

Production of transgenic rice plants showing reduced heading date and plant height by ectopic expression of rice MADS-box genes

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Abstract

Plant architecture is an important factor that controls several characteristics, such as light interception, harvest index, and lodging. Plant architecture is controlled by a group of regulatory genes. It was previously found that ectopic expression of some MADS-box genes resulted in early flowering and a dwarf habit in *Arabidopsis* and tobacco (*Nicotiana tabacum*) plants. To explore ways to alter heading time and heading height, we expressed ectopically several *OsMADS* (*Oryza sativa* MADS-box) genes in rice plants. The *OsMADS* cDNA clones were connected to the maize *ubiquitin* promoter in the sense orientation, and the constructs were introduced into rice plants by the *Agrobacterium*-mediated transformation. Ectopic expression of the rice MADS-box genes hastened flowering to varying extents. RNA-blot analyses revealed that the phenotypes of early flowering and dwarfism were correlated with the transcript level of the transgene. In addition, weak expression of *OsMADS1* using the *nopaline synthase* (*nos*) promoter, shortened the heading time by a few days and resulted in a mild reduction of heading height without any pleiotropic effects. The present study suggests that the rice MADS box genes could be used as sources of early-flowering and dwarfing traits in monocot species.

Introduction

The transition from vegetative to reproductive development is a dramatic morphological change during the plant life cycle. Physiological studies of flowering have led to the creation of a multifactorial model, in which floral induction is regulated by various developmental and environmental factors (reviewed by Bernier et al. 1993). Genetic studies of several early- and late-flowering mutants in *Arabidopsis thaliana* have shown that floral induction can be delayed or promoted by multiple genetic pathways (reviewed by Peeters and Koornneef 1996). A complex regulatory network of early- and late-flowering genes is involved in the regulation of floral induction. The study of late-flowering mutants has resulted in identification of floral-promotion genes, such as *LUMINIDEPEN-*

DENS (*LD*), *CONSTANS* (*CO*), *FCA*, and *FHA* (Lee et al. 1994; Putterill et al. 1995; Macknight et al. 1997; Guo et al. 1998). Characterization of early-flowering mutants identified a floral repression genes, *FLOWERING LOCUS C* (Michaels and Amasino 1999).

In response to floral induction, the inflorescence meristem becomes committed to flowering. *LEAFY* (*LFY*) and *APETALA1* (*API*) in *Arabidopsis* are responsible for promoting the formation of floral meristems (reviewed by Ma 1998). Consequential expression of floral organ-identity genes specifies floral morphogenesis (reviewed by Theissen and Seadler 1999). In particular, a majority of the regulatory genes involved in flower development share a conserved MADS domain, which is found in a wide variety of plants as well as in yeast and animals (reviewed by

Shore and Sharrocks 1995). The MADS box genes are also present in monocotyledonous (monocot) plants, including rice, maize, sorghum, and orchid (reviewed by Schmidt and Ambrose 1998). Loss-of-function analysis has been used to study the roles of some MADS box genes, such as maize *ZAG1* and *Silky*, and rice *OsMADS3* and *OsMADS4* (Mena et al. 1996; Kang et al. 1998; Schmidt and Ambrose 1998).

Understanding the genetic basis of flower development in a model plant, such as rice, should enable us to manipulate the transition to flowering in various cereal plants. We previously demonstrated that a rice MADS box gene, *OsMADS1*, could trigger the formation of floral meristems in tobacco plants that overexpressed the gene (Chung et al. 1994). Ectopic function of other rice MADS box genes, *OsMADS5*, *OsMADS7*, and *OsMADS8*, also promoted floral formation in transgenic tobacco plants (Kang and An 1997; Kang et al. 1997). Similarly, *API* and *AGAMOUS* (*AG*), initiated the formation of floral meristems (Mandel and Yamovsky 1995; Mizukami et al. 1997).

Ectopic function of *MdMADS2*, an apple MADS box gene, hastened flowering in tobacco (Sung et al. 1999). Here, we provide evidence that constitutive expression of the *API/AGAMOUS-LIKE9* (*AGL9*) group (Purugganan et al. 1995) of the rice MADS box genes can promote the formation of the floral meristems in transgenic rice plants, resulting in early-flowering and dwarf phenotypes.

Rice is one of the world's most important food crops and the primary food for more than a third of the world's population (David 1991). Analyzing the genes that control heading date (flowering time) is of practical importance because of the effects of heading date on plant adaptation to different cultivation areas and crop seasons. Dwarfism is also one of the most important breeding objectives because of its characteristics relating to plant type, such as light interception, harvest index, fertilizer response, and lodging resistance (Futsuhara 1986). Here, we report the usefulness of *API/AGL9* group of MADS box genes in rice breeding as sources of early-flowering and dwarf traits.

Materials and methods

Bacterial strains

Escherichia coli JM83 was used as the host for routine cloning experiments. *Agrobacterium tumefaciens* LBA4404, containing the *Ach* chromosomal background and a disarmed helper Ti-plasmid pAL4404,

was used for rice transformation (Hoekema et al. 1983).

Construction of binary vectors

The cDNA clones of *OsMADS1* (accession number L34271), *OsMADS5* (U78890), *OsMADS6* (U78782), *OsMADS7* (U78891), *OsMADS8* (U78892), and *OsMADS14* (AF058697) were inserted into multiple cloning sites of pGA1611 (Kang et al. 1998) in the sense orientation. The resulting plasmids were pGA1634, pGA1625, pGA1627, pGA1630, pGA1631, and pGA1909, respectively. In the constructs, rice MADS box genes were placed under the control of the maize *ubiquitin* (*ubi*) promoter. The fusion between the CaMV 35S promoter and the *hygromycin phosphotransferase* (*hph*) gene was used as a plant selectable marker. To direct a weak expression of *OsMADS1*, the cDNA clone was placed under the control of the *nopaline synthase* (*nos*) promoter, making pGA1896 (An et al. 1986).

Rice transformation

All plasmids were transformed into *A. tumefaciens* LBA4404 by the freeze and thaw method (An et al. 1986). *Japonica* rice varieties, Dongjin, Nakdong, Milyang 99, Daesan, Hwayoung, Milyang 109, Palgong, Singeumho, and Milyang 151, were used for transformation by the *Agrobacterium* cocultivation method as described previously (Jeon et al. 1999; Lee et al. 1999). All transgenic rice plants were generated on media that contained 40 mg/l hygromycin B. In this study, we called the primary transgenic lines the T₁ generation, and their progeny the T₂ generation.

DNA and RNA blot analyses

Genomic DNA was isolated from leaves of mature plants, as described previously (Dellaporté et al. 1983). Genomic DNA (5 µg) was digested with *Hind*III and separated on a 0.7% agarose gel. Total RNA was isolated from leaves of mature plants by an RNA isolation kit (Tri Reagent, MRC, Cincinnati, OH). Total RNA (5 or 10 µg) was fractionated on a 1.3% agarose gel, and all procedures of gel-blot analysis were carried out as described previously (Kang et al. 1998).

Table 1. Phenotypes^a for height and heading date of transgenic plants.

Gene	Line	Growth room ^b		Field ^c	
		plant height (cm)	heading date (days)	plant height (cm)	heading date (days)
Wild type ^d	–	63.4 ± 5.5	49–50	105.5 ± 2.6	87–89
Control ^e	–	67.4 ± 4.8	49–50	100.2 ± 8.2	87–89
<i>OsMADS1</i>	1634-1	9.6 ± 0.8	39–40	29.3 ± 9.3	79–81
	1634-2	30.1 ± 10.1	42–44	54.0 ± 12.8	81–84
	1634-3	19.2 ± 8.4	39–42	49.2 ± 10.9	80–82
	1634-5	22.5 ± 1.3	41–42	42.4 ± 11.1	80–82
<i>OsMADS5</i>	1625-2	38.7 ± 6.3	43–45	66.5 ± 3.6	81–84
	1625-3	48.5 ± 2.8	45–49	76.1 ± 2.6	86–88
	1625-5	41.1 ± 4.6	43–47	73.2 ± 1.7	83–85
	1625-7	50.1 ± 2.3	47–49	86.9 ± 5.3	85–88
<i>OsMADS7</i>	1630-1	40.7 ± 8.5	42–46	ND ^f	ND
	1630-3	38.0 ± 4.9	40–42	ND	ND
	1630-6	41.2 ± 5.0	43–35	ND	ND
	1630-7	50.9 ± 10.0	46–50	ND	ND
	1630-8	43.1 ± 5.7	45–47	ND	ND
<i>OsMADS8</i>	1631-1	41.0 ± 5.2	43–45	ND	ND
	1631-2	41.6 ± 4.8	41–45	ND	ND
	1631-3	46.1 ± 4.3	45–47	ND	ND

^aThe data were an average of five to eight plants whose progeny all were hygromycin-resistant.

^bExperiment was carried out in the controlled-growth room with 10 h of light per day.

^cExperiment was carried out in the field at the Yeongnam Agriculture Experiment Station (35.5 °N). Seeds were germinated on 10 May 1998, and then transplanted in the field on 1 June 1998.

^dWild-type Nakdong.

^eHygromycin-sensitive segregates.

^fNot determined.

Growth conditions and analysis of phenotype

To examine the effect on heading date, all transgenic rice plants were grown at 10 h of light, in controlled-growth rooms that were typically 30 °C during the day and 20 °C at night. Throughout the study, heading date was recorded as the day the first panicle emerged. The phenotypes of transgenic rice plants were observed at Yeongnam Agriculture Experiment Station (of 35.5° N) or at Agricultural Experimental Station of Pohang University of Science and Technology (of 36° N). For anatomical observations, tissues were fixed in a solution containing 50% ethanol, 5% acetic acid, and 3.7% formaldehyde, and embedded in a Paraplast (Sigma, St. Louis, MI). The samples were sectioned to 10 μm thickness and observed under a Nikon microscope.

Results

Production of transgenic rice plants overexpressing a rice MADS-box gene

We have previously reported that *OsMADS1*, *OsMADS5*, *OsMADS6*, *OsMADS7*, *OsMADS8*, and *OsMADS14* belong to the *API/AGL9* group (Chung et al. 1994; Kang and An, 1997; Kang et al. 1997; Moon et al. 1998). To express the MADS-box genes in rice, we constructed binary vectors containing transcriptional fusions between the maize *ubi* promoter and a MADS box gene (Figure 1A). The *ubi* promoter shows strong activity in cereals such as rice, maize, oats, wheat, and sugar cane (McElroy and Brettell 1994). We introduced the constructed molecules into rice plants, using the *Agrobacterium*-mediated transformation method. Thirty-three plants with pGA1634, 21 plants with pGA1625, 20 with pGA1630, and 22 with pGA1631 were produced from independently transformed hygromycin-resistant calli. We were able

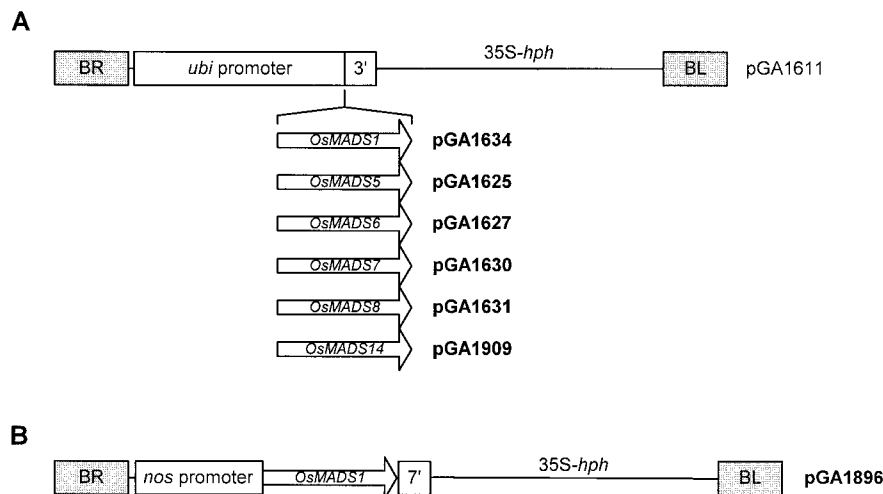


Figure 1. Maps of binary vectors used for rice transformation. A. The vectors carry rice MADS box cDNA clones under the control of the ubiquitin (*ubi*) promoter. 3' indicates the (*nos*) terminator. As a selectable marker, the vectors carry the hygromycin phosphotransferase (*hph*) gene driven by the CaMV 35S promoter. B. The pGA1896 plasmid carrying the *nos* promoter followed by the *OsMADS1* cDNA. 7' indicates the transcription termination region of the Gene 7 of pTiA6.

to produce only eight plants with pGA1627 (carrying *OsMADS6*) and two plants with pGA1909 (carrying *OsMADS14*), because most hygromycin-resistant calli transformed by these plasmids failed to regenerate normal plants.

Phenotypes of primary transgenic rice plants

We cultivated the primary transgenic plants in a controlled-growth chamber to observe morphological changes during the whole life cycle. A majority of the primary (T_1) transgenic plants expressing *OsMADS1*, *OsMADS7*, and *OsMADS8*, exhibited early-flowering and dwarf phenotypes with high frequencies of 97% (32/33), 90% (18/20), and 86% (19/22), respectively.

The *OsMADS1* transgenic lines were characterized as having their panicles embedded in the leaf sheath (Figure 2A and 2B). This phenotype was probably caused by their premature flowering. Most plants showed severe early-flowering and dwarf phenotypes, and continuously produced new tillers, thereby resulting in bushy plants (Figure 2A). We introduced *OsMADS1* into nine varieties, Dongjin, Nakdong, Milyang 99, Daesan, Hwayoung, Milyang 109, Palgong, Singeumho, and Milyang 151, and observed the same phenotypes of early flowering and dwarf in all the varieties (data not shown). This indicates that the morphological changes caused by *OsMADS1* were not genotype-dependent. Some transgenic lines that strongly expressed *OsMADS1* exhibited extreme early flowering and remarkable reductions in fertility. In

some spikelets of *OsMADS1* transgenic lines, glumes occasionally overgrew to the size of paleae or lemmas (data not shown).

The transgenic plants overexpressing *OsMADS7* or *OsMADS8* showed abnormalities, such as dark green leaves, precocious flowering, severe dwarf, and, subsequently, remarkably reduced fertility. We could not distinguish phenotypes between the *OsMADS7* and *OsMADS8* transgenic lines (Figure 2C). Unlike the *OsMADS1* transgenic lines, these transgenic plants produced fewer tillers: usually 2–4 compared to 5–8 in wild-type plants.

The phenotypes in of the *OsMADS6* or *OsMADS14* lines could be observed at the callus stage. Two weeks after the calli were transferred to the regeneration media, they became dark green on their surfaces. After another two weeks, most calli failed to develop into plantlets and, consequently, died black (Figure 3A). In contrast, transgenic calli with other MADS genes or a vector alone regenerated plantlets normally within one month (Figure 3B). Such a phenotype was observed more frequently in *OsMADS14* than in *OsMADS6* lines (data not shown). Microscopic observation and histological analysis showed that some of the *OsMADS14* transgenic calli produced several shoot- or embryo-like structures (Figure 3C). These structures eventually developed abnormal flowers that consisted of organs that resembling palea/lemma, lodicules, or carpels (Figure 3D). We suggest that ectopic function of *OsMADS6* or *OsMADS14* may promote organ dif-

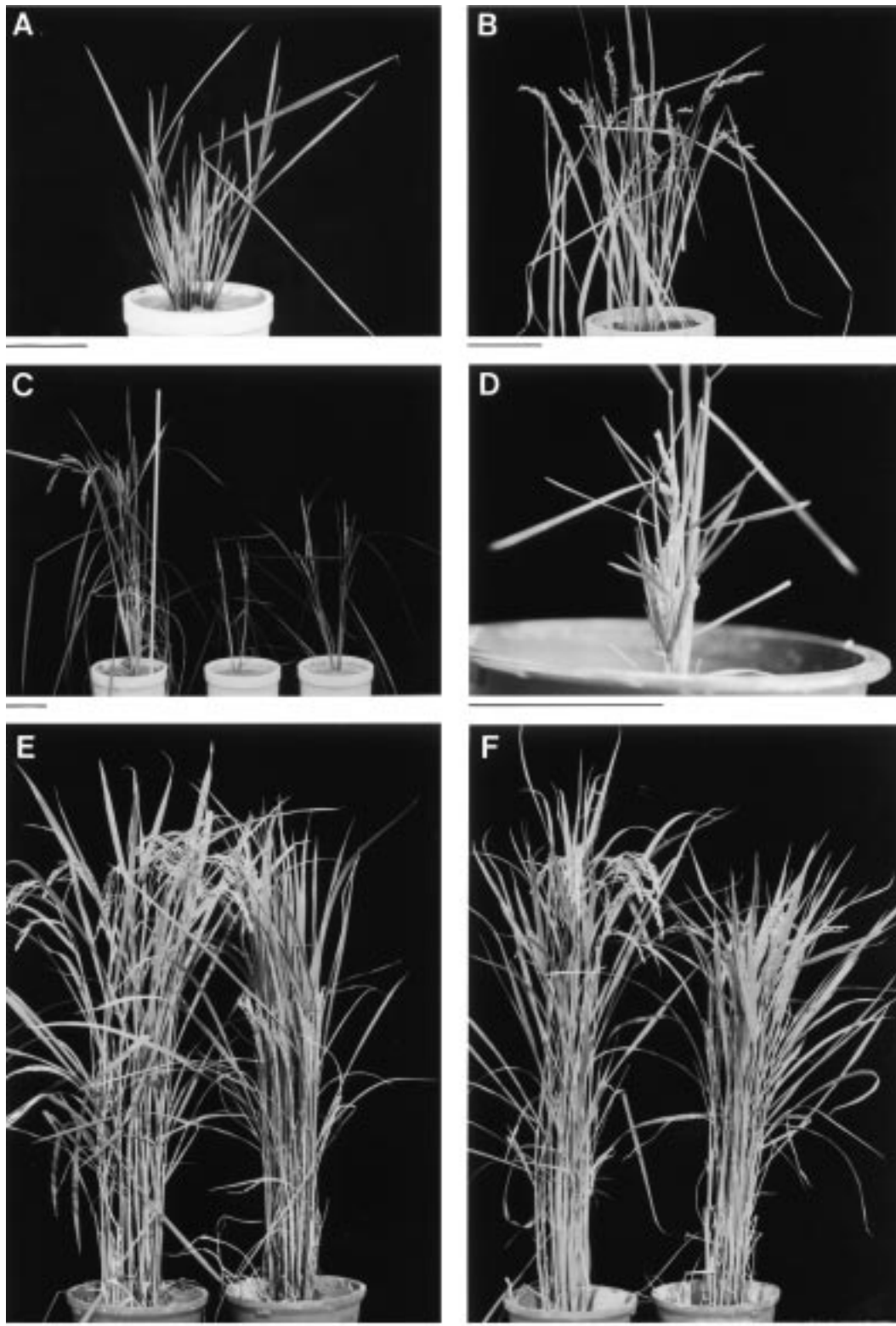


Figure 2. Phenotypes of primary transgenic lines that overexpress the *OsMADS* gene. A. Line 1634-1 (*OsMADS1*) showing strong phenotypes of early flowering and dwarf. B. Line 1634-2 showing a mild phenotype of *OsMADS1*. C. Transgenic plants overexpressing *OsMADS7* or *OsMADS8*. Left, a hygromycin-sensitive segregate as a control; middle, line 1630-3; right, line 1631-2. D. Line 1627-4 overexpressing *OsMADS6*. E. Line 1634-2 (right) grown in the field. F. Phenotypes of the *OsMADS5* line 1625-5 (right) grown in the field. Bars = 10 cm.

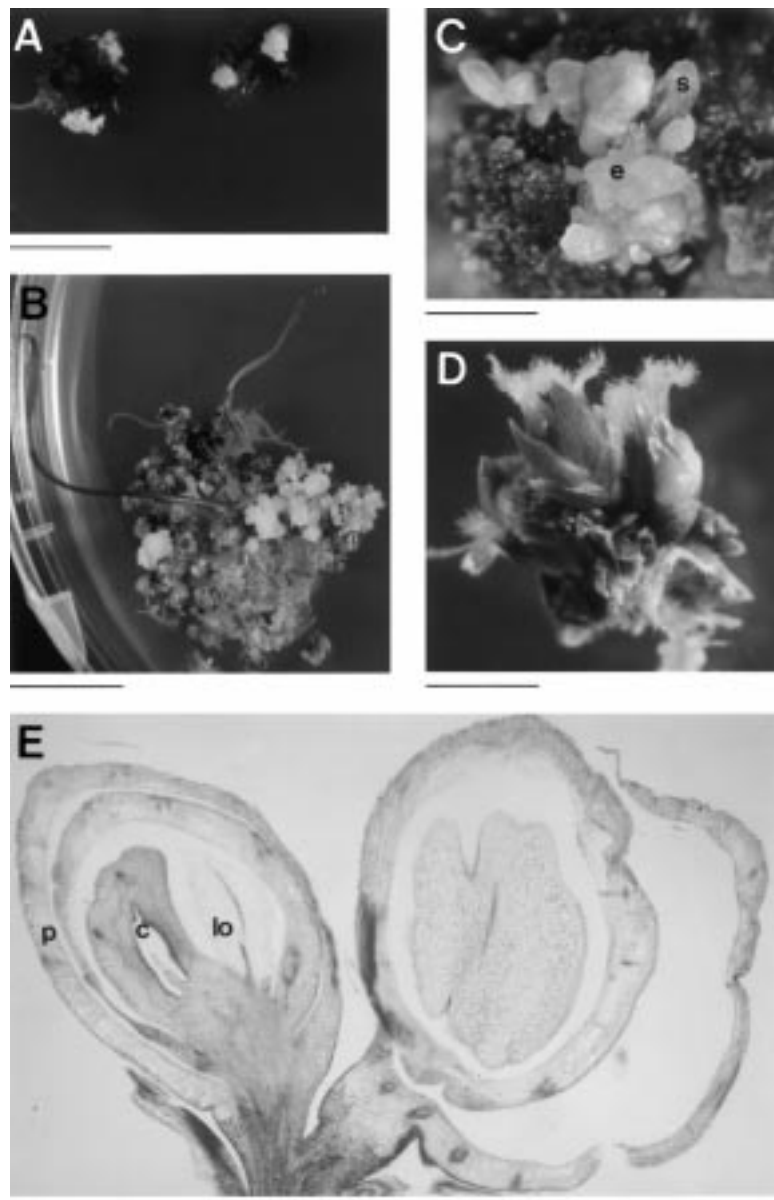


Figure 3. Phenotypes of primary transgenic lines that overexpress *OsMADS6* or *OsMADS14*. **A.** Transgenic calli with pGA1909 (*OsMADS14*) being cultivated on regeneration media (Jeon et al. 1999). **B.** Transgenic calli with pGA1633, which consisted of promoterless *GUS* and *35S-hph* (Jeon et al., 2000b). We regarded these calli as a controls. Healthy calli and several shoots were regenerated. **C** and **D.** Phenotype of the 1909 lines expressing *OsMADS14*. Embryo-like (e) and shoot-like (s) structures (**C**) and flower-like structures (**D**) are shown. **E.** Longitudinal section of the flower-like structures. The left flower consisted of structures that resembled abnormal palea/lemma (p), a lodicule (lo), and a carpel (c). Bars = 1 cm in **A** and **B**, 1 mm in **C** and **D**, and 100 μm in **E**.

ferentiation and/or inhibit cell growth from of meristematic tissues, which results in arrested cell growth. Plants were rarely regenerated from the transgenic calli, in these rare regenerated plants the transgene may have been expressed weakly. These regenerated plants were severely dwarfed and flowered much earlier than the control plants (Figure 2D). Ectopic function of *OsMADS6* or *OsMADS14* may have affected the determination of the shoot apical meristems. If the ectopic function of *OsMADS6* or *OsMADS14* were regulated appropriately, using a weak promoter or an inducible system, it would be useful for manipulating transition to flowering in crop plants as well as for studying the flowering mechanism in rice.

Unlike the other MADS box genes examined in this study, most of the transgenic plants overexpressing *OsMADS5* grew normally and flowered at the same time as the controls. Five of 21 transgenic plants were slightly shorter than the controls, but they showed no phenotypic alteration in the floral organs (data not shown).

Reduction of heading date and plant height in progeny of transgenic rice plants

The duration time for flowering, i.e., from seed germination to heading, was measured in four of the *OsMADS1* lines, four of the *OsMADS5* lines, five of the *OsMADS7* lines, and three of the *OsMADS8* lines (Table 1). T₂ plants were grown in 10 h of light per day. These transgenic plants flowered about 3 to 10 days earlier than controls. Therefore, ectopic expression of *OsMADS1*, *OsMADS5*, *OsMADS7* and *OsMADS8* promoted flowering after the mandatory vegetative growth period that was necessary for acquiring flowering competence. We did not examine the heading date from the *OsMADS6* and *OsMADS14* lines because they were sterile and heads failed to emerge.

Early-flowering transgenic plants were dwarf (Table 1). The height of the *OsMADS1* transgenic plants was significantly reduced: 10 to 30 cm in the controlled-growth room and 29 to 54 cm in the field. In contrast, the hygromycin-sensitive segregates had average respective heights of 67 and 100 cm. The wild-type plants used as a control did not show any significant difference from the hygromycin-sensitive segregates.

Two of the *OsMADS5* lines, 1625-3 and 1625-7, exhibited moderate phenotypes, while the other two

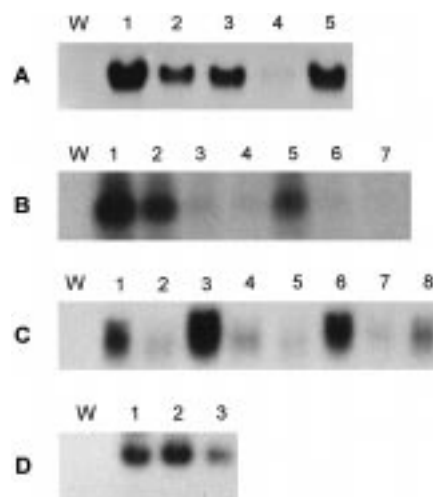


Figure 4. RNA gel-blot analysis of transgenic plants. Total RNA (5 μ g) prepared from leaves of wild-type (W) and primary transgenic plants of line 1634 (A), line 1625 lines (B), line 1630 (C), and line 1631 (D) was hybridized with gene-specific probes (Chung et al. 1994; Kang and An 1997; Kang et al. 1997). The numbers indicate independent transgenic lines. Ethidium-bromide staining of 25S and 17S rRNA demonstrated equal amounts of RNA loading (data not shown).

lines showed more severe phenotypes. Interestingly, the progeny of some *OsMADS5* lines showed more severe phenotypes than the primary transgenic plants. We think that the ectopic function of *OsMADS5* or the expression of genes necessary for the *OsMADS5* function might be mitigated by the experimental conditions for rice transformation. We do not exclude the possibility that an enhanced expression of *OsMADS5* for the next generation of the transgenic lines might result in more severe phenotypes. In general, the degree of phenotype was more severe in the *OsMADS1* lines (Figure 2E) than in the *OsMADS5* lines (Figure 2F).

The respective heights of *OsMADS7* and *OsMADS8* transgenic plants ranged between were 38 and 51 cm in the growth room. We could not examine these lines in the field because of the shortage of seeds for those lines. The degree of variability in phenotype among transgenic plants expressing different MADS box genes may have reflected the different functional mechanism between the *OsMADS* genes. For example, they may differ vary in selecting partners or choosing downstream genes.

Correlation between phenotype and transgene expression

We examined any possible correlations between degree of phenotype and the level of transgene ex-

pression. Because a constitutive promoter was used, we were able to estimate the expression level by measuring the amount of the transgene transcript in leaves of individual lines. The MADS transcripts were not detectable in leaves of wild-type plants (Chung et al. 1994; Kang and An 1997; Kang et al. 1997; Moon et al. 1989). RNA-gel blot analysis showed that expression levels differed significantly among individual primary transgenic lines (Figure 4). Line 1634-1, which showed the most severe phenotypes, exhibited the highest expression of *OsMADS1* (Figure 4). Lines 1625-2 and 1625-5, which showed strong phenotypes among the *OsMADS5* transgenic lines, contained greater amounts of the transgene transcript (Figure 4B). The other two lines, 1625-3 and 1625-7, which carried lower levels of the *OsMADS5* transcript, were moderately dwarfed.

A correlation between phenotype and transgene expression was also observed in plants overexpressing *OsMADS7* or *OsMADS8*. The line 1630-3 (*OsMADS7*) and line 1631-2 (*OsMADS8*), showed dramatic reductions in plant height and flowering time, and carried high amounts of the transcripts (Figure 4C and 4D). Therefore, phenotypic alteration was probably caused by transgene expression.

Modulation of heading height by controlled level of *OsMADS1* expression

Transgenic plants expressing the MADS-box genes by the *ubi* promoter showed strong phenotypes, with accompanying reductions in fertility and crop yield. We reasoned that a weak expression of the MADS-box genes may provide reduced flowering time and height phenotypes without pleiotropic effects.

The *nos* promoter is a weak promoter in dicot and monocot plants (An et al. 1986; unpublished data). Therefore, we constructed pGA1896, in which the *nos* promoter was placed in front of the *OsMADS1* gene (Figure 1B). Among the 30 primary transgenic plants, which were transformed with pGA1896, sixteen lines were selected for DNA-blot analysis using the *hph* gene as a probe (Figure 5A). Ten lines contained a single copy of the transgene; six lines carried two or more copies.

We also estimated the number of genetic loci of the introduced gene by scoring hygromycin resistant progeny. Seven of nine lines tested showed 3:1 segregation, indicating a single-locus T-DNA insertion. The remaining lines showed two to three loci (Ta-

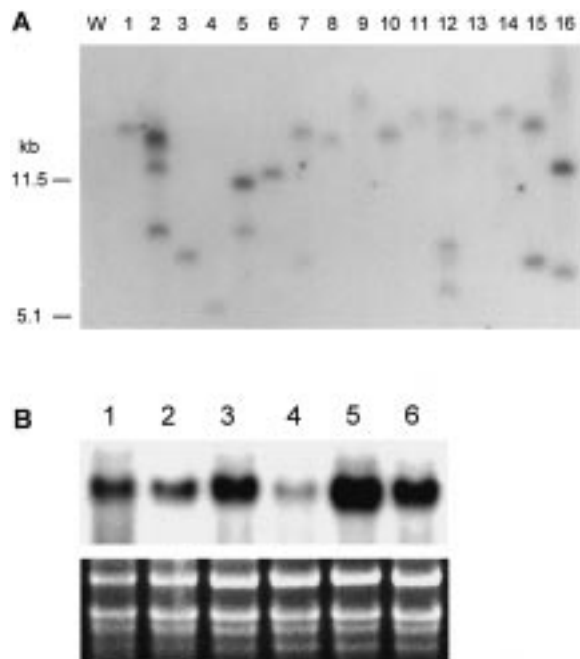


Figure 5. Molecular analysis of the pGA1896 transgenic lines. A. Genomic DNA gel-blot analysis. The blot was hybridized with the *hph*-coding region. W, wild type. The number means an independent line transformed with pGA1896. B. RNA gel-blot analysis of the pGA1896 lines (*nos* promoter-*OsMADS1*) and pGA1634 (*ubi* promoter-*OsMADS1*). 1; 1896-13, 2; 1896-12, 3; 1896-16, 4; 1896-8, 5; 1634-2, 6; 1634-8. Total RNA (10 μ g) was hybridized with the gene-specific probe of *OsMADS1*. Bottom. Ethidium bromide staining of 25S and 17S rRNA demonstrates equal amounts of RNA loading.

ble 2). In the line 1896-12, three copies of the T-DNA apparently integrated into one chromosome.

To examine the expression level of *OsMADS1* by the *nos* promoter, we compared the transcriptional ability between the *nos* and *ubi* promoters by RNA-blot analysis using mRNAs from leaves of the pGA1896 and pGA1634 lines (Figure 5B). The result demonstrated that the activity of the *nos* promoter is lower than that of the *ubi* promoter in transgenic plants, and that the lines exhibited significant variation of *OsMADS1* expression among independent lines. We selected one line (1896-1) that did not show any expression and eight lines with detectable amounts of the transgene mRNA for detailed analysis.

Forty progeny from each transgenic line were grown in the field. The transgenic lines expressing the introduced gene exhibited weakly reduced plant height and hastened heading date compared with the hygromycin-sensitive segregates and untransformed controls (Figure 6A, Table 2). Heading date was has-

Table 2. Phenotypes^a of transgenic rice plants overexpressing *OsMADS1*.

Line	T-DNA copies ^b	Genetic loci	RNA level ^c	Plant height (cm)	Panicle length (cm)	Heading date (days) ^d	Number of tillers	Yield (Seeds)
Wild type ^e	0	0	–	111.2± 2.4	24.7±1.5	106-108	14.7±1.5	1792± 275
Control ^f	0	0	–	109.4± 5.2	24.7±3.1	105-108	14.5±4.4	1814± 706
1896-1	1	(51:16) ^g	– ^h	100.9±11.5	22.7±3.3	105-107	16.7±2.1	2024± 761
1896-2	3	2-3 (60: 1)	+	100.8± 6.1	19.1±2.0	105-106	13.3±0.5	2297±1087
1896-6	1	1(84:41)	++	95.8± 4.4	18.1±1.3	100-102	13.5±5.0	1680± 840
1896-8	1	1(43:12)	+	93.3± 5.3	18.0±1.2	100-103	13.0±2.7	1685± 691
1896-9	1	1(150:40)	+	95.4± 3.7	21.1±1.4	102-105	13.6±2.3	2045± 493
1896-10	1	1 (158:33)	+	92.5± 8.4	17.8±2.9	102-103	12.7±4.1	1436± 795
1896-12	3	1(46:12)	+	109.3± 4.5	20.3±1.3	102-104	13.5±0.5	1431± 725
1896-13	1	1(234:66)	++	98.5± 7.8	19.1±4.4	101-104	13.0±3.4	1550± 574
1896-162	2	(262:17)	+++	95.9± 8.1	17.8±3.5	99-101	14.5±5.1	2033±1092

^aThe data were an average of five to ten plants whose progeny all were hygromycin-resistant. The experiment was carried out at the Agricultural Experimental Station of Pohang University of Science and Technology (36 °N).

^bThe data were determined from Figure 5.

^cAs the RNA level increased, more '+' symbols were added. The data were determined from Figure 5.

^dSeeds were germinated on 10 May 1999, and then transplanted in the field on 9 June 1999.

^eWild-type Dongjin

^fHygromycin-sensitive segregates.

^gThe ratio of hygromycin-resistant to hygromycin-sensitive T₂ plants.

^hEven though we did not detect the *OsMADS1* transcript in line 1896-1, it is likely that the line would express the *OsMADS1* mRNA at a low level, because the plants showed reduced plant height growth. The line was hygromycin-resistant and carried the introduced *OsMADS1* gene (data not shown).

tened one to six days and plant height was reduced up to 17 cm. Panicle length was reduced in 2–7 cm. This resulted in a reduced degree of heading (Figure 6B). The number of tillers, however, was not changed.

Grain yield varied significantly among individual plants. Some transgenic lines produced as much grain as did the controls, while other lines generated less seed. No clear correlation was found between yield and the level of the transgene transcript. The variation was probably due to tissue-culture effects, so that several generations may be required for stabilizing crop yield.

Discussion

Ectopic expression of six *API/AGL9* group genes (i.e., *OsMADS1*, *OsMADS5*, *OsMADS6*, *OsMADS7*, *OsMADS8* and *OsMADS14*) in rice caused plants to flower earlier. Our results coincide with previous observations that the constitutive function of *OsMADS1*, *OsMADS5*, *OsMADS7*, and *OsMADS8* directed early flowering in tobacco (Chung et al. 1994; Kang and An 1997; Kang et al. 1997). Functional mechanisms of these MADS box genes, therefore, may be conserved

between dicot and monocot species. We found that ectopic function of *OsMADS6* or *OsMADS14* directed the early-flowering syndrome at the callus stage and, subsequently, caused flowering at the callus or young-plantlet stage. These results suggest that ectopic function of the *API/AGL9* group genes triggers conversion of inflorescence shoots into flowers. Therefore, that gene function may be involved in determining floral meristems in rice plants.

Recently, we demonstrated that *OsMADS1* mutation caused the *leafy hull sterile1* (*lhs-1*) phenotype. This was manifest in the conversion of flowers into inflorescence structures in a strong phenotype, or the conversion of palea/lemma into leafy structures in a weak phenotype (Jeon et al. 2000a). Therefore, *OsMADS1* appears to be involved in several aspects of flower development, including determinations of floral meristems at early stages of reproductive development and floral-organ formation during later stages. Loss-of-function analysis should be used to evaluate the functions of the other members of the *API/AGL9* MADS box genes in rice.

It is a noteworthy that the *OsMADS* genes examined in this study were expressed preferentially in

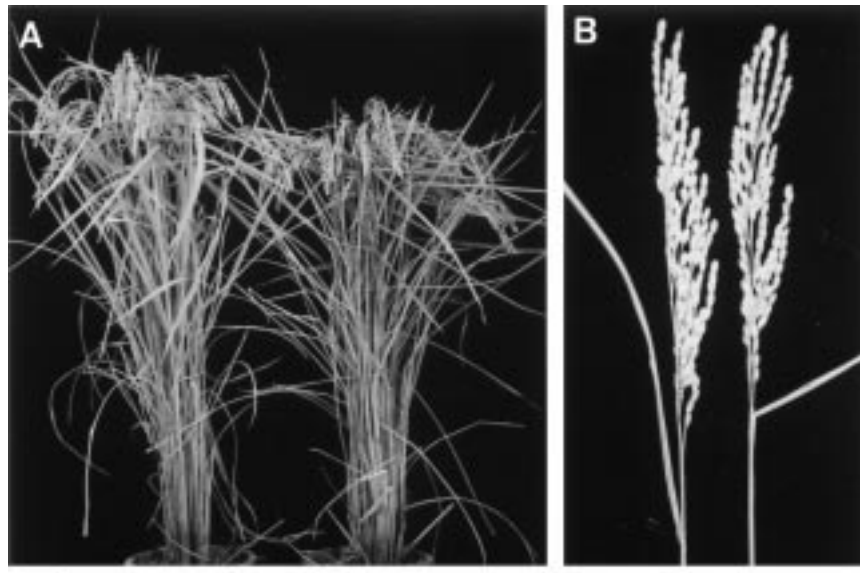


Figure 6. Phenotypes of the pGA1896 transgenic lines. A. Phenotypes of line 1896-2 grown in the field. Left, a hygromycin-sensitive segregate as a control plant. Right, a transgenic plant from line 1896-2. B. A transgenic panicle (right) of line 1896-2 compared with a control (left). Bars = 10 cm in A; and 5 cm in B.

flowers. Therefore, the early flowering induced by ectopic function of the genes might have resulted from interaction with a floral promoting factor present in the shoot apex or inflorescence meristem. Alternatively, constitutively expressed *OsMADS* proteins may have interacted with a factor that maintains shoot apical meristems. One such flower-inhibiting factor may be *TFL1*. Bradley *et al.* (1997) showed that two EST clones, *OSR29181A* and *OSS1946A*, were homologous to *TFL1*, on the basis of sequence homology. Whether the early-flowering phenotype is a direct consequence of *OsMADS* genes acting at the vegetative shoot apex remains to be determined.

Flowering time in *Arabidopsis* may be influenced by MADS box genes, zinc fingers containing transcription factors, RNA-binding protein, photoreceptors, polycomb-group genes, starch metabolism-related genes, brassinolide or GA biosynthesis-related enzymes, etc. (reviewed in by Levy and Dean 1998; Michaels and Amasino 1999). A variety of proteins play important roles in plant growth, and development, and, consequently, may direct flowering. In rice, many QTLs are involved in determining morphological and physiological traits such as plant height and heading date (reviewed by Yano and Sasaki 1977).

To date, two dwarfing genes have been identified from rice. Map-based cloning (Ashikari *et al.* 1999) and antisense strategy (Fujisawa *et al.* 1999) demon-

strated that *d1*, one of dwarfing genes, encodes an α -subunit of GTP-binding protein that is associated with gibberellin signal transduction. Sato *et al.* (1999) provided evidence that the *db* gene, another dwarfing gene of rice, is a mutation of the *OSH15* rice homeobox gene. Map locations have been determined for five QTLs, *i.e.*, *Hd-1* to *Hd-5*, that greatly affect heading date (Yano *et al.* 1997). Cloning those genes will enlarge the understanding of flowering in rice. In addition, Peng *et al.* (1999) already has shown that transgenic rice plants expressing the mutant form of the *Arabidopsis gibberellin insensitive (GAI)* gene were dwarfed.

In conclusion, we demonstrated in this study that ectopic function of *OsMADS1* should act in a dosage-dependent fashion because the degree of the phenotype is correlated with the transcriptional-activation ability of the tested promoter. We showed that a weak expression of *OsMADS1* driven by the *nos* promoter prompted desirable phenotypes in transgenic rice lines. If the expression of the *OsMADS* genes is regulated by an inducible system (Gatz 1996), one could achieve flowering at a desired time. In cases where plants are tall, with weak culms and high susceptibility to damage from wind and rain, our *nos::OsMADS1* could be used effectively to reduce plant height. *OsMADS* genes have also caused early flowering in tobacco, *Petunia* sp., and *Brassica napus*

(Chung et al. 1994; Kang and An 1997; Kang et al. 1997; unpublished data). Therefore, these genes may be useful used for controlling flowering time and plant architecture in a wide variety of other plant species.

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