9. *PLASTOCHRON3* gene regulates leaf initiation rate and termination of vegetative phase

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Leaves are generated from the shoot apical meristem (SAM) in constant phyllotaxy and plastochron patterns. These traits constitute major components of plant architecture. In regard to leaf initiation rate, we have reported two mutants with shortened plastochron, *plastochron1* (*pla1*) and *pla2* (Itoh et al. 1998, Kawakatsu et al. 2001). In addition to rapid leaf initiation, they also exhibited heterochronic phenotypes: late flowering and conversion of primary rachis branches to vegetative shoots. *PLA1* encodes CYP78A11, a member of plant specific subfamily of cytochrome P450 (Miyoshi et al. 2004). Since *pla1-2 pla2-1* double mutants showed more severe phenotypes than either single mutant, *PLA1* and *PLA2* are supposed to act redundantly in independent pathways.

Recently we have identified another recessive mutant exhibiting similar phenotypes (shortened plastochron and heterochrony) from an M2 population of cv. Kinmaze mutagenized with *N*-methyl-*N*-nitrosourea (MNU). Since this mutation was mapped on a locus independent of *PLA1* and *PLA2*, we named this mutant *plastochron3* (*pla3*).

Although mature embryos of *pla1* and *pla2* were indistinguishable from those of wild type and had three leaves, *pla3* had larger embryos than wild type (Fig. 1A, B), and four leaves were present in mature embryos. This suggests that *pla3* initiates leaves more rapidly in the embryonic phase (Fig. 1C, D).

In the vegetative phase, *pla3* showed abnormal leaf initiation pattern. *pla3* initiated leaves more rapidly than wild type, *pla1* and *pla2*: the plastochrons were 1.7, 5.0, 2.3, 1.8 days, respectively (Fig. 1E). Frequently, *pla3* showed a fusion of two leaves that were formed from adjacent shoots (Fig. 1F). Because these shoots were positioned closely and comparable in size, the SAM of *pla3* might divide during development.

In the reproductive phase, *pla3* produced several vegetative shoots instead of normal panicles as *pla1* and *pla2* did (Fig. 2A). SEM analysis revealed that after primary rachis branch primordia were formed in spiral phyllotaxy, they were soon converted into vegetative shoots. The number of ectopic shoots was larger in *pla3* than in *pla1-4* and *pla2-1*. As in *pla1* and *pla2*, *pla3* plants were dwarf due to reduced internode elongation. The pattern of internode elongation of *pla3* was similar to that of *pla1*, severely deviated from those of wild type and *pla2*. Plant height was reduced in the order of wild type, *pla1, pla2* and *pla3* (Fig. 2B). Interestingly, the extents of plastochron reduction and dwarfism are positively correlated among *pla* mutants.

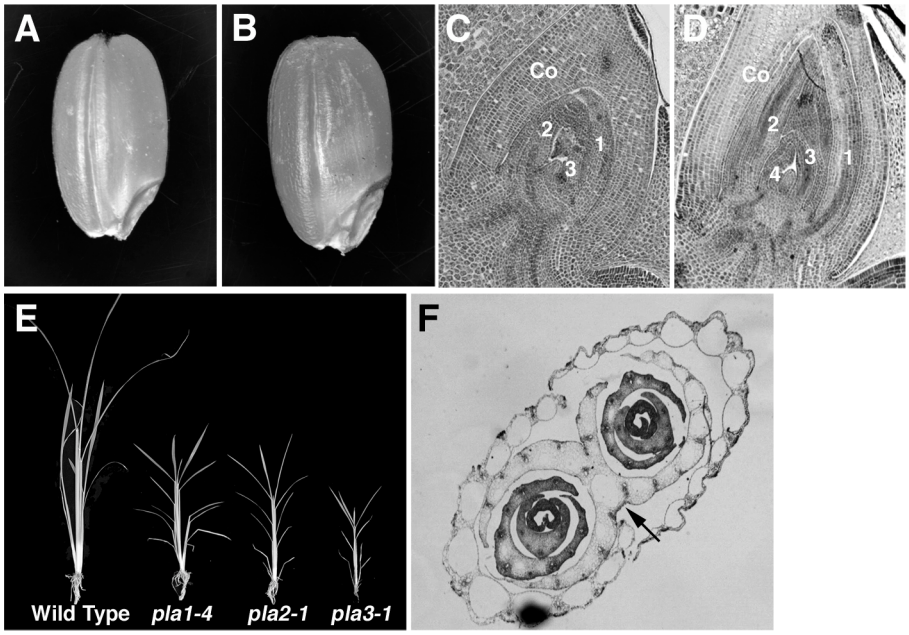

Fig. 1. Phenotypes of mature embryo and seedlings in wild type and *pla3*. A: Mature wild-type seed. B: Mature *pla3* seed. C: Plumule of mature wild-type embryo. D: Plumule of mature *pla3* embryo. E: Plants at 1 month after germination. F: Cross section of shoot apex in 1-week-old *pla3* seedling. Numerals in C and D indicate leaf numbers. Arrow in F indicates the fusion of two leaves. Co: Coleoptile.

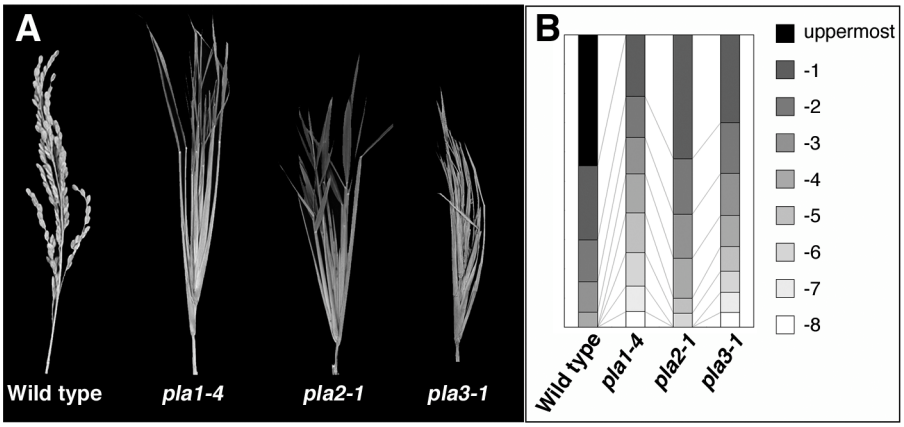

Fig. 2. Phenotypes of wild type and *pla*s in reproductive phase. A: Panicles. B: Schematic representation of internode elongation pattern. Numerals in B represent the internode numbers counted from the top.

In summary, *PLA3* gene is involved in various developmental events. *PLA3* is a negative regulator of leaf initiation and is a terminator of the vegetative phase. In addition, a possibility is suggested that *PLA3*

is also associated with meristem maintenance. At present, it is to be revealed how these three *PLASTOCHRON* genes are related with one another. Double mutant analysis and expression analysis would be necessary to address the above subject. We are now trying to isolate *PLA3* by map-based cloning. Identification of *PLA3* gene will greatly assist our understanding on the regulation of plastochron in rice.

References

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