Isolation and Functional Analysis of *Flowering Locus T* in Tree Peonies (*PsFT*)

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ABSTRACT. *Arabidopsis thaliana* Flowering locus *T* (*FT*) homologs have been shown to be sufficient to trigger flowering and to regulate flowering time in a wide range of plants. However, such a homologue for the perennial ornamental shrub tree peony has not yet been characterized. In this study, we isolated *PsFT*, which is a closely related *FT* homolog from reblooming [*Paeonia ×lemoinei* ‘High Noon’ (HN)] and nonreblooming [*P. ×suffruticosa* ‘Luo Yang Hong’ (LYH)] cultivars of tree peonies, and identified its potential role in the regulation of flowering time. The *PsFT* alleles from the two cultivars encode the same protein, which indicates that the polymorphisms observed in the coding region do not contribute to the distinct flowering phenotypes of HN and LYH. Comparative analyses of the *PsFT* expression patterns in HN and LYH indicated that *PsFT* might be associated with reblooming. Transgenic *A. thaliana* plants ectopically expressing *PsFT* exhibited a phenotype that included significantly early flowering compared with the wild-type (WT) plants. Taken together, our data provide valuable clues for shortening the juvenile periods and extending the flowering periods of perennial woody plants, such as tree peonies.

A considerable number of studies of *A. thaliana* have revealed that the flowering time is regulated by both environmental and endogenous cues. The input cues are integrated into the floral transition via several genes known as floral pathway integrators, such as *FT* (Parcy, 2005; Simpson and Dean, 2002). *FT* is a major component of florigen and has been shown to be sufficient to trigger flowering (Turck et al., 2008). In *A. thaliana*, mutations in *FT* cause a delay in flowering, and the overexpression of *FT* causes early flowering (Kardailsky et al., 1999). *FT* proteins move from the leaves to the shoot apical meristem (SAM) to induce flowering (Jaeger and Wigge, 2007). At the SAM, *FT* forms a complex with the basic leucine zipper (bZIP) protein *Flowering locus D* (*FD*) and upregulates the floral meristem identity gene *Apetala1* (*AP1*) (Abe et al., 2005; An et al., 2004).

Because of the long juvenile phase, woody plant flowering studies are important for accelerating the breeding process by shortening the juvenile phase. Based on studies of *A. thaliana*, examinations of flowering in perennial woody plants have enabled progress in unveiling the molecular mechanisms of flowering. *FT*-like genes have been identified and shown to induce flowers in *Populus* (Hsu et al., 2006; Igasaki et al., 2008). The *FT*-like *MdFT1* and *MdFT2* genes of apple (*Malus ×domestica*) have also exhibited the potential to act as floral

promoters (Kittikorn et al., 2013; Kotoda et al., 2010). The *FT* homolog (*CsFT*) of *Citrus* controls floral induction and promotes flowering in trifoliate orange (*Poncirus trifoliate*) (Chica and Albrigo, 2013a, 2013b; Endo et al., 2005). The results of these studies suggest that *FT* genes play key roles in the manipulation of flowering time, shorten the juvenile phase, and can be useful as research and breeding tools in perennial woody species (Zhang et al., 2010).

The tree peony (*Paeonia* section *moutan*) is a perennial woody shrub and an important ornamental crop that has been called the “king of flowers” in China and is cultivated worldwide (Wister, 1962). Because of its striking ornamental value, tree peony has contributed tremendously to gardens during its 1600-year history of cultivation (Gilmore et al., 2013; Li et al., 2011). In tree peony, the long juvenile phase of 4–6 years must be completed before flowering, which makes the ornamental and breeding cycle of tree peony slow. Flowering in tree peonies lasts only 4–5 d for a single flower and 20 d for the entire plant. The short and concentrated flowering period affects the economic value of the tree peony (Cheng, 2007; Kamenetsky et al., 2003). Flowering time has become one of the most relevant traits for increasing the value of tree peonies as ornamental landscape plants and promoting the peony industry.

Recently, we performed floral transcriptome sequencing and characterized a series of unigenes from tree peony that included an *FT* homolog [*PsFT* (Wu et al., 2014; Zhou et al., 2013)]. *PsFT* potentially has a role in flowering based on transcriptomic comparisons between the reblooming *P. ×lemoinei* ‘HN’ and the nonreblooming *P. ×suffruticosa* LYH. However, the *FT*

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Table 1. Phenotype of T₂ transgenic *Arabidopsis thaliana* plants with *Flowering locus T (FT)* from tree peonies (*PsFT*).

Genotype ^z	Rosette leaves at bolting (no.)	Time to bolting (d)	Leaves at flowering (no.)	Time to flowering (d)
	mean ± SE			
WT	11.17 ± 0.75	31.33 ± 0.52	16.67 ± 0.82	42.00 ± 1.10
Vector	11.50 ± 0.55	30.67 ± 1.03	15.83 ± 0.75	41.67 ± 0.82
35S:: <i>PsFT</i> -L1	4.00 ± 0.00**	20.50 ± 1.05**	5.67 ± 0.52**	27.00 ± 0.63**
35S:: <i>PsFT</i> -L2	4.16 ± 0.41**	19.67 ± 0.52**	6.00 ± 0.63**	26.17 ± 0.75**

^zWT = wild type.
**Significance at *P* = 0.01.

Fig. 1. Variations in the flowering in *Paeonia xlemoinei* 'High Noon' (HN) and *P. x suffruticosa* 'Luo Yang Hong' (LYH). (A) A reblooming HN axillary shoot in August (white arrow). (B) The axillary bud of an LYH plant surrounded by bud scale in August (white arrow). (C) The annual cycles of growth and development for HN (outer ring) and LYH (inner ring). The shading indicates the process of reblooming in HN.

homolog for tree peonies has not yet been characterized. We sought to further examine the role of *PsFT* during flowering in tree peonies and the potential benefit of *PsFT* for shortening the juvenile growth phase and manipulating the flowering time of tree peonies. In this study, we isolated *PsFT* from HN and LYH and carried out a functional analysis. We found that *PsFT* from the two cultivars of tree peony encode an identical protein and *PsFT* significantly promotes flowering in transgenic *A. thaliana*.

Materials and Methods

PLANT MATERIALS. Two cultivars of tree peony, the reblooming HN and the nonreblooming LYH, were grown in the Jiu Feng Forestry Experiment Station of the Beijing Forestry University of China. Different tissues, including the axillary buds (the scales and primordial leaves were removed), stems (distal to the buds), mature leaves (distal to the buds), and roots (fibrous roots), were collected at the same time from intact plants on 21 June 2013. The bud development process of tree peonies has previously been described (Barzilay et al., 2002; Wang and Zhang, 1987). All samples were immediately frozen in liquid nitrogen and subsequently stored at -80°C .

SYNTHESIS *PsFT* cDNA AND SEQUENCE ANALYSIS. *PsFT* cDNAs were isolated using reverse transcription polymerase chain reaction (RT-PCR) with total RNA extracted from the opened flowers. Total RNA was extracted with the RN38-EASY-spin Plus (Aidlab, Beijing, China) and treated with RNase-free DNase I (Promega, Madison, WI). Total RNA purity was assessed using a spectrophotometer (Nanodrop 2000C; Thermo Fisher Scientific, Wilmington, DE), and the quality of the RNA was visualized using agarose gel electrophoresis. First-strand cDNA was synthesized from 1 μg of DNA-free RNA using the PrimeScript RT reagent kit (Takara, Tokyo, Japan). The PCR parameters were as follows: 94°C for 5 min; followed by 30 cycles of 30 s at 94°C , 30 s at 60°C , and 1 min at 70°C ; and a final elongation

Fig. 2. Comparison of PsFT in tree peonies with its closely related homologs. (A) Alignments of the deduced protein sequences of PsFT and those of FT from other plants. The amino acids shown in black are identical across the 10 FT proteins. The asterisks and black squares represent the conserved domains of the PEBP family. (B) Phylogenetic analysis of the FT family proteins. The tree was constructed using deduced amino acid sequences, including those from *A. thaliana* (*AtFT*: NM_105222, *AtTSF*: NM_118156.1, *AtFLI*: U77674.1), rice (*Oryza sativa* Japonica Group, *Hd3a*: NM_001063395), tomato (*Solanum lycopersicum*, *SP3D*: XM_004234835), poplar (*Populus nigra*, *PnFT*: AB106111.1, *Populus tremula*, *PtFT*: DQ387859), apple (*Malus domestica*, *MdFT*: FJ555224.1), satsuma mandarin (*Citrus unshiu*, *CsFT*: AB027456), pharbitis (*Ipomoea nil*, *InFT*: EU178859), and grapevine (*Vitis vinifera*, *VvFT*: EF157728.1). *AtFLI* was used as the outgroup. The unit of the scale bar indicates the branch lengths (0.05 substitutions/site).

for 10 min at 70 °C. The primers for *PsFT* are described in Supplemental Table 1. All amplified products were cloned into the pGEM-T vector (Promega), and putative positive clones were identified using PCR with the M13F and M13R sequencing primers (Supplemental Table 1). Homologs of *PsFT* were searched using the BLAST program of the National Center of Biotechnology Information (NCBI). Phylogenetic analyses were performed based on the neighbor-joining (N-J) model (Perriere and Gouy, 1996) with 1000 bootstrap replications using MEGA 5.0 software (The Biodesign Institute, Tempe, AZ) (Tamura et al., 2011).

QUANTITATIVE REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION. Expression of the *PsFT* genes in tissues during development was evaluated using quantitative real time reverse transcription polymerase chain reaction (qRT-PCR), which was performed on a Mini option Real-Time PCR instrument (Bio-Rad, Hercules, CA) using 20 µL of SYBR Premix EX *Taq*TM (Takara). The PCR program was initiated at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 55 °C for 30 s, and 72 °C for 30 s, and completed with a melting curve analysis for each reaction. The primers used in this study are described in Supplemental Table 1. The relative expression levels were calculated using the 2^{-ΔΔCt} method (Livak and Schmittgen, 2001) and were normalized against the geometric averaging of the two reference genes *Ubiquitin (UBQ)* and *Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH)* (Wang et al., 2012). The qRT-PCR reactions were performed for three biological replicates from different plants with three technical repetitions per replicate. The data were analyzed using CFX manager 2.0 software (Bio-Rad).

TRANSGENIC *A. THALIANA* ANALYSIS. Full-length *PsFT* cDNA was amplified by PCR using the *PsFT-XbaI* and *PsFT-SmaI* pair of primers and was cloned into the binary vector pBI121 with expression driven by the cauliflower mosaic virus (CaMV) 35S promoter. The sequences of *PsFT-XbaI* and *PsFT-SmaI* are presented in Supplemental Table 1. The resulting construct, *PsFT*-pBI121, was transformed into *Agrobacterium tumefaciens* EHA105. *A. thaliana* (Col-0) transformation was performed using the floral dip method (Clough and Bent, 1998). The transformants (T₀ lines) from the dipped plants that survived in 1/2MS medium containing kanamycin (100 µg·mL⁻¹) were transplanted into a substrate consisting of vermiculite and nutrient soil (v:v = 1:1) in a growth chamber at 22 °C under long-day (LD) conditions (16 h photoperiod, light intensity: 50 µmol·m⁻²·s⁻¹). Morphological observation and expression analyses were performed on 8–10 plants from the second generation (T₂). The flowering time was measured by quantifying the number of leaves and the number of days until flowering. A one-way analysis of variance was performed with SPSS software (version 18.0; IBM, Armonk, NY).

Fig. 3. *PsFT* tissue expression analysis in the *P. x suffruticosa* 'Luo Yang Hong' (LYH) and *P. x lemoinei* 'High Noon' (HN). (A) *PsFT* transcripts in the roots, stems, leaves, and axillary buds in LYH. (B) *PsFT* transcripts in the roots, stems, leaves, reblooming axillary buds, and normal axillary buds in HN. The gene expression levels are expressed relative to roots. Data are means $\pm$ SE ($n = 3$).

Fig. 4. Analysis of the expression patterns of *PsFT* in the reblooming buds and normal buds of the *P. x lemoinei* 'High Noon'. The x-axis indicates the dates at which the shoot apices were harvested from the reblooming buds (black dot) and the normal buds (open dot) in 2013. The gene expression levels are expressed relative to the first sample. Data are means $\pm$ SE ($n = 3$).

Total RNA was extracted from the transgenic *A. thaliana* lines and the WT plants. The gene-specific primers are presented in Supplemental Table 1. PCR was performed at 94 °C for 5 min, followed by 30 cycles of 1 min at 94 °C, 1 min at the appropriate annealing temperature (Supplemental Table 1), 1 min at 72 °C, and a final elongation of 15 min at 72 °C.

VARIATION IN THE FLOWERING TIMES OF HN AND LYH. HN exhibits a stable reblooming trait under natural conditions that can cause flowering two or more times per year, whereas LYH and the majority of other peony cultivars flower once per year (Smith, 1997). Reblooming results from the sprouting of some axillary shoots and annual terminal shoots and subsequent development and reblooming after spring flowering (Fig. 1A). In LYH, after spring the axillary buds surrounded by bud scales entered the floral differentiation phase until winter dormancy (Fig. 1B). We examined the annual cycles of development and found different developmental process in both cultivars (Fig. 1C). There was a single floral transition in LYH in June and two floral transitions in HN; the first occurred in June and resulted in reblooming, and the second occurred in August ahead of the next spring flowering.

SYNTHESIS AND CHARACTERIZATION OF *PsFT* cDNAs. Based on previously obtained sequences (NCBI accession number: SRP026412 and SRP026299), we generated the full-length cDNAs of the *FT* homologues (*PsFT*) of HN and LYH using RT-PCR. The two *PsFT* cDNAs (GenBank accession numbers KP198621 and KP198622) were the same length and contained four single nucleotide polymorphisms in 519 bp open reading frames (ORFs); however, the two *PsFT* genes encoded 173-amino acid peptides with identical sequences [100% identity (Supplemental Fig. 1)]. These peptides also exhibited high levels of amino acid identity with the *FT* homologous genes of various plants, including *AtFT* in *A. thaliana*, *Hd3a* in rice (*Oryza sativa*), *PnFT* in poplar (*Populus nigra*), *MdFT* in apple, and *VvFT* in grapevine (*Vitis vinifera*) (77.71%, 79.33%, 89.66%, 89.08%, and 88.51%, respectively). The amino acid sequence alignment revealed that *PsFT* contained a conserved domain with characteristics of the phosphatidylethanolamine binding protein (PEBP) family (Kikuchi et al., 2009) (Fig. 2A). The amino acids residues Tyr [Y (site 85)] and Gln [Q (site 140)] of *FT* were identified in *PsFT*; these residues are conserved residues of the PEBP family and are essential for the functional difference between *FT* and TERMINAL FLOWER1 (TFL1) (Hanzawa et al., 2005; Huang et al., 2012).

A N-J phylogenetic tree of *PsFT* in peonies and some PEBP family in various plants was constructed based on protein sequence alignments (Fig. 2B). The phylogenetic tree revealed that *PsFT* belonged to the *FT* cluster of the PEBP family and was related to *FT* proteins in deciduous perennial woody plants, such as grapevine and poplar. These results suggested that *PsFT* is a putative ortholog of *A. thaliana FT*.

EXPRESSION ANALYSES OF *PsFT* IN HN AND LYH. *PsFT* exhibited variation in the expression patterns in the shoot apices of the HN and LYH cultivars during floral initiation throughout 2012 (Zhou et al., 2013). To further investigate *PsFT*, tissue specificity analyses of *PsFT* in HN and LYH were performed using qRT-PCR (Fig. 3). *PsFT* was detected at a high level in the flower buds and a low level in the roots, and this pattern is similar to the tissue expression patterns of poplar in June (Hsu et al., 2006). By 21 June 2013, floral induction had occurred in the axillary buds, and the vegetative growth tended to stop in LYH. At this point, *PsFT* expression in LYH was higher in the flower buds than in the leaves and stems and was expressed at a very low level in the roots (Fig. 3A). However, in HN, floral induction only occurred in the reblooming buds, which were sprouting and growing, and not in the normal axillary buds (i.e.,

Fig. 5. The ectopic expression of *PsFT* in tree peonies induced early flowering in *A. thaliana*. A randomly selected transgenic line that constitutively expressed *PsFT* exhibited an early flowering phenotype relative to the wild-type (WT) and vector controls under long-day conditions. Bar = 0.5 cm.

Fig. 6. Reverse transcription-polymerase chain reaction (RT-PCR) analyses of the accumulations of *PsFT*, *CO*, *FT*, *SOC1*, *LFY*, *AP1*, and *AP3* transcripts at the two- and four-leaf stages of two transgenic *A. thaliana* plants. The expression of these genes in the wild-type (WT) *A. thaliana* and the transgenic plants harboring empty vector was also examined as controls. The *A. thaliana* *UBQ* transcript was amplified to confirm that similar amounts of cDNA were used in the RT-PCR.

only leaf buds). *PsFT* was expressed at a high level in the reblooming axillary buds of HN and at a very low level in the normal axillary buds (Fig. 3B). *PsFT* accumulated in the leaves and stems at the same levels observed in the flower buds and was detected at background levels in the roots (Fig. 3B). These findings suggest that *PsFT* expression might be upregulated in the leaves, stems, and reblooming buds of the HN cultivar.

To investigate the association of *PsFT* with reblooming in the HN cultivar, we collected the reblooming and normal buds from the same plants and compared the *PsFT* transcript levels from induction to reblooming in 2013 (Fig. 4). In the normal buds, *PsFT* was maintained at a constantly low level during this period; however, in the reblooming buds, *PsFT* accumulated gradually to reach an ≈ 63 -fold greater level of mRNA in the flowers than in the normal buds by 16 Aug. This result revealed that high levels of the accumulation of *PsFT* were associated with reblooming.

Reblooming characteristics could effectively extend the flowering period and enhance the commercial value of tree peony (Jiang et al., 2007). To date, various forced reblooming techniques for extending the flowering period in tree peonies have been considered; these techniques include pruning, GA treatment, defoliation, and moisture stress (Liu et al., 2002; Ren et al., 2004). However, little is known about the genetic regulation of the reblooming process due to the complex genetic background and inviable male gametes in the HN cultivar (Hao et al., 2013; He and Cheng, 2006). We previously reported on changes in expression of eight unigenes, including *PsFT* that were involved in floral pathways during the floral process in 2012 and found that reblooming might be a distinctive type of floral initiation in tree peony (Zhou et al., 2013). Furthermore, in this work, we found that *PsFT* accumulated in the leaves, stems, and flower buds of the HN cultivar and that the *PsFT* levels increased continuously in the reblooming buds (Figs. 3 and 4). These findings suggest that *PsFT* might be associated with the following aspects of the floral development process in HN: bud sprouting, vegetative growth, and reblooming. Similar observations have been reported in *Populus*, in which *PtFT1* displayed peak expression in the period of floral initiation and low expression at growth cessation and bud set (Böhlenius et al., 2006), and *FT* showed a gradual but dramatic upregulation during chilling and bud burst (Rinne et al., 2011). These results indicate that *FT* is a major regulatory factor in numerous developmental processes, including vegetative growth, flowering, and fruit set (Pin and Nilsson, 2012; Wigge, 2011) of woody plants. We believe that the regulation of *PsFT* and other flowering genes under optimal environmental conditions could potentially be used to induce reblooming or even continuous blooming in HN.

ECTOPIC EXPRESSION OF *PsFT* IN *A. THALIANA*. To further examine the role of *PsFT* in floral initiation, we used a 35S: *PsFT* construct and generated transgenic *A. thaliana* plants. Ten independent kanamycin-resistant T_0 plants were obtained. All T_0 plants flowered earlier than the WT and self-pollinated to generate first-generation seeds (T_1). The seeds of each T_1 transgenic line were screened on 1/2 Murashige-Skoog (MS) containing kanamycin to generate second-generation seeds (T_2). The T_2 lines were again screened on 1/2 MS containing kanamycin, and the seedlings of the T_2 lines were used for phenotypic assessments of flowering time. All transgenic *A. thaliana* T_2 lines exhibited earlier flowering time than the WT

plants under the same growth conditions (Fig. 5A). Flowering time, in terms of numbers of leaves formed before bolting and flowering and the number of days from germination to bolting and flowering, differed significantly ($P < 0.01$) between the transgenic lines and controls (Fig. 5B). Under LD conditions, the transgenic lines bolted within 20 d of seed germination at the four-leaf (rosette) stage and flowered within 26 d of seed germination at the six-leaf stage, whereas the WT and vector controls bolted within 30 d of seed germination at the 11-leaf (rosette) stage and flowered within 41 d of seed sowing at the 16-leaf stage (Table 1). These data suggest that the ectopic expression of *PsFT* induced early flowering in transgenic *A. thaliana*.

To determine whether the early flowering phenotype was correlated with the high levels of the expression of *PsFT* in these transgenic plants, we analyzed expression of the *Constans* (*CO*), *FT*, *Suppressor of overexpression of Constans1* (*SOC1*), *Leafy* (*LFY*), *AP1*, and *Apetala3* (*AP3*) genes in the controls and transgenic *A. thaliana* using RT-PCR. As shown in Figure 6, *PsFT* expression was observed in 35S::*PsFT* transgenic plants but not in the WTs or vector controls. Low levels of *CO*, *FT*, *SOC1*, *LFY*, and *AP1* transcripts were observed at the two-leaf stage in the transgenic plants, and high levels of expression were observed at the four-leaf stage. *AP3* was detected at a background level in the two-leaf stage in transgenic plants, and this level was increased at the four-leaf stage. However, no significant expression of *CO*, *FT*, *SOC1*, *LFY*, *AP1*, or *AP3* was detected in the WT or vector control lines at the two-leaf or four-leaf stages. Thus, *PsFT* might promote the expression of *CO* and the floral integrators *FT*, *SOC1*, and *LFY* under LD conditions to further induce expression of *AP1* and *AP3*, which are involved in floral induction.

The *FT* gene has been shown to effectively control flowering time and to be useful in accelerating the breeding of woody plants via transgenic approaches (Flachowsky et al., 2009). The overexpression of the *FT*-homologous gene of apple trees induces early flowering in *A. thaliana* and apple (Tränkner et al., 2010). The ectopic expression of an *FT*-like gene from *Citrus* confers early flowering in trifoliate orange plants (Endo et al., 2005). When juvenile poplars are transformed with poplar *FT2*, the first flowering is induced within 1 year (Hsu et al., 2006). In this work, we transformed *PsFT* into *A. thaliana* and analyzed the flowering genes in the transgenic plants. Our results suggest that *PsFT* might be functionally conserved and serve the same purpose as *A. thaliana* *FT* in the regulation of flowering time. Once a transformation and regeneration system is established in *Paeonia*, *PsFT* may be useful in promoting the floral transition in tree peonies and be a valuable gene resource for modifying juvenility and thereby speeding up flowering and breeding programs for tree peonies. Furthermore, potential roles of *PsFT* in reblooming should be of particular use for extending the flowering period by manipulating the *PsFT* gene to induce the occurrence of reblooming in this ornamental crop.

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PsFT_LYH	CTCAATAACCAAAACTCTTCAACACTAGAGGAGAGCCAGCCG	CCATGCGCTAGAAATAGGGATCCCACTAGTT	70
PsFT_HN	AGAGCCAGCCG	CCATGCGCTAGAAATAGGGATCCCACTAGTT	38
	M P R N R D P L V		
PsFT_LYH	GTITGGACGTGTAGTITGGGGATGTCTTGGACCCCTTTTACCAGGACCATCTCTTTGAGGGTCACGTATACGA		140
PsFT_HN	GTITGGACGTGTAGTITGGGGATGTCTTGGACCCCTTTTACCAGGACCATCTCTTTGAGGGTCACGTATACGA		108
	V G R V V G D V L D P F T R T I S L R V T Y T		
PsFT_LYH	GTCGAGAAGTCAGTAATGGCTGTGAGCTCAGGCCCTCCCAAGTCGTTAACCAACCCAGGGTIGATATTGG		210
PsFT_HN	GTCGAGAAGTCAGTAATGGCTGTGAGCTCAGGCCCTCCCAAGTCGTTAACCAACCCAGGGTIGATATTGG		178
	S R E V S N G C E L K P S Q V V N Q P R V D I G		
PsFT_LYH	AGGGGATGATCTTAGGACCTTCTACACTCTGGTTATGTTGACCTGATGCTCCAGCCCAAGCGACCCA		280
PsFT_HN	AGGGGATGATCTTAGGACCTTCTACACTCTGGTTATGTTGACCTGATGCTCCAGCCCAAGCGACCCA		248
	G D D L R T F Y T L V M V D P D A P S P S D P		
PsFT_LYH	AACCTAAGGGAATACTTACACTGGTITGGTCAGTATATTCCTGCAACG	ACTGGGGCCAACTTTGGTCAAG	350
PsFT_HN	AACCTAAGGGAATACTTACACTGGTITGGTCAGTATATTCCTGCAACG	ACTGGGGCCAACTTTGGTCAAG	318
	N L R E Y L H W L V T D I P A T T G A N F G Q		
PsFT_LYH	AGATCGCTCGTTACGAGAGTCCACGCCCAACTGCTGGGATCCATCGTTTTTGTTTATTTTGGTTTCGACA		420
PsFT_HN	AGATCGCTCGTTACGAGAGTCCACGCCCAACTGCTGGGATCCATCGTTTTTGTTTATTTTGGTTTCGACA		388
	E I V C Y E S P R P T A G I H R F C F I L F R Q		
PsFT_LYH	ATTGGGAAGGCCAAACTGTTTACACTCCAGGTGGCGTCAGAAATTTCACACCAGAGACTTCGCTGAGCTT		490
PsFT_HN	ATTGGGAAGGCCAAACTGTTTACACTCCAGGTGGCGTCAGAAATTTCACACCAGAGACTTCGCTGAGCTT		458
	L G R Q T V Y T P G W R Q N F N T R D F A E L		
PsFT_LYH	TATAATCTCGGCTCACCGGTGGCTGCTGTTTATTTATTTGCCAAAGGGAGAGGGGGTCAGGCGGAAGAA		560
PsFT_HN	TATAATCTCGGCTCACCGGTGGCTGCTGTTTATTTATTTGCCAAAGGGAGAGGGGGTCAGGCGGAAGAA		528
	Y N L G S P V A A V Y F N C Q R E R G S G G R		
PsFT_LYH	GATATCAATATATAATCATGAGTAACAGGGATTGAA		597
PsFT_HN	GATATCAATATATAATCATGAGTAACAGGGATTGAA	CAATGCACAAGTAGATGCTGCTACTAATAATA	598
	R *		
PsFT_LYH	IGGCC		
PsFT_HN			603

Supplemental Table 1. Primers used in this study.

Primers	Sequences (5' → 3')	Annealing temperature (°C)	Fragment size (bp)
<i>PsFT</i> -F (HN)	GGGAAGGGAGGAAGAGC	54	604
<i>PsFT</i> -R (HN)	GGGCCATATTATTAGTAGCAG		
<i>PsFT</i> -F (LYH)	CTACAATAACCAAACTCTTC	54	597
<i>PsFT</i> -R (LYH)	TTCAATCCCTGTTACTCAT		
<i>PsFT</i> -F (real time)	GTGTAGTTGGGGATGTCTTGGA	55	210
<i>PsFT</i> -R (real time)	TTAGGTTTGGGTCGCTTGG		
<i>PsUBQ</i> -F (real time)	GACCTATACCAAGCCGAAG	55	142
<i>PsUBQ</i> -R (real time)	CGTTCCAGCACCACAATC		
<i>PsGAPDH</i> -F (real time)	GGTTGATCTCACTGTTAGGC	55	151
<i>PsGAPDH</i> -R (real time)	TCAGACTCCTCCCTACAAG		
M13F	GTTTTCCCAGTCACGAC		
M13R	GCGGATAACAATTTACACACAGG		
<i>PsFT</i> -F (<i>Xba</i> I)	GCTCTAGAATGCCTAGAAATAGGGATCCAC	65	542
<i>PsFT</i> -R (<i>Sma</i> I)	TCCCCCGGGTCTTCTTCCGCCTGACC		
<i>CO</i> -F (NM_121589.1)	ACGCCATCAGCGAGTTCC	59.5	479
<i>CO</i> -R	AAATGTATGCGTTATGGTTAATGG		
<i>FT</i> -F (NM_105222.2)	AGTCCTAGCAACCCCTCACCT	57	420
<i>FT</i> -R	TAGGCATCATCACCGTTTCGT		
<i>SOC1</i> -F (AY007726)	ATGGTGAGGGGCAAACTC	56	645
<i>SOC1</i> -R	TCACTTTCTTGAAGAACAAGG		
<i>LFY</i> -F (NM_125579.1)	TGAAGGACGAGGAGCTT	56	511
<i>LFY</i> -R	TTGCCACGTGCCACTTC		
<i>AP1</i> -F (NM_105581)	CATTATCTTGGGGAAGACTTGCAAG	56	450
<i>AP1</i> -R	GGAAATGCTTCATGCGGCGAAGC		
<i>AP3</i> -F (NM_115294)	GCTTGACATTGAGGAGCTGCGTCG	56	396
<i>AP3</i> -R	TAGATAGACAATGATGGCACCAGC		
<i>UQB</i> -F (NM_116090.2)	GTGGTGCTAAGAAGAGGAAGA	53	251
<i>UQB</i> -R	TCAAGCTTCAACTTCTTCTTT		

PsFT = Flowering locus T in tree peonies; *PsUBQ* = Ubiquitin in tree peonies; *PsGAPDH* = Glyceraldehyde-3-phosphate-dehydrogenase in tree peonies; *CO* = *Constans*; *FT* = Flowering locus T; *SOC1* = *Suppressor of Overexpression of Constans1*; *LFY* = *Leafy*; *AP1* = *Apetala1*; *AP3* = *Apetala3*; *UBQ* = Ubiquitin.