1-1*.

(C) and (I) *lax2-1*.

(D) and (J) *moc1-4*.

(E) and (K) *lax1-1 lax2-1* double mutant.

(F) and (L) *lax2-2 moc1-4* double mutant. The inset in (L) is a higher magnification of the double mutant panicle. Bar = 2 cm.

White and red arrowheads indicate the position of nodes in the panicle and terminal spikelets, respectively.

### Genetic Interactions of *lax2* with *lax1* or *moc1*

To clarify the genetic interactions of *lax2* with other rice mutants that exhibit a defect in AM formation, we made double mutants containing *lax2* and either *lax1* or *moc1*. *lax1* had a sparse panicle with reduced SBs and spikelets, which was similar to the *lax2* panicle, and *lax1* had tillers with slightly reduced numbers at the vegetative stage (Figures 2A to 2C and 2G to 2I) (Oikawa and Kyojuka, 2009). We made *lax1 lax2* double mutants using null alleles of *lax1*, *lax1-1*, and *lax1-6*. *lax1-1* is a deletion allele of *lax1* (Komatsu et al., 2003), and *lax1-6* has a single nucleotide deletion at the 5' end of the region encoding the conserved bHLH domain. The phenotype of the *lax1 lax2* double mutant was more extreme than that of the single mutants. In the double mutant panicle, all SBs and spikelets except terminal spikelets were absent,

whereas the number of PBs was not affected compared with the single mutants (Figure 2K, Table 2). At the vegetative stage, both single mutants shared the similar phenotype of a slightly reduced number of tillers (Figures 2B and 2C); however, there was a strong reduction in tiller branches in the double mutant (Figure 2E, Table 2). Thus, the dramatic effect on branching of the spikelets and the lack of tiller branches in the *lax1 lax2* double mutant implies that there are multiple pathways for branching and that *LAX1* and *LAX2* consistently function in these pathways both at the vegetative stage and reproductive stage, except during PB formation.

The allele of *moc1* used in our analysis, *moc1-4*, also has a sparse panicle; however, the branching pattern of the *moc1-4* panicle is different from that of the wild type, *lax1*, or *lax2*. *moc1-4* has only a few PBs, the reduction of which is mostly due to the

**Table 2.** Quantification of the Double Mutants of *lax1* and *lax2*

Line <sup>a</sup>	Genotype	<i>n</i>	No. of Tillers per Plant	No. of Primary Branches per Panicle	No. of Secondary Branches per Panicle	No. of Spikelets per Panicle
F3 No. 1	<i>LAX2/± lax1-6/lax1-6</i>	21	15.2 ± 4.1	10.5 ± 1.0	0.1 ± 0.3	38.0 ± 6.4
	<i>lax2-3/lax2-3 lax1-6/lax1-6</i>	5	1.6 ± 0.9*	11.3 ± 1.6	0.0 ± 0.0	12.6 ± 2.4*
F3 No. 2	<i>lax2-3/lax2-3 LAX1/±</i>	16	20.6 ± 5.6	9.3 ± 0.9	0.0 ± 0.1	39.5 ± 6.6
	<i>lax2-3/lax2-3 lax1-6/lax1-6</i>	10	2.6 ± 1.2*	9.6 ± 1.8	0.0 ± 0.0	9.9 ± 2.3*

Average values ± SD are shown. Asterisks indicate that the differences between the double and single mutants are statistically significant at  $P < 0.001$ , according to the Student's *t* test.

<sup>a</sup>F3 plants were derived from self-pollination of an F2 plant that was either heterozygous for *lax2-3* and homozygous for *lax1-6* or homozygous for *lax2-3* and heterozygous for *lax1-6*. The F2 plants originated from crossing *lax2-3* and *lax1-6* mutants.

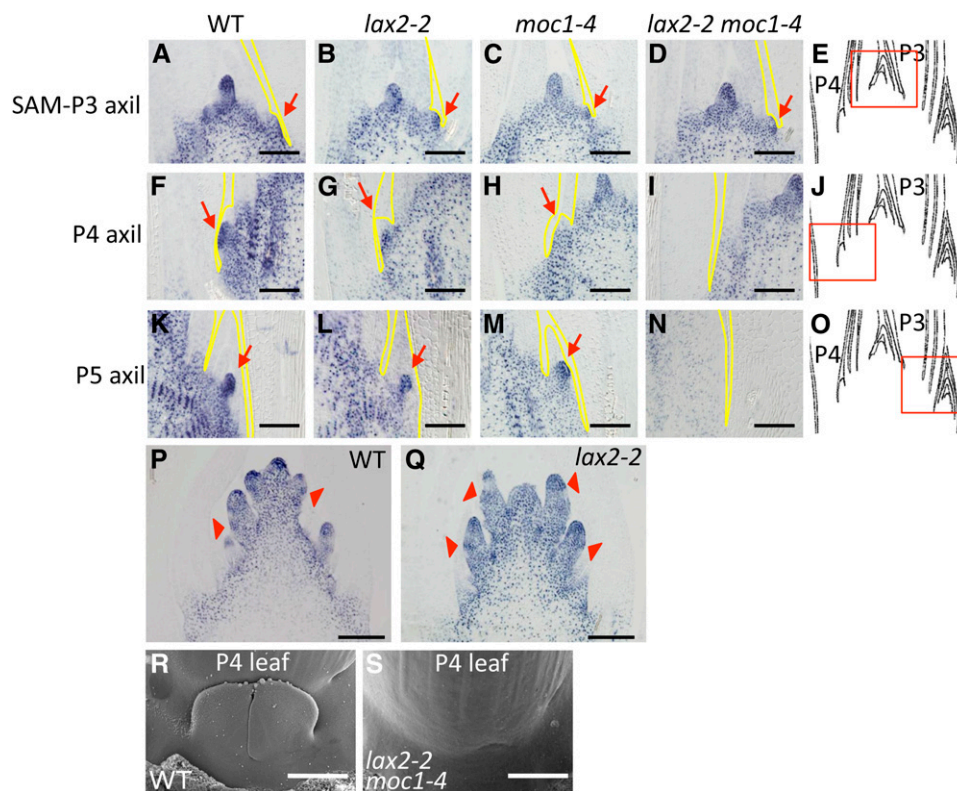
loss of the PBs in the lower part of the panicle. The numbers of SBs and spikelets on the PB are also reduced in *moc1-4* (Figure 2J). At the vegetative stage, *moc1-4* did not affect tillering (Figure 2D), although *moc1-4* contains a nonsense mutation at a conserved Trp residue within the GRAS domain and appears to be a null allele. Because the vegetative branching phenotype in *moc1* is strongly affected by the genetic background, we tried to make a *lax2 moc1* double mutant in the same genetic background as the corresponding single mutants. We used *lax2-2* and *moc1-4*, both of which were derived from Nipponbare. The phenotype of the *lax2-2 moc1-4* double mutant was more extreme than that of either single mutant. In the double mutant panicle, all PBs, SBs, and spikelets were absent (Figure 2L), and there were no tiller branches at the vegetative stage (Figure 2F). Thus, the dramatic effect on branching of the panicle and tiller branches in the *lax2 moc1* double mutant implies that there is another pathway for

branching in which *MOC1* is involved and that *LAX2* and *MOC1* function in vegetative and reproductive branching, including during PB formation.

### LAX2 Is Involved in the Maintenance of the AM

The reduced branching at both the vegetative and reproductive stages in *lax2* single and double mutants was caused by a defect in the formation of the AM, suggesting that *LAX2* is involved in the initiation or maintenance of the AM. To confirm this, we observed the expression of the OSH1 protein (a marker of the meristem) and the morphology of the AM in single and double mutants of *lax2* and *moc1*.

At the vegetative stage in a wild-type plant, a population of cells that expresses OSH1 can be detected at the axil of the second and third youngest leaf primordia (P2-P3) as a bulge



**Figure 3.** Anti-OSH1 Immunostaining of the Wild Type, *lax2*, *moc1*, and *lax2 moc1* Double Mutant and Scanning Electron Microscopy Image of the Wild Type and *lax2 moc1* Double Mutant.

(A), (F), and (K) Vegetative shoot of wild-type (WT) Nipponbare.

(B), (G), and (L) Vegetative shoot of *lax2-2*.

(C), (H), and (M) Vegetative shoot of *moc1-4*.

(D), (I), and (N) Vegetative shoot of the *lax2-2 moc1-4* double mutant.

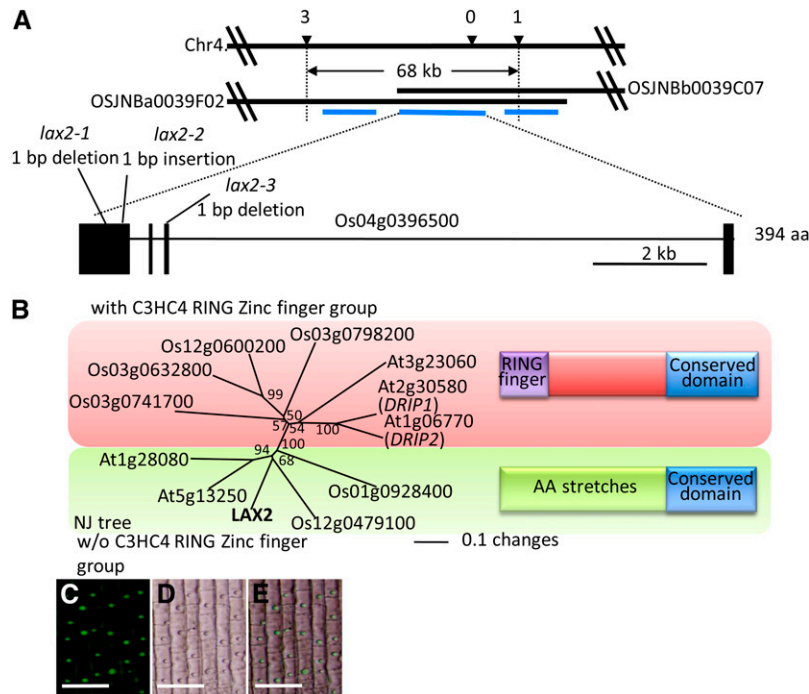
(E), (J), and (O) Position of photograph in shoot in each row is shown in red boxes.

(P) Developing wild-type Nipponbare inflorescence with SB primordia.

(Q) Developing inflorescence of *lax2* at the same stage as in (P).

(R) and (S) Scanning electron microscopy images of the wild type (R) and double mutant (S) at the base of P4 leaves.

P3, P4, and P5 represent the 3rd, 4th, and 5th youngest leaf primordia, respectively (Steeves and Sussex, 1989). Red arrows indicate the position of AMs marked by morphology and OSH1 expression. Red arrowheads indicate the position of incipient AMs identified by the condensed OSH1 signals. Black and white bars indicate 100  $\mu$ m and 1 mm, respectively.



**Figure 4.** Molecular Cloning of *LAX2*.

**(A)** Positional cloning of *LAX2*. Black horizontal bars represent chromosomal segments or BAC clones corresponding to the region of interest on chromosome 4. Numbers above the top bar represent the number of recombinants between markers and the *lax2* mutation after screening an F2 population derived from crosses between the *lax2* mutation and an *indica* cultivar. Blue lines represent the positions of predicted genes. The detailed gene structure of *LAX2* is shown at the bottom. Boxes and lines connecting boxes represent exons and introns, respectively. The gene structure presented here is modified from that predicted in the Rice Annotation Project Database or The Institute for Genomic Research database based on our experiments. aa, amino acids.

**(B)** Phylogenetic analysis of amino acid sequences of conserved domains shared with *LAX2* from rice and *Arabidopsis*. The alignment used to construct the phylogenetic tree was made using ClustalX (Jeanmougin et al., 1998) and is shown in Supplemental Figure 2 online. The phylogenetic tree was constructed by the NJ method (Saito and Nei, 1987) using PAUP\* 4.0 software. Proteins sharing the conserved domain with *LAX2* are divided into two groups, one with a zinc finger motif at the N-terminal side of the conserved domain (red box) and one without a zinc finger (green box). Numbers adjacent to each branch point of the tree indicate bootstrap support of that branch after 1000 replicates. The bar in the figure represents the degree of amino acids changes.

**(C) to (E)** Subcellular localization of *LAX2*:GFP fusion protein in transgenic rice plants. GFP fluorescence, differential interference contrast, and merged images are shown in **(C)**, **(D)**, and **(E)**, respectively. Bars = 50  $\mu$ m.

(Steeves and Sussex, 1989), depending on the sampling stage within a plastochron (Figures 3A and 3E). Similar structures were observed in both *lax2-2* and *moc1-4* single and double mutants (Figures 3B to 3D). These bulges possibly include cells that give rise to an incipient AM. At the P4 leaf axil, the AM is formed and grows in wild-type plants and in *lax2* and *moc1* single mutants (Figures 3F to 3H and 3J). At the P5 leaf axil, the AM grows continuously and is covered by prophyll in wild-type plants and in *lax2* and *moc1* single mutants (Figure 3K to 3M and 3O). However, in the *lax2 moc1* double mutant, the bulge with OSH1-expressing cells, although seen initially at the P2-P3 leaf axil, did not proliferate at the P4 leaf axil (Figure 3I) and was absent at the P5 leaf axil (Figure 3N). The lack of the AM in the *lax2 moc1* double mutant was also observed by scanning electron microscopy analysis (Figures 3R and 3S). This phenotype is quite consistent because there is never a tiller in the *lax2 moc1*

double mutant. This observation negates the possibility that the initiation of the SAM is stochastically arrested in the mutant and indicates that the double mutant is defective in the maintenance of the AM. Thus, at the vegetative stage, both *LAX2* and *MOC1* are involved in the maintenance of the AM.

At the reproductive stage, AM primordia that give rise to PBs formed normally in the *lax2* mutant as in the wild type (Figures 3P and 3Q). The dense staining of OSH1 indicated by red arrowheads in Figures 3P and 3Q reside just above the place of OSH1 downregulation both in the wild type and *lax2* mutant. These places are also marked by compact cells produced by frequent cell divisions and correspond to incipient meristems destined to become SBs or spikelets (Komatsu et al., 2003; McSteen et al., 2007). Considering that the number of lateral branches formed on the PBs, such as SBs or spikelets, was reduced in *lax2*, *LAX2* is also involved in the maintenance of AMs at the reproductive stage.

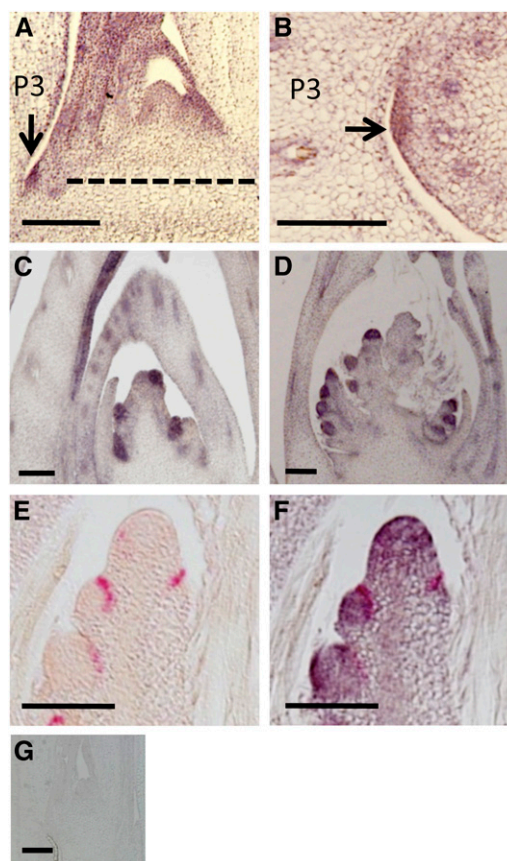
### LAX2 Encodes a Novel Nuclear Factor

We cloned *lax2* by a map-based method. *lax2* mutations were roughly mapped on chromosome 4. We screened recombinants between two genetic markers that reside 68 kb apart on the physical map and identified four recombinants between these markers. Three genes were predicted to exist between two genetic markers, and among them we found mutations in gene Os04g0396500 for all three *lax2* mutant alleles (Figure 4A). The putative *LAX2* gene consisted of four exons and three introns and encoded a plant-specific conserved domain. The cDNA clone of the putative *LAX2* driven by its own promoter was introduced into the *lax2* mutant, and the characteristic *lax2* phenotype (i.e., in which the SB does not form on the PB) was complemented (see Supplemental Figure 1 and Supplemental Table 1 online). Based on these results, we conclude that Os04g0396500 is *LAX2*.

Using the predicted amino acid sequence of *LAX2*, we identified six genes with homology to *LAX2* in the rice genome and five genes from the *Arabidopsis* genome (Figure 4B; see Supplemental Data Set 1 and Supplemental Figures 2A and 2B online). We conducted a phylogenetic analysis of the amino acid sequences of conserved domains from rice and *Arabidopsis* that are shared with *LAX2*. Proteins containing the conserved domains shared with *LAX2* were divided into two groups based on their conservation of amino acid residues. Members of one group had a C3HC4-type RING zinc finger motif at the N-terminal side of the conserved domain, while members of the other group did not have this motif; instead, the other group had numerous stretches of the same amino acid residues, including stretches of Arg, Ala, Glu, His, Pro, Gly, Ser, and Thr, at the N-terminal region (Figure 4B; see Supplemental Figure 2B online).

To identify the molecular function of *LAX2*, we investigated the cellular localization of *LAX2* by expressing *LAX2*:green fluorescent protein (GFP) fusion proteins driven by a 35S constitutive promoter of *Cauliflower mosaic virus* in the root cells of stable rice transformants (Figures 4C to 4E). GFP fluorescence was localized exclusively in the nucleus, suggesting that *LAX2* is a nuclear protein, although we could not find the typical nuclear localization signal in the predicted *LAX2* amino acid sequence.

We then examined the in situ expression of *LAX2*. In the vegetative shoots, *LAX2* expression was observed at the axil of the P3 leaf, at the location where the AM is formed, as well as in young leaves (Figures 5A, 5B, and 5G). In the reproductive stage, *LAX2* expression was observed at all the AMs that give rise to PBs, SBs, and spikelets (Figures 5C and 5D). The *lax1* and *lax2* mutants had similar phenotypes during the vegetative and reproductive stages. Furthermore, the *lax1 lax2* double mutant resulted in a more extreme *lax* panicle phenotype than either single mutant, without markedly affecting the number of PBs (Figures 2H, 2I, and 2K). Based on these findings, we hypothesized that *LAX1* and *LAX2* act at a related process in AM formation. To test this, we observed whether the spatiotemporal expression domains of *LAX1* and *LAX2* overlapped using two-color in situ hybridization (Figures 5E and 5F). The domain of *LAX1* mRNA localization, visualized in red in Figures 5E and 5F, resides at the margin of the *LAX2* expression domain, visualized in purple in Figure 5F. At the tip of the primary branch meristem, we also observed *LAX2* expression, and this is consistent with the fact that PBs are also branches made at the



**Figure 5.** In Situ mRNA Accumulation Pattern of *LAX2*.

- (A) In situ hybridization with a *LAX2*-specific probe on a longitudinal section of a shoot during vegetative development. The dashed line indicates the approximate position of the cross section shown in (B).  
 (B) In situ hybridization with a *LAX2*-specific probe on a cross section of a shoot during vegetative development.  
 (C) In situ hybridization with a *LAX2*-specific probe on a longitudinal section of an inflorescence meristem with PB primordia.  
 (D) In situ hybridization with a *LAX2*-specific probe on a longitudinal section of an inflorescence meristem with secondary PB primordia.  
 (E) In situ hybridization with a *LAX1*-specific probe on a longitudinal section of a primary branch meristem with SB primordia.  
 (F) Two-color in situ hybridization with *LAX1*- and *LAX2*-specific probes on a longitudinal section of a primary branch meristem with SB primordia. *LAX2* expression is detected by a purple color and is visualized on the same section in (E). *LAX1* mRNA localization is visualized by red color.  
 (G) In situ hybridization with a *LAX2* sense probe on a longitudinal section of the vegetative shoot.  
 Bars = 100  $\mu$ m. Arrows indicate *LAX2* expression at the vegetative AM.

inflorescence. *LAX1* is directionally trafficked to the body of the AM, from its mRNA localization domain, which is the boundary between the AM and SAM (Oikawa and Kyoizuka, 2009). Thus, it is possible that the functional domains of *LAX1* and *LAX2* proteins largely overlap.

Because the *LAX1* protein and *LAX2* mRNA localization domains largely overlap, we investigated the possibility that *LAX1*

and LAX2 regulate the expression of *LAX2* and *LAX1*, respectively, by in situ hybridization (see Supplemental Figure 3 online) and RT-PCR analysis (see Supplemental Figure 4 online). We found that expression of *LAX1* was not abolished in the *lax2* mutant, indicating that the expression of *LAX1* does not depend on the expression of *LAX2* (see Supplemental Figure 3 online). We also found that *LAX2* mRNA was not abolished in *lax1* mutant (see Supplemental Figure 4 online). This indicates that *LAX2* expression does not require *LAX1*, although there is a possibility that *LAX1* affects *LAX2* expression at a very subtle level, which cannot be distinguished by RT-PCR.

### LAX2 Physically Interacts with LAX1, and the Interaction Requires the Conserved Domain

The sites where LAX1 and LAX2 function in the AM overlap, and both proteins localize in the nucleus; therefore, it is possible that LAX1 and LAX2 physically interact. To test this, we conducted a yeast two-hybrid assay (Figure 6A). Yeast cells carrying bait with the full-length *LAX2* cDNA and the empty prey vector grew on the selective medium, suggesting the presence of a transcriptional activation domain in LAX2, at least in yeast cells. Consequently, we used a bait construct with a partial *LAX2* cDNA lacking the region corresponding to the 113 amino acids at the N terminus of LAX2. We confirmed that yeast cells carrying this bait and empty prey vector barely grew on the selective medium. We then transformed yeast cells with this bait and the prey with *LAX1* cDNA. The transformants grew on the selective medium, suggesting an interaction between LAX1 and LAX2 in yeast cells.

We then examined the LAX2 domain that is responsible for the interaction with LAX1. A series of truncated *LAX2* cDNAs was cloned into the bait constructs and assayed for interaction with LAX1 by observing growth on selective medium. Constructs lacking the region corresponding to the 270 amino acids at the N terminus or the 15 amino acids at the C terminus did not interact with LAX1, indicating that the interaction of LAX2 and LAX1

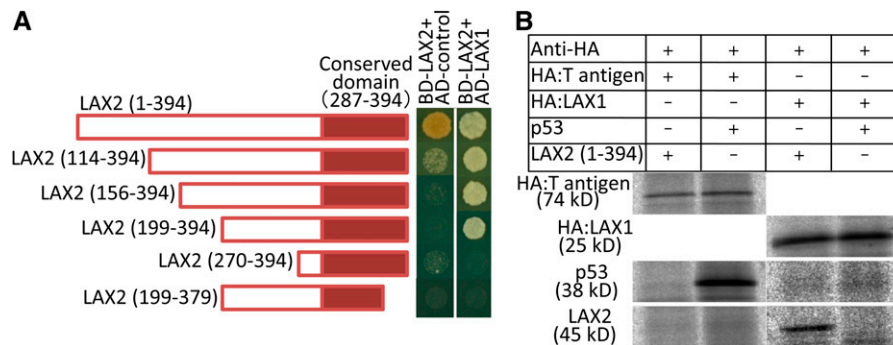
requires regions 199 to 269 and 380 to 394 of LAX2. This suggests that the C-terminal region of LAX2, which includes the conserved domain, is involved in the interaction. Because LAX1 also shows strong transcriptional activation in yeast when *LAX1* cDNA is used in the bait construct (see Supplemental Figure 5 online), we could not switch *LAX2* and *LAX1* cDNA inserts in the bait and prey construct, respectively, to confirm the interaction. Instead, we confirmed the interaction by an in vitro binding assay (Figure 6B). HA-tagged LAX1 protein and LAX2 were synthesized in vitro in the presence of <sup>35</sup>S-labeled Met. These proteins were incubated and immunoprecipitated using anti-HA antibody. LAX2 coprecipitated with HA-LAX1, while it did not coprecipitate with the HA-T antigen. This assay confirmed that LAX1 and LAX2 interact in vitro.

### DISCUSSION

*LAX2* functions in the aboveground branching of the rice plant throughout development. The phenotype of *lax2* mutants is similar to that of *lax1* mutants during both the vegetative and reproductive stages. The synergistic genetic interaction of *lax2* with *lax1* and *moc1* in branching (as illustrated by the *lax1 lax2* and *lax2 moc1* double mutant phenotypes) indicates that there are multiple pathways in which LAX1, LAX2, and MOC1 function for branching (Figure 7). LAX2 encodes a nuclear protein with a plant-specific conserved domain, and it physically interacts with LAX1 through a region containing this domain. So far, many factors that regulate AM formation have been identified, but the biochemical relation between these factors is not well characterized. Thus, we propose that LAX2 is a novel factor that acts synergistically with LAX1 in rice to regulate AM formation.

### LAX2 Plays a Role in the Maintenance of AM

*lax2* mutants have a sparse panicle that results in a reduced number of SBs and spikelets. *lax2* also affects vegetative



**Figure 6.** Molecular Interaction between LAX1 and LAX2.

**(A)** Interaction between LAX1 and LAX2 was tested by a yeast two-hybrid assay. The full-size *LAX2* cDNA and a deletion series of *LAX2* cDNAs were fused with a DNA binding domain construct (BD), and the full-size cDNA of *LAX1* was fused with an activation domain construct (AD). Yeast cells were grown on -Leu, Trp, His medium, and the interactions were monitored by growth on the selective medium. Numbers in parentheses are the positions of amino acid residues included in each construct. Red boxes indicate the position of the conserved domain.

**(B)** In vitro binding of LAX1 and LAX2. In vitro-synthesized HA-tagged T antigen and HA-tagged LAX1 along with LAX2 (1 to 394) and p53 proteins in the presence of <sup>35</sup>S-Met were used as bait and prey, respectively. After incubation of bait and prey proteins, bait proteins were immunoprecipitated using anti-HA antibody. LAX2 and p53 proteins were coprecipitated with HA-LAX1 and HA-T antigen, respectively.



development in that *lax2* mutants have fewer tillers. This is consistent with the double mutant phenotype of *lax2* combined with either *lax1* or *moc1*, in which a monocult phenotype is observed. The reduction or absence of branching in *lax2* plants could be due to either loss or arrest of the AM. Morphological, histological, and scanning electron microscopy observations did not provide evidence of arrested axillary shoots that give rise to tillers, SBs, or spikelets in *lax2* single or double mutants. Interestingly, immunohistochemical analysis of the *lax2* mutant inflorescence shows that AMs that give rise to SBs or spikelets are marked by anti-OSH1 antibody on PBs, similar to the wild type. Furthermore, in the *lax2 moc1* double mutant, a population of OSH1-positive cells at the flank of the SAM is observed by both histological and immunohistochemical analysis. Thus, we propose that *LAX2* is involved in the maintenance of the AM.

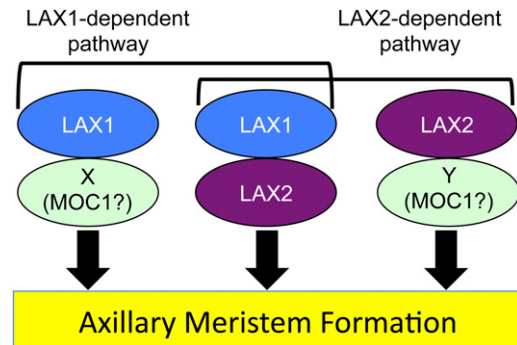
*lax1* mutants are reported to be defective in the maintenance of the AM during the vegetative and reproductive stages (Komatsu et al., 2003; Oikawa and Kyojuka, 2009). During the vegetative stage, the phenotype of the *lax1* and *lax2* single mutants is relatively weak, as tillers are occasionally present. However, in the *lax1 lax2* double mutant, no tillers form during the vegetative stage. In the reproductive stage, either the *lax1* or *lax2* mutation partially affects the formation of SBs and spikelets on PBs. However, in the double mutant, the formation of SBs and spikelets on PBs is completely suppressed. The similar phenotypes of single *lax1* and *lax2* mutants and the strong synergistic genetic interaction between the *lax1* and *lax2* mutation in the double mutant suggest that both *LAX1* and *LAX2* are involved in the maintenance of the AM throughout rice development.

By contrast, the formation of PBs in the inflorescence does not depend on *LAX1* or *LAX2*, although both *LAX1* and *LAX2* are expressed at the site of PB formation (Figure 5C) (Komatsu et al., 2003). It is possible that there is a mechanism that forms AMs destined to become PBs in the inflorescence that is independent of *LAX* genes. Alternatively, PB formation might not require the full activity of the *LAX* genes, which promote the maintenance of lateral shoots because immediately after their formation, PBs start to elongate as independent main shoots.

### Genetic Pathways That Regulate the Maintenance of AMs in Rice

The striking similarity of *lax1* and *lax2* mutants suggests an epistatic relationship between *lax1* and *lax2*. However, the phenotype of the double mutant is synergistically enhanced compared with the single mutant, suggesting that *LAX1* and *LAX2* act in independent pathways in AM formation rather than a direct or indirect regulation of one gene by the other. In support of this, *LAX1* or *LAX2* expression was not abolished in the *lax2* or *lax1* mutant, respectively (see Supplemental Figures 3 and 4 online). Thus, we propose that there are multiple pathways that regulate the formation of the AM through the action of *LAX1* and/or *LAX2* (Figure 7).

*MOC1* is another factor that regulates the formation of AM in rice (Li et al., 2003). In the *moc1* mutant, the number of branches is reduced during both the vegetative and reproductive stages. The combination of the *moc1* and *lax1* mutations results in the complete loss of branching (Komatsu et al., 2003). This phenotype is similar to that of *moc1 lax2* double mutants. We propose



**Figure 7.** A Model for the Functions of *LAX1*, *LAX2*, and *MOC1*.

*LAX2* is a novel nuclear factor that is involved in branching events throughout rice development. There are multiple pathways for AM formation where *LAX1* and *LAX2* are involved. *LAX2* acts in two pathways, *LAX1* dependent and *LAX1* independent. In the *LAX1*-dependent pathway, *LAX1* and *LAX2* form a dimer. Both *LAX1* and *LAX2* can also function independently. Factors X and Y could be redundant factors of *LAX2* and *LAX1*, respectively, and they could be partners of *LAX1* and *LAX2*, respectively. Alternatively, it is also possible that factor X and factor Y could correspond to *MOC1* because the phenotype of *lax1 moc1* and *lax2 moc1* double mutants are similar. In this case, *MOC1* may or may not form dimers with *LAX1* or *LAX2*. Once the activity of two proteins from the group consisting of *LAX1*, *LAX2*, and *MOC1* is removed by mutations in the corresponding genes, the formation of the AM is almost completely suppressed at all developmental stages, except PB formation. Thus, there is expected to be another layer of regulation for the formation of PB.

that there are multiple pathways for the formation of the AM, in which three factors (*MOC1*, *LAX1*, and *LAX2*) have overlapping functions (Figure 7). Once two of the three factors are removed by mutation of the corresponding genes, the process of AM formation is strongly suppressed at all developmental stages except PB formation. In the formation of PBs, *MOC1* seems to have a more critical role than *LAX1* or *LAX2*. It is interesting that the number of PBs is not reduced in *lax1* and *lax2* single mutants and in their double mutants. However, both *LAX1* and *LAX2* are also involved in the formation of PBs because *lax1 moc1* or *lax2 moc1* double mutants completely lose the PB. Thus, for the formation of PBs, there should be an additional layer of the regulation as discussed before.

### *LAX2* Encodes a Novel Nuclear Protein That Acts Together with *LAX1* in Branching

The molecular cloning of *lax2* revealed that it encodes a nuclear protein with a plant-specific conserved domain. Proteins containing this domain either have a C3HC4-type RING zinc finger motif or have stretches of the same amino acids, such as Arg, Ala, Glu, His, Pro, Ser, and Thr (see Supplemental Figure 2B online). The C3HC4-type RING zinc finger is often found in the single-subunit E3 ubiquitin ligase family (Kuroda et al., 2002; Stone et al., 2005). In *Arabidopsis*, there are ~400 RING domain-containing proteins, which may function as single-subunit E3 ligases (Stone et al., 2005), and some of these proteins are known to target transcription factors (Hardtke et al., 2000;

Osterlund et al., 2000; Xie et al., 2002; Seo et al., 2003; Yang et al., 2005; Zhang et al., 2005; Dong et al., 2006). Among the proteins that share a conserved domain with LAX2, the function of two genes, *DRIP1* and *DRIP2*, which encode the C3HC4 RING zinc finger, has been reported in *Arabidopsis* (Qin et al., 2008). These two genes target DREB2A, the key transcriptional factor for the response to water stress, to mediate DREB2A degradation by a mechanism that tightly modulates its protein level and thereby regulates gene expression in response to water stress.

LAX2 does not have a C3HC4-type RING zinc finger; thus, it is unlikely that LAX2 has E3 ligase activity. It has been shown that DRIP1 and DRIP2 interact with DREB2A through a region that includes a LAX2-conserved domain (Qin et al., 2008). We have also shown that LAX2 localizes to the nucleus and interacts with LAX1 through a region that includes this domain. Thus, it is possible that this domain is responsible for protein–protein interactions. The *in situ* mRNA accumulation of LAX2 partially overlaps with that of LAX1, although the mRNA expression domain of LAX2 is broader than that of LAX1 (Komatsu et al., 2003). It is noteworthy that LAX1 moves directionally to the newly formed AM primordium where LAX2 mRNA resides. Thus, it is possible that LAX1 and LAX2 protein localizations largely overlap. These observations suggest that LAX1 and LAX2 form a dimer and that the dimer is responsible for AM formation, at least in part.

Because LAX2 does not have a typical DNA binding domain, it is unlikely that LAX2 acts as a sequence-specific DNA binding protein. It is also unlikely that LAX2 regulates LAX1 expression. Rather, we propose that LAX2 acts as a cofactor of LAX1. LAX1 is a bHLH-type transcription factor, and its function is regulated at both the transcriptional and posttranscriptional levels (Komatsu et al., 2003; Oikawa and Kyojuka, 2009). Furthermore, BA1, a LAX1 ortholog in maize, is a substrate of the PINOID-like kinase BIF2 *in vitro* (Skirpan et al., 2008), suggesting that BA1 is regulated by BIF2 at the posttranscriptional level. Although LAX1 and LAX2 have a unique role in the maintenance of the AM, it is possible that LAX2 modulates the activity of LAX1 at the posttranscriptional level.

## METHODS

### Plant Material and Growing Conditions

The rice single-gene recessive mutants *lax2-1*, *lax2-2*, *lax2-3*, *lax1-1*, *lax1-6*, and *moc1-4* and wild-type rice (all *Oryza sativa* subsp *japonica*) were used in this study. *lax2-1* was obtained from an ethyl methanesulfonate–mutagenized population of Zhonghua 11. *lax2-1* was provided by Qian Qian of the China National Rice Research Institute. *lax2-2* and *moc1-4* were screened from a population generated by tissue culture of Nipponbare followed by regeneration. *lax2-3* and *lax1-6* were generated by chronic  $\gamma$ -ray irradiation of Norin 8 and Nihonmasari, respectively, with a dose rate of 0.4 Gy/d from transplanting until the mature stage. Plants were grown in a paddy field or in pots in a greenhouse under standard growth conditions. Zhonghua 11, Nipponbare, or Norin 8 was used as the wild type for the observation of phenotypes.

### Immunohistochemical Staining

Vegetative and reproductive shoots were fixed in FAA (1:1:18 [formaldehyde to glacial acetic acid to 70% ethanol]) for 24 h at 4°C and then

dehydrated in a graded ethanol series. Dehydrated samples were embedded in Paraplast Plus (Oxford Labware), sectioned into 5- to 8-mm-thick sections with a rotary microtome, and used for immunostaining. Immunostaining was conducted based on the method described by Donlin et al. (1995), except that anti-OSH1 antibody and anti-rabbit IgG linked with alkaline phosphatase (ZYMED Laboratories) were used at 1:150 and 1:600 dilutions, respectively.

### Scanning Electron Microscopy Analysis

For scanning electron microscopy, seedlings were harvested and young leaves were excised to expose P5 leaf axils. These samples were observed directly under an N-SEM system (S-3000N; Hitachi).

### Map-Based Cloning of *lax2*

Map-based cloning was conducted using a mapping population made by crosses between the *lax2* mutant and an *indica* cultivar; the *lax2* mutation was mapped on chromosome 4. Using more than 1000 mutant individuals from the mapping population, we screened recombinants between two genetic markers that reside 68 kb apart on the physical map and identified four recombinants between these markers. This region corresponds to BAC clones OSJNBa0039F02 and OSJNBb0039C07. Mutations in *lax2* were determined by PCR amplification and sequencing of genes predicted within these regions. We confirmed that LAX2 cDNA driven by the LAX2 promoter complements the mutant phenotype. LAX2 cDNA was amplified with primers LAX2F1 (5'-GCGTCGACGAGGGTGAGAGG-3') and LAX2R2 (5'-TACCGGCAAAAGTTGTTAGAAG-3'), cloned into the pCRII Blunt TOPO vector (Invitrogen), and verified by sequencing. This plasmid was designated as pLAX2full. The promoter region of LAX2 was amplified using the primers LPM1-pm-3108+Asc-F (5'-AGGCGCGCC-TATGGCACAGGGTGGTTAGTG-3') and the 3' primer LPM1-pm+531-R (5'-CAGCATGTGCGTGGGTATC-3'). The fragment was cloned into the pCRII Blunt TOPO vector and verified by sequencing. A *Bam*HI-*Eco*RI fragment of this vector was cloned into pBluescript SK+ (Agilent Technologies). The resulting vector was digested with *Bam*HI-*Xba*I and ligated with a *Bam*HI-*Spe*I fragment of pLAX2full. The resulting vector and the pZH2Bik (Kuroda et al., 2010) were digested with *Asc*I-*Sac*I and ligated to produce vector pL2C. The pZH2Bik and pL2C vectors were introduced into scutellum-derived calli of *lax2* by *Agrobacterium tumefaciens*-mediated transformation under selection with hygromycin at 50 mg/L (Toki, 1997). Transformants were used to analyze the panicle phenotype.

### Phylogenetic Analysis

The alignment used to construct the phylogenetic tree was made using ClustalX (Jeanmougin et al., 1998), and the sequences were manually adjusted to optimize the alignment as shown in Supplemental Figure 2A online. The parameters used in the construction of the alignment are as follows. For the pairwise alignment parameters, we used a GAP extension penalty of 0.75 and a GAP opening penalty of 35.00. For the multiple alignment parameters, we used a GAP extension penalty of 0.3 and a GAP opening penalty 15.00. We also used the BLOSUM 30 protein weight matrix. The phylogenetic tree was constructed by the NJ method (Saitou and Nei, 1987) using PAUP\* 4.0 software. Statistical support for nodes is shown as bootstrap values obtained by 1000 trials.

### Subcellular Localization of LAX2

The pEGFP plasmid (Clontech) was digested with *Nco*I and *Xba*I, and pCAMBIA1305 was partially digested with *Nco*I and *Nhe*I. The EGFP fragment was then cloned into pCAMBIA1305 to produce the pCAMBIA1305-EGFP vector. To examine the subcellular localization of LAX2, the

*LAX2* coding sequence driven by the 35S promoter of *Cauliflower mosaic virus* was cloned into the pCAMBIA1305-EGFP vector in which *LAX2* was fused in-frame to EGFP. The plasmid was introduced into wild-type callus, using the method described previously. The roots of transgenic plants were examined using an Olympus BX51 fluorescence microscope.

### In Situ Hybridization

In situ hybridization was performed as previously described by Kouchi and Hata (1993). For the *LAX2* probe, the full-length cDNA clone was used as a template for in vitro transcription. Hybridizations were conducted at 53°C overnight; slides were then washed four times at 50°C for 10 min each. An excess amount of sense transcript was used as the negative control. For two-color in situ hybridization, a DIG-labeled full-length *LAX1* antisense RNA probe and a fluorescein-labeled *LAX2* antisense probe were made and used for hybridization. Conditions of hybridization and the procedure for the two-color in situ hybridization were the same as those of the single-color method except that after the development of the first color using Fast Red TR (Sigma-Aldrich), slides were heat-treated at 70°C for 8 h to inactivate the enzyme and then used to detect the second color using the standard NBT/BCIP method.

### Yeast Two-Hybrid Assay

We used the Matchmaker yeast two-hybrid system (Takara). The selection of transformants with bait and prey plasmids was performed on SD plates lacking Trp and Leu. Interactions were monitored by growth on SD plates lacking His, Trp, and Leu.

The bait and prey plasmids used in this experiment were constructed as follows. Regions of *LAX1* and *LAX2* cDNA spanning the entire open reading frame and a deletion series of the *LAX2* cDNA were amplified using the primers listed in Supplemental Table 2 online and cloned into pGBKT7 with *EcoRI* and *XhoI*, and into pGADT7 with *EcoRI* and *Sall*. All of the PCR-derived fragments were sequenced after cloning, and an absence of errors in PCR amplifications was confirmed.

### In Vitro Binding Assay

HA-*LAX1*, *LAX2*, HA-T antigen, and p53 proteins for coimmunoprecipitations were expressed from T7 promoters in the TnT in vitro transcription/translation system (Promega) in the presence of <sup>35</sup>S-Met. After incubation of bait and prey proteins, bait proteins were immunoprecipitated using the anti-HA antibody derived from the 3F10 clone (Roche Diagnostics). The binding buffer was as follows: 25 mM Tris-Cl, pH 7.5, 100 mM NaCl, 10% glycerol, 0.2% Nonidet P-40, 5 mg/mL BSA, 2 mM EDTA, and 1 × complete protease inhibitor cocktail [Roche Diagnostics]. After washing six times with binding buffer, samples were boiled in the presence of SDS-PAGE sample buffer and loaded onto SDS-PAGE gels. After electrophoresis, gels were fixed, dried, and developed with contacting imaging plates for BAS2000 (Fuji Film).

### Detection of *LAX2* Transcripts in *lax1-6*, *lax2-2*, *moc1-4*, and *lax2-2 moc1-4* Mutants

Total RNA was extracted using TRIzol reagent (Invitrogen) from 3-week-old seedlings of the wild type and mutants. RT-PCR was performed using Omniscript (Qiagen). Primers used to amplify *LAX2*, *MOC1*, *Os ACT1*, and *Os TB1* are shown in Supplemental Table 2 online. We performed RT-PCR at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, for 30 (*LAX2* and *Os TB1*), 32 (*MOC1*), and 26 (*Os ACT1*) cycles, respectively. PCR products were run on agarose gels, stained with ethidium bromide, and visualized on a UV transilluminator. Data were obtained using RNA extracted from more than five biologically independent seedlings for the wild type and all mutants.

### Accession Numbers

Sequence data from this article can be found in the GenBank/EMBL database under accession numbers AB669025 (*LAX2*), AB115668 (*LAX1*), AB088343 (*OsTB1*), AY242058 (*MOC1*), and AK058421 (*OsACT1*).

### Supplemental Data

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

**Supplemental Figure 1.** Complementation of *lax2* Mutants by *LAX2* cDNA Driven by Its Own Promoter.

**Supplemental Figure 2.** Multiple Alignments of Amino Acid Sequences of Conserved Domains Shared with *LAX2* from Rice and *Arabidopsis*.

**Supplemental Figure 3.** In Situ Expression of *LAX1* in Mutants and the Wild Type.

**Supplemental Figure 4.** RT-PCR Analysis of Genes Expressed at AM in Mutants and the Wild Type.

**Supplemental Figure 5.** *LAX1* and *LAX2* Have Transactivation Activity in Yeast.

**Supplemental Table 1.** Quantification Data of the Complementation of the *lax2* Mutant.

**Supplemental Table 2.** Primers Used in This Experiment.

**Supplemental Data Set 1.** Alignment Used to Generate the Phylogeny Presented in Figure 4B.

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### AUTHOR CONTRIBUTIONS

H.T., Y.Z., S.H., M.O., S.S.-S., T.O., H.X., X.F., H.Y., F.C., J.K., and Y.S. designed and conducted the experiments. Q.Q., M.N., and H.K. generated the plant materials. Y.S., H.Y., and F.C. wrote the article.

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