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ABERRANT PANICLE ORGANIZATION 1 temporally regulates meristem identity in rice

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Abstract

We report a recessive mutation of rice, *aberrant panicle organization 1* (*apo1*), which severely affects inflorescence architecture, floral organ identity, and leaf production rate. In the wild-type inflorescence, the main-axis meristem aborts after forming 10–12 primary branch primordia. However, in *apo1*, the main-axis meristem was converted to a spikelet meristem after producing a small number of branch primordia. In addition, the branch meristems in *apo1* became spikelet meristems earlier than in wild type. Therefore, in the inflorescence, the *apo1* mutation caused the precocious conversion of the meristem identity. In the *apo1* flower, lodicules were increased at the expense of stamens, and carpels were formed indeterminately by the loss of meristem determinacy. Vegetative development is also affected in the *apo1*. Leaves were formed rapidly throughout the vegetative phase, indicating that *APO1* is also involved in temporal regulation of leaf production. These phenotypes suggest that the *APO1* plays an important role in the temporal regulation of both vegetative and reproductive development.

Keywords: Rice; Aberrant panicle organization 1; Inflorescence; Organ identity; Meristem; Phyllotaxy; Plastochron

Introduction

Plants exhibit a myriad of inflorescence structures that are specified by the spatial and temporal arrangement of branches and flowers (Coen and Nugent, 1994; Weberling, 1989). In the case of *Arabidopsis*, inflorescence meristems and coflorescences continue to produce flowers until senescence occurs; for that reason, they have indeterminate properties. Petunia inflorescence meristem yields two types of meristems of similar sizes: one of them grows out to form a flower; the other remains as an inflorescence meristem, engendering the typical branching pattern of cymose inflorescences (Souer et al., 1998). On the other hand, rice inflorescence is categorized as raceme, in which spikelets are attached to lateral branches, not directly to the main axis. Rice inflorescence is also called a panicle because of the conical inflorescence shape. The main-axis meristem (frequently called the rachis) aborts after producing 10 or more primary branches (Ikeda et al., 2004). Abortion of the main-axis meristem is also observed in other taxa such as *Convallaria majalis* in Liliaceae and *Delphinium elatum* in Ranunculaceae (Weberling, 1989). Therefore, the architecture of rice inflorescence is determined fundamentally by the number of primary branches (length of main axis) and the number of spikelets on each primary branch (length of primary branch).

Molecular genetic approaches have identified numerous genes involved in controlling inflorescence architecture over the last decade, mainly using two model plants: *Arabidopsis thaliana* and *Antirrhinum majus*. Floral meristem identity genes such as *LEAFY* (*LFY*)/*FLORICAULA* (*FLO*), *APETALA1* (*AP1*)/*SQUAMOSA* (*SQUA*), *CAULIFLOWER* (*CAL*), and *FRUITFULL* (*FUL*) confer floral fate on lateral meristems (Coen et al., 1990; Ferrandiz et al., 2000; Huijser et al., 1992; Kempin et al., 1995; Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). On the other hand, *TERMINAL FLOWER 1* (*TFL1*)/*CENTRORADIALIS* (*CEN*) prevent the inflorescence meristem to be transformed

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to floral meristem by delaying the up-regulation of floral meristem identity genes (Alvarez et al., 1992; Bradley et al., 1996, 1997; Ratcliffe et al., 1998, 1999).

Once a floral meristem is established, three classes of floral organ identity genes begin to function. Each floral organ identity gene functions in two adjacent whorls. The combinational activities of these genes specify the floral organ identity known as the ABC model (Coen and Meyerowitz, 1991; Mandel and Yanofsky, 1995; Weigel and Meyerowitz, 1994). Genes are also revealed that mediate floral meristem identity genes and organ identity genes. The *UNUSUAL FLORAL ORGAN* (*UFO*) and *FIMBRIATA* (*FIM*) are reported as functioning in specifying the floral identity of the meristem and controlling the expression of floral organ identity genes (Levin and Meyerowitz, 1995; Simon et al., 1994; Wilkinson and Haughn, 1995).

In contrast to remarkable advancement in the understanding of eudicots, genetic mechanisms of monocot (grass) inflorescence formation remain poorly understood. *BRANCHED SILKLESS 1* (*Bd1*) in maize and *FRIZZY PANICLE* (*FZP*) in rice that are required for establishment of the floral meristem from the spikelet meristem (Colombo et al., 1998; Chuck et al., 2002; Komatsu et al., 2001, 2003). *Tassel seed 6*, *indeterminate spikelet 1* (*ids1*), and *reversed germ orientation 1* (*rgo1*) in maize are mutations that abolished the determinate fate of the spikelet meristem, thereby increasing the number of florets in each spikelet (Chuck et al., 1998; Irish, 1997; Kaplinsky and Freeling, 2003). Mutations in the *LAX PANICLE* (*LAX*) and *MONOCULM 1* (*MOC1*) genes of rice cause a lack of axillary meristem. Consequently, inflorescences have few spikelets (Komatsu et al., 2001; Li et al., 2003). Other subjects have not been studied: the mechanisms regulating the developmental fate of main-axis meristem and that specifying the branch length.

Floral organ identity has been studied in rice. Rice homologs of *Arabidopsis* class A, B, and C genes show specific expression patterns that are similar to those of *Arabidopsis* genes (Kyozuka et al., 2000; Nagasawa et al., 2003), suggesting that the ABC model is applicable to rice, except for carpel identity. Carpel identity is specified by *DROOPING LEAF* (*DL*) gene (Nagasawa et al., 2003; Yamaguchi et al., 2004). Therefore, one interesting subject is the elucidation of the way in which expression of floral identity genes is regulated in rice.

This study characterized the *aberrant panicle organization 1* (*apo1*) mutant, which pleiotropically affects developmental events in rice. The *apo1* mutation decreases the number of spikelets because of precocious conversion of inflorescence meristem into spikelet meristem and exhibits aberrant floral organ identity. We also demonstrate that the *APO1* gene acts as temporal regulator in the vegetative phase. Using genetic and double mutant analysis, we demonstrate that *APO1* affects class C gene expression and interacts with *FON1* and *FON2* gene during floral development. *APO1* temporally regulates the meristem identity throughout the life cycle.

Materials and methods

Plant materials

We identified three single recessive rice (*Oryza sativa* L.) mutants that showed abnormal inflorescence architecture. They were allelic, and designated *aberrant panicle organization 1-1* (*apo1-1*), *apo1-2*, and *apo1-3*. The *apo1-1* and *apo1-2* were derived from the M_2 population of cv. Taichung 65 (T65) and *apo1-3* from that of cv. Kinmaze mutagenized with *N*-methyl-*N*-nitrosourea. These three mutants were from different genetic backgrounds. For that reason, we used the original cultivar of each mutant as a control.

We constructed double mutants among *apo1*, *fzp*, *floral organ number 1* (*fon1*), and *fon2* to analyze *APO1* interaction with other flower-associated genes. The *fzp* mutant is defective in the conversion of spikelet meristem to floral meristem, resulting in flowerless inflorescence comprising only branches (Komatsu et al., 2001); both *fon1* and *fon2* mutants show an increased number of floral organs (Nagasawa et al., 1996). The F_1 plants were allowed to self-pollinate; the double mutant plants were selected from the F_2 population.

Mapping of APO1 locus

Heterozygous *APO1*/*apo1-3* plant (japonica type) was crossed with pollens of cv. Kasalath (indica type). We extracted DNAs from leaves of 21 *apo1* plants found in the F_2 population. By using these DNAs, we made linkage analysis between *apo1* mutation and molecular (CAPS and STS) markers.

Histological analysis

For paraffin sectioning, samples were fixed overnight at 4°C in FAA (formalin:glacial acetic acid:70% ethanol; 1:1:18), and dehydrated in a graded ethanol series. Following substitution with xylene, we embedded the samples in Paraplast Plus (Oxford Labware, St. Louis, MO) and sectioned them at 8 μm in thickness using a rotary microtome. Sections were stained with 0.05% toluidine blue and observed with a light microscope.

For scanning electron microscopy (SEM), samples were fixed overnight at 4°C in FAA. After dehydration in a graded ethanol series and substitution with 3-methyl-butyl-acetate, the samples were critical-point-dried, sputter-coated with platinum, and observed under a scanning electron microscope (S-4000; Hitachi Ltd., Tokyo) at an accelerating voltage of 10 kV.

Samples were fixed overnight in FAA for clearing of seedlings and young inflorescences. After dehydration in a

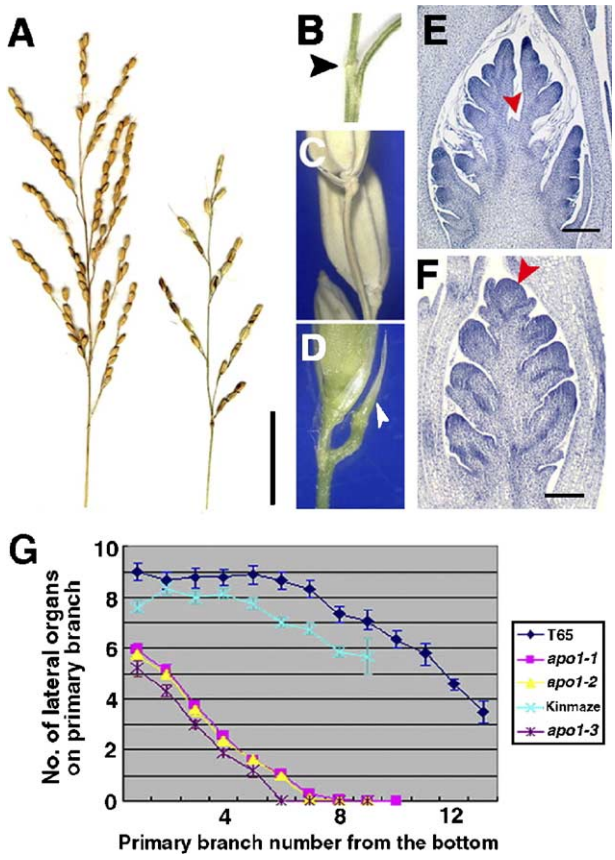

Fig. 1. Inflorescence phenotypes in wild type and *apo1*. (A) Mature inflorescence of wild type (left) and *apo1-3* (right). (B) The vestige of main-axis meristem (arrowhead) in wild type. (C) Terminal spikelet of *apo1-1*. Vestige is not observed. (D) Incomplete terminal spikelet (arrowhead) of *apo1-3* consisting of rudimentary glumes and an empty glume. (E) Longitudinal section of wild-type young inflorescence showing aborted main-axis meristem (arrowhead). (F) Longitudinal section of *apo1-1* young inflorescence. The main-axis meristem is converted into terminal spikelet (arrowhead). (G) Change in the number of lateral organs on each primary branch. The reduction of lateral organs is acropetally enhanced in *apo1*. Scale bars, 5 cm in panel (A), and 100 μm in panels (E) and (F).

graded ethanol series, samples were transferred into BB4-1/2 clearing fluid (Herr, 1982). The cleared samples were observed using a microscope (IX70; Olympus Optical Co., Tokyo) equipped with Nomarski differential interference contrast optics.

In situ hybridization

Tissues were fixed with 4% (w/v) paraformaldehyde and 0.25% glutaraldehyde in 0.1 M sodium phosphate buffer, dehydrated through a butanol series and embedded in Paraplast Plus. Microtome sections (8 μm thick) were applied to glass slides treated with vectabond (Vector Laboratories Inc., Burlingame, CA). Digoxygenin-labeled antisense probes were prepared from the coding region of *OSH1* without the poly(A) region. Digoxygenin-labeled RNA probes used to detect *SPW1* and *RAG* mRNAs were prepared as described by Nagasawa et al. (2003) and Kyozuka et al. (2000), respectively. Hybridization and immunological detection with alkaline phosphatase were performed as described in Kouchi and Hata (1993). Only the results with antisense probes are shown because the sense probes of these genes showed no specific hybridization signals.

Results

For mapping the *APO1* locus, we examined the linkage between *apo1* mutation and CAPS and STS markers. As a result, *APO1* locus was mapped on chromosome 6 between two STS markers, R3879 (96.5–100 cM) and C11635 (109.5 cM).

Phenotypes of inflorescence in apo1 mutant

The three *apo1* mutants, *apo1-1*, *apo1-2*, and *apo1-3*, were characterized by their small inflorescence with the short main axis and small numbers of primary branches and spikelets (Fig. 1A and Table 1). Size reduction of inflorescence components was the most prominent in *apo1-2* and the mildest in *apo1-3* (Table 1). The reduced number of primary branches suggests the precocious termination of the main-axis meristem.

In wild type, we can observe a small protrusion as a vestige of main-axis meristem at the base of the uppermost primary branch (Fig. 1B). By contrast, *apo1-1* and *apo1-2* had no vestiges in most inflorescences (Fig. 1C). In *apo1-3*, about 40% of the inflorescences had a vestige of the main-axis meristem at the base of the uppermost spikelet. At the same position, in another 40% of *apo1-3* inflorescences, incomplete spikelets comprising glumes and a vestige of spikelet meristem were formed (Fig. 1D). The remaining 20% of *apo1-3* inflorescences had no vestige of main-axis meristem as in the *apo1-1* and *apo1-2*.

We examined a young inflorescence to determine the cause of the disappearance of meristem vestige in *apo1*.

Table 1
Effect of *apo1* mutations on inflorescence traits (average ± SEM)

Genotype	Length of main axis (mm)	No. of primary branches	No. of secondary branches	Total no. of flowers
Wild type (T65)	186.6 ± 6.0	13.4 ± 0.4	31.4 ± 1.0	132.6 ± 8.9
apo1-1	178.6 ± 2.1	10.8 ± 0.1	2.8 ± 0.2	39.0 ± 0.4
apo1-2	159.0 ± 2.5	9.7 ± 0.1	2.5 ± 0.3	33.5 ± 1.3
Wild type (Kinmaze)	149.6 ± 8.3	8.5 ± 0.3	19.5 ± 1.6	102.8 ± 6.7
apo1-3	119.7 ± 4.5	8.1 ± 0.3	2.0 ± 0.2	26.9 ± 1.2

The main-axis meristem of wild type aborts after generating ten or more primary branch primordia (Fig. 1E). On the contrary, most *apo1-1* and *apo1-2* main-axis meristems were converted to spikelet meristems after producing a small number of primary branch primordia (Fig. 1F). That is, the main axis had a terminal spikelet. The *apo1-3* had a comparable number of primary branches to that of the wild type and terminal spikelets infrequently (Table 1). These results suggest that *apo1* main-axis meristem precociously loses its ability to form primary-branch primordia because of the conversion to spikelet meristem. We infer that incomplete spikelets lacking floral organs in *apo1-3* were resulted from the abortion of a spikelet meristem just after producing glumes.

We observed a similar tendency in primary branches. We measured the length of the primary branch and counted the lateral organs. The three *apo1* mutants had short primary branches (data not shown) and had fewer lateral branches and spikelets than wild type (Fig. 1G). These phenotypes were acropetally enhanced. The basal primary branches were short and set a small number of spikelets because of the early conversion of branch meristems to spikelet meristems. An extreme case of this tendency was observed in the upper several primary branches. They comprised only one terminal spikelet (Fig. 1G) because the branch meristem was immediately converted to a spikelet meristem before producing lateral meristems. Consequently, the total number of spikelets in *apo1* was reduced to 20% of that in wild type (Table 1).

Short axes and few lateral organs were commonly observed in the main axis and primary branches. Accordingly, in *apo1*, the meristem identity is converted precociously to that of advanced stages. These results suggest that *APO1* gene suppresses the change from inflorescence meristem to spikelet meristem. Among the three *apo1* alleles, *apo1-2* appeared to be the strongest allele; *apo1-3* was the weakest.

The apo1 mutation causes altered inflorescence meristem

Because *apo1* young inflorescence differed greatly from that of wild type, we analyzed initial stages of main-axis meristem using SEM. After the transition to the reproductive phase, the main-axis meristem in wild type differentiates bracts and primary-branch primordia in spiral phyllotaxy with a divergence angle of about 138° (Fig. 2A). In contrast, all the *apo1* mutants had aberrant phyllotaxy. The primary branches occasionally showed 1/2 alternate (distichous) phyllotaxy (Fig. 2B). Most inflorescences showed spiral phyllotaxy with variable divergence angle around 160° (Fig. 2C). In addition, the spiral direction sometimes changed from clockwise to counterclockwise or vice versa (Figs. 2D and E). Phyllotactic abnormality was observed only in primary branches; the phyllotaxy of the secondary branches and vegetative leaves was normal.

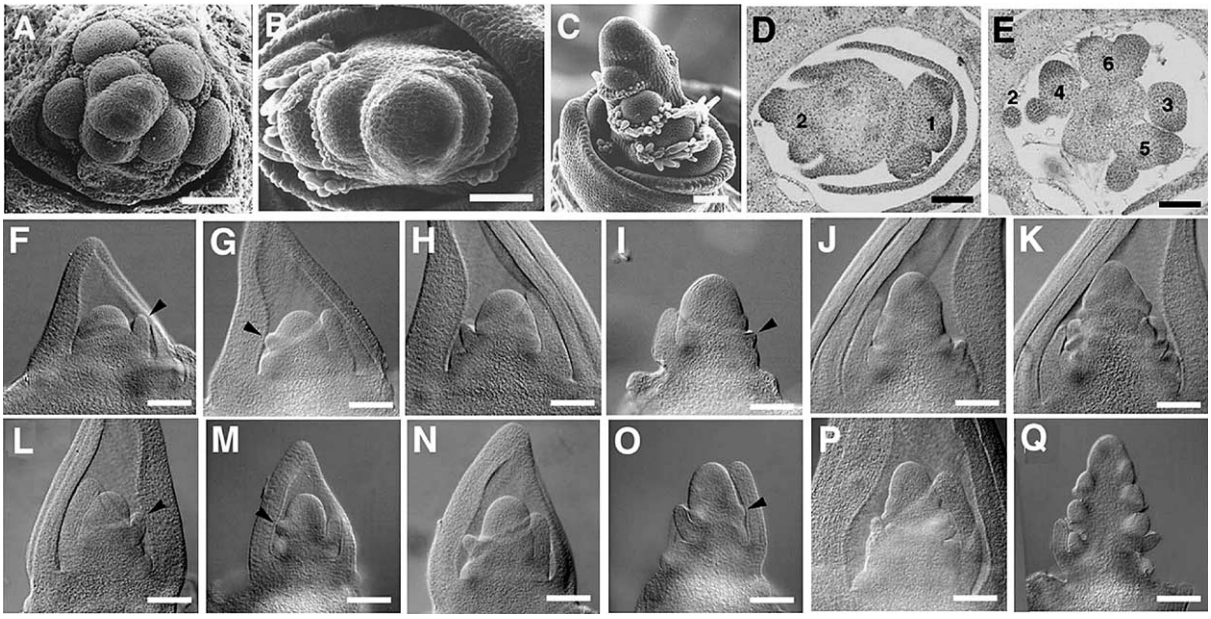

Fig. 2. Inflorescence development in wild type and *apo1*. (A) SEM image of wild-type inflorescence showing spiral phyllotaxy of primary-branch primordia with divergence angle of about 138°. (B) SEM image of *apo1-2* inflorescence showing distichous phyllotaxy of primary-branch primordia. (C) SEM image of *apo1-3* inflorescence showing spiral phyllotaxy of primary-branch primordia with divergence angle of about 160°. (D, E) Serial transverse sections of an *apo1-1* young inflorescence. The direction of spiral changes from clockwise (D) to counterclockwise (E). (F–K) Cleared inflorescence apices of wild type. (L–Q) Cleared inflorescence apices of *apo1-2*. (F and L) Main-axis meristem just after the transition from vegetative phase. The flag leaf primordia (arrowheads) are seen. (G and M) Main-axis meristem forming first bract primordium (arrowhead). (H and N) Main-axis meristems enlarging their size. (I and O) The largest main-axis meristem. Second-bract primordia is formed. (J and P) Main-axis meristem forming a few primary branches. (K and Q) Main-axis meristem at the late stage of primary branch primordium formation. Scale bars, 50 μm in panels (A) and (E), and 100 μm in panels (F)–(Q).

We observed the early stage of main-axis meristem of *apo1-2* using a clearing method to examine the cause of phyllotactic change of primary branches. Stages of inflorescence development were taken from a previous study (Ikeda et al., 2004). At stage In1, in which the main-axis meristem was established, the *apo1* main-axis meristem shape differed markedly from that of wild type (Figs. 2F and L). The main-axis meristem of *apo1-2* was 20% higher and 15% narrower than that of the wild type. This tendency was maintained during development (Figs. 2G–K and M–R). At stage In2, when the wild-type meristem became largest, enlargement of main-axis meristem was not remarkable in *apo1* compared to the wild type (Figs. 2G–I and M–O). At stage In3, during which primary-branch primordia were formed in succession, the *apo1* young inflorescence was slender compared to the wild type (Figs. 2J, K, P, and R). These observations indicate that the small size or aberrant shape of main-axis meristem may cause phyllotactic change in *apo1* mutants.

APO1 affects the rate of lateral organ initiation in vegetative phase

At the transition from the vegetative to the reproductive phase, the *apo1* meristem deviated from that of the wild type, suggesting that the *APO1* gene may function in vegetative growth as well as in the reproductive growth. We examined vegetative development in detail. Fig. 3A shows wild type and *apo1* plants 42 days after germination. The *apo1* plants had more leaves and tillers (branches) than the wild type. Examination of the number of leaves at various stages showed that rapid emergence of leaves occurred constantly in *apo1* throughout the vegetative phase (Fig. 3B). Consequently, the plastochron was shortened. Finally, *apo1* produced 1.5-fold more leaves than the wild type (Fig. 3B).

Three foliage leaves are formed in rice during embryogenesis (Fig. 3C). We sectioned mature embryos to determine whether plastochron reduction occurs there. The *apo1* embryo frequently had four leaves (Fig. 3D). Even in the case of three leaves present in the embryo, the third leaf of *apo1* was much larger than that of the wild type. Therefore, rapid emergence of leaves demonstrably occurs from the embryonic stage.

Meristem size affects the rate of leaf initiation, as indicated in *pla1* mutant (Itoh et al., 1998). We observed the vegetative meristem in detail. We were unable to discriminate the *apo1* meristems from wild-type meristem until 60 days after germination (Figs. 3E and G). Subsequently, the wild-type meristem grew, but the *apo1* meristem showed no remarkable enlargement (Figs. 3F and H). Little enlargement of *apo1* meristem at the late vegetative phase engendered the small size of initial main-axis meristem. These results indicate that rapid initiation of leaves in *apo1* does not result from the large meristem size.

Transition from vegetative to reproductive phase in *apo1* was delayed by about 1 week compared to the wild type.

APO1 affects floral organ identity

Although rudimentary glumes, empty glumes, lemma, and palea were normal, floral organ development was

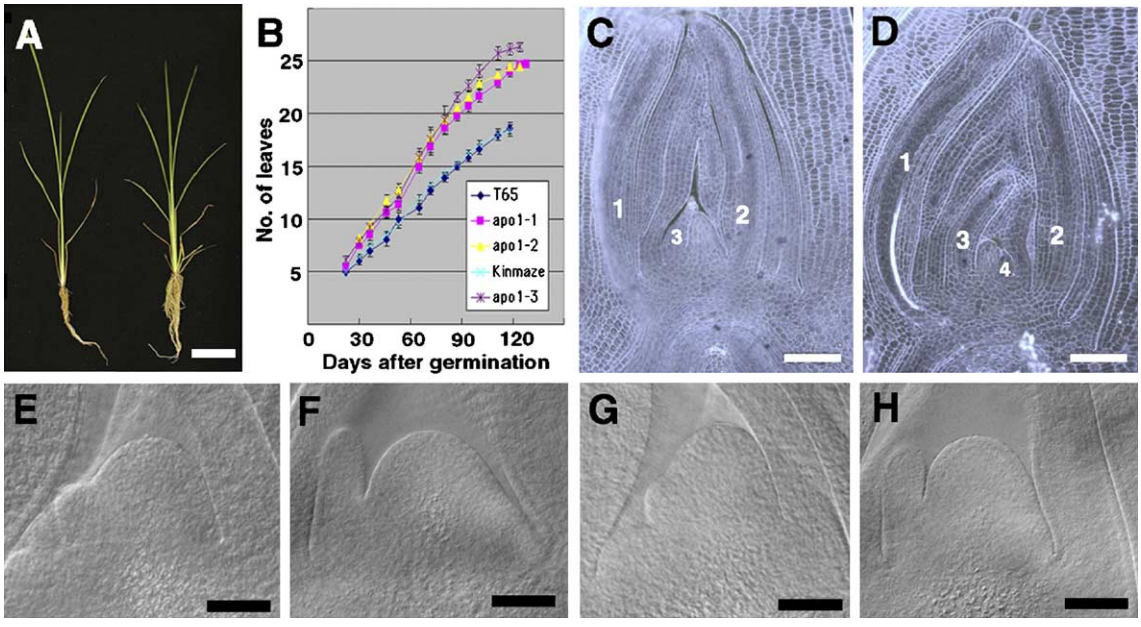

Fig. 3. Vegetative phenotypes of wild type and *apo1*. (A) Wild type (left) and *apo1-3* (right) plants 6 weeks after germination. (B) Increase in the number of leaves in wild type and *apo1*. Vertical bars indicate standard error. (C and D) Longitudinal sections of wild type (C) and *apo1-2* (D) mature embryos. Numerals in the figures show leaf numbers. (E and F) Cleared shoot apices of wild type 60 and 79 days after germination, respectively. (G and H) Cleared shoot apices of *apo1-3* 60 and 79 days after germination, respectively. Scale bars, 5 cm in panel (A), 100 μm in panels (C) and (D), and 50 μm in panels (E)–(H).

affected severely in *apo1* (Fig. 4). Wild-type flowers comprise two lodicules, six stamens, and one pistil composed of one carpel and one ovule (Figs. 4A and E). Organs that correspond to sepals are lost in grasses. For convenience, lodicule, stamen, and carpel whorls are designated as whorl 2, whorl 3, and whorl 4, respectively. The *apo1* flowers showed wide variation in the organ identity and the number of organs (Figs. 4B, C, and D). The remarkable characteristics were the increase of lodicules, decrease of stamens, and indeterminate carpel formation (Table 2). In addition to the normal two lodicules on the lemma side (Figs. 4E and F), *apo1* formed additional ones first on the palea side and then on the lemma side (Fig. 4F). By contrast, stamens were decreased, and confined to the lemma side when less than three stamens remained (Fig. 4G). Mosaic organs of lodicule and stamen were often observed (Figs. 4B and H). The numbers of lodicules, lodiculous stamens, and stamens varied greatly from 2 to 8, from 0 to 4, and from 0 to 5,

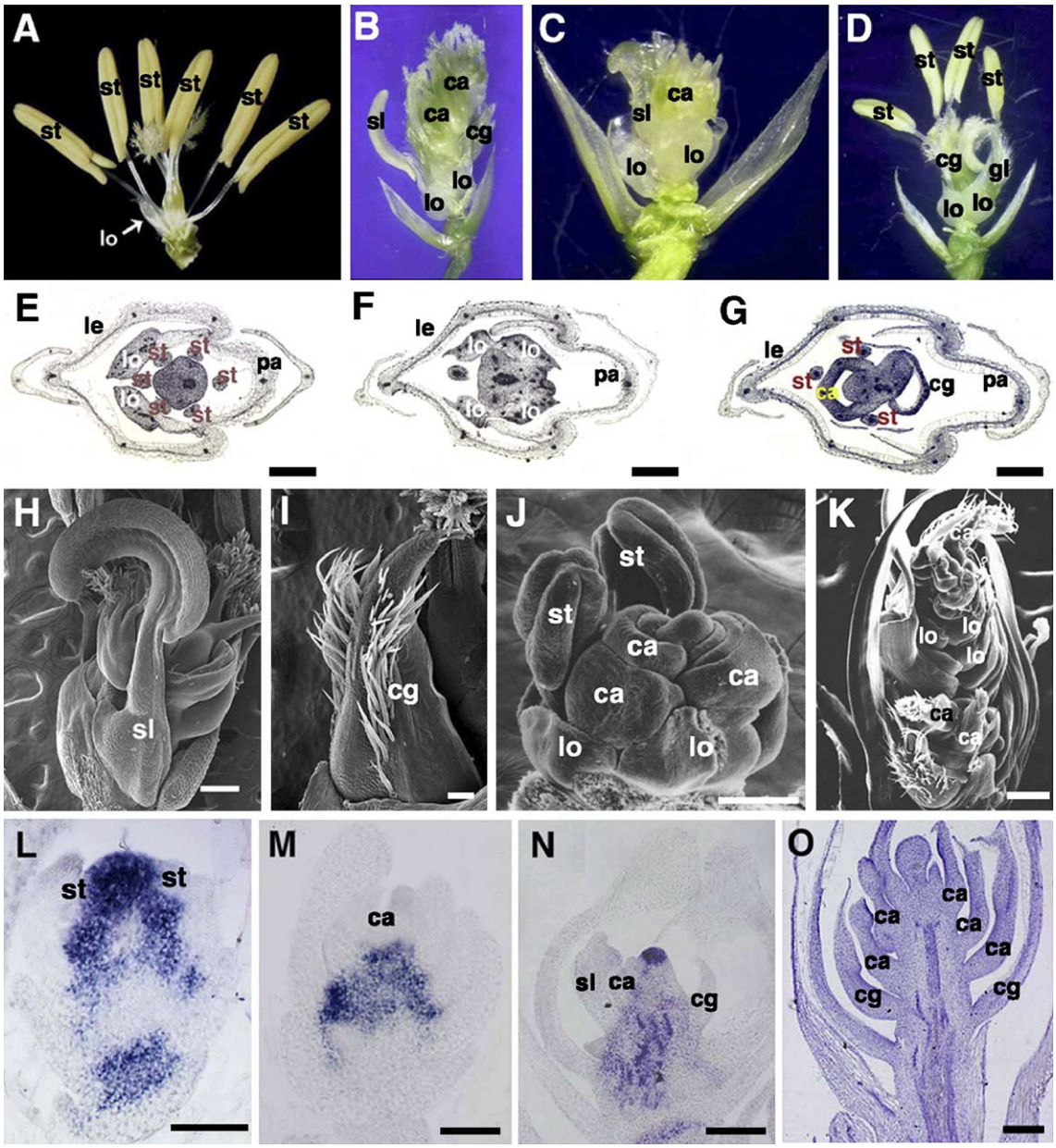

Fig. 4. Floral phenotypes of wild type and *apo1*. (A) Wild-type mature flower with two lodicules, six stamens, and one pistil. (B–D) *apo1-1*, *apo1-2*, and *apo1-3* mature flowers, respectively. (E) Cross section of wild-type mature flower. (F and G) Serial cross sections of an *apo1-1* flower showing lodicules transformed from stamens on the palea side (F) and three stamens biased to the lemma side (G). (H) Mosaic organ between lodicule and stamen. (I) Mosaic organ between glume and carpel. (J) Indeterminate carpel formation in alternate phyllotaxy. (K) Ectopic lodicule formation inside a few carpels. (L) Longitudinal section of *apo1-2* flower showing carpels bearing no ovules. (M and N) *OSH1* expression in wild type flower showing down-regulation after carpel formation. (O) Prolonged *OSH1* expression in *apo1-2* flower after several carpel formation. lo: lodicule, st: stamen, ca: carpel. sl: staminoid lodicule, cg: carpeloid glume, gl: glume-like organ, le: lemma, pa: palea. Scale bars, 1 mm in panels (E)–(G), 300 μm in panels (H) and (K), and 100 μm in panels (I), (J), and (L)–(O).

Table 2
Floral organ number (average ± SEM) in wild type and *apo1*

Genotype	No. of flowers examined	Lodicule	Staminoid lodicule	Stamen	Carpeloid glume	Carpel
Wild type	50	2.0 ± 0	0	6.0 ± 0	0	1 ± 0
apo1-1	82	3.9 ± 0.1	1.6 ± 0.1	1.5 ± 0.2	0.7 ± 0.1	Numerous
apo1-2	45	4.7 ± 0.4	1.4 ± 0.2	1.1 ± 0.3	2.0 ± 0.4	Numerous
apo1-3	52	3.4 ± 0.2	0.8 ± 0.1	3.4 ± 0.2	0.2 ± 0.1	A few

respectively. However, the total number of these organs was conserved to be about eight, which was the sum of wild-type lodicules and stamens (Table 2). Therefore, *apo1* mutations caused a partial or complete homeotic transformation of stamens into lodicules. Among the three alleles, the homeotic transformation of stamens to lodicules was most frequently observed in *apo1-2*, and most infrequently in *apo1-3*. (Table 2, Figs. 4C and D).

Inside the third (stamen) whorl and outside the carpel whorl, one or more organs were formed which have no clear wild-type counterpart. They appeared to be a mosaic organ between the carpel and glume, because they were green, sometimes had trichomes on their surface, and tipped occasionally with stigmatic tissues (Fig. 4I). Carpels were indeterminately formed in the central region, indicating a loss of floral determinacy (Figs. 4B and C). The *apo1-1* and *apo1-2* frequently produced more than ten carpels, but in *apo1-3*, only a few carpels were produced (Fig. 4 and Table 2). The SEM observation of early flower development showed that the floral meristem of *apo1* successively produced a number of carpel primordia in distichous phyllotaxy (Fig. 4J). In some flowers of strong *apo1-2* allele, ectopic lodicules were formed after a few carpels and then followed by several carpels (Fig. 4K). To confirm the loss of floral determinacy in *apo1*, we examined the expression of the class knox gene *OSH1*, a molecular marker of meristematic indeterminate cells in rice (Sato et al., 1996). In the wild type, the *OSH1* expression disappeared from the apex when the carpel primordium covered the ovule primordium (Figs. 4L and M). In the *apo1* floral meristem, *OSH1* expression was maintained even after generating several carpel primordia (Fig. 4N). This result indicates that floral meristem determinacy was lost in *apo1*.

Most carpels in the strong alleles, *apo1-1* and *apo1-2*, did not subtend ovules, and thereby cause sterility (Fig. 4O). Although the weak *apo1-3* flowers had a few ovules, most of them were not functional. Floral abnormality appeared to be acropetally enhanced in an inflorescence.

Homeotic gene expression in apo1

Homeotic transformation of the third whorl organs (stamens) to second whorl organs (lodicules) and the loss of floral determinacy suggest the down-regulation of class C gene in *apo1*. In rice, as a class C gene, *OsMADS3/RAG* is known, which is a rice homolog of *Arabidopsis AGAMOUS* gene. It is expressed in the third and fourth whorls of developing flowers (Kang et al., 1998; Kyozuka et al., 2000). We examined the expression pattern of *OsMADS3* by in situ hybridization. *OsMADS3* was expressed strongly in whorls 3 and 4 of wild-type flower, as reported (Fig. 5A) (Kyozuka et al., 2000). On the contrary, *OsMADS3* expression was down-regulated in the *apo1* flower (Fig. 5B). This result suggests that class C gene expression is affected in *apo1*.

As Table 2 shows, the total number of lodicules and stamens was not affected in *apo1*. For that reason, we inferred that class B genes are not affected by the *apo1* mutation. The *SUPERWOMAN 1* (*SPW1*) gene is an *AP3* homolog of rice. It is expressed in the whorl 2 (lodicules) and whorl 3 (stamens) (Fig. 5C) (Nagasawa et al., 2003). The expression of *SPW1* was restricted to whorls 2 and 3 as in the wild type (Fig. 5D). Accordingly, *APO1* gene would not regulate the expression of class B (*SPW1*) gene.

Interactions between APO1 and flower-associated genes

We constructed double mutants to determine whether *APO1* interacts genetically with other genes involved in the control of inflorescence and floral development. First, we examined a double mutant between *apo1-3* and *fzp* in which

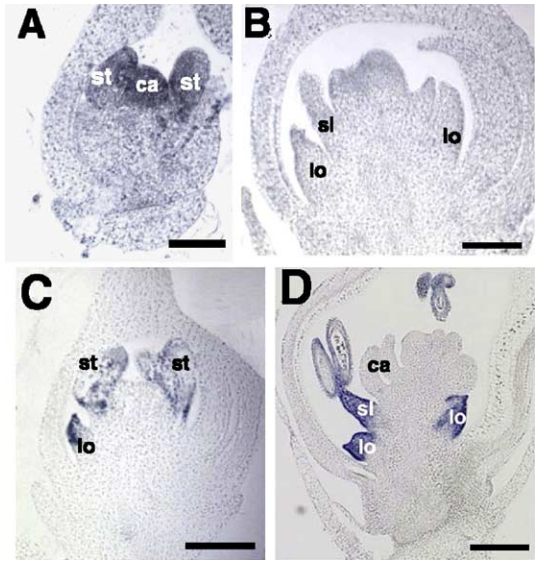

Fig. 5. Floral homeotic gene expression in wild type and *apo1* flowers. (A) Expression of *RAG* in wild-type flower in third (stamen) and fourth (carpel) whorls. (B) Down-regulation of *RAG* expression in *apo1-2* flower. (C) Expression of *SPW1* in second (lodicule) and third (stamen) whorls of wild-type flower. (D) Expression of *SPW1* in second and third whorls of *apo1-2* flower. ca: Carpel, st: stamen. lo: lodicule. Scale bars, 50 μm.

spikelet meristems are not converted to floral meristems, but instead form lateral spikelet meristems at the axils of rudimentary glumes without affecting the number and length of primary branches (Komatsu et al., 2003). The *fzp apo1-2* double mutant exhibited an inflorescence having a small number of primary branches and short primary branches lacking flowers (Fig. 6A). SEM observation revealed that the phyllotaxy of *fzp apo1* primary branches was 1/2 alternate (data not shown). Therefore, the *frz apo1-2* double mutant showed additive phenotypes of the two mutants; *APO1* and *FZP* function independently.

Next, we examined the interaction between *APO1* and *FON1*/*FON2* genes. The *fon1* and *fon2* plants have a large floral meristem, and more numerous floral organs, six to eight stamens, and two or three carpels (Fig. 6B) (Nagasawa et al., 1996). The *fon1 apo1-2* flower showed a phenotype that enhanced the *apo1-2* abnormalities: many lodicules up to eight, almost no stamens, glume-like organs inside the stamen whorl and more carpels than those of *apo1-2* (Fig. 6C). The *apo1-1 fon2-2* double mutant showed a similar phenotype as *fon1 apo1-2* (Fig. 6D). These phenotypes suggest that the expression of class C gene (*RAG*) in the double mutant is more strongly suppressed than in *apo1*.

We observed early flower development. The size and shape of floral meristem differ greatly for *apo1-2* and *fon1*. Although meristem fasciation was not observed in each single mutant (Figs. 6E and F), the floral meristem of the *fon1 apo1-2* double mutant producing carpel primordia was severely fasciated (Fig. 6G). Therefore, *APO1* and *FON1*/*FON2* are considered to control the proliferation of cells in the floral meristem redundantly.

Discussion

This study demonstrated that *apo1* mutation causes pleiotropic defects throughout the life cycle. Phenotypes include short plastochron, abnormal phyllotaxis of primary branches, precocious transition from inflorescence into spikelet meristem, transformation of stamens to lodicules, and loss of floral determinacy. All these phenotypes suggest that *APO1* gene is related to temporal regulation of development in the life cycle.

Function of APO1 in reproductive phase, a temporal regulator

The short inflorescence and a reduced number of spikelets in *apo1* are attributable to precocious conversion of an inflorescence meristem to a spikelet meristem in both main axis and branches. Therefore, *APO1* is considered to be a temporal regulator of meristem identity. The *apo1* phenotype partially resembles *cen* in *Antirrhinum* and *tfl1* in *Arabidopsis*, in which the inflorescence meristem is precociously converted into a terminal flower (Bradley et al., 1996; Shannon and Meeks-Wagner, 1991). Constitutive expressers of *RCN1* or *RCN2*, rice *TFL1/CEN* homologs, showed modified inflorescences (Nakagawa et al., 2002) in which secondary branches and spikelets were increased. However, the number of primary branches was rather decreased and the length of primary branch seemed unaffected. It was also reported that they showed a prolonged vegetative phase (Nakagawa et al., 2002). On the contrary, the *apo1* mutant was not early flowering, but

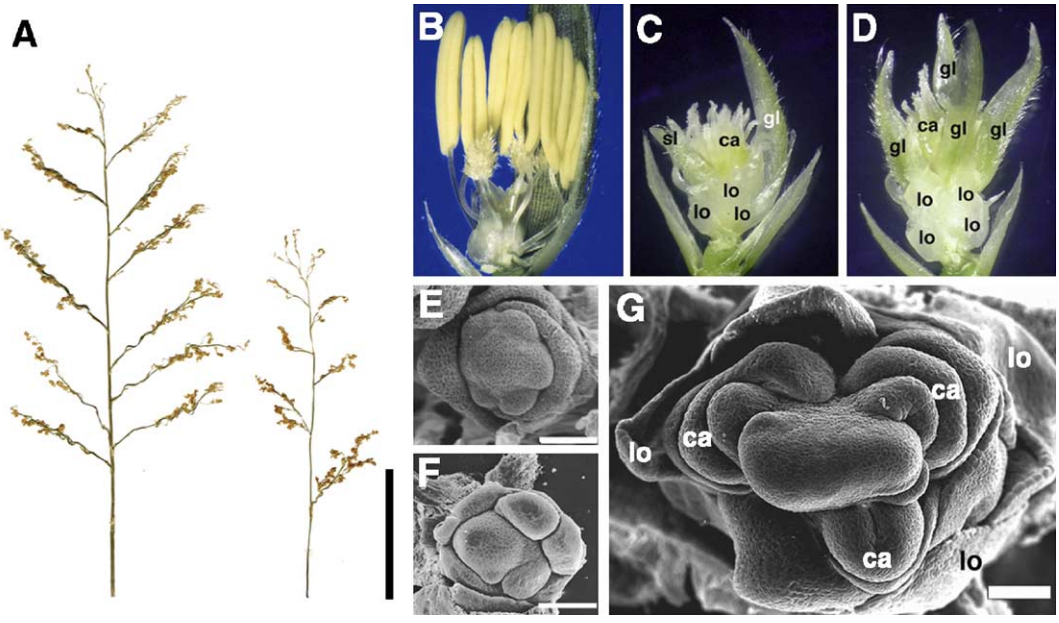

Fig. 6. Double mutant phenotypes of *fzp apo1-2*, *fon1 apo1-2*, and *apo1-1 fon2-2*. (A) Mature inflorescence of *fzp* (left) and *fzp apo1-2* (right). (B) A *fon2-2* flower with two lodicules, seven stamens, and two carpels. (C) A *fon1 apo1-2* flower with eight lodicules, one staminoid lodicule, one glume-like organ, and numerous carpels. (D) An *apo1-1 fon2-2* flower with seven lodicules, four glume-like organs, and numerous carpels. (E–G) SEM images of *fon1* (E), *apo1-2* (F), and *fon1 apo1-2* (G) flowers, respectively. In panel (G), floral meristem is severely fasciated. lo: lodicule, st: stamen, ca: carpel. sl: staminoid lodicule, gl: glume-like organ. Scale bars: 5 cm in panel (A) and 50 μm in panels (E)–(G).

rather slightly late flowering. In addition, *RCN1* and *RCN2* do not affect floral organ identities. Accordingly, *APO1* might have a role in inflorescence development that is distinct from that of *RCN1* and *RCN2*.

The *APO1* also regulates flower development. A possible model of homeotic gene expression is proposed in Fig. 7. Homeotic transformation of stamens to lodicules and loss of floral determinacy are commonly observed in all the alleles. In addition, the number of stamens was negatively correlated with that of carpels (Table 2). These phenotypes are interpreted as prolonged expressions of class A and *DL* genes, which would be caused by the reduction of class C gene expression, *OsMADS3*. The down-regulation of class C gene in *apo1* is further supported by the phenotypes of *apo1-1 fon2-2* and *fon1 apo1-2* flowers: more lodicules, glume-like organs in whorl 3 and indeterminate carpel formation. In the strong *apo1* allele, *DL* expression might be transiently down-regulated, as deduced by the lodicule formation after a few carpels. For those reasons, *APO1* can be regarded to be a temporal regulator of floral homeotic genes. This fact is supported by the fact that the loss-of-function mutants of *OsMADS3* formed *apo1*-like flowers: the formation of lodicules in place of stamens (Kang et al., 1998). It is noted that *APO1* and *OsMADS3* are positioned on different chromosomes.

In rice, loss of floral determinacy is also observed in *dl* mutant (Nagasawa et al., 2003). However, indeterminate carpel formation in *apo1* indicates that *DL* expression is not down-regulated; in addition, loss of determinacy in *apo1* flowers would be caused by down-regulation of class C gene (*OsMADS3*). In *Arabidopsis*, the class C gene is considered to be involved in floral meristem determinacy in cooperation with meristem-related genes such as *WUS* (Laux et al., 1996; Lenhard et al., 2001; Lohmann et al., 2001). Floral meristems were fasciated in the *fon1 apo1* and *apo1 fon2* double mutants. This fasciation suggests that *APO1*, *FON1*, and *FON2* cooperatively regulate cell proliferation and determinacy in rice floral meristem.

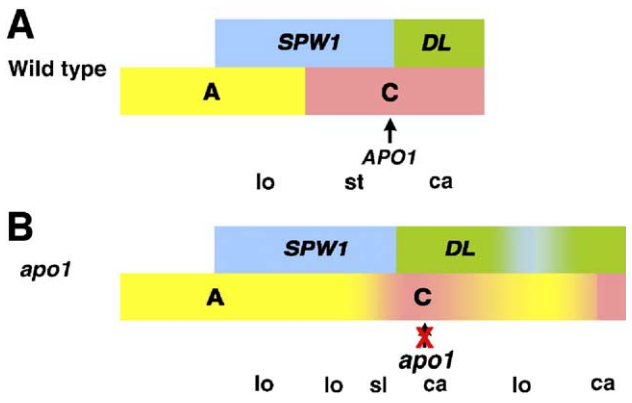

Fig. 7. A model of floral homeotic gene functions in *apo1* mutant. (A) Homeotic gene expression in wild-type flower (revised from Nagasawa et al., 2003). (B) Homeotic gene expression in *apo1* flower. Class C gene (*RAG*) is down-regulated and the domain of class A gene is enlarged, causing transformation of stamens to lodicules and loss of floral meristem determinacy. *DL* expression might be occasionally down regulated in the central region, as inferred from the lodicule formation after a few carpels. lo: lodicule, st: stamen, ca: carpel, sl: staminoid lodicule.

Regarding the regulation of floral homeotic genes, *FIM* in *Antirrhinum* and *UFO* in *Arabidopsis* are reported (Levin and Meyerowitz, 1995; Wilkinson and Haughn, 1995). Their recessive mutants are similar to *apo1* in affecting floral organ identity such as the production of mosaic organs. In addition, *ufo* also produces terminal flowers (Wilkinson and Haughn, 1995) as in rice *apo1*. However, *fim* severely disrupts the normal expression of both class B and class C genes, and *ufo* mainly affects class B expression (Simon et al., 1994; Samach et al., 1999; Wilkinson and Haughn, 1995). In contrast, rice class B gene *SPW1* was expressed correctly in *apo1*. Therefore, *APO1* gene seems differ from *FIM* and *UFO* in the respect of target floral homeotic genes.

Class C gene expression seemed to be suppressed in *fon1 apo1-2* and *apo1-1 fon2-1* double mutants. This type of suppression is comparable with the phenotypes of *ufo clv* double mutants in *Arabidopsis*, in which class B genes are more strongly down-regulated (Levin and Meyerowitz, 1995). Therefore, although the target homeotic gene would be different, both *APO1* and *UFO* genes would function in the regulation of homeotic genes in cooperation with genes associated with meristem size or cell proliferation in SAM.

Function of APO1 in vegetative phase, a plastochron regulator

The *APO1* gene also functions in the vegetative phase through regulation of the rate of leaf initiation (plastochron). The rapid leaf production and rapid conversion of meristem identity in the reproductive phase enable us to deduce a fundamental function of the *APO1* gene. That is, *APO1* gene is related to developmental timing in the life cycle.

To date, *plastochron 1* (*pla1*) mutant has been reported in rice, which shows a short plastochron (Itoh et al., 1998; Miyoshi et al., 2004). However, *pla1* differs from *apo1* in many aspects. The leaf number is nearly doubled in the case of *pla1*, but in *apo1*, leaves are 1.5-fold more numerous than in wild type. The *pla1* shows the transformation of primary inflorescence branches to vegetative shoots, and large SAM from the early vegetative stage, whereas *apo1* does not have larger SAM than the wild type, and shows no vegetative trait in the reproductive phase. In addition, *PLA1* expression is normal in *apo1* mutant (data not shown). Therefore, *APO1* would regulate the leaf initiation rate independently of *PLA1*.

Interestingly, until the late vegetative stage, *apo1* SAM was comparable to the wild-type SAM, although plastochron was shorter than that of wild type throughout the vegetative phase. *APO1* gene would control the proliferation of leaf founder cells without affecting SAM size and shape. Mutants showing abnormal plastochron and phyllotaxy thus far reported had SAMs with aberrant size and shape (Itoh et al., 1998, 2000; Jackson and Hake, 1999; Veit

et al., 1998). Further analysis of *apo1* mutant may reveal a novel mechanism regulating leaf initiation rate.

The *apo1* phenotypes in the vegetative and reproductive phases strongly suggest that *APO1* is associated with temporal regulation of development. Mutants that show temporal modification of developmental program are called heterochronic mutants. A number of heterochronic mutants have been reported, such as *Teopod 1*, *Teopod 2*, *Teopod 3*, and *glossy15* in maize, *mori1* and *pla1* in rice, and *hasty* in *Arabidopsis* (Asai et al., 2002; Bassiri et al., 1992; Dudley and Poethig, 1991; Evans et al., 1994; Itoh et al., 1998; Lawson and Poethig, 1995; Miyoshi et al., 2004; Telfer and Poethig, 1998). They have been mainly discussed in relation to phase changes: juvenile to adult and vegetative to reproductive, because mutants that affect the developmental timing in vegetative phase also modify vegetative-reproductive phase change. In *apo1*, however, the transition to reproductive phase was not accelerated in spite of rapid leaf production. This indicates that *apo1* can be regarded as a kind of heterochronic mutants, but it would not be involved in phase changes. Accordingly, *APO1* would be categorized differently from genes affecting phase change.

Developmental fates of inflorescence meristems

Rice has two types of inflorescence meristems with different fates, namely, main-axis meristem and branch meristem. In rice, the main-axis meristem is not converted to a spikelet meristem, but aborted. In contrast, normal branch meristems are transformed to spikelet meristems; they set terminal flowers. In *apo1*, however, the main-axis meristem and branch meristem were converted into spikelet meristems. It is unlikely that the *APO1* is involved in meristem abortion, because terminal flowers on the main axis were formed earlier than the timing of the meristem abortion in the wild type. In addition, both abortion and conversion were observed in the weak *apo1-3* allele. Furthermore, incomplete spikelets comprising only rudimentary glumes and empty glumes that subtended the trace of the meristem are sometimes produced in *apo1-3*. We consider that these incomplete spikelets arose from the meristem abortion that occurred just after glume formation. We observed *fzp* plants, in which floral meristems are not formed (Komatsu et al., 2003). Thereby, we confirmed that the terminal meristem of each branch aborted after producing several lateral meristems (data not shown). Therefore, the branch/spikelet meristems have an abortion program that is usually hidden by the conversion program. We examined the presence of a terminal spikelet or the vestige of main axis meristem in four grass species. Terminal spikelets were observed on the main axes of *Avena fatua* and *Bromus unioloides*, vestiges of main-axis meristems were detected in *Setaria viridis*, and both terminal spikelets and meristem vestiges were observed in *Lolium multiflorum* (data not shown). Accordingly, the fate of the main-axis meristem would not be conserved in grasses, rather arbitrarily determined in each species, depending on which program is predominant, either abortion or conversion.

Phyllotaxis

The *apo1* mutant exhibited phyllotactic change of primary branches from spiral to distichous, but vegetative leaves showed normal 1/2 alternate phyllotaxy. Phyllotactic modification in *apo1* seems to be correlated with the meristem size and shape because in the vegetative phase except for the late stage, *apo1* SAM did not differ largely from the wild-type SAM, whereas the early inflorescence meristem was small and slender. Although the *bellringer* (*blr*) mutant is reported in which phyllotactic change of floral primordia is not accompanied by the change of inflorescence meristem size (Byrne et al., 2003), correlation between phyllotaxy and SAM size and shape is also observed in maize *abphyl1* mutant (Jackson and Hake, 1999). Changes in both size and shape of SAM are apparently important for phyllotaxy because the meristem enlargement unaccompanied by shape change, affects plastochron but not phyllotaxy, as shown in *pla1* mutant (Itoh et al., 1998). Therefore, the phyllotaxy modification in *apo1* is likely caused by the small and slender meristem.

Lemma–palea asymmetry of rice flower

The *apo1* flowers showed asymmetry of stamens along lemma–palea axis. This asymmetry would be a reflection of organizational nature in normal flowers that has not been an issue of discussion. In the second (lodicule) whorl of rice and many grass flowers, polarity along lemma–palea axis is apparent because two lodicules are confined to lemma side, and one lodicule to be formed on the palea side is considered to be missing during grass evolution. Stamens on the palea side were more severely affected by *apo1* mutation than those on lemma side, indicating that the third (stamen) whorl is also polarized along the lemma–palea axis. In the wild-type rice flower, one stamen positioned on the lemma side seems to have retarded growth (data not shown), suggesting unsynchronized initiation of stamen primordia along the lemma–palea axis. We can also observe polarization along the lemma–palea axis in the fourth whorl (carpel/pistil). Carpel primordia always initiate from the lemma side of floral meristem, and the chalaza of ovule is attached to the palea-side carpel. These facts indicate that all whorls of the rice flower are organized asymmetrically along the lemma–palea axis. To date, asymmetry along the lemma–palea axis has not been discussed explicitly (Clofford, 1961; Coccucci and Anton, 1988), nor has the genetic mechanism controlling the asymmetry been elucidated. The above asymmetry presents an interesting subject for elucidating the developmental mechanism of flowers and evolution of grass flowers.

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