EgLFY, the *Eucalyptus grandis* homolog of the *Arabidopsis* gene LEAFY is expressed in reproductive and vegetative tissues

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The EgLFY gene cloned from *Eucalyptus grandis* has sequence homology to the floral meristem identity gene LEAFY (LFY) from *Arabidopsis* and FLORICAULA (FLO) from *Antirrhinum*. EgLFY is preferentially expressed in the developing eucalypt floral organs in a pattern similar to that described previously for the *Arabidopsis* LFY. *In situ* hybridization experiments have shown that EgLFY is strongly expressed in the early floral meristem and then successively in the primordia of sepals, petals, stamens and carpels. It is also expressed in the leaf primordia of adult trees. The expression of the EgLFY coding region under control of the *Arabidopsis* LFY promoter could complement strong lfy mutations in transgenic *Arabidopsis* plants. These data suggest that EgLFY plays a similar role to LFY in flower development and that the basic mechanisms involved in flower initiation and development in *Eucalyptus* may be similar to those occurring in *Arabidopsis*.

**Key words:** *Eucalyptus*, flowering, LEAFY, reproductive development.

**INTRODUCTION**

In the model species *Antirrhinum* and *Arabidopsis*, the apical meristem switches from vegetative to floral development as plants enter the reproductive phase (Coen and Meyerowitz, 1991; Hempel et al., 1994).

In *Antirrhinum* and *Arabidopsis*, the shoot apical meristem (SAM) initiates lateral primordia that develop into either shoots or flowers. The development of flowers instead of shoots is mediated by the action of floral meristem identity genes which include LEAFY (LFY) in *Arabidopsis* (Weigel et al., 1992) and its homologue FLORICAULA (FLO) in *Antirrhinum* (Coen et al., 1990). Inactivation of the FLO gene in *Antirrhinum* causes formation of indeterminate shoots in place of flowers and in *Arabidopsis* lfy mutants the structures that would normally develop into flowers develop into structures intermediate between shoots and flowers. FLO and LFY share 70% amino acid identity and each has a proline rich region and an acidic domain, which indicates their possible role as transcriptional activators (Coen et al., 1990). In *Arabidopsis*, LFY has been found to activate homeotic genes, which regulate floral organogenesis (Weigel and Meyerowitz, 1993). Both LFY and FLO are expressed in the...
floral meristem prior to initiation of floral organ primordia while expression at later stages of floral development in both species is less conserved (Coen et al., 1990; Weigel et al., 1992). In *Antirrhinum*, *FLO* expression is also observed in the leaf-like bracts which sub tend the flower (Coen et al., 1990). *LFY* might act in suppressing bract formation in wild-type *Arabidopsis* since in *lfy* mutants lack of functional *LFY* RNA leads to ectopic bract formation (Weigel et al., 1992).

In contrast to what is observed for *Arabidopsis*, the apical meristem in eucalypts (*Eucalyptus* spp, Myrtaceae) generally remains vegetative. Lateral meristems, formed in the axils of the leaves, may give rise to a leafy shoot or to an inflorescence in response to inductive environmental conditions, such as day-length and temperature, if the tree is sufficiently mature (Drinnan and Ladiges, 1991). The *E. grandis* inflorescence is determinate and converts directly to a floral meristem(s). Both the inflorescence and flower meristems are completely enveloped by a pair of bracts which protect the primordia. While eucalypt flower buds and flowers are obviously structurally different from those of *Arabidopsis* and *Antirrhinum*, the pattern and timing of organ development is similar in the three species (see figure 1). Within the bracts enclosing the eucalypt inflorescence (figure 1C), the flower is initiated on the sides of the floral meristem as four protrusions, corresponding to sepals, which enlarge, elongate and rapidly fuse, forming the outer layer of the protective structure known as the calicine operculum (Pryor and Knox, 1971; Drinnan & Ladiges, 1991; Steane et al., 1999). The four primordia from the second whorl, which normally give rise to petals in *Arabidopsis*, arise similarly in *Eucalyptus*, forming the inner (coroline) operculum (figure 1D). Stamen primordia, often in the number of several hundreds, arise in tightly packed whorls surrounding the central gynoecium and correspond to the third whorl of *Arabidopsis* and other plants. The gynoecium generally consists of four to five carpels in the innermost whorl (figure 1D). Early during reproductive development the bracts covering the flowers are shed. Depending on the *Eucalyptus* species, the calicine operculum also dehisce during early floral development (Steane et al., 1999; Drinnan & Ladiges, 1991). At anthesis, the coroline operculum is shed and the prominent stamens surrounding the single style are clearly visible (figure 1E).

As some common developmental features exist between the flower ontogenesis in eucalypts and model species such as *Arabidopsis* and *Antirrhinum*, it may be suggested that the key floral regulatory genes, described for these model species, would be conserved in eucalypts (Southerton et al., 1998a,b). Nevertheless, it is expected that these genes would display some altered patterns of expression consistent with the unique structural features of the eucalypt flower.

Orthologs of *FLO/LFY* have been cloned and characterized in several woody perennial species such as Monterey pine (*Pinus radiata*; Mellerowicz et al., 1998; Mouradov et al., 1998), *Populus trichocarpa* (Rottmann et al., 2000) kiwifruit (*Actinidia delicosa*; Walton et al., 2001) and grape vine (*Vitis vinifera*; Carmona et al., 2002). Additionally, Southerton et al. (1998) described the cloning of a *LFY* homolog from *Eucalyptus globulus* and suggested that the biological function of *LFY* may be conserved in woody species. However, its specific role in the characteristic features of tree reproductive development has not yet been elucidated. Furthermore, partial or total *FLO/LFY*-like sequences have been reported from other basal angiosperms and gymnosperms (Frohlich and Meyerowitz, 1997; Frohlich and Parker, 2000), although in these cases functional information is not available.

We are currently studying genes involved in the early stages of floral development in woody tropical angiosperm trees. In this paper we describe the cloning of the *Eucalyptus grandis* *LFY/FLO* putative homolog (named *EgLFY*). We also describe and analyze its expression pattern during eucalypt reproductive and vegetative development. The *EgLFY* gene appears to be the functional homolog of *LFY* as deduced from data on its expression patterns during eucalypt reproductive development and from complementation experiments with *Arabidopsis lfy* mutants.

**MATERIAL AND METHODS**

*Plant Material:* Samples of vegetative and reproductive tissues of *Eucalyptus grandis* (var. Coffes Harbour) were collected in the fields of the Escola Superior de Agricultura Luiz de Queiroz, at the University of São Paulo (Piracicaba, SP, Brazil). Young expanding leaves were also used for isolation of genomic DNA. RNA-blot and *in situ* hybridization and SEM analyses were performed on plant tissues collected and fixed in different developmental stages during two growing seasons.

*Cloning of *EgLFY:* Genomic DNA for PCR amplification, Southern analysis and construction of genomic libraries was isolated by the traditional CTAB-based method (Sambrook et al., 1989). Total RNA samples for cDNA library construction and Northern Blot were isolated from eucalypt leaves, vegetative apices and from a mix of inflorescences at different developmental stages using the Rneasy plant minikit (QIAGEN) following the supplier’s instructions.
The genomic clones of *EgLFY* were isolated by screening 165,000 plaques from an *E. grandis* genomic library (22 x 10⁶ pfu) constructed with partially *Sau*3A-digested genomic DNA, using the Packagene Lambda Packing Systems (Promega). For this screening, we have used a biotin-labeled probe (North2South chemiluminescent system, Pierce) using the entire *Arabidopsis* LFY cDNA from plasmid pDW124 (Weigel et al., 1992) as a template. Two adjacent BamHI fragments (E28B with 2Kbp and E6B with 6Kbp) spanning the entire genomic *EgLFY* sequence were subcloned into pBluescriptKS (Clontech). Subclones were prepared by nested deletions (Zhu and Clark, 1995) and sequenced on an ABI Prism 377 (Perkin-Elmer/Applied Biosystems) automated sequencer using the DYEenamic ET terminator Cycle Sequencing Kit (Amersham/Pharmacia Biotec, USA) coupled with M13 reverse and forward primers following the manufacturer instructions.

A cDNA library was constructed using total RNA from a mix of *E. grandis* inflorescences at different developmental stages. The poly-A fraction of RNA was isolated and the first strand of cDNA was synthesized using the SuperScript cloning system (Life Technologies). The cDNA library screening was performed using a PCR-based strategy (Sussman et al., 2000) and the LFY-specific degenerated primers L1: 5’-CGGAYAIIAYAARCCIAARATGMICAYTA-3’ and L4: 5’-CGGATCCGTGICKIRYKIGTIG-ACRTA-3’.
absolute ethanol, where they were stored at 4°C until needed. The collected plant material was immediately fixed in 4% paraformaldehyde under vacuum for 24 h and dehydrated with a mix of inflorescences at different developmental stages, separated in a denaturing agarose gel (Sambrook et al., 1989) and hybridized to an \textit{EgLFY} probe.

The \textit{EgLFY} probe used in both Southern and Northern experiments was a 235bp PCR product obtained from the 3’ transcribed region of the gene, using primers E13: 5’- TGCGGAGCTTGTTGGGACA-3’ and E25: 5’- CTTCTCCTCAAGTCCAATC-3’, and an \textit{EgLFY} cDNA as a template. PCR reactions were performed in a final volume of 25 μL with an initial 3 min denaturation at 96°C, followed by 40 cycles of 96°C for 40 sec; 45°C for 30 sec and 72°C for 2 min. The PCR product was purified using the Concert Kit (Gibco-Life Sciences). The probe was labeled with fluorescein using the DCP-Star GeneImage System (Pharmacia-LKB). After polymerization, serial sections of 5-8 μm were obtained from the resultant pDW132E plasmid pEGLFY and blunt-ended using DNA polymerase I (Klenow fragment). An intermediate pDW132E vector was prepared by cloning the polished fragment described above into the SmaI site of pDW132, containing the \textit{LFY} promoter (Weigel et al., 1992). The correct orientation of the cloning process was checked by endonuclease digestion.

The resultant material was dried under CO₂ in a Balzer’s critical point drier and further dissected, when necessary. The samples were mounted in metallic stubs with carbon conductive adhesive tape, coated with colloidal gold and observed at 10-20kV using a ZEISS DSM 940 A or a LEO 435 VP scanning electron microscope, at the University of Sao Paulo (ESALQ-NAP/MEPA).

Sequence comparisons: The trimmed partial \textit{EgLFY} genomic and cDNA sequences obtained were aligned using Clustal W (Thompson et al., 1994), before being checked for similarity with sequences already deposited in public databases using BLASTX (Altschul et al., 1997). Nucleotide and protein sequences of different \textit{LFY} homologs were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) and aligned with Clustal W (Thompson et al., 1994). Distance matrices were obtained from the alignments and comparative trees were built using TreeView (Page, 2000).

\textbf{Complementation of the Arabidopsis lfy-26 mutant:} The XbaI–Smal \textit{EgLFY} fragment, carrying the coding region of \textit{EgLFY}, with its endogenous start and stop codons, was obtained from plasmid pEGLFY and blunt-ended using DNA polymerase I (Klenow fragment). An intermediate pDW132E vector was prepared by cloning the polished fragment described above into the Smal site of pDW132, containing the \textit{Arabidopsis LFY} promoter (Weigel et al., 1992). The correct orientation of the cloning process was checked by endonuclease digestion. The \textit{Psrl-Spel} fragment from the resultant pDW132E (\textit{LFY::EgLFY}) vector was blunt-ended with Klenow and cloned into the plant transformation vector pSKI015 (a gift from D. Weigel, Salk Institute, LA Jolla CA, USA), which contains the BAR gene, allowing selection with the herbicide Basta (Sylvet), constituting the pSKI015E T-DNA. The \textit{Arabidopsis} plants (Columbia ecotype) transgenic for pSKI015E T-DNA were obtained by using \textit{Agrobacterium tumefaciens}-mediated transformation, as described by Bechtold et al. (1998). Putatively transformed seeds were selected upon germination on sand wetted with a Basta (Sylvet) solution at 500 μL.mL⁻¹. Homozygous (Basta-resistant) lines were obtained by selfing the primary transformants.
segregation ratio of resistant:sensitive was used to estimate the number of transgene insertions. T2 lines, homozygous for the LFY::EgLFY T-DNA locus, were identified by sowing 200–300 T2 seeds, derived from different T1 plants under selective conditions. Transgenic and non-transgenic plants were grown in growth chambers at 23°C under illumination with fluorescent lights: long day (LD) conditions (16 h of light / 8 h of dark) or short day (SD) conditions (8 h of light / 16 h of dark). Finally, LFY::EgLFY transformants in the Columbia ecotype were crossed to the strong lfy-26 mutant allele in the Landsberg erecta background (wild-type and mutant Arabidopsis seeds were obtained from the ABRC seed stock at the Ohio State University, Columbus, Ohio, USA). To genotype F2 plants at the LFY locus, CAPS (Cleared Amplified Polymorphic Sequences; Konieczny and Ausubel, 1993) markers that distinguished between Columbia and Landsberg were used (URL:http://www.salk.edu/LABS/phio-w/caps.html). Transgenic and non-transgenic Arabidopsis flowers and inflorescences at different developmental stages were photographed under a stereomicroscope or analyzed by SEM.

**RESULTS**

The EgLFY gene is an expressed Eucalyptus grandis homolog of LFY: The EgLFY gene contains two introns (figure 2) and encodes a putative protein with high sequence similarity to FLO/LFY-like proteins (figure 3). The deduced protein sequence of EgLFY is 95.2% identical to the previously published ELF1 gene, the LFY homolog in E. globulus (Southerton et al., 1998b). The EgLFY gene encodes a putative protein of 359 amino acids, which is 67% identical to Arabidopsis LFY and 71% identical to the FLO protein (figure 3). These three protein sequences are most similar in their C-
terminal regions. Beyond Arg-177, EgLFY is 80% identical to LFY and 84% identical to FLO. In this region, a stretch of 30 amino acids is identical in all three proteins, and a total of 156 amino acids in which virtually all changes are conservative replacements. N-terminal of Arg-177, the EgLFY protein is 55% identical to LFY and 58% identical to FLO. The EgLFY protein sequence contains a highly acidic region between glutamates 163 and 174, a short leucine zipper of leucines 45, 52 and 59, and a basic region between Arg-145 and His-153, all features observed in similar positions in the LFY and FLO sequences. EgLFY differs from LFY and FLO in that it lacks the proline rich region at its N-terminus and contains a serine and alanine rich region between Ser-335 and Ala-349.

The number of loci that hybridize with an EgLFY probe was investigated by Southern hybridization. This experiment was performed due to the report by Southerton et al. (1998) that *E. globulus* has a second LFY-like homolog that appears to be a pseudogene. Figure 4A shows a Southern blot of *E. grandis* DNA, probed with the EgLFY probe. Two hybridizing bands were detected at low-medium stringencies (washes in 2xSSC at 40°C). Nevertheless, these additional bands could not be detected in Southern blot experiments when higher stringencies were used (0.1xSSC at 65°C; data not shown). Thus, the presence of a second LFY-like gene in the *E. globulus* genome can not be ruled out. The Northern blot experiments (figure 4B) were always performed at high stringency and the cross-detection of transcripts of LFY-like loci other than EgLFY was unlikely. The Northern blot results (figure 4B) indicate that the expression of EgLFY is restricted to adult plants and that EgLFY is preferentially expressed in reproductive tissues.

**EgLFY is expressed in the tip of leaf primordia of adult trees and during floral organ development:** The expression pattern of EgLFY in vegetative and reproductive tissues was determined more precisely by *in situ* hybridization of longitudinal sections of vegetative and reproductive meristems of *E. grandis* (figure 5). No hybridization signal was detected in the shoot apical meristems of juvenile (6 months-old) plants (figure 5A), agreeing with the Northern blot results. In both apical and lateral vegetative meristems of adult (6 years-old) plants, the EgLFY transcripts were detected at the tip of the leaf primordia. No signal was detected in the shoot apical meristem itself (figures 5B and 5C). During reproductive development, EgLFY expression was detected only in young floral buds, similar to the expression of the FLO and LFY genes in *Antirrhinum* and *Arabidopsis*, respectively. Eucalypt tissues tended to stain light brown during fixation, noticeably in oil glands and epidermal cells. However, the characteristic purple color generated from alkaline phosphatase substrates observed during the detection of the digoxigenin-labelled antisense probes was easily distinguished from the non-specific staining. No labeling other than background was observed in serial sections probed with sense probes (figure 5G). The patterns of EgLFY expression

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**Figure 3.** Phylogenetic relationships of EgLFY with other LFY/FLO homologs. The deduced amino acid sequence of EgLFY was compared with (accession nos. in parentheses): PTLF from *Populus balsamifera* (U93196); PRLFY from *Platanus racemosa* (AF106842); TOFL from tomato (AF197934); APLFY from apple (BAB83097); PEgLFY from pea (AAC49782); NLTLFY from tobacco (U16172); ZFL1 from maize (AY179883); OSLFY from rice (AB005620); FLO from snapdragon (M55525); LFY from *Arabidopsis* and ELF1 from *E. globulus* (AF34806). Bootstrap support values (for 1000 replicates) are indicated when over 50.

**Figure 4.** A. Southern blot of genomic DNA from *Eucalyptus grandis* probed with EgLFY. Lane 1, digested with *XhoI*; lane 2, digested with *PstI*. B. Northern blot made with total RNA extracted from a mix of inflorescences in different developmental stages (IF), vegetative apices of juvenile plants (VG), young leaves of adult plants (LV) and probed with EgLFY. The same blot was re-probed with a heterologous *Arabidopsis* ubiquitin sequence (AtUBI) to show uniform loading and transfer of all lane contents.
in the floral buds of *E. grandis*, were similar to those described for *ELF1* expression in *E. globulus* and *E. macandra* (Southerton et al., 1998b) and a selection of the patterns observed at different floral stages are shown in figure 5. In developing flowers of *E. grandis* *EgLFY* was first detected uniformly in early floral meristems, before the onset of the floral organ primordia, (figure 5D). Later, the *EgLFY* hybridization signal was preferentially detected in areas corresponding to the developing floral primordia (figures 5E, 5H and 5I). Expression was briefly observed in sepal primordia and then in petal primordia (figures 5E and 5F). *EgLFY* expression declined in the sepals as they enlarged and fused, and was then observed in the petal primordia. As the petal primordia enlarged, expression became restricted to the center of the floral meristem, where the carpels form, and in the stamen primordia (figures 5H and 5I). Afterwards, expression declined in the petals and no hybridization signal was detected anymore in the operculum tissues. Expression was maintained during stamen development and in the region of the developing gynoecium, particularly in the developing ovules (data not shown). *EgLFY* expression was not detected in fully developed floral buds, but these tissues were extremely difficult to section and contained high levels of phenolic compounds and oils that interfered with proper *in situ* hybridization.

**Figure 5.** *In situ* localization of *EgLFY* transcripts during vegetative and reproductive growth of *E. grandis*. All sections are longitudinal. All hybridizations were done with the antisense probe, except when mentioned otherwise. The hybridization signal with the *EgLFY* probe was observed as a purple precipitate. A: vegetative meristem of a juvenile (6 months-old) plant. No signal was detected above background. B: *EgLFY* transcripts were detected at the tip of leaf primordia (lp) of the apical vegetative meristem of an adult (6 years-old) plant. C: Lateral vegetative meristem of an adult (6 years-old) plant showing no hybridization signal in the meristem and *EgLFY* transcript accumulation in the young leaf primordia. D: The early inflorescence meristems (arrows) expressed *EgLFY*. E: The *EgLFY* expression was detected in flower meristems and sepal primordia (s). F: Flower meristem hybridized with the *EgLFY* probe at a slightly later stage than that shown in E. G: Flower meristem at the same stage as that shown in F hybridized with an *EgLFY* sense probe. No hybridization signal was detected above background. H: Flower meristem at a late developmental stage, showing the fused calicine (k) and the coroline (c) opercula (K). This section is slightly oblique, so that the region of petal primordia fusion is not seen. The *EgLFY* expression was restricted to the site of stamen development (arrowhead) and at the center of the floral meristem. I: Flower meristem at a later developmental stage than that shown in H, the hybridization signal was more intense at the site of stamen formation (arrowhead) and in the carpel primordia (ca). og: oil gland. Bars: A, B, C: 50 µm; D: 25 µm; E and G: 20 µm; F: 15 µm; H and I: 100 µm.
The EgLFY coding region can complement transgenic Arabidopsis lfy mutants: When the EgLFY coding region was fused downstream to the Arabidopsis LFY promoter and introduced into the strong-phenotype lfy-26 Arabidopsis mutant, complete restoration of the wild type development was observed (figure 6). The early arising (basal) flowers in the Arabidopsis lfy-26 mutants were replaced by bracts adjacent to secondary inflorescence shoots, whereas later arising flowers were replaced by small bracts, in whose axils abnormal flowers developed (figures 6B and 6C; Weigel et al., 1992). These abnormal flowers contained sepals and carpels but no petals or stamens, these later being usually homeotically substituted by more sepals and carpels, respectively (figures 6C and 6D; Weigel et al., 1992). In contrast, wild-type flowers typically contain four sepals, four petals, six stamens, and two carpels. The lfy-26 floral phenotype was largely complemented by the LFY::EgLFY transgene. The main shoot of these plants developed flowers in both basal and apical positions, and most of these contained all four floral organ types (figures 6E and 6F).

**DISCUSSION**

We have isolated an expressed eucalypt LFY homolog named EgLFY. The EgLFY gene contains two introns that occur in identical positions to those found in all the described LFY/FLO homologs clones to date (Frohlich and Parker, 2000).

\begin{figure}
\centering
\subfloat[A: Wild type inflorescence (Columbia ecotype) showing flower buds at different developmental stages. B: main inflorescence axis of a lfy-26 mutant. Solitary flowers are replaced by a cauline leaf (bract) adjacent to a lateral inflorescence axis or an abnormal flower. C and D: SEM images of abnormal flowers of the lfy-26 mutant. Note the cauline leaf in C and the homeotic conversion of petals and stamens in sepal-like organs and carpel-like organs, respectively. E: A homozygous transgenic (LFY::EgLFY) lfy-26 mutant showing a wild type phenotype, indicating the complementation of the lfy mutation by the expression of EgLFY. F: A higher magnification view of the inflorescence of the same plant shown in E. Bars: A, B and F: 3 mm; C: 300 µm; D: 250 µm; E: 3cm.]
\end{figure}
and its sequence and expression patterns are very similar to those described for most dicot LFY/FLO homologs in the literature. Expression of EgLFY driven by the Arabidopsis LFY promoter is able to restore the wild type phenotype of transgenic Arabidopsis lfy-26 mutants. These close structural and functional similarities strongly suggest that EgLFY is the functional eucalypt homologue of LFY/FLO. LFY/FLO homologs similar to EgLFY have also been isolated from other plants (Frolich and Parker, 2000). Weigel and Nilsson (1998) have reported that transgenic hybrid aspen (Populus tremula x P. tremuloides) constitutively expressing the Arabidopsis LFY cDNA flowers precociously and shows similar phenotypes to Arabidopsis transformed with the same construct. Similarly, Peña et al. (2001) also reported the early flowering of citrus plants overexpressing a LFY homolog. These data add further weight to the hypothesis that floral regulatory mechanisms, and hence regulatory genes, are conserved among the angiosperms. The putative protein encoded by EgLFY shares a number of sequence motifs with other characterized LFY/FLO proteins (Frolich and Parker, 2000). The acidic domain is not conserved with respect to sequence and occurs in a region of relatively poor sequence conservation among the LFY homologs. The putative EgLFY protein, as well as its E. globulus homolog (Southerton et al., 1998) is shorter at the N-terminal end when compared to other LFY/FLO homologs and thus lacks the proline rich region suggesting that this motif may not be functionally significant. None of these protein sequence motifs has yet been demonstrated to be functionally important in any of the floral meristem identity genes. It is of interest to note that eucalypts probably have two EgLFY-like genes, although one of these is probably now inactive (Southerton et al., 1998). This duplication is probably a general phenomenon within the genus, and suggests that eucalypts may have experienced ancient genome duplications and many of their genes might be expected to be present in at least two copies (Southerton et al., 1998). In addition to being expressed in floral primordia in a pattern similar to LFY and FLO, the EgLFY gene is strongly expressed in leaf primordia forming on vegetative meristems of adult plants, but not in the shoot apical meristem itself. The overall pattern of expression of EgLFY is, however, similar to other described LFY/FLO homologues (Coen et al., 1990; Weigel et al., 1992; Southerton et al., 1998; Peña et al., 2001; Carmona et al., 2002).

Experiments by Hempel et al. (1994) and Blázquez et al. (1997) using in situ hybridization and GUS reporter gene expression driven by the LFY promoter have now also established vegetative expression of LFY in both vegetative apices and young leaves of three different ecotypes of Arabidopsis grown under short day conditions. The Arabidopsis LFY gene is the earliest of the known floral identity genes to be expressed, and directly activates at least one of the later genes, APETALA1 (Wagner et al., 1999). Plants carrying fusions of the LFY promoter to the GUS marker gene were used to demonstrate that LFY expression responds both to the long-day flowering pathway and to gibberelic acid (GA). Furthermore, deletion of a putative MYB transcription factor binding site within the LFY promoter prevented activation by GA, but not by the long-day pathway (Blázquez and Weigel, 2000). We have failed to identify any putative MYB transcription factor binding site within the EgLFY promoter (data not shown). However, exogenous application of paclobutrazol reduced the concentration of endogenous GA in apical tissues of different Eucalyptus species and enhanced the reproductive activity of grafted trees (Moncur and Hasan, 1994), suggesting that in Eucalyptus, high concentrations of GA inhibits the flowering process, as opposed to what is observed in Arabidopsis. It would be interesting to investigate whether paclobutrazol can interfere with EgLFY expression.

Although the available information suggests that overexpression of LFY is sufficient to promote the conversion of shoots into flowers in woody species such as Populus spp. (Weigel and Nilsson, 1995) and Citrus spp. (Peña et al., 2001), the role of the endogenous FLO/LFY homologs and their function during meristem development are poorly understood. Genetic studies in Eucalyptus are difficult because of the long time to flowering of trees and no characterized flowering mutants have been described in this genus. Nevertheless, recent advances in the transformation of Eucalyptus species (unpublished data from our own lab) and the large-scale cloning of a number of other floral gene homologues (https://forests.esalq.usp.br) may allow us to use reverse genetic approaches and to define more clearly the role played by EgLFY in Eucalyptus vegetative and floral tissues.

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