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Isolation and functional analysis of the *CjNdly* gene, a homolog in *Cryptomeria japonica* of *FLORICAULA/LEAFY* genes

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Summary We report the isolation and characterization of *CjNdly*, a homolog in Japanese cedar (*Cryptomeria japonica* D. Don) of the *FLORICAULA/LEAFY* (*FLO/LFY)* genes. We determined the entire nucleotide sequence of *CjNdly*, including short 5′- and 3′-untranslated regions. The deduced amino acid sequence was similar to those of the products of the *FLO/LFY* genes from other species. The nucleotide sequence showed the closest homology to that of the *NEEDLY* gene in *Pinus radiata* D. Don. Although no proline-rich region has been reported previously in homologous gene products from gymnosperms, we found such a region at the amino-terminal end of the deduced amino acid sequence encoded by *CjNdly.* We detected the expression of *CjNdly* in both reproductive and vegetative tissues and organs of *C. japonica*. Heterologous expression of *CjNdly* in transgenic tobacco plants induced precocious flowering of regenerating shoots on agar-solidified medium and flowers with an abnormal phenotype, namely, petal-like stamens. Our findings suggest that the *CjNdly* gene may have important roles in flower development in Japanese cedar, resembling those of its angiosperm homologs.

Keywords: flower development, Japanese cedar, meristem identity gene, NEEDLY gene, pollinosis.

Introduction

The development of plants is characterized by juvenile and adult phases. After a relatively short juvenile phase, herbaceous annual plants progress to the adult phase, during which flowering occurs. By contrast, perennial woody plants, have a much longer juvenile phase, in some cases persisting for decades, which poses a major obstacle to the genetic improvement of trees. Thus, biotechnological control of flowering should facilitate breeding of woody plants.

Japanese cedar (*Cryptomeria japonica* D. Don) is one of the most important coniferous tree species in Japan. However, pollinosis caused by Japanese cedar is a cause of serious medical and social problems (Yasueda et al. 1983). In Japan, many strategies are being developed to deal with this problem. The efforts of biologists have included selection of pollen-free mutants of Japanese cedar and the generation of transgenic rice plants whose grains have immunotherapeutic effects (Takagi et al. 2005). To supplement these approaches, an understanding of the genetic mechanisms underlying the transition from the juvenile to the reproductive phase in Japanese cedar may accelerate efficient selection of pollen-free elite clones.

The *LEAFY* (*LFY*) gene plays a central role in the flower-development pathway as a flower meristem-identity gene. *LFY* was isolated from *Arabidopsis thaliana* (L.) Heynh (Weigel et al. 1992) and identified as a homolog of *FLORICAULA* (*FLO*) from *Antirrhinum majus* L. (Coen et al. 1990). The angiosperms and gymnosperms examined to date possess a single *LFY* gene and two distinct *LFY* genes, respectively. Stimulation of the expression of *LFY* in meristems during plant development is a key step in the transition to reproductive development (Blázquez et al. 1997). *Arabidopsis* plants with mutations in the *LFY* gene form leaf-like shoots at the sites of flower formation in wild-type plants and they are unable to make the transition to floral development. By contrast, overexpression of *LFY* in transgenic plants can induce early flowering (Weigel and Nilsson 1995, Blázquez et al. 1997). When aspen (*Populus tremuloides* Michx.) was transformed with an *LFY* gene, the time to flowering was reduced from years to months (Weigel and Nilsson 1995). In the case of gymnosperm trees, *NEEDLY* (*NLY*), whose gene is a homolog of *FLO/LFY* genes, was isolated from *Pinus radiata* D. Don. (Mouradov et al. 1998). The *NLY* gene belongs to a different clade from typical *LFY* homologs on phylogenetic trees. Both *LFY* and *NLY* genes and their homologs have been isolated from various plants, including woody plants such as aspen, apple, eucalyptus, pine and tropical cedar (Mellerowicz et al. 1998, Mouradov et al. 1998, Southerton et al. 1998, Rottmann

et al. 2000, Wada et al. 2002, Dornelas and Rodriguez 2006). However, to our knowledge, there are no reports of *LFY* homologs in Japanese cedar.

We isolated the coding region of the *CjNdly* gene, a *LFY* (*NLY*) homolog from *C. japonica*, and examined its pattern of expression in Japanese cedar by reverse transcription-polymerase chain reaction (RT-PCR). In addition, we examined the function of the gene in transgenic tobacco plants, in which a chimeric *CjNdly* gene was overexpressed under the control of a constitutive promoter. We discuss the possible roles of the *CjNdly* gene in gymnosperms, as well as the potential biotechnological utility of the gene for the efficient production of lumber and in efforts to limit Japanese cedar pollinosis.

Materials and methods

Plant materials

Japanese cedar (*C. japonica*) trees growing in an experimental field of the Forestry and Forest Products Research Institute (Ibaraki, Japan, 36°0′ N, 140°0′ E, 22 m elevation) were selected for study. Developing apices of lateral shoots were excised at a distance of 2.0–3.0 cm from the tip and collected on May 23, 2000. The excised apices were immediately frozen in liquid nitrogen and stored at about –195 °C. For RT-PCR, total RNA was prepared from the samples as described by Futamura et al. (2006). *Nicotiana tabacum* cv. Samsun NN was used for transformation as described elsewhere (Yamada et al. 2003).

Bacterial strains and vectors

We used the pT7blue T-Vector (Novagen, Darmstadt, Germany) and the pGEM-T Easy Vector (Promega, Tokyo, Japan) for cloning and analysis of nucleotide sequences. *Escherichia coli* strains MV1190 and DH5α were used as recipients for transformation, genetic manipulations and nucleotide sequencing. *Escherichia coli* strain HB101 was used as the recipient in bacterial transformations, and pRK2013 was used as the helper plasmid for triparental mating (Bevan 1984). *Agrobacterium tumefaciens* strain LBA4404 was used for the transformation of tobacco.

Isolation of total DNA and total RNA from C. japonica

Total DNA and total RNA were isolated from frozen tissues of *C. japonica* as follows. A 0.5 g sample of plant material was placed in liquid nitrogen and powdered. After the liquid nitrogen had evaporated, 5 ml of washing buffer (0.1 M HEPES-buffered saline (pH 8.0), 2% 2-mercaptoethanol, 1% polyvinylpyrrolidone (M.W. 40,000) and 0.05 M ascorbic acid) was gradually added to the powdered sample in a centrifuge tube. After gentle mixing and rotation of the tube for 10 min at room temperature, the mixture was centrifuged at 11,000 *g* for 5 min at room temperature. The DNA in the pellet was extracted by a modified version of the CTAB method, as described by Wagner et al. (1987) and Bousquet et al. (1990). Total RNA was prepared with an RNeasy Plant Mini Kit (QIAGEN, Tokyo, Japan).

Amplification by PCR and sequencing

To obtain a fragment of the *CjNdly* gene, we performed PCR and inverse-PCR using total DNA from *C. japonica* and Takara Ex Taq (TaKaRa BIO, Otsu, Japan). We amplified a DNA fragment that corresponded to the putative third exon of the *CjNdly* gene. Degenerate primers for PCR were designed on the basis of the amino acid sequences, MRHYVHCYA and WYVPTKLRQ, that are conserved in FLO/LFY-like proteins (Figure 1). The nucleotide sequences of the primers were: primer-FW1, sense primer, 5′-ATGMGRCATTATGTKCAYTGYTATGC-3′; and primer-RV1, antisense primer, 5′-TGHCKWARTTTWGTGGGRACATACCA-3′. Amplification by PCR was performed under the following conditions: 5 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C; and final extension at 72 °C for 6 min. We succeeded in amplifying a DNA fragment of 225 bp. This fragment, without the primer sequences, was designated *cjfll-1* (DDBJ Accession no. AB037662).

To obtain 5′- and 3′-sequences adjacent to *cjfll-1*, we first performed inverse-PCR with primers corresponding to the fragment. We digested total DNA with the restriction enzyme *Kpn* I. Then, after self-ligation, the fragment was used as the template for PCR. Primers for PCR corresponded to the putative third exon and had the following nucleotide sequences: 5′-TGGGACATAGAGGGTGTTTTTAATAGAAATGAAAA-G-3′ (CJ-F2-F); and 5′-TATATTCTTCTGAGGTGATTGGATTGCTCATTGTCT-3′ (CJ-F2-R). Amplification by inverse-PCR was performed under the following conditions: 5 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 66 °C and 1 min at 72 °C; and final extension at 72 °C for 5 min. We obtained an amplified DNA fragment of about 3500 bp that contained the putative first intron, second exon and 3′-untranslated region, but not the putative first exon of the *CjNdly* gene.

To identify the sequence of the first exon, we performed rapid amplification of cDNA ends (5′-RACE) with total RNA that had been isolated from the shoot apex of a young tree. First-strand cDNA was synthesized with a 5′-RACE kit (5′-RACE Version 2.0; GIBCO-BRL Life Technologies, Rockville, MD), and a DNA fragment that contained the putative first exon was amplified with the gene-specific primers: GSP1, 5′-TATATTCTTCTGAGGTGATTGGATTGCTCA-3′; GSP2, 5′-GCCACCTTTGCAGGTTTTGATATTCAATGG-3′; and GSP3, 5′-GATCACTTGCCCTCACATCCTTAGAGGTGC-3′. GSP1 was based on the sequence that corresponded to the putative third exon, and both GSP2 and GSP3 were based on the sequence of the putative second exon. Synthesis of first-strand cDNA from total RNA and subsequent PCR with the gene-specific primers were performed according to the instructions from Gibco-BRL. The 5′-RACE procedure yielded an amplified DNA fragment of about 700 bp that included the putative first exon and the 5′-untranslated region. Finally, we amplified DNA fragments that contained all of the putative exons and introns by PCR using the first-strand cDNA and total DNA prepared from the shoot apex. The nucleotide sequences of the primers used for PCR were: EX1F3, 5′-TAAATTGTTTTAATGGATGGTGAG-3′; and EX3R1, 5′-CTATTGGCATTCTTTGCTTCTCTC-3′.

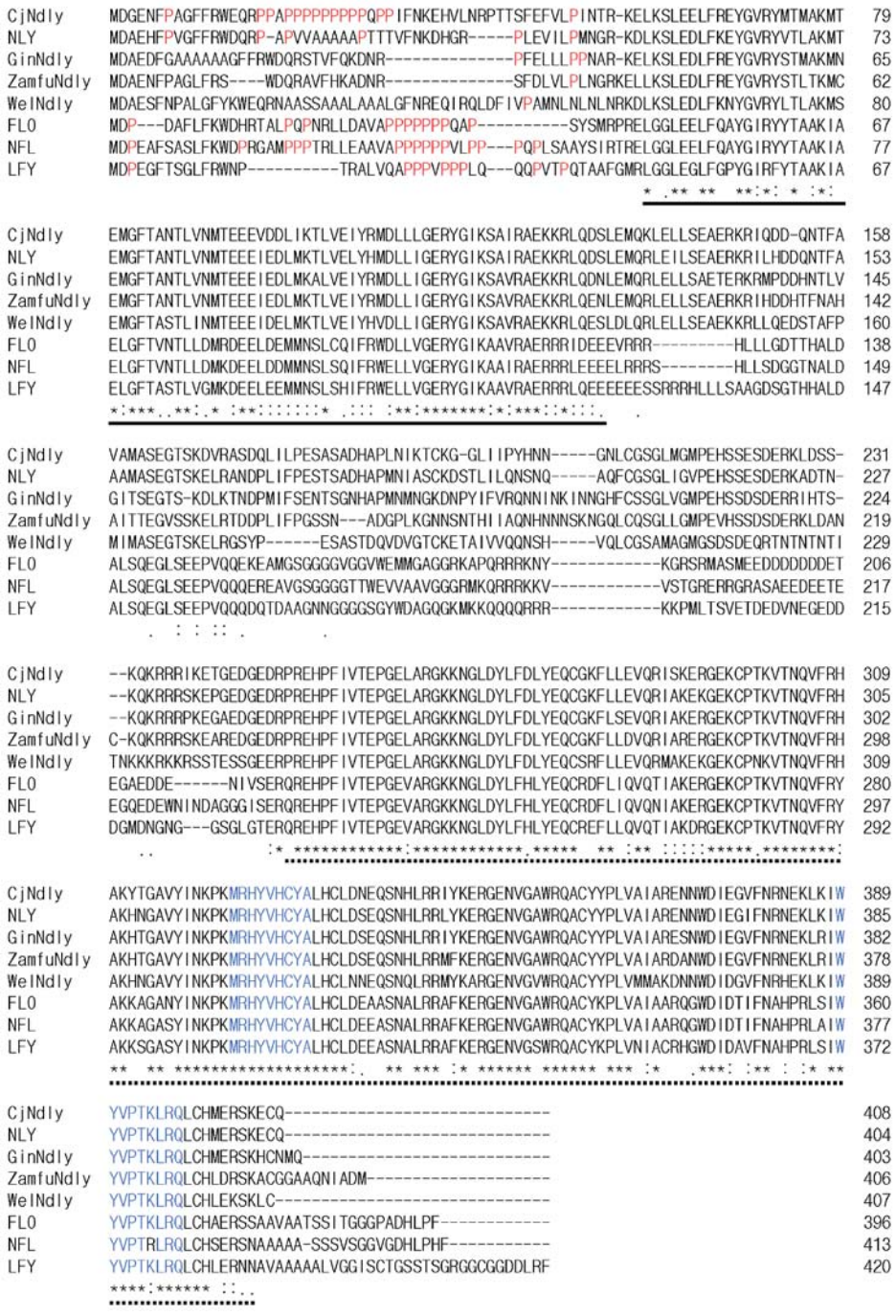

Figure 1. Comparison of sequences of deduced FLO/LFY proteins: CjNdly from *Cryptomeria japonica*; NEEDLY (NLY) from *Pinus radiata*: GinNdly from *Ginkgo biloba*; ZamfuNdly from *Zamia furfuracea*; WelNdly from *Welwitschia mirabilis*; FLO from *Antirrhinum majus*; and NFL (NFL1) from *Nicotiana tabacum*. Asterisks indicate identical residues; colons indicate conserved residues; and periods indicate semi-conserved residues. Black and dashed underlining indicates amino- and carboxyl-terminal conserved regions, respectively. Proline residues at the amino-terminal end are indicated in red. Conserved sequences used for the design of PCR primers are indicated in blue.

Each PCR product was cloned into the pT7blue T-Vector or the pGEM-T Easy Vector. The cloned products of PCR were sequenced with an ABI PRISM BigDye Terminator Cycle Sequencing FS Ready Reaction Kit and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Tokyo, Japan). To exclude errors that might have arisen during PCR, we sequenced at least five clones of each fragment that had been obtained by PCR in every case. The deduced amino acid sequence derived from the cloned *CjNdly* fragment was aligned and compared with those deduced from other *FLO/LFY* genes using the ClustalW and TreeView software programs.

Analysis of expression by semi-quantitative RT-PCR

We performed semi-quantitative RT-PCR with total RNA from various tissues of Japanese cedar as described by Futamura et al. (2006). Before synthesis of first-strand cDNA, total RNA was treated with RQ1 RNase-free DNase (Promega Corporation, Tokyo, Japan) to remove any contaminating total DNA. Reverse transcription was performed with a Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics, Mannheim, Germany) with the oligo(dT) primer supplied with the kit. We used 600 ng of total RNA for the synthesis of first-strand cDNA in a 20 µl reaction mixture and one-tenth of the reaction

mixture as the template for subsequent PCR with gene-specific primers. To analyze *CjNdly* expression, we used Cjndly-1 (5′-TTGATCTCTATGAGCAGTGTGG-3′) and Cjndly-2 (5′-GGCATTCTTTGCTTCTCTCC-3′) as primers. We monitored the expression of the putative 40S ribosomal protein S2 by RT-PCR, with FWD (5′-GTGTAAGGTCACGGGCAAGT-3′) and REV (5′-AAGGCGATTTTGAGAAACGA-3′) as primers. The conditions for PCR were: incubation for 3 min at 94 °C; and then 32 cycles for *CjNdly* or 25 cycles for the gene for the 40S ribosomal protein S2 of 30 s at 94 °C, 30 s at 53 °C and 45 s at 72 °C.

Construction of plasmids for transformation of tobacco plant

The *Xho* I-*Bam*H I fragment of *CjNdly*, including three putative exons and two putative introns, was generated by PCR with primers 5′-cgctcgagATGGATGGTGAGAATTTCCCT-3′ and 5′-cgggatccCTATTGGCATTCTTTGCTTCT-3′, where small letters and underlining indicate an artificial *Xho* I site and an artificial *Bam*H I site, respectively. This fragment was cloned into the pT7blue T-Vector to yield pT7CjNdly, which was then sequenced. The fragment of *CjNdly* from pT7CjNdly was cloned into pART7 (Gleave 1992), which had been digested with *Xho* I and *Bam*H I, and the product was designated pART7CjNdly. We cloned a fragment that consisted of the CaMV 35S promoter, the *CjNdly* fragment and the 3′-untranslated region of a gene for octopine synthase (ocs) from pART-7CjNdly, which had been digested with *Not* I into *Not* I-digested pART27 to generate pART27CjNdly, which we used for transformation of tobacco.

Transformation of tobacco

Leaf discs from *N. tabacum* were transformed as described previously (Yamada et al. 2003). Transformed plants were grown on Murashige and Skoog medium with 100 mg l^{-1} kanamycin under long-day conditions (18 h photoperiod) at 25 °C. Antibiotic-resistant plants were maintained as transgenic lines and plantlets were transplanted to soil.

Results

Cloning and analysis of the expression of CjNdly

We succeeded in isolating the coding region of a *FLO/LFY* homolog from Japanese cedar by PCR, inverse-PCR and 5′-RACE. We postulated, before attempting to isolate the gene, that a homolog(s) of *FLO/LFY* would be present in the genome of Japanese cedar and that the structure of the gene (number of exons and positions of intron in the gene) would be similar to that in other species. We designed one set of degenerate primers for PCR on the basis of conserved sequences (as shown in Figure 1) in the third exon of two *FLO/LFY* homologs, namely, *NLY* and *PRFLL* (Mellerowicz et al. 1998, Mouradov et al. 1998). With these primers and total DNA from Japanese cedar as the template, we amplified a 225-bp fragment of DNA, which we designated *Cjfll*. The nucleotide sequence of this fragment was similar to the corresponding regions of the two *FLO/LFY* homologs. We used the sequence of this fragment to design additional primers for inverse-PCR, which yielded a fragment of about 3500 bp. The sequence of this fragment suggested that it corresponded to the second and third exons of the *FLO/LFY* homolog of Japanese cedar. We cloned an additional DNA fragment (705 bp) that corresponded to the first exon by 5′-RACE. After a final cloning, we succeeded in amplifying a DNA fragment of 3730 bp that contained three putative exons and two putative introns, which we designated *CjNdly* (DDBJ Accession No. AB074568).

The coding region of *CjNdly* was 1227 bp long and encoded 408 putative amino acids. The position and length of each intron were predicted from comparisons with sequences of two amplified DNA fragments, namely, one amplified from total DNA as template and one amplified with first-strand cDNA as template. The positions of the two putative introns of *CjNdly* corresponded to those of introns in other *FLO/LFY* genes. The first intron was 2154 bp long and was located between the first and second nucleotides of the 165th codon (Gly). The second intron was 349 bp long and was located between the third nucleotide of the 301st codon (Lys) and the first nucleotide of the 302nd codon (Val). Alignment of the amino acid sequences deduced from the *CjNdly* gene and other *FLO/LFY* genes revealed that the amino- and carboxyl-terminal conserved regions were also present in the CjNdly protein (Figure 1). Moreover, the deduced amino acid sequence of CjNdly included a proline-rich region in its amino-terminal end, as observed in the products of some angiosperm *FLO/LFY* genes, but not, as far as we know, in those of gymnosperm genes.

We examined the expression of *CjNdly* by RT-PCR with total RNA from several tissues and organs of *C. japonica* (Figure 2). The transcript was detected at high level in seeds, cotyledons, roots of seedlings and in both developing and mature female strobili. In addition, low but unambiguous expression was evident in leaves and roots of saplings and in developing male strobili. By contrast, no transcripts were detected in stems of saplings, mature male strobili or pollen. These results indicate that *CjNdly* is expressed in both reproductive and vegetative tissues and organs.

Abnormal development of flowers on transgenic tobacco plants that expressed a CjNdly transgene

To analyze the biological function of the *CjNdly* gene, we expressed the open reading frame of the PCR-amplified fragment of *CjNdly*, which included the two introns, in transgenic tobacco. We inserted the sequence into an expression vector between the CaMV 35S promoter and an ocs-3′ terminator, and then introduced the entire construct into tobacco by *Agrobacterium*-mediated transformation. After several rounds of selection, we obtained 36 independent T_0 plants that harbored the chimeric *CjNdly* gene. Some of the transgenic plants had normal vegetative morphology without abnormal floral phenotype. However 22 of the selected transgenic plants exhibited phenotypic changes, as indicated in Figure 3. Although the structure of flowers varied to some extent among the *CjNdly*-transgenic plants, the most typical differences in flower mor-

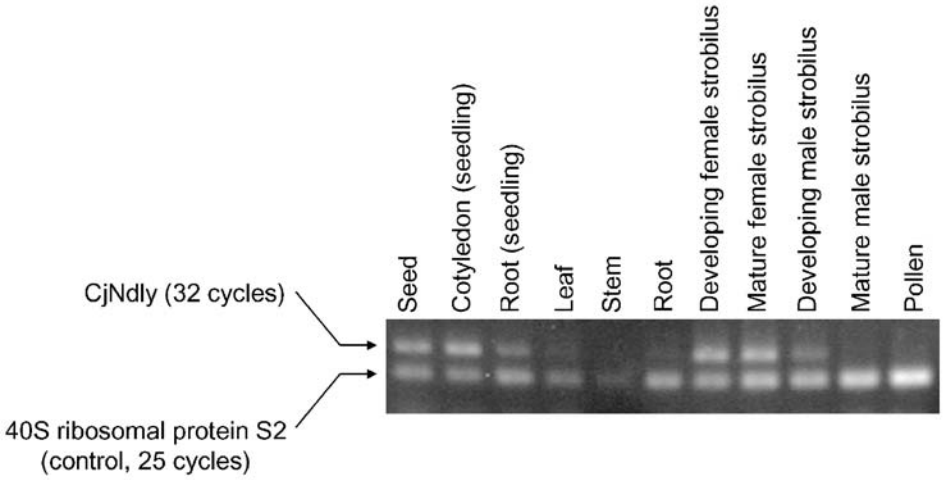

Figure 2. Analysis by RT-PCR of the expression of *CjNdly* in various tissues and organs of *Cryptomeria japonica*. Transcripts of the gene for 40S ribosomal protein S2 from each tissue and organ were amplified as controls.

phology between wild-type and *CjNdly*-transgenic plants were evident in the structures of their stamens. The *CjNdly*-transgenic plants had petal-like stamens in most of their abnormal flowers (Figure 3B). Occasionally, some of the stamens had fused with one another (Figure 3D). As a result, the abnormal flowers of transgenic plants sometimes had three or four petals compared with the five petals of wild-type flowers. In rare cases, two flowers had fused with each other (Line 29; data not shown) and secondary flowers grew from the axils of petals (Figure 3E). Some of these features in the abnormal flowers were also observed by Shindo et al. (2001) in transgenic *Arabidopsis* plants that expressed a chimeric *GpLFY* gene, a *FLO/LFY* homolog in the gymnosperm *Gnetum parvifolium* (Warb.). Furthermore, we observed solitary flowers immediately after the differentiation of unrooted shoots that regenerated from transgenic calli during culture on agar-solidified medium (Figure 3F). These results indicate that ectopic expression of *CjNdly* in transgenic tobacco plants influences both the temporal and morphological regulation of flower formation.

Discussion

We succeeded in cloning the *CjNdly* gene from *C. japonica*. This gene is a homolog of *FLO/LFY* genes that encode for transcription factors regulating floral-meristem identity. Several *FLO/LFY* genes have been isolated from gymnosperms, and the deduced proteins constitute a separate clade that comprises two divergent groups (Gymnosperm I and Gymnosperm II clades; Frohlich and Parker 2000, Himi et al. 2001). Gymnosperm I is a sister clade of angiosperm *FLO/LFY* genes, and Gymnosperm II is the sister clade of Gymnosperm I and the angiosperm clade. As indicated in Figure 4, CjNdly is closely homologous to NLY (Gymnosperm II), which was derived

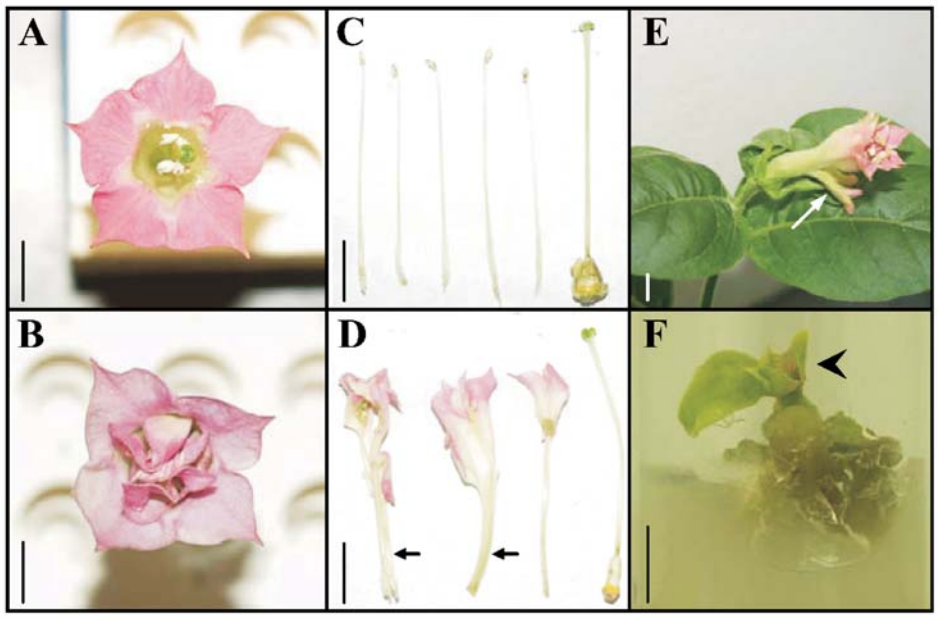

Figure 3. Phenotypes of flowers on transgenic tobacco plants that harbored a chimeric *35S::CjNdly* construct. (A) Flower of wild-type plant. (B) Flower of a typical transgenic plant (Line 31). (C) The pistils and stamens from the flower shown in A. (D) The pistils and abnormal petal-like stamens from the flower shown in B. Two petals were fused in some transgenic flowers (black arrows). (E) Secondary flower (white arrow) growing from the axil of a petal (Line 44). (F) A flower (arrowhead) that emerged directly from regenerating tissue. Bars = 1 cm.

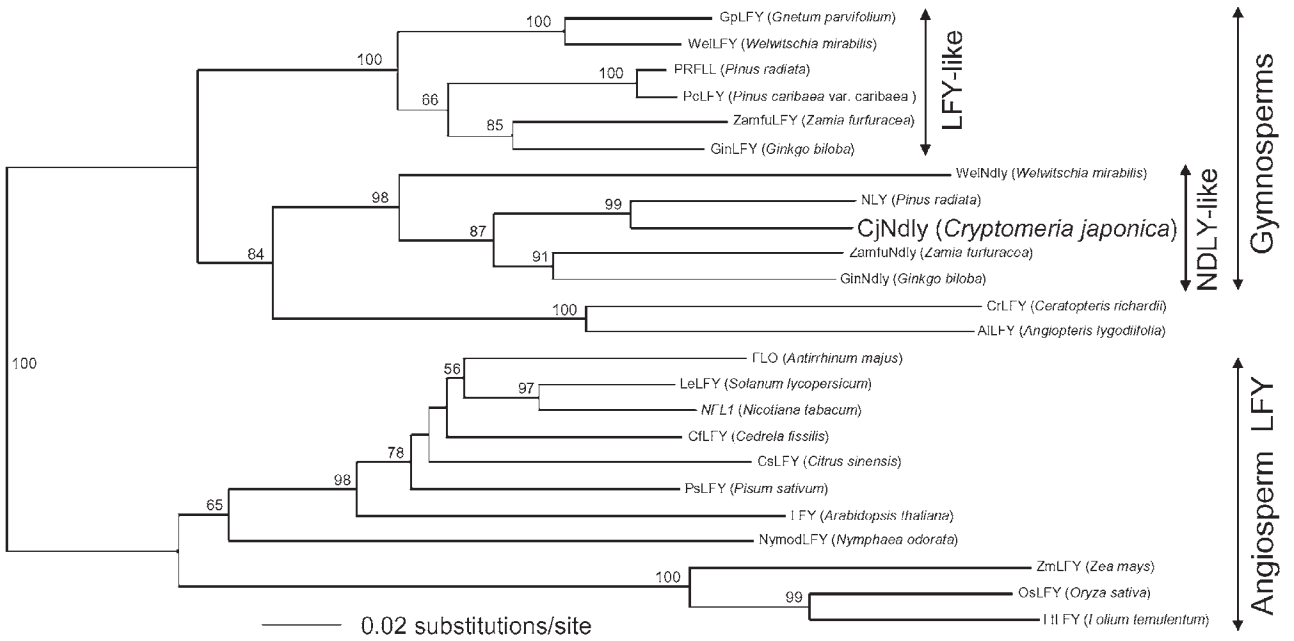

Figure 4. Phylogenetic tree of FLO/LFY sequences. The deduced amino acid sequence of CjNdly was compared with those of other FLO/LFY homologs. Only sequences with bootstrap support greater than 50% are indicated.

from *Pinus radiata*. It has been suggested that *FLO/LFY* is a single-copy gene in all extant angiosperms studied to date. By contrast, gymnosperm genomes contain two *FLO/LFY*-like genes. In the *P. radiata* genome, there are two homologs, *NLY* and *PRFLL*. Recently, we have isolated a partial fragment of an additional clone of *FLO/LFY* homolog (*CjLFY*) from *C. japonica* by RT-PCR (648 bp, data not shown). Phylogenetic analysis of the deduced amino acid sequence of the clone revealed its classification in Gymnosperm I, and it is related closely to PRFLL from *P. radiata*. (84.7% identity to PRFLL at the amino acid level). This result indicates that, like other gymnosperms, the Japanese cedar genome has two *FLO/LFY* homologs.

Most angiosperm FLO/LFY proteins have a proline-rich region within about the first 40 amino acids. In addition, two conserved regions, located at the amino (N) and carboxyl (C) regions of the proteins, respectively, are present both in angiosperm and gymnosperm FLO/LFY proteins (Figure 1). Even though, to our knowledge, no proline-rich region has previously been found in gymnosperms, the *CjNdly* gene encodes an apparent proline-rich region at the N-terminus of its product (proline residues in the N-terminal end are indicated in red in Figure 1). Coen et al. (1990) suggested that the proline-rich region plays an important role in transcriptional activation. Recently, Maizel et al. (2005) revealed that, in some LFY homologs, it is the C-terminal domain (dashed underlining in Figure 1), and not the proline-rich region, that is the minimal functional region for binding to the enhancer sequences of floral homeotic genes, such as *APETALA1* and *AGAMOUS*. Furthermore, it appears that a conserved histidine residue (His^{308} in LFY) in the C-terminal domain plays an essential role in DNA-binding activity (Maizel et al. 2005). This residue is also conserved in CjNdly (His^{325}), as it is in other FLO/LFY homologs with the exception of a moss FLO/LFY homolog, PpLFY, which is unable to bind to a canonical LFY-binding site in vitro (Maizel et al. 2005). Although the role of the proline-rich region remains unclear, several features observed in the deduced amino acid sequence of CjNdly indicate that this protein might act as a regulator of transcription, as do other angiosperm and gymnosperm homologs of FLO/LFY.

We used semi-quantitative RT-PCR to analyze the expression of *CjNdly* in various tissues and organs. In previous studies, the expression of some *FLO/LFY* homologs was found in the reproductive and vegetative tissues of both angiosperms and gymnosperms. In the case of *P. radiata*, *NLY* is expressed in male (pollen-corn bud) and female (seed-corn bud) organs (Mouradov et al. 1998). In addition, *NLY* transcripts were also detected in vegetative shoot buds and in needles of seedlings but not in roots. Another *FLO/LFY* homolog, *PRFLL* in *P. radiata*, which appears to be involved in the determination of male cone primordium identity, is preferentially expressed in male corn primordia (Mellerowicz et al. 1998). We found that *CjNdly* was expressed in eight of the eleven tissues and organs analyzed, which included both reproductive and vegetative tissues (Figure 2). The expression of *CjNdly* was detected in both male and female strobili, even though no expression was detected in mature male strobili. These results suggest that *CjNdly* has important roles in flower meristem identity in the development of both male and female floral organs.

We detected the strong expression of the *CjNdly* gene in

non-flowering tissues, such as seeds, cotyledons and roots of seedlings (Figure 2). The apparent expression of *FLO/LFY*-like genes in non-flowering tissues has been reported in papaya, pine, apple, pear and fern (Mouradov et al. 1998, Himi et al. 2001, Wada et al. 2002, Esumi et al. 2005, Yu et al. 2005). Although the roles of these genes in non-flowering tissues remain unknown, it is likely that potential *LFY*-target genes, without roles in flowering, are expressed in non-flowering tissues, such as roots and leaves. Several studies have shown that the expression of some genes that are not, apparently, involved in flowering is affected by ectopic expression of *LFY* in transgenic plants and calli (Wagner et al. 2004, William et al. 2004).

We introduced the *CjNdly* sequence into the tobacco genome to examine its effects on the development of flowers and to investigate the effect of a gymnosperm *CjNdly* gene on angiosperm flowering. Although we observed normal flowers on some of the transgenic plants, more than 60% of our transgenic tobacco plants had flowers with abnormal morphology. One major characteristic of the flowers on our *CjNdly*-transgenic plants was petal-like stamens. Sepals and carpels (pistils) seemed barely affected by the ectopic expression of *CjNdly.* Petaloid stamens were also observed on transgenic *Arabidopsis* plants with a chimeric *NLY* gene and with a chimeric *GpLFY* gene and on transgenic tobacco with a chimeric *NFL1* gene (Mouradov et al. 1998, Ahearn et al. 2001, Shindo et al. 2001). These results indicate that, even though the *CjNdly* gene was isolated from a gymnosperm, it can act as a meristem-identity gene in angioperms, as do the *NLY* and *GpLFY* genes isolated from gymnosperms.

Most transgenic plants with a *FLO/LFY*-like gene under the control of the CaMV 35S or *Arabidopsis* LFY promoter exhibit precocious flowering. By contrast, early flowering was not apparent in most of our transgenic plants during cultivation in soil. However, in some cases, we observed the direct formation of floral organs from regenerating tissue during the culture of calli on agar-solidified medium (Figure 3F). This type of early flowering was also observed on root explants when *Arabidopsis* was transformed with an inducible *LFY* gene and with *CaMV35S::LFY* gene (Wagner et al. 2004). These observations suggest that *CjNdly* is sufficient for the induction of flower formation in angiosperms without prior formation of vegetative organs, as is the case for *LFY.* Actual functions of *CjNdly* in *C. japonica* are still poorly understood. Complementation of the *Arabidopsis lfy* mutant with *CjNdly* and *CjLFY* may provide more information about the role of these genes.

Because the *CjNdly* gene was associated with flowering in tobacco, this gene may play a role in the flowering of Japanese cedar. An increased understanding of flower development in Japanese cedar should eventually contribute to biotechnological manipulations that will lead to production of reproductively incompetent trees (Mouvadov and Teasdale 1999). Increased vegetative growth of trees can be achieved, at least in part, by inhibition of reproductive growth, an approach that might contribute to enhanced timber production, because trees divert significant amounts of nutrients and energy to the development of reproductive organs. Furthermore, if we can control pollen production by inhibiting the development of male strobili via the control of meristem-identity genes, such as *CjNdly*, we might be able to reduce the serious problem of Japanese cedar pollinosis.

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