The gene **FLORAL ORGAN NUMBER1** regulates floral meristem size in rice and encodes a leucine-rich repeat receptor kinase orthologous to *Arabidopsis* CLAVATA1

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**Summary**

The regulation of floral organ number is closely associated with floral meristem size. Mutations in the gene **FLORAL ORGAN NUMBER1 (FON1)** cause enlargement of the floral meristem in *Oryza sativa* (rice), resulting in an increase in the number of all floral organs. Ectopic floral organs develop in the whorl of each organ and/or in the additional whorls that form. Inner floral organs are more severely affected than outer floral organs. Many carpel primordia develop indeterminately, and undifferentiated meristematic tissues remain in the center in almost-mature flowers. Consistent with this result, **OSH1**, a molecular marker of meristematic indeterminate cells in rice, appears to control the distance between floral primordia initiation events.

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**Introduction**

The floral meristem in angiosperms produces floral organs in numbers and patterns that are characteristic of each species. The number of floral organs, together with their shape and patterns, contributes to the diversification of flowers. Genetic and molecular mechanisms that regulate floral organ number have been well studied in *Arabidopsis thaliana* by means of a number of mutants. Two classes of gene affect floral organ number. The first class includes genes that regulate meristem activity and maintenance, such as **CLA VATA (CLV)**, **FAS CI A TA (FAS)** and **ULTRAPETALA (ULT)** (Clark et al., 1993; Clark et al., 1995; Fletcher, 2001; Kayes and Clark, 1998; Leyser and Furner, 1992); loss of function of these genes causes enlargement of flower meristems and results in an increase in floral organ number. The second class includes genes such as **PERIANTHIA (PAN)**, of which loss of function give rises to an increase mainly in the number of perianth organs (Running and Meyerowitz, 1996). Mutation of **PAN** does not affect meristem size; instead, **PAN** appears to control the distance between floral primordia initiation events.

The **clv1**, **clv2** and **clv3** mutations cause enlargement of the vegetative and inflorescence meristems, in addition to the floral meristem, leading to abnormal phyllotaxy, inflorescence fasciation and enlargement of floral meristems (Clark et al., 1993; Clark et al., 1995; Kayes and Clark, 1998). Genetic analysis has shown that these three genes act in the same pathway to regulate meristem size. **CLV1** encodes a receptor-like protein consisting of an extracellular leucine-rich repeats (LRR) domain, a small transmembrane domain, and a cytoplasmic serine/threonine kinase domain (Clark et al., 1993; Clark et al., 1995; Kayes and Clark, 1998; Leyser and Furner, 1992); loss of function of these genes causes enlargement of flower meristems and results in an increase in floral organ number. The second class includes genes such as **PERIANTHIA (PAN)**, of which loss of function give rises to an increase mainly in the number of perianth organs (Running and Meyerowitz, 1996). Mutation of **PAN** does not affect meristem size; instead, **PAN** appears to control the distance between floral primordia initiation events.
1998; Schoof et al., 2000). Mutations in the CLV genes fail to repress WUS activity, resulting in expansion of the WUS expression domain and enlargement of the meristem. Conversely, WUS positively regulates the expression of CLV3 and maintenance of the stem cell domain. This regulatory feedback system is required to maintain appropriate meristem size throughout Arabidopsis development.

In spite of much progress in our understanding of the regulation of floral organ number and maintenance of the meristem in Arabidopsis, the regulation of such events in monocots is poorly understood. The fasciated ear2 (fea2) gene is exceptionally well characterized in Zea mays (Taguchi-Shiobara et al., 2001). Mutations in fea2 cause enormous enlargement of the inflorescence meristem but have a more modest effect on floral meristems and organ number. The fea2 gene encodes an LRR receptor-like protein that is most closely related to Arabidopsis CLV2. This finding indicates that the CLV signaling pathway for regulating meristem maintenance is conserved in monocots as well as in dicots. In rice (Oryza sativa), two loci responsible for floral meristem size and number have been identified, FLORAL ORGAN NUMBER1 (FON1) and FON2 (Nagasawa et al., 1996). Floral organ number is also increased in rice by antisense suppression of the genes OsLRK1 and OsFOR1, which encode an LRR kinase-like protein and a polygalacturonase-inhibiting protein, respectively (Kim et al., 2000; Jang et al., 2003).

In this report, we describe in detail a new allele, fon1-2, which is stronger than the fon1-1 (formerly fon1) allele previously reported by Nagasawa et al. (Nagasawa et al., 1996). In fon1-2, the floral meristem is severely affected and accumulates a large number of cells. Consequently, unlike in fon1-1, the number of all organs in the floret increase and the effect of the mutation is more evident in the outer whorls than in the inner whorls in fon1-2. We isolated FON1 by positional cloning and found that this gene encodes an LRR receptor-like kinase that is orthologous to CLV1 of Arabidopsis. Our results suggest that the CLV signal transduction pathway for regulating floral meristem size and number is conserved in rice. Although FON1 is expressed in all of the meristems responsible for development of the aerial part of rice, marked changes are not observed in meristem size or in the phenotypes of the vegetative or inflorescence organs even in the strong fon1-2 mutant, suggesting that there may be genetic redundancy in the maintenance of both vegetative and inflorescence meristems in rice.

Materials and methods

Plant materials

The rice strains used in this study were Oryza sativa L. spp. japonica. A new mutant with an increased number of floral organs was found in M2 plants of a derivative strain from Kinuhikari that had been mutagenized with N-methyl-N-nitrosourea. An allelism test revealed that this new mutant (fon1-2) was allelic to fon1-1 [previously described as fon1 by Nagasawa et al. (Nagasawa et al., 1996)]. Kinuhikari and Taichung65 (T65) were used as wild-type strains for comparing phenotypes and for in situ analysis.

Scanning electron microscopy (SEM) and meristem size measurement

For observation by SEM, young panicles and flowers were fixed using the methods of Itoh et al. (Itoh et al., 2000). Samples were dried at their critical point, sputter-coated with platinum, and observed with a scanning electron microscope (model S-4000; Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV. For observation by Nomarski microscopy, shoot and floral apices were fixed by the methods of Itoh et al. (Itoh et al., 2000). After clearing them in benzyl-benzoate-four- and-a-half fluid, specimens were observed with a microscope equipped with Nomarski differential interference-contrast optics (model M-2; Olympus, Tokyo, Japan). The width and height of the meristems were measured by the methods of Nagasawa et al. (Nagasawa et al., 1996).

Positional cloning of FON1

The fon1 locus was mapped by using an F2 population of fon1-1 and Kasalath (ssp. indica). First, the locus was mapped to a region between CAPS markers R1028 and E10139 on the distal end of the long arm of chromosome 6 by using 119 fon1 homozygotes. Then, by using 2,419 F2 plants, the locus was narrowed to a region between two closely linked CAPS markers, M11 (5'-AGACCTGATACGATGGC- GAAC-3', 5'-TCTCTTCATGGTTGAACTAG-3'; AflII digestion) and M54 (5'-CCACCCGACCTCTACGG-3', GTGGCCGTCACGT- CACC-3'; HhaI digestion), which were designed by comparing genomic sequences of the japonica and indica. This region of 150 kb in length was present in two YAC contigs, AP003614 and AP003769.

Ten putative genes were identified by using a gene prediction program, Rice Automated Annotation System (http://ricegias.dna.affrc.go.jp). Because a gene highly similar to Arabidopsis CLV1 was found among these ten putative genes, the genomic sequences of the FON1 candidate gene in the fon1-1 and fon1-2 mutants were determined by direct method of sequencing after PCR amplification. Primers were selected on the basis of the CLV1 gene using the database of the rice genomic sequence. A genomic DNA fragment including the FON1 candidate was isolated from the genomic library. For complementation, a 7.7 kb fragment including 2.2 kb of sequence directly upstream of the initiation codon was cloned into a binary vector and introduced into fon1-1 by Agrobacterium-mediated transformation (Hiei et al., 1994). A FON1 cDNA was synthesized and amplified from poly(A)+ RNA isolated from young panicles. Exon-intron structures were determined by comparing the genomic and cDNA sequences.

In situ hybridization

To detect FON1 transcripts unambiguously, two probes derived from independent regions of the gene were used: the first region (probe A; 437 bp), consisting of the last 241 bp of the coding region and 196 bp of the 3' UTR, was amplified with the primers 5'-ACTG-GGTGCCGAAAGGTGAC-3' and 5'-AGATCATTAGCCCGGAA-3'; the second region (probe B; 396 bp), consisting of 381 bp of the 5' UTR and 15 bp of the coding region, was amplified with the primers 5'-ACCCCTACTAGTCAACCG-3' and 5'-GAGAGTAGGGAGGCA-TTGTGA-3'. The amplified DNA fragments were cloned into T/A cloning vector (Novagen, Madison) and used for RNA synthesis and labeling. The FON1 expression patterns detected with these two probes coincided with each other. OSH1 and DL probes were prepared as described in the original papers (Sato et al., 1996; Yamaguchi et al., 2004).

Plant materials were fixed and dehydrated by the methods of Itoh et al. (Itoh et al., 2000). They were embedded in Paraplast Plus (Oxford Labware, St Louis) after replacement with xylene. Microtome sections (8 μm) were mounted on glass slides coated with Vectabond (Vector Laboratories, Burlingame). RNA probes were labeled with digoxigenin using a DIG labeling kit (Roche, Mannheim). In situ hybridization and immunological detection of the signals were carried out by the methods of Kouchi and Hata (Kouchi and Hata, 1993).
Results

**FON1 is necessary for regulating floral organ number**

Rice flowers have a pistil in the central whorl and six stamens around it. In the next outer whorl, two lodicules corresponding to petals in dicots are produced. These floral organs are subtended by a palea and a lemma (Fig. 1A). Mutations in the *fon1* locus cause an increase in the number of floral organs. In *fon1-1*, the number of pistils increased to two or three in all flowers examined, but there were small or no changes in the number of stamens, lodicules, palea or lemma (Figs 1, 2). However, the *fon1-2* mutation affected all floral organs (Fig. 1C). More than 70% of the *fon1-2* flowers had four or five pistils, and in a few extreme cases the flowers had nine pistils (Fig. 2). Increases in stamen number, varying from seven to 12, were observed in almost all *fon1-2* flowers. Extra lodicules or palea/lemma-like organs were observed in about a third of *fon1-2* flowers. Thus, the *fon1-2* allele seems to have a stronger mutation than the *fon1-1* allele. In both alleles, the effects were more evident in the inner than in the outer floral organs.

Genetic analysis revealed that the *fon1-2* mutation was inherited as a recessive trait. First, the numbers of floral organs in all flowers (n=50) in F1 plants generated between the wild type and *fon1-2* were the same as the number of floral organs in wild-type flowers (i.e. one lemma, one palea, two lodicules, six stamens and one pistil). Second, segregation of wild type and *fon1* mutant flowers in the F2 population was in accordance with a 3:1 ratio (36 wild type, 11 *fon1-2*: 2:0.64, P>0.7). We found no flowers that showed an increase in the floral organ number out of 400 flowers of the F2 plants examined that were heterozygous for the *fon1* locus.

Flower organ development in earlier stages

To study the abnormalities in *fon1* flowers in detail, we examined the phenotypes of the strong *fon1-2* mutant flowers in the early stages of development by SEM. In wild-type rice, the lemma initiated at the flank of the flower meristem close to the inflorescence axis and the palea initiated at the flank opposite to the lemma (Fig. 3A). By contrast, ectopic palea/lemma-like organs developed on the lateral side (Fig. 3D) or on the palea side of the meristem in *fon1-2* (Fig. 3E). In the former case, a pair of palea/lemma-like organs developed on the inside of the palea, suggesting that an additional whorl had formed (Fig. 3D). In the latter case, an additional palea/lemma-like organ developed on the inside of the palea (data not shown) or two palea-like organs developed next to each other in the original whorl (Fig. 3E). These palea-like organs, which were arranged in parallel, were thinner than wild-type palea in the mature flower. In wild type, two lodicules formed at the flank of the meristem adjacent to the lemma. In *fon1-2*, extra lodicules, usually two, were produced at the flank adjacent to the palea (data not shown). Six stamens developed in a concentric whorl in wild-type flowers. In many of the *fon1-2* flowers, by contrast, extra stamens were produced in the same whorl as the original stamens (Fig. 3D). The maximum number of stamens formed within the same whorl was ten. In other *fon1-2* flowers, extra stamens developed in two concentric whorls, suggesting that an extra whorl for stamens was produced in these flowers (Fig. 3F).

Carpel development was markedly affected by the *fon1* mutations. In wild type, the carpel primordia arose at the flower meristem near the lemma and developed from the flank of the meristem towards the opposite side, enclosing the meristem (Yamaguchi et al., 2004). After the carpel primordia fused at the top, two styles developed (Fig. 3B,C). In *fon1-2*, however, the floral meristem was enlarged and became a long oval dome-like structure before carpel initiation (Fig. 3F). The carpel primordia first arose at the flank of the floral meristem near the lemma, but extra carpel primordia for the second pistil subsequently arose in the opposite flank of the meristem (Fig. 3G). Thus, these two initial sets of ectopic carpel primordia arose in an alternate phyllotaxy, as described previously (Nagasawa et al., 1996). The region between the two independent carpel primordia remained morphologically undifferentiated and maintained meristem activity (see below). Subsequently, carpel primordia arose repeatedly and
indeterminately. Eventually, many pistils stood together in large numbers (Fig. 3H,I). The pattern of carpel appearance was largely irregular after the third set of carpels emerged, and the floral meristems remained even after the production of many sets of carpels (Fig. 3H-J).

A few fon1-2 flowers produced a small secondary floret adjacent to the stamens (Fig. 3K). This secondary floret consisted of a pistil, a few stamens, and a pair of palea and lemma that enclosed the reproductive organs. This structure might be associated with a splitting of the flower meristem after the initiation of palea primordia in the primary flower: after separation, the detached meristem could result in the development of a secondary flower independent of the primary flower. Floral organ identity was affected by the fon1-2 mutation at only a low frequency. For example, lodicules were transformed into palea/lemma-like organs (data not shown) or an anther was partially transformed into stigma-like organs (Fig. 3L).

To verify the results of this phenotype analysis, we examined the expression patterns of two genes, DROOPING LEAF (DL) and OSH1. DL regulates carpel identity in rice and is expressed exclusively in carpel primordia (Yamaguchi et al., 2004). DL expression in wild type was detected in carpel primordia, which then developed into a pistil enclosing an ovule (Fig. 3M), whereas DL transcripts in fon1-2 were detected in the multiple sets of carpel primordia, which developed into independent pistils (Fig. 3N). OSH1 is a molecular marker of meristematic indeterminate cells in rice and is expressed in the floral meristem (Sato et al., 1996; Yamaguchi et al., 2004). In wild type, OSH1 expression was downregulated when the floral organs began to initiate and completely disappeared when the carpel primordia began to develop (Fig. 3O). However, expression of OSH1 continued in the region around the carpel primordia in fon1-2 (Fig. 3P). Together with the phenotypic analysis by SEM, this result suggests that the floral meristem maintains its activity and loses its determinacy in fon1-2, even after the initiation of several sets of carpel primordia.

fon1 affects predominantly floral meristem size

The control of floral organ number is closely associated with...
floral meristem size in Arabidopsis clv mutants (Clark et al., 1993; Clark et al., 1995; Kayes and Clark, 1998). We examined meristem sizes in the strong fon1-2 allele. At an early flowering stage, the floral meristem of fon1-2 was apparently larger than that of wild type (Fig. 4A,B; Table 1). After initiation of the stamens, the fon1-2 floral meristem became considerably larger and increased in length along the palea-lemma axis (Fig. 3D,F). The expression pattern of OSH1 suggested that the number of meristematic cells increased in the fon1-2 floral meristem (Fig. 4C,D). Consistent with the floral phenotype, the floral meristem size of fon1-2 was larger than that of fon1-1.

However, no difference was observed in the size of vegetative meristems of wild type, fon1-2 and fon1-1 mutants (Fig. 4E,F; Table 1). The vegetative growth phenotypes of fon1-2 were indistinguishable from those of wild type. The inflorescence meristem size of fon1-2 was slightly larger than that of wild type (Fig. 4G,H). Probably associated with this slight increase in inflorescence meristem size, the number of primary rachis branches increased by about 1.4-fold in fon1-2 (15.0±0.9), when compared with wild type (10.7±1.7) (Fig. 4I,J). However, marked changes such as fasciation were not observed in fon1-2. Thus, the strong fon1-2 mutation does not profoundly affect the size of vegetative and inflorescence meristems or the phenotypes of the plant bodies derived from them. These results suggest that FON1 regulates mainly the floral meristem in rice.

Isolation of FON1

To elucidate the molecular function of FON1, we set out to isolate the gene by positional cloning (Fig. 5A). First, the fon1 locus was mapped to the distal end of the long arm of rice chromosome 6. Using about 2400 F2 plants, we subsequently confined the fon1 locus to a region of about 150 kb. Using the rice genomic sequence database and the RiceGAAS program, which efficiently predicts putative genes in rice, we identified ten genes in this region. Among them, we identified a putative gene that encodes an LRR-type receptor kinase similar to Arabidopsis CLV1 (Clark et al., 1997).

clv1 mutants share several characteristics with fon1 mutants, such as an increase in floral organ number and enlargement of the floral meristem. Thus, the CLV1-like gene was a strong candidate for the FON1 gene. We therefore determined the nucleotide sequence of this candidate and identified the nucleotides that cause amino acid substitution in both fon1-1 and fon1-2 (Fig. 5B-D). The mutant phenotype of fon1-1 was rescued when a 7.7 kb genomic fragment containing the candidate gene was introduced into fon1-1 rice plants (Fig. 1D). These results clearly indicate that the CLV1-like gene identified by our positional cloning approach was derived from the fon1 locus.

FON1 encodes a CLV1-like receptor kinase

We determined the positions of introns in FON1 by sequencing the RT-PCR product generated with FON1-specific primers and the predicted open reading frame. FON1 encodes a putative protein of 994 amino acids (Fig. 5B), comprising a putative hydrophobic signal peptide, LRRs, a transmembrane domain and a cytoplasmic serine-threonine kinase domain. This overall protein structure of FON1 closely resembles that of CLV1 (Clark et al., 1997). The number of LRRs (22) and the two cysteine pairs that flank the LRR domain are conserved in both proteins (Fig. 5C). Between FON1 and CLV1, the amino acid identities of the LRR and kinase domains are 55.1 and 73.2%, respectively (Fig. 5B-D). In fon1-1, proline (903) is substituted for leucine. This amino acid is located just downstream of subdomain IX of the kinase domain (Die’vart and Clark, 2003), and is shared by other plant LRR-type receptor-like kinases such as CLV1, BRI1 and HAR1 (Clark et al., 1997; Li and Chory, 1997; Nishimura

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Table 1. Sizes of the floral and shoot apical meristems

Fig. 4. Meristem sizes and inflorescences in wild-type and fon1-2. (A.C,E,G,I) Wild type. (B.D,F,H,J) fon1-2. (A.B) Floral meristems. (C,D) Expression of OSH1 in the floral meristem. The expression domain is expanded in fon1-2, but the spatial pattern does not differ from that in wild type. (E,F) Vegetative shoot apical meristems. (G,H) Inflorescence meristems. (I,J) Inflorescence phenotypes after seed production. (A.B.E-H) Micrographs viewed under Nomarski optics. Scale bars: 100 μm in A-H; 5 cm in I,J.
et al., 2002). In fon1-2, glycine (205) is substituted for serine in the sixth repeat of the LRR domain. This amino acid substituted in fon1-2 is the same amino acid that is altered in clv1-4 (G201E), one of several strong alleles of CLV1 that has a dominant-negative effect (Die'vart et al., 2003).

There are numerous LRR-type receptor-like kinases in plant
Arabidopsis genes other than HAR1 in legumes such as encoding receptor-like kinases responsible for hypernodulation.

Spatial expression patterns of FON1 in this clade, which strongly suggests that FON1 constitutes a small clade with Arabidopsis CLV1 in rice. Together with proteins known to belong to this class, the kinase domains were used to construct a phylogenetic tree by the neighbor-joining method (Fig. 5E). The result indicated that FON1 is an ortholog of Arabidopsis CLV1 in rice.

Spatial expression patterns of FON1

To elucidate the function of FON1, we examined its mRNA expression patterns by in situ hybridization. FON1 transcripts were initially detected in the shoot meristem in the embryo (Fig. 6A). Transcripts were subsequently detected specifically in the shoot apical meristem, but not in any other regions of the vegetative apex (Fig. 6B). After transition to the reproductive phase, FON1 transcripts were detected in the primary and secondary rachis branch meristems (Fig. 6C), which correspond to the inflorescence meristems. Subsequently, FON1 was expressed in the floral meristems from the initial stage of floral development (Fig. 6D). FON1 continued to be expressed in the floral meristem (Fig. 6E), and its expression persisted even after the initiation of carpel primordia (Fig. 6F). Thus, FON1 is expressed in all of the meristems responsible for development of the aerial part of rice. FON1 transcripts were uniformly distributed throughout all types of meristem. This expression pattern differs from that of CLV1, which is mainly localized to L3 cells in Arabidopsis. Therefore, we analyzed FON1 expression again using two probes derived from independent regions of the gene and confirmed with both probes that FON1 was expressed uniformly throughout the meristems.

In addition to the meristems, FON1 was also expressed in floral organ primordia, such as the palea (Fig. 6D), lemma (Fig. 6D), lodicules (Fig. 6E,F), stamens (Fig. 6E,F), carpels (Fig. 6F) and ovules (data not shown). As a control, no signal was detected in floral meristems and floral organ primordia when sense RNA was used as a probe (Fig. 6G).

Discussion

Mutations at the fon1 locus cause enlargement of the floral meristem, resulting in an increase in the number of floral organs. Molecular cloning of the causative gene has shown that FON1 encodes an LRR-type receptor kinase; furthermore, evolutionary analysis clearly indicates that FON1 is an ortholog of Arabidopsis CLV1. Together with the finding that the CLV2 ortholog FEA2 regulates female inflorescence size in maize (Taguchi-Shiobara et al., 2001), our results indicate that the CLV signaling system that regulates meristem size in Arabidopsis is conserved in the grass family.

FON1 regulates maintenance of the floral meristem

The increase in floral organ number in the fon1 mutants may be closely associated with enlargement of the floral meristem. The meristem size is much larger in fon1-2 than in fon1-1, and the increase in floral organ number is consistently much greater in fon1-2 than in fon1-1. Furthermore, the extent of meristem enlargement in fon1-2 is much greater at later stages of floral development than at earlier stages. Consistent with this, the increase in floral organ number is more evident in the inner than in the outer whors.

Floral organs increase in two ways: first, additional floral organs are formed in the same whorl; and second, floral organs develop in the additional whors that form. In the innermost whors, carpel primordia arise repeatedly among the carpel primordia that have already been produced. This repetitive production of carpel primordia is associated with the indeterminate nature of floral primordia. For example, OSH1, which marks meristematic cells, continues to be expressed in the center of the floral meristem even after the carpel primordia have been produced, confirming that determinacy is lost in the fon1 floral meristem. Although floral meristem determinacy is affected in loss-of-function mutants of both DL and class CLV2 ortholog FEA2 regulates female inflorescence size in maize (Taguchi-Shiobara et al., 2001), our results indicate that the CLV signaling system that regulates meristem size in Arabidopsis is conserved in the grass family.
H.-Y.H., unpublished), stamens and carpels, whose identities are regulated by these genes, develop normally in the fon1 mutants. Therefore, the loss of determinacy in fon1 seems to be independent of the function of DL and class C genes. The continued expression of OSH1 in the center of floral meristems in the fon1 mutant and the uniform expression of FON1 throughout the meristem also suggest that the control of floral meristem determinacy by FON1 is indirect.

FON1 is expressed in all types of meristem in the aerial part of rice, from the shoot apical meristem in embryos to the floral meristem. Unlike the floral meristem, however, the vegetative and inflorescence meristems show no or only slight abnormalities in fon1 mutants. Because fon1 is inherited as a recessive trait, there may be genetic redundancy in the pathways that maintain the vegetative and inflorescence meristems. Because complete loss-of-function mutants of CLV1 show weak clv phenotypes in Arabidopsis, the strong clv phenotypes are thought to be caused by a dominant-negative effect of the mutated protein, and the existence of an RLK-X protein that functionally overlaps with CLV1 has been assumed in Arabidopsis (Die’vart et al., 2003). Analogous to this, it is possible that the gene corresponding RLK-X in rice is not expressed in the floral meristem, but is expressed and functions in the vegetative and inflorescence meristems. In the latter case, the mutation in the FON1 proteins may not cause a dominant-negative effect in the fon1 mutants. This hypothesis is consistent with both the recessive nature of the fon1 mutation and the reduced effect of the mutation in the vegetative and inflorescence phases. The phylogenetic tree shows that three genes encoding LRR receptor kinase are close to FON1 (Fig. 5E). These genes are possible candidates for sharing functional redundancy with FON1 in the vegetative meristem.

Spatial expression patterns of FON1
FON1 transcripts are distributed throughout the whole meristem and in all types of meristem. This pattern of expression is in high contrast to that of CLV1, which is predominantly expressed in the L3 cell layer of the meristems (Clark et al., 1997). CLV1 functions on perception of CLV3 ligands that are secreted from stem cells in the L1 and L2 layers (Rojo et al., 2002; Lehard and Laux, 2003). CLV1 transfers a signal that negatively regulates WUS, which is expressed in a putative organizing center within the CLV1 expression domain (Brand et al., 2000; Fletcher et al., 1999; Mayer et al., 1998; Schoof et al., 2000). Thus, CLV1 functions in a signaling pathway that communicates between two distinct domains, the stem cell region and an organizing center in the meristem. Expression of FON1 throughout the whole meristem would therefore seem to be irrelevant to the communication between two independent domains.

Although there is no evidence that homologs of CLV3 and WUS are expressed and function in rice in a way similar to that in Arabidopsis, we can propose two hypotheses for FON1 function on the basis of the Arabidopsis CLV signaling system. If LRR-receptor kinases work as heterodimers as proposed by Die’vart et al. (Die’vart et al., 2003), it is possible that a putative protein partner of FON1 may function only in the L3 layer; indeed, whereas CLV1 is expressed in a domain-specific manner, CLV2, is expressed ubiquitously in Arabidopsis (Jeong et al., 1999). Alternatively, mature FON1 proteins may be localized to the L3 layer in rice through post-transcriptional regulation. Both hypotheses would result in the distribution of a functional receptor complex of FON1, similar to the distribution of CLV1 expression. However, we do not rule out a third hypothesis that the signaling system in rice differs from that in Arabidopsis. In this hypothesis, the presence of a WUS homolog in an organizing center is not postulated; instead, it is assumed that the FON1 receptor in stem cells transfers the signal from the ligand to a putative factor that suppresses stem cell proliferation in the L1 and L2 layers without passing through an organizer (domain-autonomous regulation). Finally, it is also possible that the difference in the expression pattern between FON1 and CLV1 is not essential for the function of both proteins because expression of CLV1 under the control of the widely expressed ELECTA promoter does not cause any changes in the meristem size in Arabidopsis (Die’vart et al., 2003).

To understand the genetic mechanism that regulates meristem maintenance in rice, it will be necessary to isolate the genes that function in this pathway. The fon2 mutant, the flowers of which have an increased number of floral organs similar to those of fon1 (Nagasawa et al., 1996), may provide clues towards identifying these genes. In addition, it will be essential to determine whether a WUS-like organizer functions in rice meristems.

FON1 transcripts are expressed in the primordia of all floral organs including lemma, palea, lodicules, stamens and carpels, from their inception to later floral stages. This expression of FON1 seems to be specific to rice, because CLV1 is not expressed in the floral organ primordia of Arabidopsis (Clark et al., 1997). The sizes and identities of floral organs were almost normal, even in the strong fon1-2 mutant. Therefore, even if FON1 regulates the development of floral organs, the fon1 mutation may be masked by other genes that share functional redundancy with FON1. We observed slight abnormalities in the floral organs at low frequency. These defects seem to be side-effects of the enlargement of the meristem and do not seem to be associated with the function of FON1 expressed in these floral organs, because the weaker allele fon1-I also produces flowers with abnormal floral organ identities at a similar frequency (Nagasawa et al., 1996). It will be of great interest to examine whether FON1 is involved in the regulation of floral organ size.

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