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A Recessive Heterochronic Mutation, *plastochron1*, Shortens the Plastochron and Elongates the Vegetative Phase in Rice

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We describe two recessive alleles of a rice heterochronic gene, *plastochron1-1* (*pla1-1*) and *pla1-2*, that reduce the length of the plastochron to approximately half that of the wild type. Because the onset of the reproductive phase in *pla1* was not temporally affected, the number of leaves produced in the vegetative phase was nearly twice that produced in the wild type. Panicle development was severely disturbed in *pla1* mutants. In *pla1-1*, many primordia of primary rachis branches were converted into vegetative shoots. These ectopic shoots repeated the initiation of panicle development and the conversion of primary rachis branches into shoots. In the weak allele *pla1-2*, however, only the basal one or two primordia developed as vegetative shoots, and the remaining primordia developed to produce a truncated panicle. These results indicate that both vegetative and reproductive programs are expressed simultaneously during the reproductive phase of *pla1*; however, the degree varied depending on the strength of the allele. Accordingly, *pla1* is a heterochronic mutation that extends the vegetative period. The shoot apical meristem of *pla1* was larger than that of the wild type, although the shape was not modified. An in situ hybridization experiment using the histone *H4* gene as a probe revealed that cell divisions are accelerated in the *pla1* meristem. The *PLA1* gene is considered to regulate the duration of the vegetative phase by controlling the rate of leaf production in the meristem.

INTRODUCTION

Plant development generally is divided into four distinct phases: embryogenesis, early vegetative phase (juvenile phase), late vegetative phase (adult phase), and reproductive phase (Conway and Poethig, 1993). In almost all plant species, two phase changes are recognized easily by the distinct morphological changes that occur in plants from the embryonic phase to the juvenile vegetative phase and from the adult vegetative phase to the reproductive phase. A clear change between the juvenile and adult vegetative phases has been reported for English ivy, maize, and Arabidopsis (Hackett, 1985; Zimmerman et al., 1985; Poethig, 1988, 1990; Lawson and Poethig, 1995; Telfer et al., 1997). However, in several species including rice, the phase change from juvenile to adult is not clear because it is continuous and is not accompanied by gross visible changes.

Whatever the case may be, the plant life cycle is driven by phase-specific genetic programs that are partially overlapping (Bongard-Pierce et al., 1996). Mutations that delay or advance phase change have been reported for several plant species. Late-flowering mutants can be interpreted as prolonging the vegetative phase and delaying the reproductive phase. Recently, using trichome distribution or epidermal characters as phase-specific markers, researchers have described several late-flowering and dwarf mutations that prolong the juvenile phase (Evans and Poethig, 1995; Telfer et al., 1997).

Heterochronic mutations affecting the timing of developmental events may be of major significance from an evolutionary viewpoint (Gould, 1982; Lord and Hill, 1987). If a single mutation modifies the expression of a gene that controls phase change or the temporal pattern of organ development, a conspicuous change of body plan results. In plants, several heterochronic mutations that affect vegetative development and markedly alter shoot architecture have been identified (Poethig, 1988). Several dominant mutations in maize, including *Teopod1* (*Tp1*), *Tp2*, *Tp3*, and *Corngrass*, are considered to prolong the juvenile phase and change plant body organization (Galinat, 1966; Poethig, 1988; Dudley and Poethig, 1991). In the above-mentioned mutants, various morphological and physiological traits of juvenile leaves are ectopically expressed during the adult phase. However, these genes are not considered to regulate the production of leaf primordia.

Several mutations modifying phyllotaxy and the plastochron, which also affect the shoot architecture, have been reported. The maize *abphyl* mutant shows variable phyllotaxy, including helical, decussate, and bijugate arrangements associated with an enlarged apical meristem (Greyson and Walden, 1972; Greyson et al., 1978). The rice *shoot organization1*

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(*sho1*) mutant shows random phyllotaxis, a short plastochron, and aberrant leaves (Tamura et al., 1992). However, these phenotypes of *sho1* are restricted to the juvenile phase, and normal phenotypes are recovered in the adult phase. Therefore, *sho1* affects juvenile shoot architecture but not the phase change. In the *puzzle box* mutant of tobacco, irregular plastochron and dwarfism have been reported (Trull and Malmberg, 1994). Furthermore, the floral phenotypes of *puzzle box* mutants suggest that inflorescence and floral programs are expressed together. Accordingly, a modified plastochron may cause heterochrony as well as the alteration of vegetative shoot architecture.

Here, we describe novel heterochronic mutations of rice, *plastochron1-1* (*pla1-1*) and *pla1-2*, that result in a shortened plastochron and ectopic expression of the vegetative program during the reproductive phase. These mutations are unique because they are recessive and affect both plastochron length and the duration of the vegetative phase.

RESULTS

Inheritance Mode of *pla1* Mutations

In an M_2 line of rice cultivar Fukei 71 mutagenized with γ-ray irradiation, two of 10 plants showed rapid leaf emergence and abnormal heading. Because the mutant plants set almost no seeds, they were recovered from seeds set on heterozygous plants. The frequency of mutants (25.1% for 222 of 884 plants) indicated that this mutation is single and recessive.

Recently, another mutant exhibiting similar phenotypes (rapid leaf emergence and abnormal panicle) was isolated from an M_2 population of cultivar Kinmaze chemically mutagenized with *N*-methyl-*N*-nitrosourea (MNU). Because the frequency of mutant plants resulting from seeds set on heterozygous plants was $\sim$25% (23 of 90 plants), this mutation was also single and recessive. The allelism test for these two mutations indicates that they are allelic.

Because the most striking feature in the vegetative phase was the rapid emergence of leaves, the mutation derived from γ-ray irradiation was designated *pla1-1* and that derived from MNU treatment as *pla1-2*.

Leaf Emergence and the Shoot Apical Meristem in *pla1*

The shape of the mature *pla1-1* and *pla1-2* embryos is identical to that of the wild-type sibling, which has three leaves in the mature embryo. Immediately after germination, *pla1* seedlings could not be distinguished from those of wild-type siblings. Accordingly, *pla1* does not seem to affect embryonic and juvenile development. Seedlings of *pla1* were first distinguished from those of the wild type 7 days after germination by the rapid emergence of leaves and the small leaf blade size. At 2 weeks after germination, seven leaves had emerged in *pla1-1* seedlings, whereas five leaves had emerged in their wild-type siblings (Figure 1). Because three leaves had already differentiated in both *pla1* and wild-type embryos, four leaves were newly produced and emerged in *pla1* within 2 weeks after germination, whereas only two leaves emerged in the wild type. Rapid emergence of leaves in *pla1-1* was constantly observed throughout the vegetative phase (Figure 2).

Similarly, rapid leaf emergence was observed in *pla1-2*, being nearly twice that of wild-type plants (Figures 1 and 3). However, when the number of leaves in the two *pla1* mutants was plotted against the number of leaves in the respective wild-type plants at the same stage (days after germination), the leaf production rate in *pla1-2* was slightly lower than that in *pla1-1* (Figure 3).

At any stage of the vegetative phase at which a new leaf blade was fully emerged, the number of immature and primordial leaves was nearly constant in both *pla1* (four or five leaves) and wild-type plants (three or four leaves), indicating that rapid leaf emergence in *pla1* reflected the rapid rate of leaf initiation (plastochron). Therefore, the plastochron of *pla1* in the vegetative phase is estimated to be reduced to

Figure 1. Phenotypes of Wild-Type, *pla1-1*, and *pla1-2* Seedlings at 2 Weeks after Germination.

The wild-type seedling shown at left is the sibling wild-type seedling of *pla1-1*. The wild-type sibling of *pla1-2* has the same phenotype.

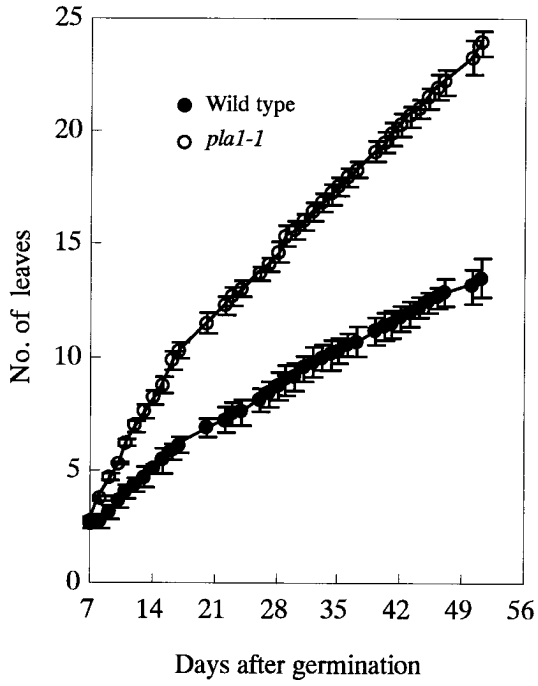

Figure 2. Increase in the Number of Leaves Emerging during the Vegetative Phase in Wild-Type and *pla1-1* Plants.

Closed circles represent wild-type plants; open circles represent *pla1-1* plants. Vertical bars indicate ±SD.

half that of the wild type. However, the duration of the vegetative phase from germination through the emergence of the flag leaf, which is the last vegetative leaf and is easily distinguished from the other leaves by its short blade, was the same in both types. Thus, *pla1-1* and *pla1-2* did not affect the timing of reproductive phase initiation, although the number of phytomers in the vegetative phase was doubled.

Next, we observed the shoot apical meristems. At 3 weeks after germination, the meristems were much larger in *pla1-1* and *pla1-2* (Figures 4B and 4C) than in the corresponding wild-type plants (Figure 4A), although meristem shape was comparable. In addition, we examined the change of shoot apical meristem size during the vegetative phase. In both *pla1-1* and the wild type, meristem size increased with the increased number of days after germination (Figures 5A and 5B). *pla1-1* had a longer and wider meristem than did the wild type at any stage after germination, but no difference was observed in the changing pattern of meristem shape between *pla1-1* and the wild type. Thus, *pla1-1* requires approximately twice the number of leaves to reach the same developmental stage as does the wild type.

Also in *pla1-2*, the shoot apical meristem was larger than that in the wild type. However, the extent of enlargement in *pla1-2* relative to the wild type was less than that in *pla1-1.* In contrast, the *pla1-1* meristem was 1.5-fold taller and wider than was that of the wild-type sibling; *pla1-2* had a 1.2- to 1.3-fold larger meristem than did the wild type. Thus, as for meristem size, *pla1-2* showed a less severe phenotype than did *pla1-1.*

Cell Division Activity in the Shoot Apical Meristem of *pla1*

In an in situ hybridization experiment that used as a probe the rice histone *H4* gene, which is specifically expressed in the S phase of the cell cycle, *pla1-1* meristems showed more hybridization signals than did the wild type. In the wild type, hybridization signals were observed in zero to two cells in many meristems at a median longitudinal plane through the site of leaf primordia insertion (Figure 6A). The mean number of cells expressing histone *H4* per median longitudinal section was 1.9 (Figure 7). On the other hand, in *pla1-1*, the number of cells with hybridization signals per median longitudinal section was nearly twice (3.8 cells on average in *pla1-1*) that of the wild type (Figures 6B and 7). At maximum, the signals were detected in eight cells. In the *pla1-2* meristem, the signals also were observed in more cells than in those of the wild-type meristem (Figure 6C). These results show that the shoot apical meristem of *pla1* has higher cell division activity than does that of the wild type, which is reflected in the rapid production of leaf primordia in the mutant.

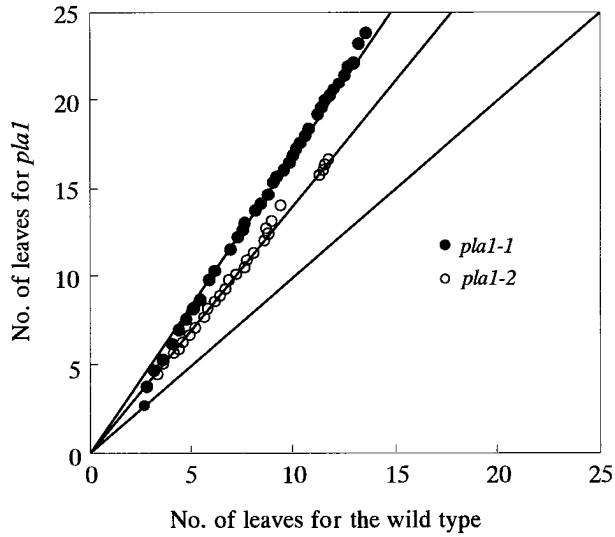

Figure 3. Increase in the Number of Leaves for *pla1-1* and *pla1-2* Relative to the Respective Wild Type.

At various developmental stages, the number of leaves for *pla1* mutants is plotted against that for the respective wild types. Closed circles represent *pla1-1* plants; open circles represent *pla1-2* plants.

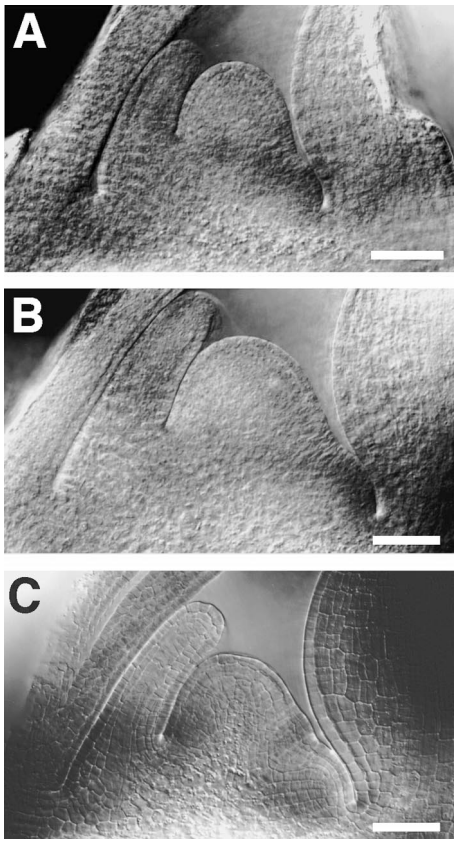

Figure 4. Shoot Apical Meristems of Wild-Type and *pla1* Plants 3 Weeks after Germination.

(A) Wild-type meristem viewed using Nomarski differential interference optics.
(B) *pla1-1* meristem viewed using Nomarski differential interference optics.
(C) *pla1-2* meristem viewed using Nomarski differential interference optics.
Bars = 50 μm.

Leaf Size during the Vegetative Phase

The three parameters of leaf size—length of leaf blade, width of leaf blade, and length of leaf sheath—were reduced in *pla1-1* (Figure 8). In *pla1-1*, the maximum width and length of the leaf blade were nearly half those of the wild type (Figures 8A and 8B), but the length of leaf sheath was not markedly reduced (Figure 8C).

Figure 8 also shows that the pattern of change of leaf size with position was conserved in wild type and *pla1-1*. In the wild type, the length of the leaf blade increased from the first through 10th leaves and decreased in the subsequent leaves. Also in *pla1-1*, the 18th leaf had the longest leaf blade, and the subsequent leaves became shorter (Figure 8B). Because three leaves were produced during embryogenesis, the wild-type plant produced seven leaves after germination before it produced the longest leaf blade, whereas *pla1-1* differentiated 15 leaves. Therefore, in *pla1-1*, twofold more leaves were produced between germination and the stage showing the longest leaf blade. However, both *pla1-1* and the wild type reached the longest leaf blade stage at the same time (days after germination), because the plastochron of *pla1-1* was halved. This tendency was also recognized in the leaf sheath length (Figure 8C). Therefore, although *pla1-1* needs twice as many leaves (phytomers) to pass some developmental phases, the pattern of leaf size change is conserved in *pla1-1* and the wild type, suggesting that the onset of the adult phase is not altered in *pla1-1*.

Phenotype of *pla1* in the Reproductive Phase

In rice, internode elongation is activated by the onset of reproductive growth. In wild-type plants, the apical four or five internodes are elongated. In *pla1-1* and *pla1-2*, eight to 10

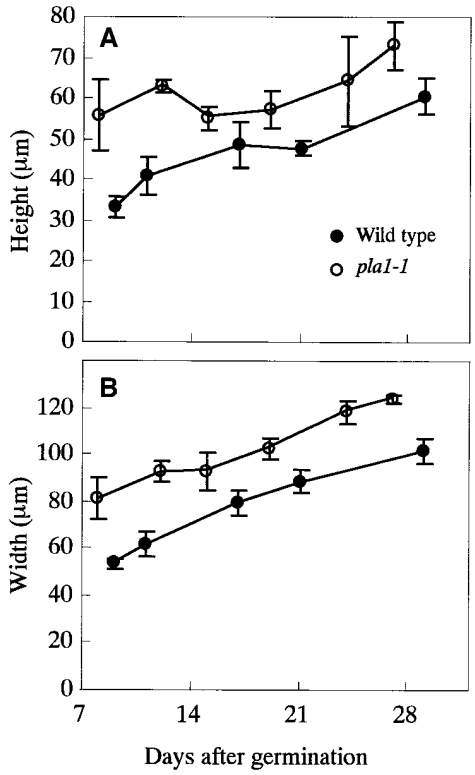

Figure 5. Changes in Shoot Apical Meristem Size in the Wild Type and *pla1-1*.

Closed circles represent wild-type plants; open circles represent *pla1-1* plants. Vertical bars indicate ±SD.
(A) Change in meristem height.
(B) Change in meristem width.

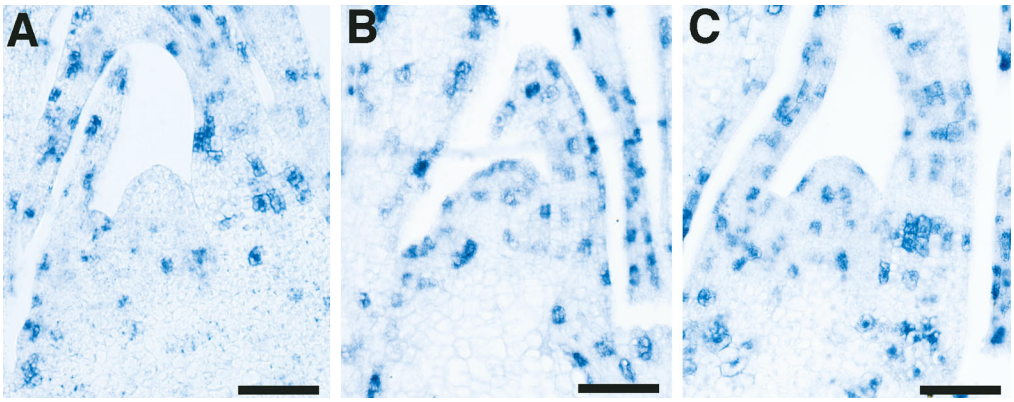

Figure 6. Histone *H4* Expression in Wild-Type and *pla1* Meristems at 7 Days after Germination.

Hybridization was with the histone *H4* antisense RNA probe.
(A) Wild type.
(B) *pla1-1.*
(C) *pla1-2.*
Bars = 50 μm.

internodes were elongated, but each internode was much shorter than that of the wild type, which resulted in dwarf plants. Therefore, in *pla1*, internodes as well as leaf characters were also modified; there were increased numbers of elongated internodes and short internodes.

In both *pla1-1* and *pla1-2*, reproductive development was conspicuously modified (Figure 9). Although both mutants had the same tendency, *pla1-1* showed a more severe phenotype than did *pla1-2.* After the emergence of the flag leaf, several vegetative shoots were produced instead of a panicle in *pla1-1* (Figure 9). These ectopic shoots differed from the normal tillers (lateral shoots) in two ways. Their phyllotaxy was helical instead of alternate, which is the phyllotaxy exhibited by normal tillers, and usually no tillers were produced from several nodes below the flag leaf in both *pla1* and the wild type. In addition, the bract was enormously elongated at the base of each ectopic shoot. Scanning electron microscopy, however, showed that in *pla1-1*, the primary rachis branch primordia were produced normally in a helical phyllotaxy, although the bracts were enlarged (cf. Figures 10A and 10C). The cross-section from a later stage of these primordia, which corresponds to the young panicle of the wild-type plants, showed an unexpected feature. In the basal part of the young panicle of *pla1-1*, shoots were differentiated in a helical phyllotaxy, with each shoot surrounded by a large bract (Figure 10D). This helical phyllotaxy is observed in the wild-type plant only at the differentiation of the primary rachis branches of the panicle (Figure 10A). In each of these ectopic shoots, normal leaves were produced in an alternate phyllotaxy (Figure 10D). These findings indicate that in *pla1-1*, primordia of primary rachis branches were converted into vegetative shoots. As shown in Figure 10E, hairs were produced from the main axis of *pla1-1* panicles. In the wild type, these hairs were observed specifically in young panicles when the primordia of secondary rachis branches were being differentiated (Figure 10B). Therefore, both reproductive and vegetative programs were simultaneously in operation during the reproductive phase of *pla1-1.*

The ectopic shoots of *pla1-1* repeated reproductive growth (differentiation of primary branch primordia in a helical phyllotaxis) and ectopic shoot production. In many *pla1-1* plants, ectopic shoots eventually differentiated small panicles with elongated bracts in November, even when grown under

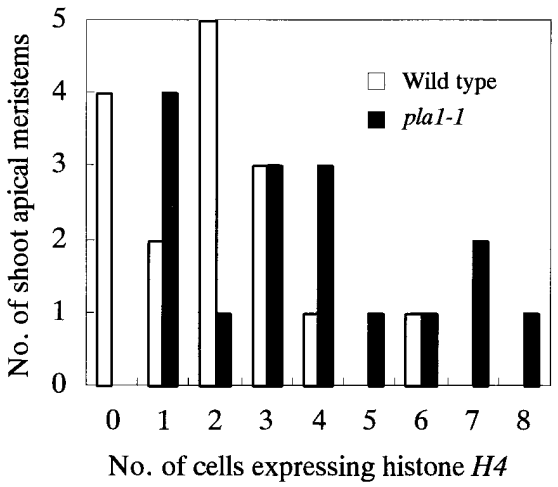

Figure 7. Frequency Distribution of Cells Expressing Histone *H4* Transcripts in the Wild-Type and *pla1-1* Shoot Apical Meristems.

Open bars represent the wild type; filled bars represent *pla1-1.*

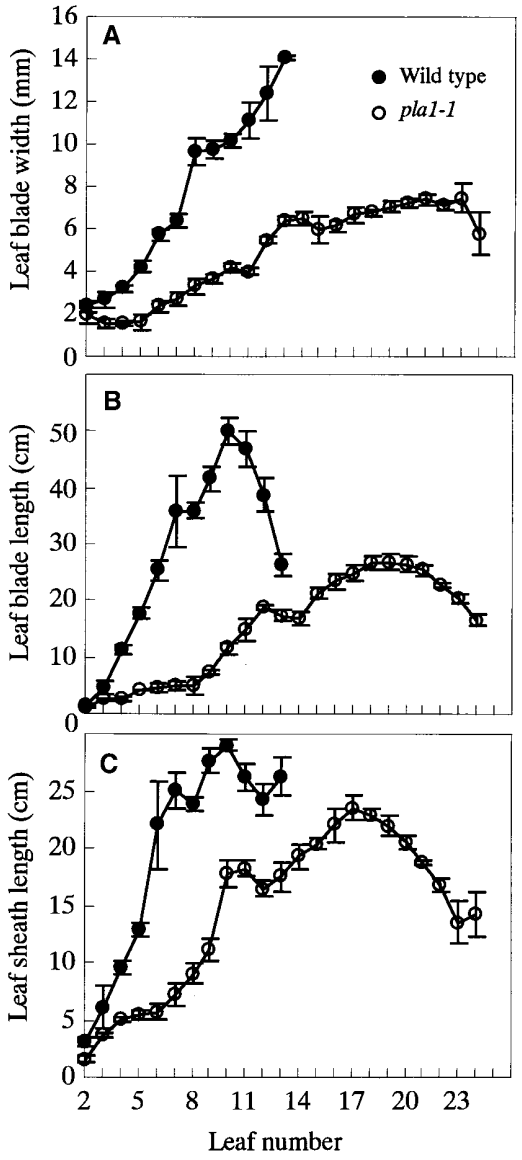

Figure 8. Change of Leaf Size in the Vegetative Phase of the Wild Type and *pla1-1.*

Closed circles represent wild-type plants; open circles represent *pla1-1* plants. Vertical bars indicate ±SD.
(A) Width of leaf blade.
(B) Length of leaf blade.
(C) Length of leaf sheath.

constant temperature (Figure 10F). The flowers apparently were normal, although most of them were sterile. These results suggest that short days might induce normal panicle development. However, extreme short-day (10 hr of light per 14 hr of darkness) treatment was not effective in promoting panicle emergence in *pla1-1.*

In *pla1-2*, the heading of panicles was observed at a normal developmental stage. However, several abnormalities were recognized in the panicle. The basal one or two rachis branch primordia were converted into vegetative shoots, and bracts were elongated as in *pla1-1*, but the other primordia followed the normal developmental course of the primary rachis and set flowers (Figure 9). These flowers were apparently normal and set several seeds per panicle. Therefore, in *pla1-2*, vegetative and reproductive programs were overlapping only in the early phase of panicle development, and the extension of the vegetative phase was not as severe as in *pla1-1.*

In *pla1*, more phytomers were required to pass through a developmental phase, and the program for the vegetative phase was ectopically expressed during the reproductive phase, resulting in the conversion of primary rachis branches into vegetative shoots. In *pla1*, no abnormalities were detected during embryogenesis and in the timing of reproductive phase initiation. Accordingly, *pla1* was considered to be a heterochronic mutation prolonging the (adult) vegetative phase. The wild-type gene *PLA1* was considered to regulate

Figure 9. Phenotypes of Panicles in Wild-Type and *pla1* Plants.

A wild-type panicle at left shows ~10 primary rachis branches and nearly 100 flowers (caryopses). For *pla1-1* (center), several vegetative shoots are produced rather than primary rachis branches. For *pla1-2* (right), a truncated panicle is formed with one vegetative shoot (arrow) and enlarged bracts (arrowhead).

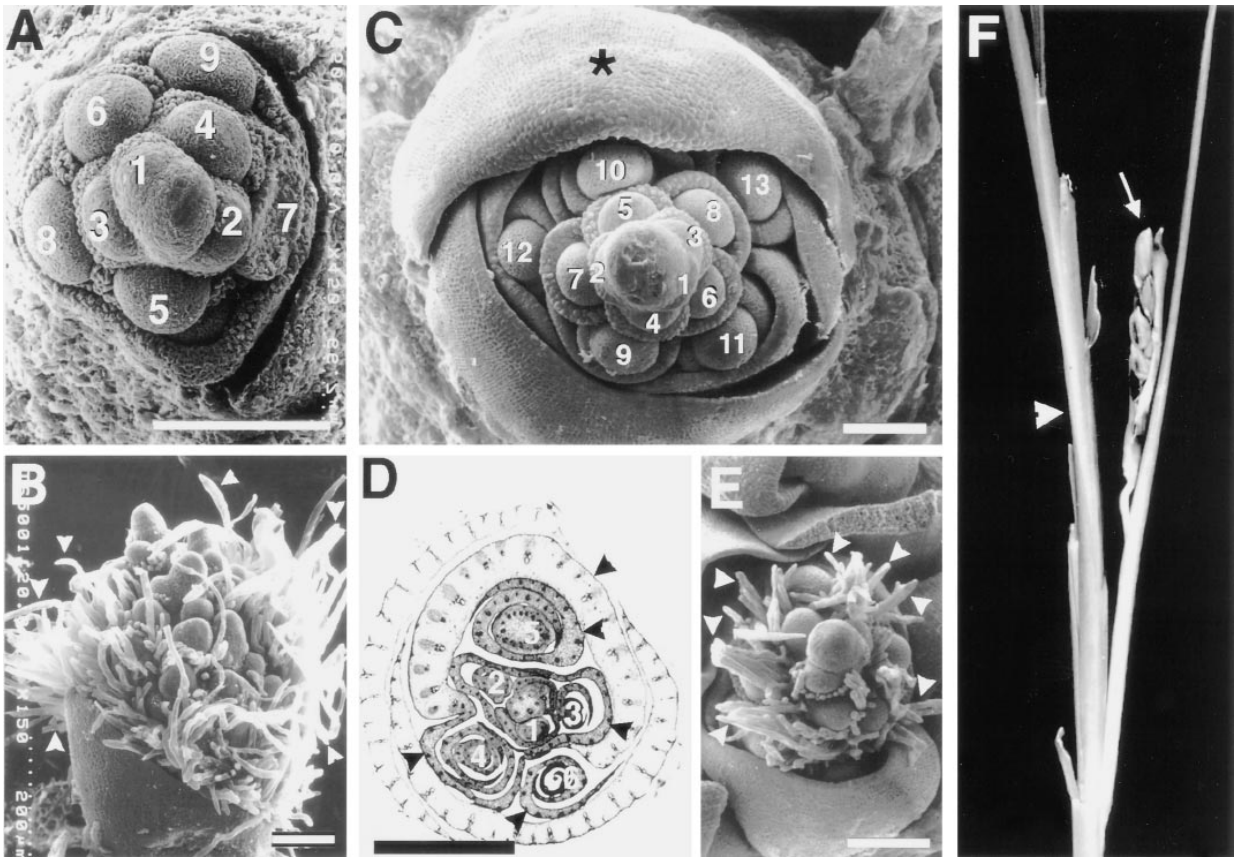

Figure 10. Phenotypes of Wild-Type and *pla1-1* Plants during the Reproductive Phase.

(A) Scanning electron microscopy of wild-type primary rachis branch primordia (numbered sequentially from the latest one) produced in helical phyllotaxy.
(B) Scanning electron microscopy of a wild-type young panicle at the stage of secondary rachis branch differentiation. Many hairs (arrowheads) are seen.
(C) Scanning electron microscopy of *pla1-1* primary rachis branch primordia (numbered sequentially from the latest one) produced in helical phyllotaxy, as shown in **(A)**. The asterisk indicates an enlarged bract.
(D) Cross-section of *pla1-1* young panicle showing ectopic shoots, which are numbered sequentially from the latest one and arranged in helical phyllotaxy, and enlarged bracts (arrowheads).
(E) Scanning electron microscopy of a *pla1-1* young panicle showing hairs (arrowheads) characteristic of a wild-type panicle at the stage of secondary rachis branch differentiation, as shown in **(B)**.
(F) Side view of a *pla1-1* panicle (arrow) with apparently normal flowers. An ectopic shoot (arrowhead) was also produced.
Bars = 100 μm in **(A)** to **(E)**.

the duration of (adult) vegetative phase by controlling the plastochron.

DISCUSSION

pla1 Is a Unique Heterochronic Mutation

Phenotypes of *pla1* are unique and interesting when considering plant development and shoot architecture. Although *pla1*-like mutants have not been reported in other species, one similar mutant designated as *leafy head* (*lhd*) has been reported in rice (Hu, 1961). The phenotypes of *lhd* and *pla1* are similar and are characterized by a short plastochron, ectopic shoot development above the flag leaf node, and dwarfism. These phenotypic similarities suggest an allelic relationship between *pla1* and *lhd*; unfortunately, the *lhd* mutant was lost, and we could not test for allelism.

In this study, we identified two alleles with different backgrounds. Although it is not exactly clear how the difference in background genotypes affects the mutant phenotypes, the

background effect is not expected to be large because the phenotypes of the two parental cultivars are similar, except for Fukei 71, which is a dwarf compared with Kinmaze. In the vegetative phase, the leaf emergence rate relative to each wild-type sibling is higher in *pla1-1* than in *pla1-2*; in the reproductive phase, more primary branch primordia are converted into vegetative shoots in *pla1-1* than in *pla1-2.* In other words, the rate of leaf production in the vegetative phase is correlated with the number of ectopic shoots in the reproductive phase. This suggests that the *PLA1* gene regulates the plastochron and the duration of the vegetative phase in a coordinate manner.

In *pla1*, the vegetative phase is prolonged, resulting in the simultaneous expression of both vegetative and reproductive programs and in a unique shoot architecture, which is quite different from that of normal rice. Therefore, *pla1* exemplifies the role of a heterochronic mutation in radically modifying plant form.

To date, several heterochronic mutations have been identified in maize (Galinat, 1966; Poethig, 1988; Bertrand-Garcia and Freeling, 1991), Arabidopsis (Zagotta et al., 1992), and pea (Wiltshire et al., 1994). Of these, the most intensively studied are those of maize. Dominant heterochronic mutations of maize, *Tp1*, *Tp2*, and *Tp3*, show a prolonged vegetative (juvenile) phase, increased number of leaves, a reduction of leaf and panicle size, dwarfism, and overlapping of vegetative and reproductive development. The life span is markedly extended in *pla1-1* plants by the repetitive production of ectopic shoots in the reproductive phase but is not affected in *Tp* (Bassiri et al., 1992). In *pla1*, the vegetative program may be functioning during the reproductive phase more strongly than it functions in *Tp* mutants, which do not produce ectopic shoots. Thus, the duration of the reproductive period may depend on the activity of the vegetative program, and the life span can also be altered by heterochronic mutations.

In *pla1*, a short plastochron brings about a large number of phytomers before some developmental stages, such as the stage exhibiting the longest leaf blade and the onset of the reproductive phase, are reached. These observations indicate that the number of phytomers does not determine the timing of phase change (Poethig, 1988; Dudley and Poethig, 1991). *Tp* mutations affect the timing of phase change but not the rate of leaf production (plastochron), suggesting that these two events are independently regulated. However, the fact that *pla1* modifies both processes suggests that *PLA1* functions in the cascade shared by both processes.

In this study, we were unable to determine how plastochron and heterochrony are linked. Because *pla1* is considered to function throughout the adult vegetative phase, it would not specify directly the end of vegetative phase. Recently, the *viviparous8* (*vp8*) mutation of maize was shown to increase the rate of leaf initiation early in shoot development and to prolong the juvenile vegetative phase (Evans and Poethig, 1997). In *vp8* plants, the juvenile-to-adult phase change is delayed, but the end of the adult phase is not affected, which results in a truncated adult phase and normal reproductive organs. Thus, the phenotypic difference between *pla1* and *vp8* is due to the difference in their functional phases—*pla1* in the adult phase and *vp8* in the juvenile phase. The correlation of plastochron and heterochrony is also suggested by the *puzzle box* mutant of tobacco, which shows a random plastochron and is considered to show heterochrony in the reproductive phase (Trull and Malmberg, 1994). It may be that developmental rate affects the duration of the developmental phase.

Although most mutations that extend the vegetative (juvenile) phase are dominant (Freeling et al., 1992), *pla1* is a recessive mutation. Dominant *Tp* mutants may ectopically express the juvenile program in the adult phase, and thus the wild-type *Tp* gene may regulate the juvenile program such that it operates in an adequate space and time. Loss-of-function mutations in *pla1*, however, cause a short plastochron and an elongated vegetative (adult) phase. Thus, the wild-type gene, *PLA1*, most likely suppresses the overproduction of leaf primordia and turns off the vegetative program at the appropriate time. It is expected that the phasic development of plants is regulated by a number of functionally diverse genes.

Independent Regulation of Embryonic, Vegetative, and Reproductive Phases

We could not distinguish the *pla1* embryo from the wild-type embryo. This suggests that the *pla1* embryo follows a normal developmental course. When germinated, *pla1* seedlings are easily distinguished from wild-type seedlings as early as 1 week after germination by the rapid emergence and small leaf size. Therefore, *pla1* is considered to start to function immediately after the onset of the adult vegetative phase and is constantly expressed throughout the vegetative phase. Although the juvenile phase is not explicitly distinguished from the adult phase in rice, the juvenile phase is not prolonged in *pla1* because the plastochron of the first three leaves is not modified, and the number of days required until the production of the longest leaf is unaffected. This feature is conspicuous because the *Tp* mutations are related mainly to the juvenile phase (Poethig, 1988). Therefore, *PLA1* is estimated to be a regulator of the vegetative period, functioning at the stage later than *Tp*-like genes.

The onset of the reproductive phase, as determined from the emergence of the flag leaf in *pla1*, is comparable to that of the wild type, indicating that *pla1* does not affect the onset of the reproductive phase. Similarly, in maize, *Tp* and *vp8* mutations are estimated to prolong the juvenile phase but not to affect the onset of reproductive development (Lawson and Poethig, 1995; Evans and Poethig, 1997). Accordingly, in plants, vegetative and reproductive phases are independently regulated, resulting in the coexistence of vegetative and reproductive programs due to the extension of the vegetative phase in *pla1* mutant.

Many homozygous *pla1-1* plants grown under constant temperature produced panicles in November, which is 2

months later than wild-type plants produced them. This delayed production suggests that in *pla1-1*, extreme short days might be a signal to turn off the vegetative program. However, short-day treatment is not effective in inducing normal panicles. Therefore, some endogenous factor, such as the repetition of ectopic shoot production, may specify the end of the vegetative phase. In a weak allele, such as *pla1-2*, normal development of primary branches is observed, except in the basal one or two primordia, which are converted into shoots. This suggests that in *pla1-1*, the primary branch primordia positioned in the upper part of the panicle will be ready to develop normally if the number of ectopic shoots is reduced. In wild-type plants, the end of the vegetative phase and the onset of the reproductive phase are usually synchronized. The loss of synchrony in *pla1* further confirms that they are under different genetic control.

Modified Shoot Apical Meristems Alter the Plastochron

Recently, many mutations affecting the shoot apical meristem and shoot architecture have been identified in Arabidopsis (Medford, 1992; Medford et al., 1992; Barton and Poethig, 1993; Clark et al., 1995, 1996; McConnell and Barton, 1995; Laux et al., 1996). An enlarged shoot apical meristem in *clavata1* causes abnormal phyllotaxis and an increase in the number of floral organs (Clark et al., 1995). An analogous mutation of rice also enlarges meristem size and increases the number of floral organs (Nagasawa et al., 1996). In contrast, *wuschel* and a weak allele of *shoot meristemless* of Arabidopsis reduce floral meristem size and floral organ number (Clark et al., 1996; Laux et al., 1996). These Arabidopsis mutants are considered to be associated with the abnormalities in the central zone of the meristem (Leyser and Furner, 1992). Other mutants with modified phyllotaxis, such as *sho1* of rice (Tamura et al., 1992) and *abphyl* of maize (Greyson et al., 1978), also have wide meristems. Thus, meristem size and/or shape may regulate the number of organ primordia and phyllotaxy.

In *pla1*, the shoot apical meristem is consistently enlarged. The enlarged apical meristem via activated cell divisions results in a short plastochron. However, a dominant negative mutation of the cell cycle Cdc2 kinase showing reduced cell division rate did not affect the rate of leaf production (Hemerly et al., 1995). Although the extent of the reduction in the cell division rate of this mutant is not clear, localized and/or gross alterations of the cell division rate may be required for the modification of the rate of leaf primordia production. As Figures 6 and 7 indicate, enhanced cell division in the shoot apical meristem would result in rapid leaf primordia production.

In summary, *pla1* is unique because it is a recessive mutation and causes a short plastochron and the transformation of primary rachis branches into vegetative shoots as a result of strong expression of the vegetative program in the reproductive phase. These phenotypes may be derived from enhanced cell division in the shoot apical meristem. The phenotypes of *pla1* and the analogous maize mutants indicate that a single heterochronic mutation is sufficient for altering basic body plan.

METHODS

Plant Materials and Measurement

Two single recessive mutants of rice (*Oryza sativa*) that has a short plastochron were identified: one was from an M_2 population of cultivar Fukei 71 mutagenized with 20 kiloroentgens of γ-ray, and the other was from an M_2 population of cultivar Kinmaze mutagenized with *N*-methyl-*N*-nitrosourea (MNU). Because the two mutants are from different genetic backgrounds, we used wild-type siblings of each mutant as controls for characterizing mutant phenotypes.

Plants were grown in a greenhouse at 28°C during the day and 23°C at night. To evaluate the effect of day length, we applied short-day treatment (10 hr of light and 14 hr of darkness) for 2 months, beginning July 10.

When every new leaf blade had emerged completely from the sheath of the previous leaf, five plants at the same developmental stage were sampled, and the number of leaves was counted for each plant. Using the same five plants for each sampling time, we sampled shoot apices and leaves for measuring the shoot apical meristem and leaf sizes. Plastochron was estimated by the rate of leaf emergence, which was represented by days elapsed between the complete emergence of two successive leaf blades.

The width of the shoot apex was measured just above the youngest leaf primordium insertion, and the height of the shoot apex is given as the shortest distance from the line used for measuring the width to the tip of the apex.

Paraffin Sectioning

For paraffin sectioning, samples were fixed in FAA (formalin–glacial acetic acid–70% ethanol [1:1:18]) and dehydrated in a graded ethanol series. After substitution with xylene, we embedded the samples in Paraplast Plus (Oxford Labware, St. Louis, MO) and sectioned them at 10 μm by using a rotary microtome. Sections were stained with 0.05% toluidine blue O and observed with a light microscope (model AX-80; Olympus, Tokyo, Japan).

Clearing of the Shoot Apex

Shoot apices were fixed in FAA for ~16 hr at 4°C and dehydrated in a graded ethanol series. After they were cleared in the benzyl-benzoate-four-and-a-half fluid devised by Herr (1982), we observed shoot apices with a microscope equipped with Nomarski differential interference contrast optics (model IMT-2; Olympus).

Scanning Electron Microscopy

Young panicles were fixed with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2, for ~16 hr at 4°C. After rinsing with 0.1 M sodium phosphate buffer, pH 7.2, they were postfixed in 1% osmium tetroxide for 3 hr at 4°C and rinsed with the buffer. Samples were then dehydrated in a graded ethanol series, and 100% ethanol was

replaced with 3-methylbutyl acetate. Samples were critical point dried, sputter coated with platinum, and observed with a scanning electron microscope (model S-4000; Hitachi, Tokyo, Japan) at an accelerating voltage of 10 kV.

In Situ Hybridization

Shoot apices were fixed in 4% paraformaldehyde and 0.25% glutaraldehyde in 0.1 M sodium phosphate buffer for ~20 hr at 4°C. They were then dehydrated in a graded ethanol series, replaced with xylene, and embedded in Paraplast Plus (Oxford Labware). Microtome sections at 8 μm thick were applied to slide glasses coated with Vectabond (Vector Laboratories, Burlingame, CA). Digoxigenin-labeled antisense and sense probes were prepared from the full-length histone *H4* cDNA of rice. In situ hybridization and immunological detection of the hybridization signals were performed by the methods of Kouchi and Hata (1993).

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