Formation of Corymb-like Inflorescences Due to Delay in Bolting and Flower Development in the corymbosa2 Mutant of Arabidopsis

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Among the wild-type ecotypes of Arabidopsis thaliana whose shape of inflorescence is categorized as raceme, the ecotype Landsberg harboring the er (er) mutation shows a corymb-like inflorescence, namely, a compact inflorescence with a flattened arrangement of flower buds at the tip. The fact that the ER gene encodes a receptor-like protein kinase implies the presence of a signaling cascade responsible for the inflorescence morphology of flowering plants. We report here the characterization of another mutant with a corymb-like inflorescence, named corymbosa2 (crm2), and the isolation of the CRM2 gene. While the er mutation causes a severe reduction in the length of pedicels, the crm2 mutation results in a significant delay in the initiation of internode elongation and in the development of flowers, despite having little effect on the timing of floral induction. Consequently, the number of flower buds is apparently increased at the tip of crm2 inflorescence. The crm2 er double mutant shows an additive phenotype. These results suggest that CRM2 and ER may act in different ways to generate wild-type inflorescence. The CRM2 gene was isolated by positional cloning and appears to encode a polypeptide with no significant homology to known sequences.

Key words: Arabidopsis thaliana — Bolting — Corymb-like inflorescence — corymbosa2 — Flower development — Inflorescence development.

Abbreviations: BAC, bacterial artificial chromosome; CaMV, cauliflower mosaic virus; CAPS, cleaved amplified polymorphic sequence; GUS, β-glucuronidase; NLS, nuclear localization signal; ORF, open reading frame; RT-PCR, reverse transcription-PCR; SAM, shoot apical meristem; SEM, scanning electron microscopy; SSLP, simple sequence length polymorphism.

The nucleotide sequence reported in this paper has been submitted to GenBank under accession number AF327068 (CORYMBOSA2).

Introduction

The inflorescence shoot architecture of flowering plants can be divided into various types according to anatomical criteria such as the identity of the shoot apical meristem (SAM), the pattern of lateral flower primordia initiation, and the degree of internode elongation. In Arabidopsis thaliana, transition of a vegetative shoot meristem to an inflorescence meristem is accompanied by continuous production of flowers on the flanks of the SAM with a spiral phyllotaxy and by rapid elongation of pedicels (the stalk of a single flower) and stem internodes. The resulting shape of the inflorescence is referred to as raceme (Weberling 1989). However, the molecular basis corresponding to such terminology remains largely unexplored.

The SAM is a key source of cells for shoot growth. A number of genes involved in determination of meristem identity and in regulation of meristem activity have been identified in Arabidopsis. The TERMINAL FLOWER1 (TFL1) gene plays a critical role in the maintenance of indeterminate inflorescence meristems (Alvarez et al. 1992). While CLAVATA (CLV) genes are involved in promoting the progression of meristem cells toward organ initiation, SHOOT MERISTEMLESS (STM) antagonistically functions in preventing meristem cells from being incorporated into organ primordia (Barton and Poethig 1993, Clark et al. 1996, Laux and Schoof 1997). CLV1 has been shown to encode a receptor-like kinase with an extracellular domain composed of tandem leucine-rich repeats (Clark et al. 1997), and this suggested the exciting possibility that a cell-cell communication of information on cell proliferation and differentiation at shoot meristems might be mediated by receptor-ligand interactions in a similar manner to growth-factor signaling cascades in animal systems. Based on genetic evidence, a small secreted peptide encoded by CLV3 has been identified as a likely candidate for the CLV1 ligand (Fletcher et al. 1999). Furthermore, wuschel (wus) mutations, which result in premature termination of shoot and floral meristems as with stm, appear to be epistatic to clv mutations, suggesting that WUS may function as a downstream target of CLV genes (Laux et al. 1996).

On the other hand, there is little genetic information on the regulatory mechanisms of phyllotactic patterns of flower primordia and the lengthening of pedicels and stem internodes, both of which also contribute to formation of the gross morphology of inflorescence. Mutation in the ERECTA (ER) locus, which is known to be harbored by the wild-type Arabidopsis ecotype Landsberg erecta (Ler), results in a compact inflorescence, short pedicels, and upright blunt siliques. As a consequence, a bunch of flower buds at the tip of inflorescences continuously forms an umbellate flat surface referred to as corymbos inflorescence. The ER gene codes for a receptor-like kinase similar in overall structure to that of CLV1 (Torii et
al. 1996). However, it is not known how the ER gene product performs signal transduction. To understand the molecular mechanisms regulating the inflorescence shoot architecture, the genes involved must be identified. Toward this goal, we have isolated a series of Arabidopsis mutants, named corymbosa (crm), which show corymb-like inflorescences (Komeda et al. 1998). Here we describe the phenotypic characterization of the crm2 mutant in comparison with er. We show that the corymb-like phenotype of crm2 is manifested in a different fashion from that of er. Cloning of the CRM2 gene revealed that it encodes a novel protein.

Results

Overview of the corymb-like phenotype

In wild-type Columbia (Col) plants of A. thaliana, the apical inflorescence meristem and flowers at early stages are covered with flowers at later stages (Fig. 1A). This is simply due to a rapid upward growth of pedicels in unison with flower organ development. In er mutants, the length of pedicels as well as stem internodes is apparently reduced, and this results in the corymb-like phenotype of the tip of the inflorescence (Fig. 1B, F). The crm2 mutant was isolated by screening for mutants with a similar appearance of inflorescence as that of er mutants (Komeda et al. 1998). The flattened arrangement of flower buds in the crm2-1 allele is shown in Fig. 1C. We observed that, while the total number of flowers at stages 7–13 (stages defined by Smyth et al. 1990) at the tip of the inflorescence was normal in er alleles, it was increased in the crm2-1 allele compared to that of the wild type (Fig. 1E–G). Furthermore, in contrast to er alleles, in which the length of fully elongated pedicels is about half of that in the wild type, crm2-1 appeared to have a moderate effect on the length of pedicels. The number of floral organs was unaffected in crm2-1, as with er alleles. These observations indicate that the corymb-like phenotype in crm2-1 is attributable in part to the increased number of flower buds at the tip.

To examine the relationship between CRM2 and ER in inflorescence development, crm2 er double mutants were constructed. For the er mutation, the er-105 allele, which has an

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<th>Table 1 Measurements of wild-type and crm2-1 plants</th>
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<tr>
<td>Wild type</td>
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<tr>
<td>Number of rosette leaves</td>
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<td>Number of cauline leaves</td>
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<td>Days to 1-cm bolting a</td>
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<td>Days to first-flower opening a</td>
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<td>Total number of lateral meristems at day 32 b</td>
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<td>Length of stem internodes (mm) c</td>
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a Days from seed germination are shown.

b Total number of leaves and flowers visible without dissection and using a dissecting microscope on the primary shoot was scored.

c The mean length of the first ten internodes between nodes bearing a single flower

Mean values ± SD in 20 wild-type (Col) and crm2-1 plants are shown.
insertion of a 4-kb DNA fragment of unknown origin in the ER gene and putatively represents a null allele (Torii et al. 1996), was used. We did not detect a novel mutant phenotype in double-mutant seedlings at the vegetative stage. After bolting, crm2-1 er-105 double mutants showed an extremely exaggerated corymb-like phenotype at the tip of the inflorescence (Fig. 1D), suggesting that the effects of crm2 on inflorescence development is additive to er.

The crm2 mutation delays the timing of bolting and flower development

Under standard long-day conditions (see Materials and Methods), the number of rosette leaves was normal in the crm2-1 allele. On the other hand, crm2-1 produced approximately two cauline leaves more than the wild type (Table 1). These results suggest that the crm2 mutation affects the flowering time. To determine whether or not the timing of floral induction is delayed in crm2-1, expression of the flower-meristem-identity gene APETALA1 (AP1) was examined by reverse transcription (RT)-PCR. AP1 is known to be expressed throughout stage 1 and 2 flower primordia and in the first and second whorls of developing flowers but not in vegetative and inflorescence meristems (Mandel et al. 1992). We could not
detect clear difference between the time courses of AP1 expression in *crm2-1* and wild-type plants (Fig. 2A).

On the other hand, *crm2-1* started to bolt approximately 9 d later than did the wild type (Table 1). The period from germination to anthesis (stage 13) of the first flower in *crm2-1* was also much longer than that in the wild type (Table 1; Fig. 2B). However, the production rate of stage 13 flowers by the primary inflorescence meristem in *crm2-1* was higher than that in wild-type plants (1.6 vs. 1.2 flowers per day for the first 5 d in the wild type; Fig. 2B). These results indicate that the *crm2* mutation causes accumulation of flower buds at the tip of inflorescences due to a significant delay in the initiation of bolting and in the development of floral organs but has little effect on the timing of floral induction. Consistent with this, the total number of visible flowers just before bolting was apparently increased in *crm2-1* (Fig. 3A, B). However, the total number of lateral meristems including leaves and flowers was unaffected in *crm2-1* in comparison with that in the wild type of the same day (Table 1). At day 36, when all of the flowers in *crm2-1* were at young bud stages and they were still in the center of the rosette (Fig. 3B), wild-type plants already had flowers after anthesis, developing siliques, and elongating internodes. Furthermore, the growth of stem internodes after bolting was decelerated in *crm2-1* (Fig. 2C). The final length of an individual internode was also reduced in *crm2-1* (Table 1; Fig. 3C, D).

Examination of apical inflorescence meristems by scanning electron microscopy (SEM) revealed that the spiral arrangement of flower primordia at its flanks was unaffected in *crm2-1* (Fig. 4A, B). Sections of inflorescence apices revealed that the number of flowers at early stages was increased in *crm2-1* (Fig. 4C, D), while the apical meristem in the *crm2-1* allele was normal in size and shape (Fig. 4E, F).

**Reduced fertility in crm2**

In addition to the corymb-like phenotype, *crm2-1* exhibited reduced fertility (Fig. 3C, D). Although mature siliques with wild-type length were occasionally produced, the length of siliques and the seed set varied along the stem. Because of the reduced fertility and/or the delayed development of flowers after stage 13, these flowers were also observed in the outermost whorl of a compact flower-bud cluster in the *crm2 er* double mutant (Fig. 1D), instead of developing siliques in the *er* single mutant. We measured the lengths of floral organs of stage 13 flowers (Table 1). Unlike sepals, petals, and carpels, which exhibited normal lengths in *crm2-1*, the average length of *crm2-1* stamens was shorter than that of wild-type stamens. On the other hand, *crm2-1* apical inflorescence meristems continued to produce flowers for a prolonged period compared to the wild type (Fig. 2B). As a result, the *crm2-1* plants finally had more flowers than did the wild type and exhibited a final stature comparable to or higher than that of the wild type, in spite of a reduction in the length of each internode. These phenotypes may be accounted for by reduced fertility, which often causes a prolonged proliferative activity of apical inflorescence meristems (Hensel et al. 1994).

**Positional cloning of CRM2**

The *crm2* mutation segregates as a monogenic recessive (Komeda et al. 1998). Fine mapping experiments revealed that the *CRM2* locus is located within an 80-kb region between the markers T13K14.160−RsaI and g13838−1.4 on chromosome IV, which we designed based on the genomic sequence data in GenBank (see Fig. 5A). We determined DNA sequences of all of the predicted open reading frames (ORFs) in this region by using the *crm2-1* genome and found only a 3-bp deletion in a putative ORF in the bacterial artificial chromosome (BAC) clone T13K14 (Fig. 5B).
To determine whether it corresponds to the CRM2 gene or not, a 6-kb wild-type genomic DNA fragment encompassing the ORF (Fig. 5B) was used for complementation experiments. After transformation of wild-type plants with this fragment, they were crossed with homozygous crm2-1 mutants. Our results showed that all of the F2 populations derived from four independent crosses failed to segregate for kanamycin-resistant plants with the mutant phenotype (data not shown). Sequence determination of the endogenous CRM2 gene of the kanamycin-resistant F2 plants (see Materials and Methods) revealed that some of them were homozygous for the crm2-1 allele and phenotypically wild type (Fig. 6A).

We further transformed wild-type plants with an antisense construct containing a part of this ORF (Fig. 5B) fused in reverse orientation to the cauliflower mosaic virus (CaMV) 35S RNA promoter. Five of sixteen transgenic lines displayed phenotypes similar to the original crm2 phenotype (Fig. 6B). Taken together, these results indicate that this ORF is the CRM2 gene.

Analysis of the CRM2 gene

Isolation of the corresponding cDNA by RT-PCR revealed that the CRM2 gene contains nine introns (Fig. 5B). It encodes a polypeptide of 942 amino acids with a predicted molecular mass of 104 kDa (Fig. 5C). The crm2-1 mutation is located at the second exon and it causes a deletion of a lysine (K) residue in the CRM2 gene product (Fig. 5B, C). A BLAST search using the CRM2 polypeptide sequence revealed no homology with another protein.
known proteins. However, we found that a homologous sequence is tandemly arrayed with the CRM2 gene in the BAC clone T13K14. The CRM2 polypeptide sequence has 64.2% identity overall with a deduced polypeptide of its neighboring homolog (T13K14.80; accession number T10634). The PSORT algorithm (Nakai and Kanehisa 1992) recognized a potential nuclear targeting sequence (KKKK, amino acid residues 68 to 71), which is converted into KKK in crm2-1. The deduced polypeptide of T13K14.80 also contains a nuclear targeting sequence (KRKK, amino acid residues 56 to 59) in the corresponding position (KKKK) of the CRM2 protein (data not shown). Motif analysis using the Pfam HMM database revealed a region of the CRM2 protein between amino acids 11 and 85 that displays similarity with the double-stranded RNA-binding motif (Fig. 5C).

Because we detected no positive signals of CRM2 expression by RNA gel blot analysis, we carried out RT-PCR to determine where the CRM2 gene is expressed. The CRM2 transcripts were mainly detected in young seedlings, root tissue and flower buds with shoot apices (Fig. 7). Similar expression patterns were also observed on the T13K14.80 gene (Fig. 7).

![Fig. 7 RT-PCR analysis of CRM2 and its neighboring homolog T13K14.80. Total RNA was isolated from 7-day-old seedlings (Se), root tissue (R), rosette leaves (L), stem internodes (St), flowers containing shoot apical meristems (F) and siliques (Si). One µg of total RNA was used in each reaction. The PCR products were blotted onto nylon membranes and hybridized with each labeled probe. As a control, the TUBULIN β9 (TUB9; Smustad et al. 1992) transcript was amplified.](image)

### Discussion

There is an astonishing variety of forms of flower-bearing branching systems, which contain different modifications of the same basic plan often observed within the same family. As one approach toward unravelling the molecular basis for these divergent forms of inflorescence in flowering plants, we focused on the inflorescence morphology in *A. thaliana*. As described in detail by Weberling (1989), the raceem inflorescence of the family Cruciferae, to which Arabidopsis belongs, is characterized by elongation of stem internodes of its main axis and development of pedicels in each flower. Although *er* and *crm2* mutant inflorescences take on the form of a raceem at later stages of development, these continuously keep a corymb-like flattened arrangement of flower buds at the tip. Our analysis revealed that the corymb-like form in *crm2* is generated by an essentially different mechanism from that in *er*.

Fig. 8 illustrates how corymb-like phenotypes are manifested in these mutants. Because the timing of bolting and flower opening was normal in *er*-105, which represents a putative null allele (data not shown), the *er* phenotype is primarily attributable to a reduction in the pedicel length. On the other hand, the concurrent delay in flower organ development and bolting in *crm2-1* resulted in the accumulation of flower buds at the tips of inflorescences. Although we could not detect any differences between *AP1* expression profiles in the wild type and *crm2-1*, *crm2-1* produced more cauline leaves than did the wild type, suggesting that the timing of floral induction may be only slightly delayed. These phenotypes might be interpreted, at least in part, by the delayed cell division rate at the cellular level. It is less likely that the rate of initiation of flower primordia is increased in *crm2-1*, because the total number of leaves and flowers was unchanged in young flowering plants of the mutant compared to wild-type plants of the same day (Table 1). However, the interpretation of the *crm2* phenotype is somewhat complicated by the fact that the rate of production of stage 13 flowers was higher in the mutant than that in the wild type (Fig. 2B). Assuming that the rate of initiation of flower primordia is normal in *crm2-1*, this result suggests that the duration of flower organ development may be gradually restored in late flowers and, accordingly, the phenotype must become moderated. To clarify this point, further analysis of the development of individual flowers will be needed. Smyth et al. (1990) defined 12 stages of Arabidopsis flower development from initiation until the bud opens. Although the possibility that the duration of certain stages in *crm2* may be particularly prolonged cannot be ruled out, we observed no obvious accumulation of flower buds of specific stages in the mutant bud cluster (Fig. 1, 4), suggesting that the *crm2* mutation may affect all stages of flower development.
The *crm2* phenotype suggests a tight continuity between internode elongation and flower organ development in Arabidopsis. However, considering the fact that growth of stem internodes can occur without flower development in the *leafy* mutant of Arabidopsis (Schultz and Haughn 1991) and that some mutants have been identified with a defect in internode elongation but not in flower organ development (Fridborg et al. 1999, Hanzawa et al. 2000), a causal relationship between the delay in bolting and that in flower organ development is unlikely. Reduction in the final length of an individual internode in *crm2-1* (Table 1; Fig. 3C, D) may reflect the effect of the mutation on the elongation rate of stem internodes after bolting (Fig. 2C). However, as a consequence of flower production for a prolonged period, which may be related to reduced fertility, the final stature of *crm2* plants is comparable to or higher than that of the wild type. Taken as a whole, CRM2 is likely to be involved in the acceleration of growth of flower organs and stem internodes. It is well known that gibberellins (GAs) play a critical role in many aspects of plant development, including internode elongation and flower opening, as a plant growth regulator (Pharis and King 1985). Although *crm2* plants do not exhibit pleiotropic phenotypes caused by GA deficiency and the *crm2* phenotype cannot be restored by exogenous application of GAs (data not shown), suggesting that *crm2* plants have normal levels of endogenous GAs, the possibility cannot be excluded that CRM2 may be involved in GA signal transduction pathways.

We cloned the CRM2 gene by a chromosome walk. Final proof of identification of the CRM2 gene was obtained by complementation of the *crm2* mutant phenotype with the wild-type gene. RT-PCR experiments revealed that the CRM2 transcripts are present mainly in seedlings, roots and flower buds. We could not detect any signals of the CRM2 transcript by in situ hybridization analysis. Given the mutant phenotype, CRM2 may be expressed in shoot and root meristems, although the *crm2-1* allele showed normal growth of the root tissue (data not shown). The *crm2-1* allele contains a 3-bp deletion corresponding to one amino acid that is located in a putative nuclear localization signal (NLS) of the deduced protein. Expression of the mutated transcript was detected in *crm2-1* plants (data not shown), suggesting that it may be translated. It remains to be determined whether the CRM2 protein is indeed targeted to the nucleus or not. If so, deletion of the NLS in *crm2-1* might prevent nuclear localization of the mutated CRM2. Whatever the functional significance of the amino acid deleted in *crm2-1* is, the possibility that the mutated CRM2 protein still retains its function to some degree and results in the observed phenotype cannot be ruled out. A homologous sequence was found to be present next to the CRM2 locus. This raises another possibility that the growth of internodes and the development of flowers in *crm2-1* may be accomplished by redundant functions of this homologous gene. Identification of additional mutant alleles of CRM2 and its homolog will be of considerable importance in defining the functions of these gene products during inflorescence development. Motif analysis of the CRM2 protein also shows homology to the double-stranded RNA-binding motif at the N-terminal region. Double-stranded RNA-binding proteins are involved in diverse cellular functions and provide further examples of post-transcriptional gene regulation by RNA-binding proteins (Burd and Dreyfuss 1994). It remains to be determined whether CRM2 binds double-stranded RNA or not.

In conclusion, our study revealed the importance of the timing of bolting and the balance between the rate of flower primordia initiation and that of flower opening in determining the inflorescence morphology. Now that the CRM2 gene has been cloned, it should provide a useful tool for further study and genetic manipulation of the inflorescence shoot architecture of other plant species.

**Materials and Methods**

**Plant material and growth conditions**

The original *crm2-1* allele was identified among the M2 progeny of fast neutron-irradiated seeds in the Columbia (Col) ecotype (Lehle Seeds, Round Rock, TX, U.S.A.), as previously described (Komeda et al. 1998). The mutant was backcrossed more than three times to the wild type before analysis. For the *er* mutant, the *er-105* allele in the Col background (Torii et al. 1996) was used for phenotypic and genetic analyses.

All plants were grown on rockwool bricks supplemented with a vermiculite in a growth chamber at 22°C under illumination with white fluorescent light. The photoperiodic cycle was 16 h light/8 h dark of long-day conditions.

**Microscopy**

For light microscopy, tissues were fixed in FAA (45% [v/v] ethanol, 5% [v/v] formaldehyde, and 5% [v/v] acetic acid) overnight at room temperature, dehydrated in a graded ethanol series, and embedded in Technovit 7100 resin ( Heraeus Kulzer GmbH, Wehrheim, Germany). Sections, 5 μm thick, were cut on a microtome (Yamato-Koki, Anaka, Japan), attached to slide glass, and stained for 30 s in a 0.1% (w/v) toluidine blue solution. They were examined and photographed using a microscope (Nikon, Tokyo, Japan).

For SEM, samples were fixed in FAA and dehydrated in a graded ethanol series and isoamyl acetate. They were then critical-point-dried with liquid CO₂. The dried materials were mounted and coated with gold-palladium in a sputter coater (Hitachi, Tokyo, Japan). Specimens were viewed under a scanning electron microscope (JEOL, Tokyo, Japan) with an accelerating voltage of 25 kV.

**Positional cloning**

The CRM2 locus was mapped on chromosome IV by segregation analyses using cleaved amplified polymorphic sequence (CAPS) markers (Konieczny and Ausubel 1993) and simple sequence length polymorphism (SSLP) markers (Bell and Ecker 1994). Further fine mapping experiments delimited the locus to BAC clones T13K14 (accession number AL080254) and F21C20 (accession number AL080254). The primers we designed for CAPS on these BAC clones (see Fig. 5A) and restriction enzymes used were as follows: T13K14-10-MseI-1, 5'-ATTCG AGCAA CATGA GTGCC-3; T13K14-10-MseI-2, 5'-TCTCG AGCTG CAGAT TCACG-3; T13K14.80-HapII-1, 5'-TCTCC ATTTC CTCTT TGACC-3; T13K14.80-HapII-2, 5'-TAAGT AGGCT TTTTG ACGAG-3; T13K14.160-Rsal-1, 5'-ACATC CTCAA GAACA TTAAG C-3; T13K14.160-Rsal-2, 5'-ACCCG TCTTG AATT
ATCAT CTC-3; T13K14.180-Del-1, 5'-ATGGC GGGCA TGCAA CTAC-3; T13K14.180-Del-2, 5'-TCTTC CTGTG ATTC TCCAGC-3; T7J7.10-EcoR1-1 (accession number AL021960); 5'-TGGTA GTTAT GCCGGT GCCAG-3; T7J7.10-EcoR1-2, 5'-AGCTG CAACT TATCT CGCGG-3; T6K22.80-Ear1-1 (accession number AL031187); 5'-CATGA ACTTA TGATG ATCC AA-3; and T6K22.80-Ear1-2, 5'-GCCAT TGATG ATCC AG-3.

Nucleotide sequences of putative open reading frames within the T13K14 region in crm2-1 were determined by using an ABIPRISM377 DNA Sequencer (Applied Biosystems/Perkin Elmer, CA) and were compared to the wild-type sequences in GenBank using the BLAST program (www.ncbi.nlm.nih.gov/BLAST/).

**RNA extraction and RT-PCR analysis**

Total RNA was extracted according to the SDS-phenol method (Takahashi et al. 1992). RT-PCR was conducted by using a Takara RNA LA PCR Kit (AMV) Ver.1.1 (Takara, Kyoto, Japan) with 1 μg of total RNA. For the first-strand cDNA synthesis, an oligo (dT)-adaptor was annealed in 0.8% (w/v) agarose gels, transferred to GeneScreen Plus (NEN Life Science Products, Boston, MA, U.S.A.). Blots were hybridized at 42°C for 16 h in 6X SSPE and 1% (w/v) SDS for 3 h and 1% (w/v) SDS for 20 min, and once with 0.1x SSC for 20 min.

**Plant transformation**

For transgenic complementation, a 6-kb fragment containing the CRM2 genomic sequence was amplified by PCR using the wild-type genomic DNA as a template and sequence-specific primers with regard to the CaMV 3SS RNA promoter. The resulting construct was introduced into wild-type plants (Col).

**Acknowledgments**

We thank Masashi Hashimoto for help with plant transformation experiments and Masahiko Ozaki for help with mapping crm mutations. This work was supported in part by a grant-in-aid from the Ministry of Education, Science and Culture of Japan.

**References**


Schult, E.A. and Haugln, G.W. (1991) LEAFY, a homeotic gene that regulates...

(Received December 10, 2001; Accepted January 4, 2002)