

Isolation and functional characterization of three TERMINAL FLOWER 1-like genes from *Platanus acerifolia*

Sisi Zhang (✉ zhangsis@webmail.hzau.edu.cn)

Wuhan Institute of Landscape Architecture <https://orcid.org/0000-0003-1463-9262>

Qin Zhou

Huazhong Agriculture University College of Horticulture and Forestry Sciences

Xingyu Yang

Wuhan Institute of Landscape Architecture

Jianqiang Wang

Wuhan Institute of Landscape Architecture

Jie Jiang

Wuhan Institute of Landscape Architecture

Miaomiao Sun

Guangzhou Institute of Forestry Landscape Architecture

Yanjun Liu

Wuhan Institute of Landscape Architecture

Chaoren Nie

Wuhan Institute of Landscape Architecture

Manzhu Bao

Huazhong Agriculture University College of Horticulture and Forestry Sciences

Guofeng Liu

Guangzhou Institute of Forestry and Landscape Architecture

Research Article

Keywords: London plane, flowering regulation, PEBP, TFL1, BFT

Posted Date: March 2nd, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2620144/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Plant Cell Reports on April 6th, 2023. See the published version at <https://doi.org/10.1007/s00299-023-03014-9>.

Abstract

Three TERMINAL FLOWER 1 (TFL1)-like genes were isolated and characterized from London plane tree (*Platanus acerifolia*). All genes have conserved genomic organization and characteristic of the phosphatidylethanolamine-binding protein (PEBP) family. Sequence alignment and phylogenetic analysis indicated that two genes belong to the TFL1 clade, designated as PlacTFL1a and PlacTFL1b, while another one was grouped in the BFT clade, named as PlacBFT. qRT-PCR analysis showed that all three genes primarily expressed in vegetative phase, but the expression of PlacTFL1a was much higher and wider than that of PlacTFL1b, with the latter only detected at relatively low expression levels in apical and lateral buds in April. PlacBFT was mainly expressed in young stems of adult trees followed by juvenile tissues. Ectopic expression of any TFL1-like gene in *Arabidopsis* showed phenotypes of delayed or repressed flowering. Furthermore, overexpression of PlacTFL1a gene in petunia also resulted in extremely delayed flowering. In non-flowering 35:PlacTFL1a transgenic petunia plants, the FT-like gene (PhFT) gene was significantly up-regulated and AP1 homologues PFG, FBP26 and FBP29 were significantly down-regulated. Yeast two-hybrid analysis indicated that only weak interactions were detected between PlacTFL1a and PlacFDL, and PlacTFL1a showed no interaction with PhFDL1/2. These results indicated that the TFL1-like genes of *Platanus* have conserved roles in repressing flowering, but probably via a distinct regulatory mechanism.

Key Message

TFL1 -like genes of the basal eudicot *Platanus acerifolia* have conserved roles in maintaining vegetative growth and inhibiting flowering, but may act through distinct regulatory mechanism.

Introduction

London plane tree (*Platanus acerifolia* Willd.) is an interspecific hybrid of *P. orientalis* L. and *P. occidentalis* L., which are basal eudicot species in the family Platanaceae belonging to the order Proteales (Byng et al. 2016). It is widely planted and applied in the cities as an excellent landscape plant, praised as ‘the king of street trees’. However, countless flowers and fruits with pollens and seed hairs, respectively, scatter everywhere in spring and early summer from the adult *Platanus* trees, which adversely impacts urban environment and human health and has become an increasingly serious problem in China that need to be resolved urgently (Liu et al. 2007; Lu et al. 2012). Breeding non-flowering or fruitless cultivars is desired for the species, so it is of great importance to investigate the molecular and genetic mechanisms involved in flowering regulation of *Platanus*.

It has been reported that genes of the phosphatidylethanolamine-binding protein (PEBP) family play crucial roles in controlling floral transition in plants (Wickland and Hanzawa 2015). In *Arabidopsis*, the PEBP (FT/TFL1) gene family includes six members: *FLOWERING LOCUS T* (FT), *TWIN SISTER OF FT* (TSF), *TERMINAL FLOWER 1* (TFL1), *BROTHER OF FT AND TFL1* (BFT), *ARABIDOPSIS CENTRORADIALIS HOMOLOGUE* (ATC), and *MOTHER OF FT AND TFL1* (MFT) (Kobayashi et al. 1999; Jin et al. 2020). FT

and *TFL1* encode proteins that share highly conserved amino acid residues (~ 60% identity), but they have antagonistic functions in flowering regulation: *FT* is a florigen that induces flowering, while *TFL1* represses flowering (Hanzawa et al. 2005; Ahn et al. 2006; Corbesier et al. 2007). *TSF* is the closest *FT* homolog and participates in flowering induction (Yamaguchi et al. 2005; Lee et al. 2019). *BFT* and *ATC* are *TFL1*-like genes and function as repressors of flowering (Yoo et al. 2010; Huang et al. 2012). *MFT* is regarded as ancestral to *FT* and *TFL1* genes, as its homologs exist in both basal land plants (like mosses and lycophytes) and seed plants (gymnosperms and angiosperms) (Hedman et al. 2009; Karlgren et al. 2011). In addition to promoting flowering, *MFT* also plays an important role in regulating seed germination (Yoo et al. 2004; Xi et al. 2010).

To date, *TFL1*-like genes has been identified and characterized in a wide variety of plant species including gymnosperms, monocots, and core eudicots (Wickland and Hanzawa 2015; Liu et al. 2016b). In *Arabidopsis*, *TFL1* is expressed in vegetative and inflorescence meristems to maintain their vegetative character and indeterminate state, and so to control flowering time and inflorescence architecture, respectively (Bradley et al. 1997). Loss-of-function of *TFL1* causes early flowering and determinate inflorescence by formation of a terminal flower, whereas its overexpression dramatically extends both the vegetative and reproductive phases (Ratcliffe et al. 1998). It was proposed that *TFL1* regulates inflorescence indeterminacy by repressing the flower meristem identity genes *LEAFY* (*LFY*) and *APETALA1* (*AP1*) in the center of the meristem, while *LFY* and *AP1* repress the transcription of *TFL1* in lateral floral primordia (Parcy et al. 2002). However, recent findings indicated that *LFY* is actually an activator of *TFL1* and only indirectly represses *TFL1* through *AP1* (Goslin et al. 2017; Serrano-Mislata et al. 2017). The function of flowering repression has been shown to be conserved for *TFL1*-like genes in lots of plant species (Wickland and Hanzawa, 2015); many *TFL1* homologs also have conserved function in controlling inflorescence architecture, such as *CENTRORADIALIS* (*CEN*) in snapdragon (Bradley et al. 1996), *RCN1* and *RCN2* in rice (Nakagawa et al. 2002), *VvTFL1A* in grape (Fernandez et al. 2010), *GhSP* in cotton (McGarry et al. 2016, Si et al., 2018), and *TFL1*-like members in a number of legume crops (Foucher et al. 2003; Tian et al. 2010; Repinski et al. 2012; Liu et al. 2016a; Cheng et al. 2018). In addition, more diverse functions, including those involved in shoot branching and life history strategy, were characterized in *TFL1*-like genes (Perilleux et al. 2019). For instance, *SELF-PRUNING* (*SP*), the *CEN* ortholog of tomato, regulates vegetative to reproductive switching of sympodial meristems (Pnueli et al. 1998); *StTFL1* is involved in tuberization regulation in potato (Guo et al. 2010); *AaTFL1* of *Arabis alpina* plays an important role in polycarpic development (Wang et al. 2011); *HvCEN* promotes axillary bud initiation and tillering of barley (Bi et al. 2019a); *CsCEN* gene maintains the proliferative capacity of axillary meristems by antagonizing the thorn-specifying *THORN IDENTITY1* (*T11*) gene in *Citrus* (Zhang et al. 2021); *TFL1* homolog *KSN* heterozygosity is associated with continuous flowering of *Rosa rugosa* Purple branch (Bai et al. 2021); and *DOTFL1* may controlled pseudobulb formation in the Orchidaceae family (Li et al. 2021). Although the PEBP family is extensively investigated, the functions of *TFL1*-like genes are yet to be explored in basal eudicot species, like London plane tree.

So far, there is very limited information available about the molecular mechanisms controlling flowering in basal eudicots and perennial woody species. In this study, we isolated and characterized three *TFL1*-

like genes from London plane tree, aiming to improve our understanding of flowering regulation in *Platanus* and to provide support for its genetic improvement. The gene structures, phylogenetic relationship, spatial and temporal expression patterns, and protein interaction of the three genes were investigated, and their biological functions were further characterized by overexpressing them in *Arabidopsis* and petunia. The results provide valuable information for understanding the evolution of *TFL1*-like genes in basal eudicots and for creating non-flowering and fruitless varieties of *Platanus*.

Materials And Methods

Plant materials and sample collection

Various samples were collected from two-year-old juvenile and/or over thirty-year-old adult London plane trees grown at the campus of Huazhong Agricultural University (Wuhan, China). Juvenile trees were sampled at June, including roots (JR), stems (JS), newly growing young leaves (JYL), fully expanded mature leaves (JML) and subpetiolar buds (JSB). As described in Fig. S1, the flower development of *Platanus* span two growing seasons. During the first seasons, lateral buds are formed under the petiole base (namely subpetiolar buds) on developing shoots (April-May), followed by two developmental fates. Most subpetiolar buds of adult trees (frequently located at the middle and upper part of the shoots) differentiate inflorescence and secondary shoot meristems individually in the same bud, hereinafter referred to as mixed flower buds. While some subpetiolar buds (frequently located at the bottom part of the shoots or lower shoots of the tree) can only differentiate shoot meristems without inflorescence meristems, hereinafter referred to as vegetative subpetiolar buds. To uncover the comprehensive gene expression patterns during the whole flower and fruit development process, samples from adult trees were collected along two consecutive growing seasons (from April to April of next year), including the stems (S), newly growing young leaves (YL), fully expanded mature leaves (ML), shoot apical buds (AB), lateral subpetiolar buds (SB), vegetative subpetiolar buds (VB), mixed flower buds (MB), vegetative tissues in mixed flower buds (MB-V), inflorescences in mixed flower buds (MB-F), male inflorescences (MF), female inflorescences (FF) and fruits (F). All samples were collected from three individual trees, respectively, and immediately frozen in liquid nitrogen and stored at -80°C until they were used for RNA extraction.

Isolation of *Platanus* TFL1 -like genes

Modified CTAB method was used to extract the total RNA of London plane tissues according to the procedures described by Li (Li et al. 2008). Two µg of total RNA was used for first-strand cDNA synthesis using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara) following the protocol from the manufacturer and oligo (dT) primers. Degenerate primers PlacTFL1-dF and PlacTFL1-dR were used to amplify partial coding sequences of the *PlacTFL1a* and *PlacTFL1b* and designed according to nucleotide alignments of the *TFL1*-like genes from *Vitis vinifera*, *Populus nigra*, *Malus × domestica*, *Citrus sinensis*, and *Eriobotrya japonica*. The PCR was performed by denaturing cDNA at 95°C for 3 min, followed by 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min. The

partial gDNA sequences were also obtained using the same pair of primers. 5' Tail-PCR and 3' RACE were performed to amplify the 5' and 3' terminal regions of *PlacTFL 1a*, respectively; 5'/3' Tail-PCR were carried out to obtain 5'/3' terminal sequences of *PlacTFL 1b*. Primers for amplifying the partial sequence of *PlacBFT* gene was designed according to the transcriptome sequencing data of London plane (unpublished), and 3' RACE was used to amplify its 3' terminal region. The RACE conditions were 95°C for 3 min, followed by 36 cycles of 95°C for 30 s, 60°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min. TAIL-PCR was performed using a modified method (Wang et al. 2011). All primers were designed using Primer 5 software and listed in Table S1. The full-length cDNA and gDNA sequences of the three genes were amplified and cloned into a pMD18-T vector (Takara), and 3–5 positive clones were randomly selected for sequencing.

Sequence alignment and phylogenetic analysis of *Platanus* TFL1 -like genes

Amino acid sequences of representative FT/TFL1-like proteins from *P. acerifolia*, *Arabidopsis thaliana*, *Vitis vinifera* and *Nelumbo nucifera* were aligned using Vector NTI version 11.5 (Invitrogen). Sequences of FT/TFL1 family genes for representative species (Table S2) deposited in the National Center for Biotechnology Information (NCBI) database were retrieved for the construction of a phylogenetic tree. The alignment of amino acid sequences was made with the default settings in MUSCLE implemented in MEGA version 6.0 (Kumar et al. 2004). Phylogenetic tree was constructed using MEGA v6.0 by the Neighbor-Joining (NJ) method with 1000 bootstrap replicates.

Expression analysis of *Platanus* TFL1 -like genes

Semi-quantitative RT-PCR and quantitative real-time PCR (qRT-PCR) analyses were performed to detect the expression of the *Platanus TFL 1*-like genes. Primers were designed within the non-conservative coding region and 3' UTR (untranslated region) using Primer 5.0 software to amplify the products between 90–300 bp in size (Table S1). qRT-PCR was performed in a total volume of 10 µl containing 5 µl 2×SYBR Green Master Mix, 0.2 µl of each forward and reverse primer (10 µmol/µl), 1 µl of the RT reaction mixture as template and water to a final volume. Reactions were carried out on the ABI Prism 7500 Sequence Detection System (Applied Biosystems, USA). PCR efficiency for each primer pair was determined by a standard curve generated with serially diluted cDNA. The results from instrument onboard software Sequence Detector Version 1.3.1 (PE Applied Biosystems) were further subjected to a custom-designed Microsoft Excel macro for analysis. Relative expression levels were calculated by Multiple Condition Solver REST-MCS v2 with *TPI* (*triose phosphate isomerase*) gene of *P. acerifolia* as a normalization (Lu et al. 2012). Calculations were based on three biological replicates and three technical replicates and data were shown as mean values ± SE (standard error).

Vector Construction

To produce transgenic plants overexpressing the *Platanus TFL 1*-like genes, we constructed *35S:PlacTFL 1a*, *35S:PlacTFL 1b* and *35S:PlacBFT* on the expression vector pCambia2300. The full-length

coding regions of *PlacTFL 1a/b* or *PlacBFT* genes were subcloned by *SalI* and *KpnI* or *SalI* and *BamHI* and ligation into pCAMBIA2300, which has cauliflower mosaic virus 35S promoter (*CaMV35S*) and *NOS* terminator. The amplified sequences were confirmed by restriction digestions and DNA sequencing. All generated constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 or AGL0 by the electroporation method.

Plant Transformation And Phenotype Analysis

Genetic transformation of *35S:PlacTFL 1a/b* and *35S:PlacBFT* genes into *A. thaliana* was performed using the floral dip method (Clough and Bent 1998). The seeds from infected plants were cultured on agar-solidified Murashige and Skoog (MS) medium with 50 $\mu\text{g ml}^{-1}$ kanamycin and 50 $\mu\text{g ml}^{-1}$ cefotaxime. Kanamycin-resistant seedlings were cultured in a growth incubator with a photoperiod of 16/8h (light/dark) at $22 \pm 1^\circ\text{C}$. Phenotypic alternations of transgenic lines including the numbers of rosette and cauline leaves, bolting time, and anthesis time were recorded in T_1 generation. For each gene constructions, homozygous transgenic lines defined by screening the kanamycin resistance of T_3 generation were selected for further study, except for the lines with severe phenotype that didn't produce flowers and progeny. Sixteen plants for each homozygous line were used to record phenotypic changes.

Petunia hybrida 'W115' was transformed with *35S:PlacTFL 1a* via the leaf disc method. First, 0.5% hypochlorite was used to sterilize the leaf explants for 20 minutes. Cut the leaves into approximately 0.5×0.5 cm squares and infected by *Agrobacterium tumefaciens* AGL0 containing *35S:PlacTFL 1a* expression vector for 10–15 minutes. Co-cultivate the explants in MS plates (MS medium supplemented with 2.0 mg/ml 6-BAP, 0.1 mg/ml NAA, 1.0 mg/ml zeatin, and 1.0 mg/ml folic acid) without antibiotics for 2–3 days at 25°C under dark condition, and then transfer them into selective medium plates containing 250 $\mu\text{g ml}^{-1}$ carbenicillin and 250 $\mu\text{g ml}^{-1}$ kanamycin. After the shoots appeared, excise and culture them on hormone free MS medium supplemented 1.0 mg/ml folic acid, 250 $\mu\text{g ml}^{-1}$ carbenicillin and 50 $\mu\text{g ml}^{-1}$ kanamycin until rooting. Transgenic plants were identified by PCR with a primer in the 35S promoter (*35SF*) and a *PlacTFL 1a*-specific primer (*PlacTFL 1a-vR*) (Table S1).

Expression Analysis Of Exogenous And Endogenous Genes In Transgenic Plants

To understand the functional conservation of *Platanus* TFL1-like proteins and whether key regulatory genes in *Arabidopsis* were affected by the transgene of *PlacTFL 1*-like genes, we performed qRT-PCR experiment to investigate the expression levels of *AtAP1*, *AtFUL*, *AtLFY* and *AtSOC1*. Seedlings 21 d after sowing were sampled to isolate total RNA in wild type and transgenic *Arabidopsis*. Expression of petunia *FT*-like gene (*PhFT*; GenBank accession no. GU939627) and *AP1* homologues (*PFG*, *FBP26*, and *FBP29*) were investigated using qRT-PCR in apical buds and leaves of *35S:PlacTFL 1a* transgenic petunia plants to understand the underlying mechanism of the repressed flowering phenotypes.

RNA extraction of wild type and transgenic plants were conducted by the Trizol reagent (Takara, Japan) and reverse transcription were carried out using the method described above. *AtEF1a* and *PhEF1a* were used as the endogenous reference genes to normalize the data (Mallona et al. 2010). Primers were listed in Table S1.

Yeast Two-hybrid Assays

The full-length coding sequences of London plane *TFL1*-like and *FD*-like (*PlacFDL*, GenBank accession no. MH845055.1) genes, as well as petunia *FD*-like genes (*PhFDL1* and *PhFDL2*, Appendix S1), were cloned and introduced to the bait plasmid pGBKT7 and prey plasmid pGADT7, respectively. All constructions were confirmed by sequencing. Yeast cells were transformed using the Frozen-EZ Yeast Transformation II Kit (Zymo Research Corp, USA). All baits were tested for autoactivation capacity prior to the screening for potential protein-protein interactions, and none of them showed autoactivation. Co-transformed yeast cells were selected on SD plates lacking Leu and Trp. Interactions were determined by spotting assay on selective SD media lacking Leu, Trp, His and Ade, supplemented with X- α -Gal.

Results

Isolation and phylogenetic analysis of *Platanus* TFL1 -like genes

Using 3' RACE combined with Tail-PCR methods, three *TFL1*-like genes were isolated from London plane tree. Sequence alignment and phylogenetic analysis indicated that two genes belong to the *TFL1* clade, designated as *PlacTFL1a* and *PlacTFL1b* (GenBank accession no. MG344736 and MG344737), while the other one belongs to the *BFT* clade, designated as *PlacBFT* (GenBank accession no. MG344738) (Fig. 1; Fig. 2). Comparison of their cDNA and genomic sequences revealed that *PlacTFL1a/b* and *PlacBFT* genes have identical gene structure (including four exons and three introns) and ORF (open reading frame) length (519 bp encoding 172 aa), and that the four exons of the three genes demonstrated consistent length of 198 bp, 62 bp, 41 bp and 218 bp, respectively (Fig. 1a). However, the length of introns in *PlacTFL1a*, *PlacTFL1b* and *PlacBFT* are different, and their gDNA are 1,632 bp, 1,355 bp, and 1,324 bp in length, respectively. *PlacTFL1a* and *PlacTFL1b* genes share high similarity, with the identity of 91.7% and 90.1% at nucleotide and amino acid levels, respectively, whereas *PlacBFT* was less closely related to *PlacTFL1a/b*, showing only 72.4% and 71.3% identity of nucleotide and 75.1% and 73.4% identity of amino acid with *PlacTFL1a* and *PlacTFL1b*, respectively (Fig. 1).

Multiple sequence alignment of the deduced amino acid sequences of the FT/TFL1-like proteins in *P. acerifolia* and several other species showed that *PlacTFL1a/b* and *PlacBFT* proteins had characteristic features of the TFL1-like proteins. The conserved key amino acid residues His88 and Asp144 of TFL1 (Ahn et al. 2006) were found at corresponding positions (His84 and Asp140) of *PlacTFL1a/b* and *PlacBFT* proteins, in addition, the amino acids Ser142, Ala173, Arg175, and Arg176 that were shown to be differently selected in TFL1-like proteins after the duplication resulting in FT and TFL1 clades (Wang et al. 2015) were also conserved in *PlacTFL1a/b* and *PlacBFT* (Fig. 1b). Phylogenetic analysis using the amino

acid sequences of FT/TFL1 family proteins from London plane and other 12 representative species showed that the gene family consists of TFL1-like, FT-like, and MFT-like clades. TFL1-like clade is further divided into ATC, TFL1 and BFT subclades, and ATC subclade only contains genes from core eudicots (Fig. 2). *PlacTFL1a/b* and *PlacBFT* showed the closest relationship with their corresponding orthologs of *Nelumbo nucifera*, *NnuFTL7/8* and *NnuFTL9/10*, respectively.

TFL1 -like genes display distinct expression patterns in London plane

To get insight into the potential roles of the three *TFL1*-like genes in *Platanus*, we investigate their spatiotemporal expression patterns in juvenile (two-year-old) and adult (30-year-old) London plane trees. rt-PCR was performed to pretest the expression patterns of the three genes in various tissues and ontogenetic stages. The results showed that all three *Platanus TFL1*-like genes were primarily expressed in vegetative tissues, and no expression of them was detected in different development stages of inflorescences and fruits (Fig. S2).

The expression levels were further detected in vegetative tissues and several development stages of inflorescences and fruits from juvenile and/or adult trees by qrt-PCR, which showed that *PlacTFL1a* was expressed in all sampled tissues of juvenile plants, with high expression in young leaves and stems, low expression in mature leaves and SBs, and weak expression in roots (Fig. 3a). In adult trees, *PlacTFL1a* was expressed mainly in stems, young leaves, shoot apical buds, VBs, SBs before inflorescence initiation (April and May), and vegetative tissues in MBs, but was rarely detected in mature leaves, inflorescences, and fruits (Fig. 3b). The expression levels of *PlacTFL1a* in stems, shoot apical buds, and SBs increased at May compared to April, but its expression in SBs suddenly dropped at June when the inflorescences began to differentiate. In the VBs that did not form inflorescences, *PlacTFL1a* maintains high expression levels at July, and declines during later months, till very weak or no expression being detected during dormant period (Dec to Jan); after the dormancy release, expression of *PlacTFL1a* resumed gradually again at Feb to March (Fig. 4). In the vegetative tissues of MBs, *PlacTFL1a* displayed the same expression pattern as in the VBs (Fig. 4). Compared to *PlacTFL1a*, *PlacTFL1b* showed much lower levels as well as narrower regions of expression. For instance, it was only weakly expressed in SBs and not detected in roots, stems, and young or mature leaves of juvenile trees (Fig. 3a); in adult trees, *PlacTFL1b* was mainly expressed in apical and supetiolar buds at April (Fig. 3b), with less transcripts in VBs and vegetative tissues of MBs during the growing period (Fig. 4). *PlacBFT* was also expressed in all detected tissues of juvenile plants, but predominantly in stems and roots followed by SBs and mature leaves (Fig. 3a). In adult trees, *PlacBFT* was predominantly expressed in stems (April and May), and lower expression was detected in SBs at April and VBs at March, with almost no expression in other tissues (Fig. 3b; Fig. 4).

Ectopic expression of *Platanus TFL1* -like genes in *Arabidopsis* inhibits flowering

To figure out the potential functions of *Platanus TFL1*-like genes in flowering regulation, *CaMV35S* was used to ectopically express *PlacTFL1a/b* and *PlacBFT* genes in *Arabidopsis*. Thirty-eight, fifty-one and thirty-three independent transgenic lines were achieved for *35S:PlacTFL1a*, *35S:PlacTFL1b* and *35S:PlacBFT*, respectively. Overexpression of the three *Platanus TFL1*-like genes in *Arabidopsis* resulted

in similar phenotypic alterations in delaying or repressing flowering (Fig. 5a-d; Table S3). Among the transgenic lines, five from *35S:PlacTFL 1a*, eight from *35S:PlacTFL 1b*, and three from *35S:PlacBFT* displayed the strongest phenotypes, which produced an average of 50–54 rosette leaves before bolting under long day conditions (16/8 h, day/night), with the maximum number of rosette leaves up to 75 (Fig. 5e). After bolting, the inflorescence meristems of these plants maintained the status to form secondary and tertiary inflorescences reiteratively rather than to produce flowers until death (Fig. 5f), so we can't obtain seeds and progenies from these strong phenotypic lines. For the transgenic lines with moderate or weak phenotypes, homozygous plants were chosen for further observation. We found most transgenic lines with moderate or weak phenotypes enhanced late flowering in their homozygous generation. For instance, homozygous lines #20 and #24 of *35S:PlacTFL 1a* and #19 of *35S:PlacTFL 1b* bolted with twenty to forty rosette leaves, but they always maintained at the inflorescence-producing status without flowers (Fig. 5g; Table S3). Transgenic lines with relatively weak phenotypes ultimately converted to flowering when inflorescences developed to a certain degree (Fig. 5h). In addition, overexpression of the three *PlacTFL 1*-like genes in *Arabidopsis* also showed higher plant stature, larger leaves, thicker inflorescence stems, and more cauline leaves compared with the wild-type plants (Fig. 5d-f, i; Table S3).

qRT-PCR analysis of transgenes in wild-type *Arabidopsis* (Col-0) and transgenic lines indicated that the phenotypic variations of the three *PlacTFL 1*-like transgenic plants are related in a certain extent to the expression levels of the transgenes, namely higher expression levels tend to result in more severe phenotypic changes such as later bolting and flowering time or more rosette leaves (Fig. 6; Table S3). Expression analysis of the flowering time related genes in *35S:PlacTFL 1*-like transgenic plants showed that *AP1*, *FUL*, *LFY* and *SOC1* were intensively down-regulated in 21-d-old seedlings of *35S:PlacTFL 1a* line 20 and *35S:PlacTFL 1b* line 19, especially *AP1* and *FUL* genes showed the strongest downregulating (Fig. 6). It is worth noting that only *AP1* and *FUL* genes were significantly declined in *35S:PlacBFT* line 26 (Fig. 6).

Overexpression of *PlacTFL1a* in petunia represses flowering and promotes branching

Due to the similar and strong phenotypes in *Arabidopsis* overexpressing *PlacTFL 1a/b* and *PlacBFT*, we further investigated the function of *PlacTFL 1a* by ectopic expression in petunia. After verified by PCR amplification of the transgene, twenty-five independent T₀ transgenic plants were obtained, but only three of them (#3, #6, and #23) showed evident expression via RT-PCR detection (Fig. 7a). Phenotypic observation and statistics analysis indicated that #6 and #23 lines showed obviously late flowering with more leaves and branches (Fig. 7b, c). In particular, the transgenic individual #6 maintained vegetative growth for more than one year and didn't flower after several generations of cutting propagation, whereas wild-type W115 plants usually blossom within two months after planted (Fig. 7c). Finally, no seeds and sexual progenies can be obtained from #6 that was so excluded from the statistical analysis (Fig. 7b). Transgenic line #23 showed moderate phenotype of delayed flowering. It is worth mentioning that the main shoots of #23 usually return to vegetative growth after forming the first flower (Fig. 7c, d). RT-PCR analysis revealed that plants showing severe phenotype had higher expressed levels of the transgene

compared with the plants exhibiting moderate or weak phenotype (Fig. 7), indicating that the transgene is responsible for the phenotypic alterations.

In order to study the underlying mechanism of repressed flowering in *PlacTFL1a*-overexpressing plants, expression of petunia *FT*-like gene (*PhFT*) and *AP1* homologues (*PFG*, *FBP26*, and *FBP29* genes) were investigated in apical buds and leaves of the transgenic line #6 and wild-type 'W115'. The results indicated that *PhFT* gene was upregulated in apical buds of *35S:PlacTFL1a* transgenic plant, whereas *PFG*, *FBP26* and *FBP29* genes were downregulated, especially *FBP26* was significantly repressed in both apical buds and leaves (Fig. 8). The expression tendency of up- and down-regulation in leaves was consistent with that in apical buds, although statistically significant differences was only detected for *FBP26* gene (Fig. 8a).

Interactions Between London Plane TFL1-like And FD-like Proteins

A yeast two-hybrid analysis was performed to evaluate whether the TFL1-like proteins of *Platanus* can interact like the most situation in other species with its FD-like proteins. The coding sequences of *PlacTFL1a/b*, *PlacBFT*, *PlacFDL* and *PhFDL1/2* genes were cloned into the pGADT7 (prey) and pGBKT7 (bait) domains, respectively, and used for interactional analysis. The results indicated that only *PlacTFL1a* had a weak interaction with *PlacFDL* in *P. acerifolia* (Fig. 9). Based on the ability of overexpressing *PlacTFL1a* to repress flowering in petunia, the interactions between *P. acerifolia* TFL1-like proteins and the FD-like proteins of petunia were also investigated. As a result, no interaction was detected between *PlacTFL1a* and petunia FD-like proteins (Fig. 9). However, the interaction of *PlacBFT* and *PhFDL1* was detected in one direction (Fig. 9).

Discussion

Phylogenetic evolution of the FT/TFL1 -like genes in basal eudicots

Previous phylogenetic analyses indicated that the PEBP gene family undergone two ancient duplications that generated three clades: *MFT*-like, *FT*-like and *TFL1*-like (Karlgrén et al. 2011; Liu et al. 2016b). The first duplication giving rise to the *MFT*-like and *FT/TFL1*-like clades was suggested to take place in the common ancestor of seed plants or even earlier; the second duplication happened before the divergence of seed plants, resulting in the *FT*-like and *TFL1*-like clades (Liu et al. 2016b). After the separation of gymnosperms and angiosperms, further duplications occurred in each group. In angiosperms, an early whole-genome duplication (WGD) event has produced duplicate genes in each clade, and so most angiosperms contain approximately a half-dozen PEBP genes (Table S2). According to the evolutionary history of FT/TFL1 gene family, basal eudicots like *P. acerifolia* should possess *MFT*-like, *FT*-like and *TFL1*-like homologues. Previously, our research group have identified two *Platanus* *FT*-like genes *PaFT* and *PaFTL* that exhibited the function of promoting flowering in transgenic *Arabidopsis* or tobacco plants (Zhang et al. 2011; Cai et al. 2019). In order to comprehensively understand the evolution and functions of the FT/TFL1 gene family in *Platanus*, we isolated here another three genes of the family. Sequence

alignment and phylogenetic analysis indicated that the three genes belong to *TFL1*-like clade (Fig. 1; Fig. 2).

In accordance with previous studies, our phylogenetic tree divided the PEBP proteins from 13 species (including gymnosperm, basal angiosperm, monocot, basal eudicots, and core eudicots) into MFT-like, FT-like, and TFL1-like groups. MFT-like clade was further separated into two subgroups, and two or more genes are present in most species except *Aquilegia coerulea*, *Arabidopsis*, *Fragaria vesca* and *Populus nigra* that may have lost one copy (Fig. 2). In *Platanus*, two MFT-like genes were speculated, which was supported by the transcriptome data wherein two distinct MFT-like transcripts were found (data not shown). TFL1-like clade was also grouped into two classes, BFT-like and TFL1-like, both of which consist of genes from all included basal angiosperm, basal eudicots, and core eudicots (Fig. 2), indicating that the duplication generating these two lineages should occur in the common ancestor of angiosperms. The members from rice are present only in TFL1-like other than BFT-like lineage, suggesting that monocots may have lost the BFT-like genes. Furthermore, the phylogenetic tree shows that the genes from core eudicots in the *TFL1*-like lineage form two subgroups, ATC-like and *TFL1*-like (Fig. 2), indicating that *TFL1*-like clade experienced another duplication during the evolution of core eudicots. Whereas, some species like *Populus* and *Jatropha* have lost the *TFL1*-like genes.

In basal eudicots, *Nelumbo nucifera* and *Platanus* belong to the same order, Proteales (Byng et al., 2016). In contrast to 7 PEBP members in *Platanus* (two MFT-like, three *TFL1*-like, and two FT-like), *N. nucifera* contains more FT/*TFL1* genes, including four MFT-like, four FT-like and four *TFL1*-like genes (Fig. 2), which should be resulted from a recent whole-genome duplication in *N. nucifera* (Ming et al. 2013; Wang et al. 2013). Another species of the basal eudicot, *A. coerulea*, contains one MFT-like, three *TFL1*-like, and three FT-like genes, indicating it has lost one MFT-like member and experienced further duplication of the *TFL1*-like and FT-like genes.

Divergent expression and function of the TFL1 -like genes in *P. acerifolia*

In general, gene expression pattern has significant relationship with their functions. The expression patterns of *TFL1*-like genes show obviously changes among different members and/or plant species, and their functions also exhibit significant divergence and diversity (Wickland and Hanzawa 2015). For instance, *Arabidopsis TFL1* transcripts are present in vegetative and inflorescence meristems to repress flowering and maintain inflorescence indeterminacy (Bradley et al. 1997; Serrano-Mislata et al. 2016), its paralog *BFT* is expressed in the shoot apical meristem, young leaf, and axillary inflorescence meristem (Yoo et al. 2010), whereas another paralog *ATC* was only detected in the hypocotyl of young plants, and not in the inflorescence meristem (Mimida et al. 2001). Apple *TFL1*-like genes *MdTFL1* and *MdTFL1a* are expressed in the vegetative tissues in both the adult and juvenile phases; *MdCENa* (*ATC* ortholog) is mainly expressed in fruit receptacles, cultured tissues, and roots, while *MdCENb* is silenced in most organs (Mimida et al. 2009). The three *TFL1*-like genes in *Jatropha curcas* also show distinct expression patterns: *JcTFL1a* and *JcTFL1c* are mainly expressed in the roots of juvenile plants, whereas *JcTFL1b* transcripts are abundantly accumulated in the fruits and stems (Li et al. 2015; Li et al. 2017).

Like most *TFL 1*-like genes in other species, the three *TFL 1*-like genes of London plane are preferentially expressed in vegetative tissues, but they have distinct spatiotemporal expression patterns. *PlacTFL 1a* was widely expressed in vegetative organs of both juvenile and adult plants, including stems, leaves, apical buds, VBs, and the vegetative tissues of MBs (Fig. 3; Fig. 4). The expression of *PlacTFL 1a* in SBs increased gradually prior to the inflorescence initiation (from April to May), but dramatically decreased during the inflorescence differentiation period (June), suggesting that *PlacTFL 1a* play a crucial role in maintaining the vegetative growth and repressing the reproductive development of London plane, which is further supported by the highest expression level of *PlacTFL 1a* in the VBs at July when the inflorescences are developing (Fig. 4). Based on this hypothesis, we speculate that higher expression level of *PlacTFL 1a* should be present in the VBs at June, but at that moment the subpetiolar buds maintaining vegetative status could not be distinguished visibly from those undergoing flower bud differentiation, and so not detected. It is interesting that the expression levels of *PlacTFL 1b* is significantly lower than *PlacTFL 1a* and only weak expression is detected in a few tissues (Fig. 3; Fig. 4), although they are very closely related in terms of the coding sequences, with the identity of 91.7% at nucleotide level. In general, functional evolution of genes depends on two aspects: the change of gene coding sequences and alteration of gene expression patterns. Overexpression of *PlacTFL 1a* and *PlacTFL 1b* in *Arabidopsis* resulted in comparable phenotypic changes (Fig. 5), indicating that *PlacTFL 1b* has retained its function in point of protein sequence but may have lost most functions in London plane due to expression degeneration after duplication, similar to above-mentioned apple *MdCENb* (Mimida et al. 2009). Expression pattern of *PlacBFT* is also significantly different from that of *PlacTFL 1a*, with predominant expression in stems and roots, and weak in growing SBs (Fig. 3), suggesting *PlacBFT* may have undergone subfunctionalization during evolution.

Unlike some *TFL 1*-like genes that are strongly expressed in developing inflorescences, such as *Arabidopsis TFL 1* (Bradley et al. 1997), *Antirrhinum CEN* (Bradley et al. 1996), and *HvTFL 1s* in rubber tree (Bi et al. 2019b), no *TFL 1*-like genes of London plane were expressed evidently in inflorescences with various developmental stages (Fig. 3; Fig. S2). Given that the expression of *TFL 1*-like genes in the inflorescences is related to their functions in the control of inflorescence architecture (Bradley et al. 1996; Bradley et al. 1997; Nakagawa et al. 2002; Fernandez et al. 2010; Perilleux et al. 2019), we speculate that the *TFL 1*-like genes of London plane may not involve in inflorescence development. To verify their functions, the three *TFL 1*-like genes of *Platanus* were further investigated by transgenic studies in *Arabidopsis* and petunia. Overexpression of each gene delayed or repressed flowering, increased the number of leaves and nodes in transgenic plants compared to their wild-type counterparts, as reported in other species that constitutively express *TFL 1*-like genes, confirming their highly functional conservation in flowering regulation among different plant species.

Potential mechanism of *PlacTFL 1a* function

In model plants, *TFL 1* functions via directly repressing flowering-related genes, such as *AP1*, *FUL*, and *LFY* (Bradley et al. 1997; Ratcliffe et al. 1999; Hanano and Goto 2011). In contrast to *PlacTFL 1a*, the expression of London plane *AP1* homologs (*FUL*-like genes, *PlacFLs*) increased in SBs at the stage of

inflorescence initiation (June) and maintained their expression level during the inflorescence developing process (Zhang et al. 2019), suggesting that *PlacTFL1a* inhibits reproductive development and flowering probably through repressing the expression of *FUL*-like genes in *Platanus*, which is consistent with the results reported in pear in which the expression of *TFL1*-like genes (*PpTFL1-1a* and *PpTFL1-2a*) rapidly decrease in reproductive meristems followed by upregulation of *PpAP1* and *PpFUL* genes (Bai et al. 2017). Furthermore, significant downregulation of *PFG*, *FBP26*, and *FBP29* genes were detected in *35:PlacTFL1a* transgenic petunia plants that displayed the phenotype of severely repressed flowering (Fig. 8). It has been reported previously that knockdown of *PFG* and *FBP26* genes (two *FUL* orthologs) represses the transition from vegetative to reproductive development in petunia, resulting in a phenotype exactly similar to the *35:PlacTFL1a* transgenic plant #23 in our study (Immink et al. 1999), which indicates that *35:PlacTFL1a* represses flowering at least partially through regulating the expression of *AP/FUL*-like genes in petunia. All these results support a probably conserved regulatory mechanism between *TFL1* and *AP1/FUL*-like genes in flowering regulation. However, our transgenic individual #6 showed drastically non-flowering phenotype even after several generations of propagation by cutting lasting for approximate two years, which is much more late-flowering than the *PFG* and *FBP26* downregulated plants, even than a quadruple mutant of all the petunia *AP1/FUL*-like genes, *pfg fbp26 fbp29 euap1* (Morel et al. 2019), suggesting that *35:PlacTFL1a* must have regulated other flowering-related genes besides the *AP1/FUL*-like genes. Indeed, we found a *FT*-like gene (*PhFT*) was significantly upregulated in the leaves of *35S:PlacTFL1a* transgenic line #6 (Fig. 8). However, *FT* and its orthologs in most plant species were proved to function as florigens that promote flowering (Wickland and Hanzawa 2015). Interestingly, our recent study indicated that *PhFT* (corresponding to *PhFT1* therein) might function as a repressor of flowering in petunia, because its overexpression in *Arabidopsis* resulted in significantly late flowering (Wu et al. 2019). The functions of repressing flowering have also been reported for several *FT*-like genes in other species, such as *BvFT1* in sugar beet (Pin et al. 2010), *HaFT1* in sunflower (Blackman et al. 2010), *NtFT1/2/3* in tobacco (Harig et al. 2012), and *SISP5G(2/3)* in tomato (Cao et al. 2015). In summary, *PlacTFL1a* represses flowering in petunia might through activating the flowering repressor *PhFT1* and inhibiting the flowering promoters *AP1/FUL*-like genes, and some other unknown regulators if any.

It is well known that *FT/TSF* proteins are produced in leaf and then transmitted to shoot apical meristem where they induce the formation of floral meristem through interacting with *FD* to activate the expression of downstream genes *SOC1* and *AP1* (Yamaguchi et al. 2005). *TFL1* repress flowering probably via interacting with *FD* to compete with *FT* (Hanano and Goto 2011; Ho and Weigel 2014; Zhu et al. 2020). Besides *Arabidopsis*, *TFL1*-like proteins interacting with *FD* homologues have been identified in *Rosa chinensis*, kiwifruit and so on (Varkonyi-Gasic et al. 2013; Randoux et al. 2014; Kaneko-Suzuki et al. 2018). To confirm the conservation of this regulatory mechanism, interactions between London plane *TFL1*-like proteins and *FD* homologue (*PlacFDL*) were investigated by yeast two-hybrid analysis. The results demonstrated that only *PlacTFL1a* has weak interaction with *PlacFDL*, while both *PlacTFL1b* and *PlacBFT* have no interaction with *PlacFDL* (Fig. 9). Even so, ectopic expression of both *PlacTFL1b* and *PlacBFT* in *Arabidopsis* still can delay flowering. Two hypotheses could be used to explain this result: one

possibility is that PlacTFL1b and PlacBFT are able to interact with other FD-like members in London plane, as well as *Arabidopsis* FD protein; alternatively, interaction between *Platanus* TFL1-like protein and FD-like protein is not necessary for its function in repressing flowering. The latter assumption is supported by the fact that overexpression of *PlacTFL1a* in petunia results in repressed flowering, but no interaction between PlacTFL1a as well as PlacTFL1b and petunia FD-like proteins (PhFDL1 and PhFDL2) was detected (Fig. 9), while PlacBFT does interact with one of the petunia FD-like protein PhFDL1. A recent genome-wide ChIP-seq analysis demonstrated that TFL1 may interact with other DNA-binding proteins, besides FD, to regulate the expression of downstream genes (Goretti et al. 2020). In summary, our results confirmed the function of *Platanus* TFL1-like genes in repressing flowering, but probably via a distinct regulatory mechanism.

Declarations

Author contributions LGF and BMZ designed the experiments. ZSS, ZQ, YXY, LYJ and SMM performed the experiments. ZSS, WJQ, JJ and NCR analyzed the data. ZSS and LGF wrote and revised the manuscript. All authors participated in the research and approved the final manuscript.

Funding This work was financially supported by the National Natural Science Foundation of China (31272206, 32102425, 31772345) and the Bureau of Forestry and Landscaping of Wuhan Municipality (Grant No. WHGF2022A09, WHGF2019A05). We thank all colleagues in our laboratory for discussions and technical assistance.

Availability of data and materials All data used in this research are included in this published article and its supplementary information files.

Conflicts of interest The authors declare that they have no conflict of interest.

Code availability Not applicable.

References

1. Ahn JH, Miller D, Winter VJ, Banfield MJ, Lee JH, Yoo SY, Henz SR, Brady RL, Weigel D (2006) A divergent external loop confers antagonistic activity on floral regulators FT and TFL1. *EMBO J* 25:605-614
2. Bai M, Liu J, Fan C, Chen Y, Chen H, Lu J, Sun J, Ning G, Wang C (2021) KSN heterozygosity is associated with continuous flowering of *Rosa rugosa* Purple branch. *Hortic Res* 8:26
3. Bai S, Tuan PA, Saito T, Ito A, Ubi BE, Ban Y, Moriguchi T, Wilson Z (2017) Repression of TERMINAL FLOWER1 primarily mediates floral induction in pear (*Pyrus pyrifolia* Nakai) concomitant with change in gene expression of plant hormone-related genes and transcription factors. *J Exp Bot* 68:4899-4914

4. Bi X, van Esse W, Mulki MA (2019) CENTRORADIALIS Interacts with FLOWERING LOCUS T-Like Genes to Control Floret Development and Grain Number. *Plant Physiol* 180:1013-1030
5. Bi Z, Tahir AT (2019) Cloning and functional analysis of five TERMINAL FLOWER 1/CENTRORADIALIS-like genes from *Hevea brasiliensis*. *Physiol Plantarum* 166:612-627
6. Blackman BK, Strasburg JL, Raduski AR, Michaels SD, Rieseberg LH (2010) The role of recently derived FT paralogs in sunflower domestication. *Curr Biol* 20:629-635
7. Bradley D, Carpenter R, Copsey L, Vincent C, Rothstein S, Coen E (1996) Control of inflorescence architecture in *Antirrhinum*. *Nature* 379:791-797
8. Bradley D, Ratcliffe O, Vincent C, Carpenter R, Coen E (1997) Inflorescence commitment and architecture in *Arabidopsis*. *Science* 275:80-83
9. Byng JW, Chase MW, Christenhusz MJM, Fay MF, Judd WS, Mabberley DJ, Sennikov AN, Soltis DE, Soltis PS, Stevens PF, Briggs B, Brockington S, Chautems A, Clark JC, Conran J, Haston E, Moller M, Moore M, Olmstead R, Perret M, Skog L, Smith J, Tank D, Vorontsova M, Weber A, Grp AP (2016) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot J Linn Soc* 181: 1-20
10. Cai F, Shao C, Zhang Y, Bao Z, Li Z, Shi G, Bao M, Zhang J (2019) Identification and characterisation of a novel FT orthologous gene in London plane with a distinct expression response to environmental stimuli compared to PaFT. *Plant Biol* 21:1039-1051
11. Cao K, Cui L, Zhou X, Ye L, Zou Z, Deng S (2015) Four tomato FLOWERING LOCUS T-like proteins act antagonistically to regulate floral initiation. *Front Plant Sci* 6:1213
12. Cheng X, Li G, Tang Y, Wen J (2018) Dissection of genetic regulation of compound inflorescence development in *Medicago truncatula*. *Development* 145.3:dev158766-dev158766.
13. Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16:735-743
14. Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G (2007) FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* 316:1030-1033
15. Fernandez L, Torregrosa L, Segura V, Bouquet A, Martinez-Zapater JM (2010) Transposon-induced gene activation as a mechanism generating cluster shape somatic variation in grapevine. *Plant J* 61:545-557
16. Foucher F, Morin J, Courtiade J, Cadioux S, Ellis N, Banfield MJ, Rameau C (2003) DETERMINATE and LATE FLOWERING are two TERMINAL FLOWER1/CENTRORADIALIS homologs that control two distinct phases of flowering initiation and development in pea. *Plant Cell* 15:2742-2754
17. Goretti D, Silvestre M (2020) TERMINAL FLOWER1 Functions as a mobile transcriptional cofactor in the shoot apical meristem. *Plant Physiol* 182:2081-2095
18. Goslin K, Zheng B, Serrano-Mislata A (2017) Transcription factor interplay between LEAFY and APETALA1/CAULIFLOWER during floral initiation. *Plant Physiol* 174:1097-1109

19. Guo JL, Yu CL, Fan CY, Lu QN, Yin JM, Zhang YF, Yang Q (2010) Cloning and characterization of a potato TFL1 gene involved in tuberization regulation. *Plant Cell Tiss Org* 103:103-109
20. Hanano S, Goto K (2011) *Arabidopsis* TERMINAL FLOWER1 is involved in the regulation of flowering time and inflorescence development through transcriptional repression. *Plant Cell* 23:3172-3184
21. Hanzawa Y, Money T, Bradley D (2005) A single amino acid converts a repressor to an activator of flowering. *Proc Natl Acad Sci USA* 102:7748-7753
22. Harig L, Beinecke FA, Oltmanns J, Muth J, Müller O, Rüping B, Twyman RM, Fischer R, Prüfer D, Noll GA (2012) Proteins from the FLOWERING LOCUS T-like subclade of the PEBP family act antagonistically to regulate floral initiation in tobacco. *Plant J* 72:908-921
23. Hedman H, Källman T, Lagercrantz U (2009) Early evolution of the MFT-like gene family in plants. *Plant Mol Biol* 70:359-369
24. Ho WW, Weigel D (2014) Structural features determining flower-promoting activity of *Arabidopsis* FLOWERING LOCUS T. *Plant Cell* 26:552-564
25. Huang NC, Jane WN, Chen J, Yu TS (2012) *Arabidopsis thaliana* CENTRORADIALIS homologue (ATC) acts systemically to inhibit floral initiation in *Arabidopsis*. *Plant J* 72:175-184
26. Immink RG, Hannapel DJ, Ferrario S, Busscher M, Franken J, Lookeren Campagne MM, Angenent GC (1999) A petunia MADS box gene involved in the transition from vegetative to reproductive development. *Development* 126:5117-5126
27. Jin S, Nasim Z, Susila H, Ahn JH (2021) Evolution and functional diversification of FLOWERING LOCUS T/TERMINAL FLOWER 1 family genes in plants. *Semin Cell Dev Biol* 109:20-30
28. Kaneko-Suzuki M, Kurihara-Ishikawa R, Okushita-Terakawa C, Kojima C, Nagano-Fujiwara M, Ohki I, Tsuji H, Shimamoto K, Taoka KI (2018) TFL1-like proteins in rice antagonize rice ft-like protein in inflorescence development by competition for complex formation with 14-3-3 and FD. *Plant Cell Physiol* 59:458-468
29. Karlgren A, Gyllenstrand N, Källman T, Sundström JF, Moore D, Lascoux M, Lagercrantz U (2011) Evolution of the PEBP gene family in plants: functional diversification in seed plant evolution. *Plant Physiol* 156:1967-1977
30. Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. *Science* 286:1960-1962
31. Kumar S, Tamura K, Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 5:150-163
32. Lee C, Kim SJ, Jin S, Susila H, Youn G, Nasim Z, Alavilli H, Chung KS, Yoo SJ, Ahn JH (2019) Genetic interactions reveal the antagonistic roles of FT/TSF and TFL1 in the determination of inflorescence meristem identity in *Arabidopsis*. *Plant J* 99:452-464
33. Li C, Fu Q, Niu L, Luo L, Chen J, Xu ZF (2017) Three TFL1 homologues regulate floral initiation in the biofuel plant *Jatropha curcas*. *Sci Rep* 7:43090

34. Li C, Luo L, Fu Q, Niu L, Xu ZF (2015) Identification and Characterization of the FT/TFL1 Gene Family in the Biofuel Plant *Jatropha curcas*. Springer US 2
35. Li Y, Zhang B (2021) DOTFL1 affects the floral transition in orchid *Dendrobium Chao Praya Smile*. Plant Physiol 186:2021-2036
36. Li Z, Liu G, Zhang J, Zhang J, Bao M (2008) Extraction of high-quality tissue-specific RNA from London plane trees (*Platanus acerifolia*), permitting the construction of a female inflorescence cDNA library. Funct Plant Biol 35:159-165
37. Liu G, Li Z, Bao M (2007) Colchicine-induced chromosome doubling in *Platanus acerifolia* and its effect on plant morphology. Euphytica 157:145-154
38. Liu Y, Zhang D, Ping J, Li S, Chen Z, Ma J (2016) Innovation of a regulatory mechanism modulating semi-determinate stem growth through artificial selection in soybean. PLoS Genet 12:e1005818
39. Liu YY, Yang KZ, Wei XX, Wang XQ (2016) Revisiting the phosphatidylethanolamine-binding protein (PEBP) gene family reveals cryptic FLOWERING LOCUS T gene homologs in gymnosperms and sheds new light on functional evolution. New Phytol 212:730-744
40. Lu S, Li Z, Zhang J, Yi S, Liu L, Bao M, Liu G (2012) Isolation and expression analysis of a LEAFY/FLORICAULA homolog and its promoter from London plane (*Platanus acerifolia* Willd.). Plant Cell Rep 31:1851-1865
41. Mallona I, Lischewski S, Weiss J, Hause B, Egea-Cortines M (2010) Validation of reference genes for quantitative real-time PCR during leaf and flower development in *Petunia hybrida*. BMC Plant Biol 10:4
42. McGarry RC, Prewitt SF, Culpepper S, Eshed Y, Lifschitz E, Ayre BG (2016) Monopodial and sympodial branching architecture in cotton is differentially regulated by the *Gossypium hirsutum* SINGLE FLOWER TRUSS and SELF-PRUNING orthologs. New Phytol 212:244-258
43. Mimida N, Goto K, Kobayashi Y, Araki T, Ahn JH, Weigel D, Murata M, Motoyoshi F, Sakamoto W (2001) Functional divergence of the TFL1-like gene family in *Arabidopsis* revealed by characterization of a novel homologue. Genes Cells 6:327-336
44. Mimida N, Kotoda N, Ueda T, Igarashi M, Hatsuyama Y, Iwanami H, Moriya S, Abe K (2009) Four TFL1/CEN-like genes on distinct linkage groups show different expression patterns to regulate vegetative and reproductive development in apple (*Malus x domestica* Borkh.). Plant Cell Physiol 50:394-412
45. Ming R, VanBuren R, Liu Y, Yang M, Han Y, Li LT, Zhang Q, Kim MJ, Schatz MC, Campbell M, Li J, Bowers JE, Tang H, Lyons E, Ferguson AA, Narzisi G, Nelson DR, Blaby-Haas CE, Gschwend AR, Jiao Y, Der JP, Zeng F, Han J, Min XJ, Hudson KA, Singh R, Grennan AK, Karpowicz SJ, Watling JR, Ito K, Robinson SA, Hudson ME, Yu Q, Mockler TC, Carroll A, Zheng Y, Sunkar R, Jia R, Chen N, Arro J, Wai CM, Wafula E, Spence A, Han Y, Xu L, Zhang J, Peery R, Haus MJ, Xiong W, Walsh JA, Wu J, Wang ML, Zhu YJ, Paull RE, Britt AB, Du C, Downie SR, Schuler MA, Michael TP, Long SP, Ort DR, Schopf JW, Gang DR, Jiang N, Yandell M, dePamphilis CW, Merchant SS, Paterson AH, Buchanan BB, Li S, Shen-

- Miller J (2013) Genome of the long-living sacred lotus (*Nelumbo nucifera* Gaertn.). *Genome Biol* 14:R41
46. Morel P, Chambrier P (2019) Divergent Functional Diversification Patterns in the SEP/AGL6/AP1 MADS-Box Transcription Factor Superclade. *Plant Cell* 31:3033-3056
 47. Nakagawa M, Shimamoto K, Kyojuka J (2002) Overexpression of RCN1 and RCN2, rice TERMINAL FLOWER 1/CENTRORADIALIS homologs, confers delay of phase transition and altered panicle morphology in rice. *Plant J* 29:743-750
 48. Périlleux C, Bouché F, Randoux M, Orman-Ligeza B (2019) Turning Meristems into Fortresses. *Trends Plant Sci* 24:431-442
 49. Parcy F, Bomblies K, Weigel D (2002) Interaction of LEAFY, AGAMOUS and TERMINAL FLOWER1 in maintaining floral meristem identity in *Arabidopsis*. *Development* 129:2519-2527
 50. Pin PA, Benlloch R, Bonnet D, Wremmerth-Weich E, Kraft T, Gielen JJ, Nilsson O (2010) An antagonistic pair of FT homologs mediates the control of flowering time in sugar beet. *Science* 330:1397-1400
 51. Pnueli L, Carmel-Goren L, Hareven D, Gutfinger T, Alvarez J, Ganai M, Zamir D, Lifschitz E (1998) The SELF-PRUNING gene of tomato regulates vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. *Development* 125:1979-1989
 52. Randoux M, Davière JM, Jeauffre J, Thouroude T, Pierre S, Toualbia Y, Perrotte J, Reynoird JP, Jammes MJ, Hibrand-Saint Oyant L, Foucher F (2014) RoKSN, a floral repressor, forms protein complexes with RoFD and RoFT to regulate vegetative and reproductive development in rose. *New Phytol* 202:161-173
 53. Ratcliffe OJ, Amaya I, Vincent CA, Rothstein S, Carpenter R, Coen ES, Bradley DJ (1998) A common mechanism controls the life cycle and architecture of plants. *Development* 125:1609-1615
 54. Ratcliffe OJ, Bradley DJ, Coen ES (1999) Separation of shoot and floral identity in *Arabidopsis*. *Development* 126:1109-1120
 55. Repinski SL, Kwak M, Gepts P (2012) The common bean growth habit gene PvTFL1y is a functional homolog of *Arabidopsis* TFL1. *Theor Appl Genet* 124:1539-1547
 56. Serrano-Mislata A, Fernández-Nohales P, Doménech MJ, Hanzawa Y, Bradley D, Madueño F (2016) Separate elements of the TERMINAL FLOWER 1 cis-regulatory region integrate pathways to control flowering time and shoot meristem identity. *Development* 143:3315-3327
 57. Serrano-Mislata A, Goslin K, Zheng B, Rae L, Wellmer F, Graciet E, Madueño F (2017) Regulatory interplay between LEAFY, APETALA1/CAULIFLOWER and TERMINAL FLOWER1: New insights into an old relationship. *Plant Signal Behav* 12:e1370164
 58. Si Z, Liu H, Zhu J, Chen J, Wang Q, Fang L, Gao F, Tian Y, Chen Y, Chang L, Liu B, Han Z, Zhou B, Hu Y, Huang X, Zhang T (2018) Mutation of SELF-PRUNING homologs in cotton promotes short-branching plant architecture. *J Exp Bot* 69:2543-2553
 59. Tian Z, Wang X, Lee R, Li Y, Specht JE, Nelson RL, McClean PE, Qiu L, Ma J (2010) Artificial selection for determinate growth habit in soybean. *Proc Natl Acad Sci USA* 107:8563-8568

60. Varkonyi-Gasic E, Moss SMA, Voogd C, Wang T, Putterill J, Hellens RP (2013) Homologs of FT, CEN and FD respond to developmental and environmental signals affecting growth and flowering in the perennial vine kiwifruit. *New Phytol* 198:732-746
61. Wang R, Albani MC, Vincent C, Bergonzi S, Luan M, Bai Y, Kiefer C, Castillo R, Coupland G (2011) Aa TFL1 confers an age-dependent response to vernalization in perennial *Arabis alpina*. *Plant Cell* 23:1307-1321
62. Wang Y, Fan G, Liu Y, Sun F, Shi C, Liu X, Peng J, Chen W, Huang X, Cheng S, Liu Y, Liang X, Zhu H, Bian C, Zhong L, Lv T, Dong H, Liu W, Zhong X, Chen J, Quan Z, Wang Z, Tan B, Lin C, Mu F, Xu X, Ding Y, Guo AY, Wang J, Ke W (2013) The sacred lotus genome provides insights into the evolution of flowering plants. *Plant J* 76:557-567
63. Wang Z, Ye S, Li J, Zheng B, Bao M, Ning G (2011) Fusion primer and nested integrated PCR (FPNI-PCR): a new high-efficiency strategy for rapid chromosome walking or flanking sequence cloning. *BMC Biotechnol* 11:109
64. Wang Z, Zhou Z, Liu Y, Liu T, Li Q, Ji Y, Li C, Fang C, Wang M, Wu M, Shen Y, Tang T, Ma J, Tian Z (2015) Functional evolution of phosphatidylethanolamine binding proteins in soybean and *Arabidopsis*. *Plant Cell* 27:323-336
65. Wickland DP, Hanzawa Y (2015) The FLOWERING LOCUS T/TERMINAL FLOWER 1 gene family: functional evolution and molecular mechanisms. *Mol Plant* 8:983-997
66. Wu L, Li F, Deng Q, Zhang S, Zhou Q, Chen F, Liu B, Bao M, Liu G (2019) Identification and characterization of the FLOWERING LOCUS T/TERMINAL FLOWER 1 gene family in *Petunia*. *DNA Cell Biol* 38:982-995
67. Xi W, Liu C, Hou X, Yu H (2010) MOTHER OF FT AND TFL1 regulates seed germination through a negative feedback loop modulating ABA signaling in *Arabidopsis*. *Plant Cell* 22:1733-1748
68. Yamaguchi A, Kobayashi Y, Goto K, Abe M, Araki T (2005) TWIN SISTER OF FT (TSF) acts as a floral pathway integrator redundantly with FT. *Plant Cell Physiol* 46:1175-1189
69. Yoo SJ, Chung KS, Jung SH, Yoo SY, Lee JS, Ahn JH (2010) BROTHER OF FT AND TFL1 (BFT) has TFL1-like activity and functions redundantly with TFL1 in inflorescence meristem development in *Arabidopsis*. *Plant J* 63:241-253
70. Yoo SY, Kardailsky I, Lee JS, Weigel D, Ahn JH (2004) Acceleration of flowering by overexpression of MFT (MOTHER OF FT AND TFL1). *Mol Cells* 17:95-101
71. Zhang F, Wang Y, Irish VF (2021) CENTRORADIALIS maintains shoot meristem indeterminacy by antagonizing THORN IDENTITY1 in *Citrus*. *Curr Biol* 31:2237-2242.e2234
72. Zhang J, Liu G, Guo C, He Y, Li Z, Ning G, Shi X, Bao M (2011) The FLOWERING LOCUS T orthologous gene of *Platanus acerifolia* is expressed as alternatively spliced forms with distinct spatial and temporal patterns. *Plant Biol* 13:809-820
73. Zhang S, Lu S, Yi S, Han H, Zhou Q, Cai F, Bao M, Liu G (2019) Identification and characterization of FRUITFULL-like genes from *Platanus acerifolia*, a basal eudicot tree. *Plant Sci* 280:206-218

Figures

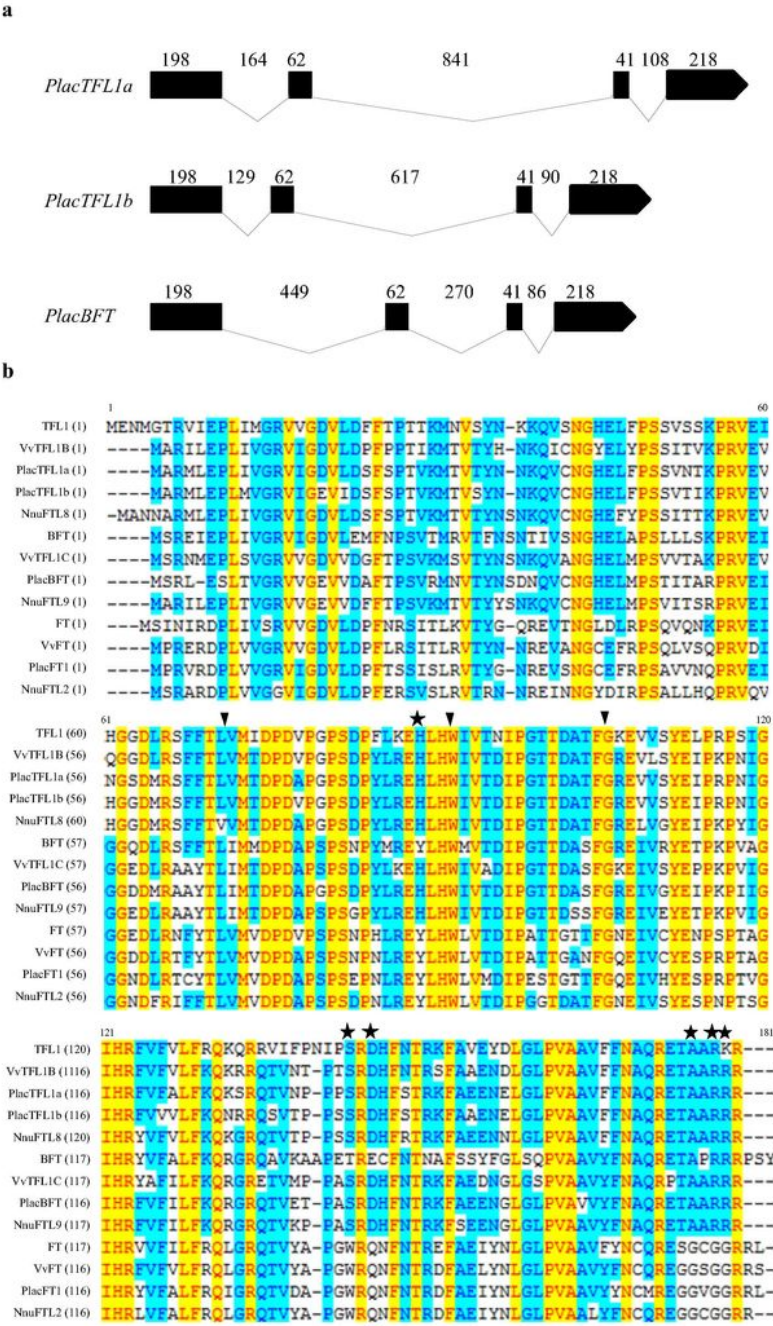


Figure 1

Gene structure and sequence alignment of FT/TFL1 family genes. **a** Comparison of structure of the three *TFL1*-like gene sequences from London plane. Numbers indicate the base pairs in the exons (black boxes) and introns (thin lines). **b** Alignment of the deduced amino acid sequences of the products of FT/TFL1 family in *Platanus acerifolia*, *Arabidopsis thaliana*, *Vitis vinifera* and *Nelumbo nucifera*. Intron positions are indicated by black arrowheads. Asterisks indicate amino acids that are critical to the definition of proteins in the FT/TFL1 family

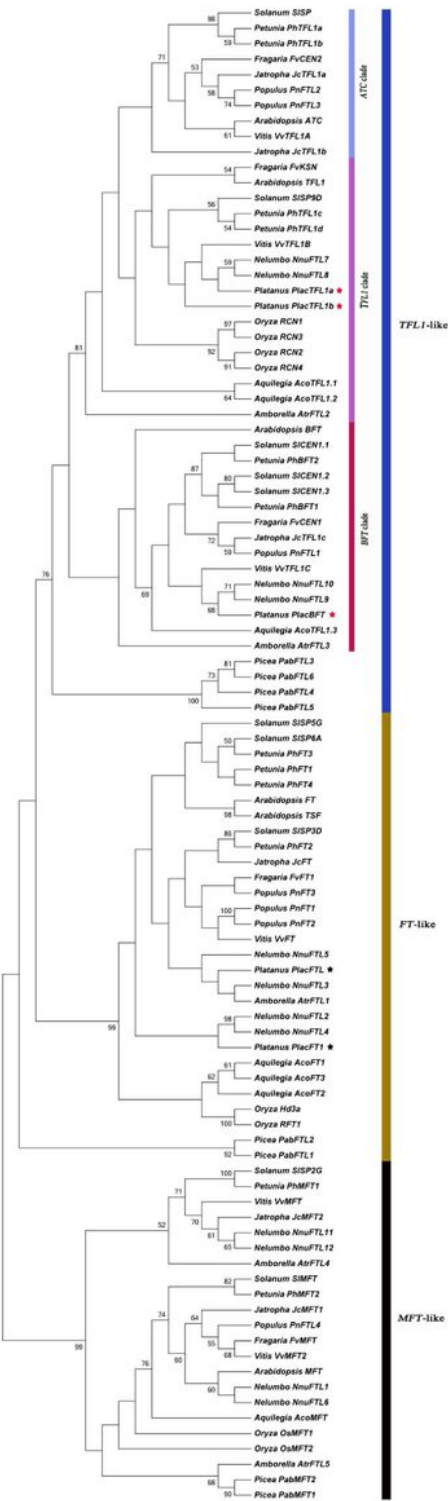


Figure 2

Phylogenetic analysis of FT/TFL1 gene family. The tree was generated with MEGA v6.0 software, using the Neighbor-Joining (NJ) method and 1000 bootstrap replicates. Bootstrap values above 50% are indicated, and *Platanus* FT/ TFL 1-like genes are marked with stars

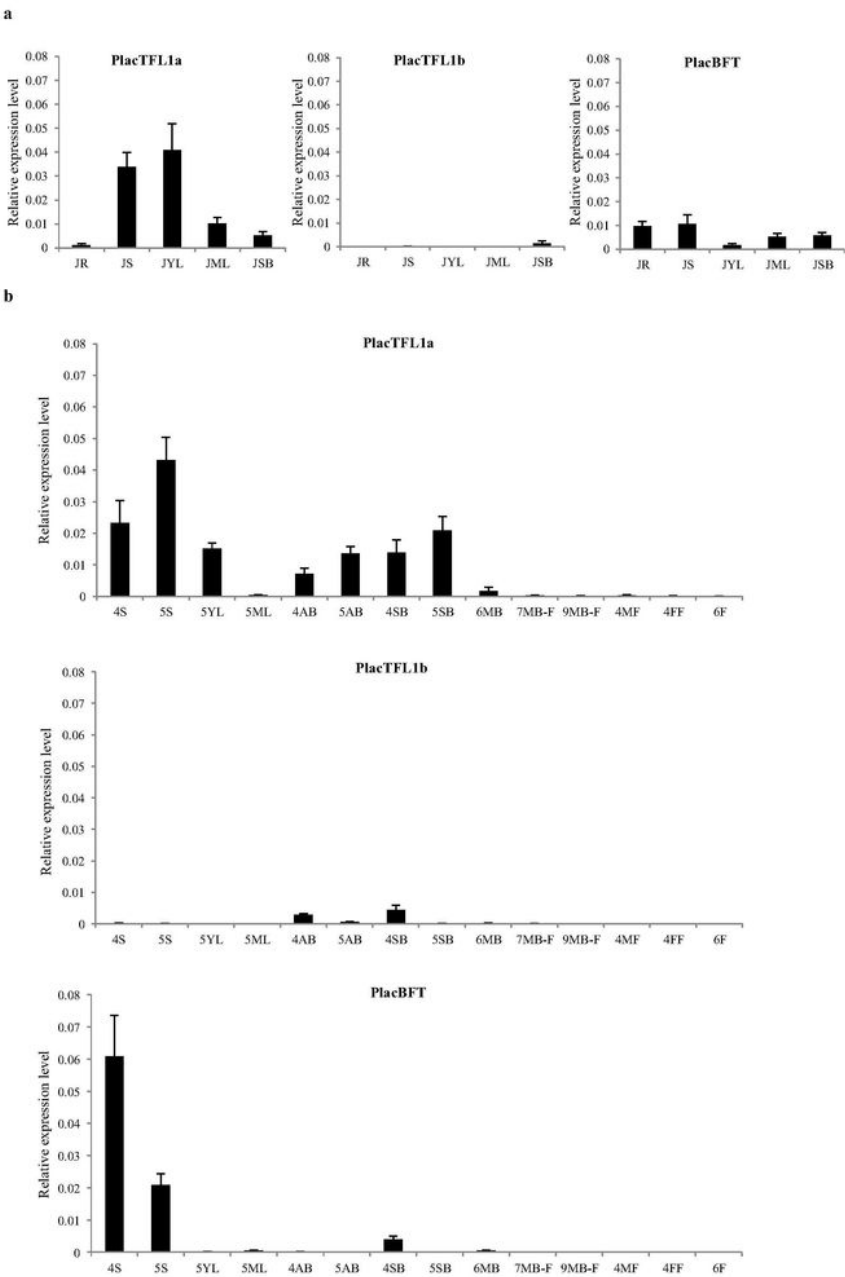


Figure 3

Expression profiling of *TFL 1*-like genes in London plane. **a** Relative expression of *TFL 1*-like genes in juvenile plants. **b**. Relative expression of *TFL 1*-like genes in adult trees. JR, roots of juvenile; JS, stems of juvenile; JYL, young leaves of juvenile; JML, mature leaves of juvenile; JSB, subpetiolar buds of juvenile; S, stems; YL, young leaves; ML, mature leaves; AB, shoot apical buds; SB, subpetiolar buds; MB, mixed flower buds; MB-F, inflorescences in mixed flower buds; MF, male inflorescences; MF-P, fleshy peduncles of male inflorescences; FF, female inflorescences. The numbers indicate the sampling month of the tissues. The level of expression was normalized to London plane *TPI* gene. Error bars represent SE for three replicates

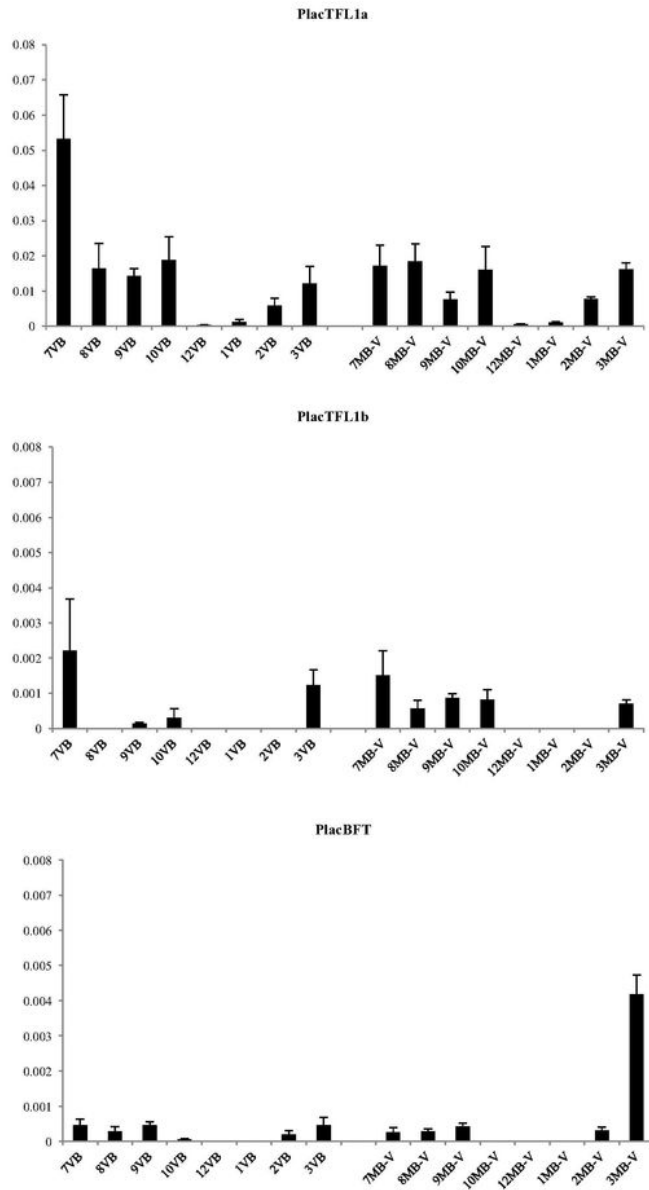


Figure 4

Expression of *PlacTFL1a/b* and *PlacBFT* genes in different stages of VB and MB-V. VB, vegetative subpetiolar buds; MB-V, vegetative tissues in mixed flower buds. The numbers indicate the sampling month of the tissues. The level of expression was normalized to London plane *TPI* gene. Error bars represent SE for three replicates

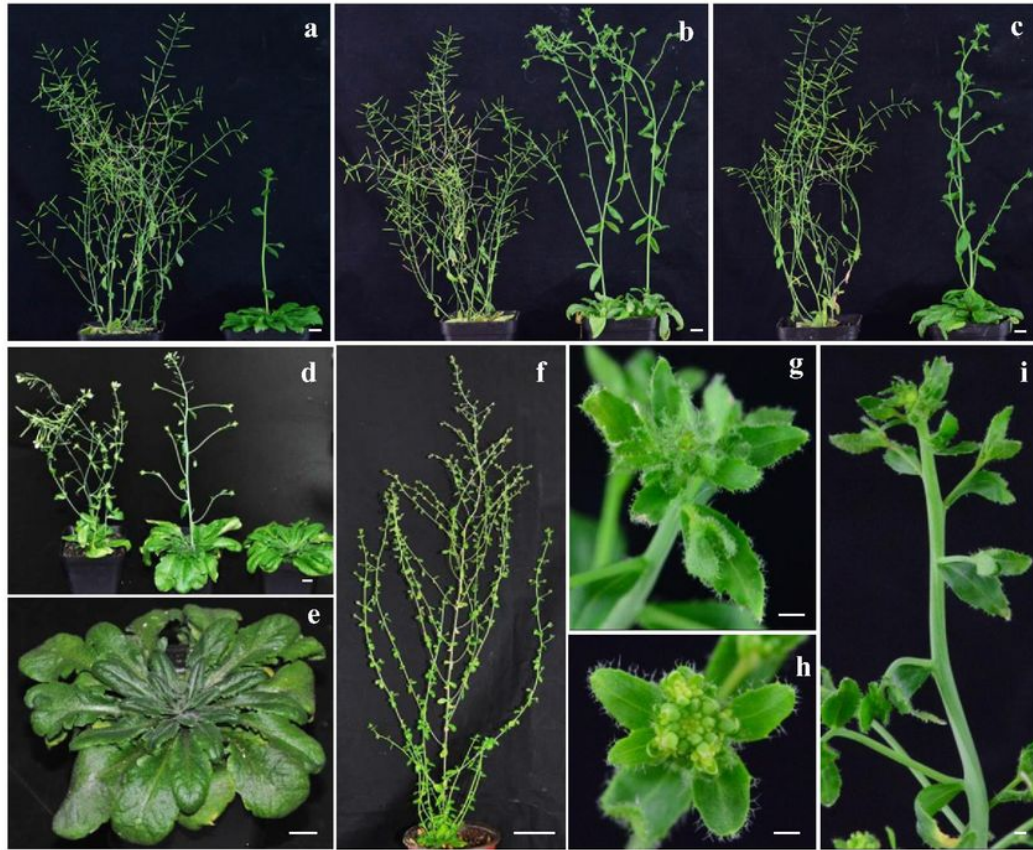


Figure 5

Phenotype analysis of transgenic *Arabidopsis* plants overexpressing *PlacTFL 1a/b* or *PlacBFT* genes. **a, b, c** Wild-type (left) and *35S:PlacTFL 1a*, *35S:PlacTFL 1b*, *35S:PlacBFT* (right) transgenic plants, respectively. **d** Wild-type (left) and *35S:PlacTFL 1a* transgenic plants with moderate (middle) and strong (right) phenotype. **e** Rosette leaves of *35S:PlacTFL 1a* transgenic plants. **f** Strong phenotypic lines of *35S:PlacTFL 1a*. **g, h** The top of inflorescences in *35S:PlacTFL 1a* transgenic plants with strong (**g**) and moderate (**h**) phenotype. **i** Inflorescence stem of *35S:PlacTFL 1a* transgenic plants. Bars: 10mm (**a-f**), 1mm (**g-i**)

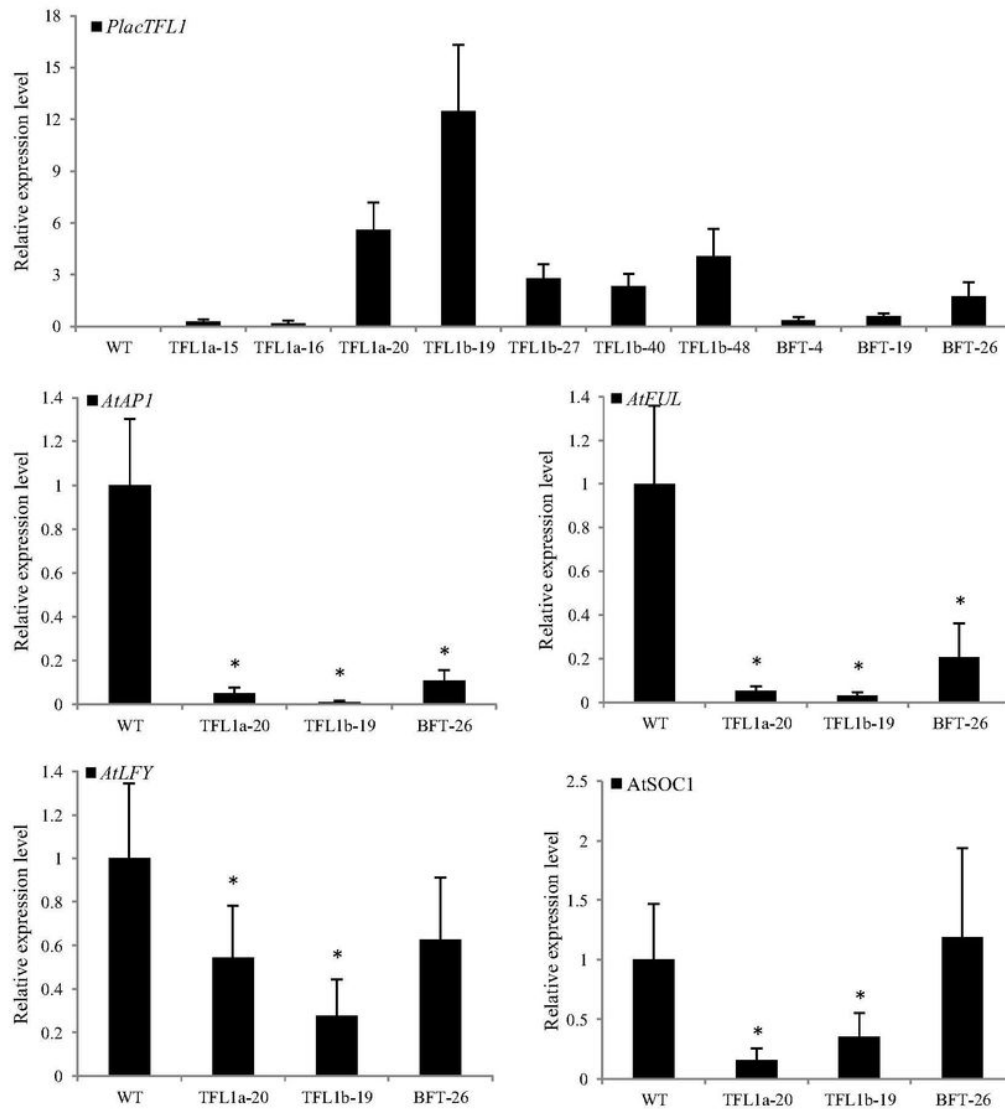


Figure 6

qRT-PCR analysis of endogenous flowering related genes in 21-d-old seedlings of *Arabidopsis* wild-type and *35S:PlacTFL1*-like transgenic lines. The numbers after the genes indicate transgenic lines of the three *35S:PlacTFL1*-like genes, respectively. Data represent the mean $\pm$ SE from three biological replicates, and *AtEF1a* was used as internal control. WT, wild-type seedlings. The asterisks indicate significant differences compared with the WT plants ($P < 0.05$)

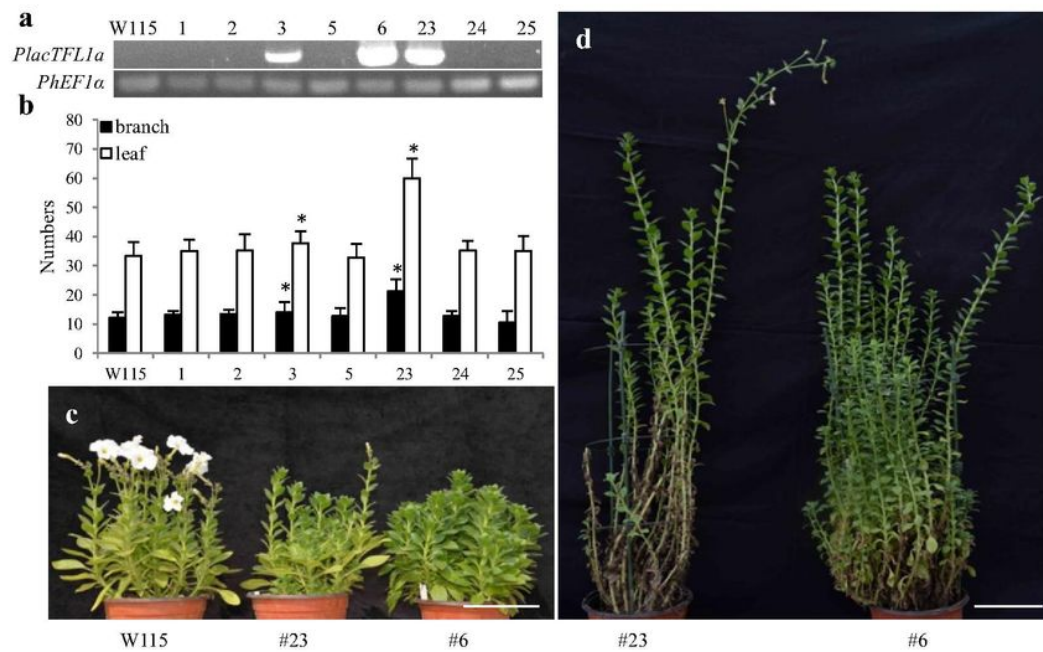


Figure 7

Phenotype analysis of transgenic petunia plants ectopically expressing *PlacTFL1a* gene. **a** RT-PCR analysis of transgenes in wild-type petunia (W115) and transgenic lines. **b** Numbers of branches and leaves on the principal shoot before flowering in W115 and T₁ transgenic lines. **c** W115 (left) and *35S:PlacTFL1a* transgenic lines #23 (middle) and #6 (right) after cultivated for two months. **d** T₀ *35S:PlacTFL1a* transgenic lines #23 (left) and #6 (right) after transplanted for seven months. Bars: 10 cm

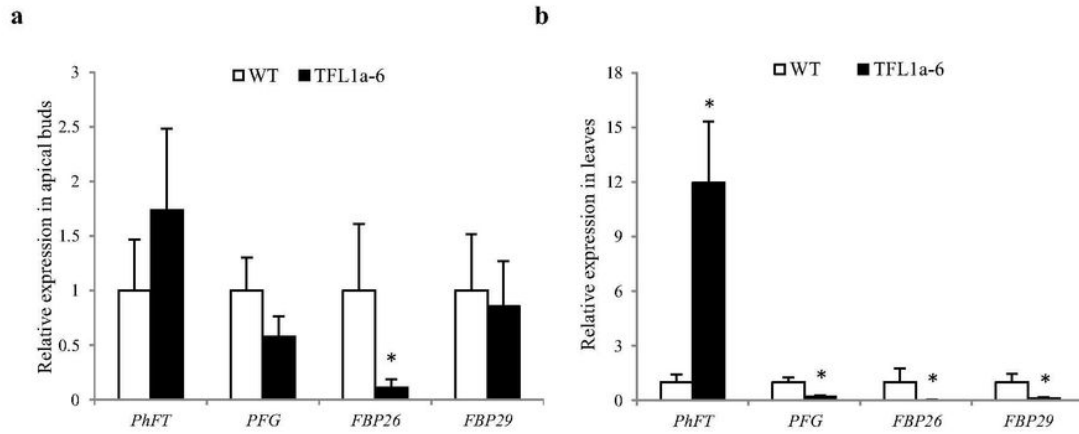


Figure 8

qRT-PCR analysis of endogenous flowering related genes in *35S:PlacTFL 1a* transgenic line (#6). **a, b** The expression levels of *PhFT*, *PFG*, *FBP26*, and *FBP29* in apical buds (**a**) and leaves (**b**) of W115 (WT) and *35S:PlacTFL 1a* transgenic line. *PhEF1a* was used as internal control; the asterisks indicate significant differences compared with the WT plants ($P < 0.05$)

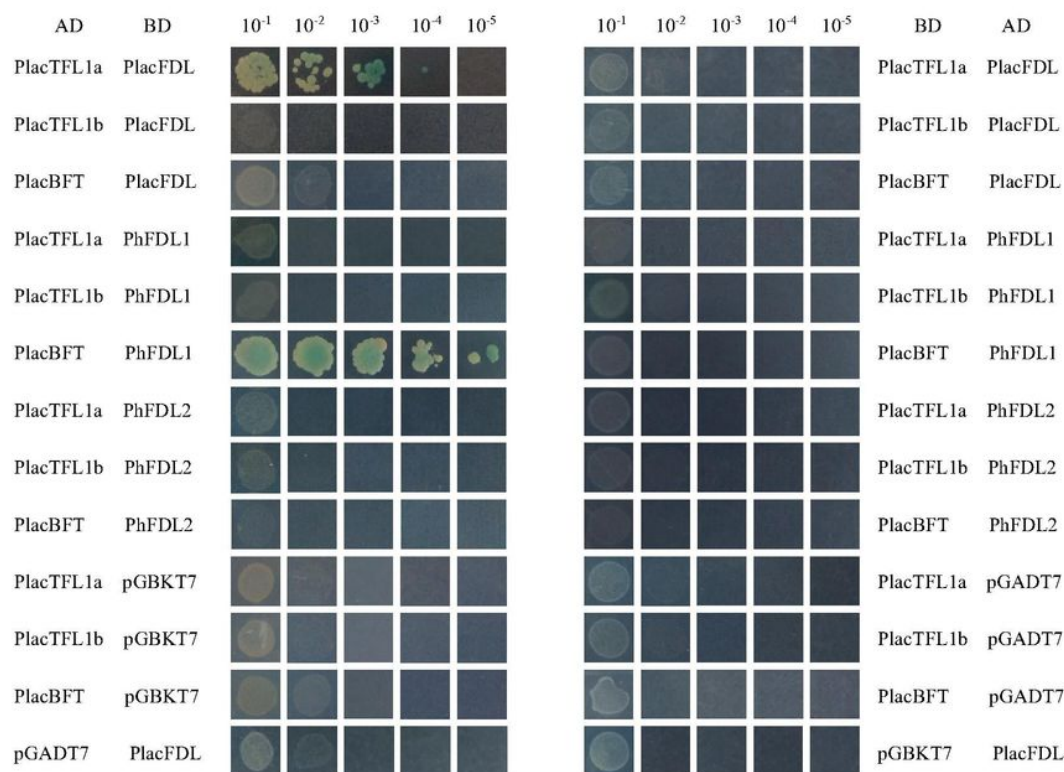


Figure 9

Interactions between *Platanus* TFL1-like proteins and the FD-like proteins. Ten-fold serial dilutions from 10⁻¹ to 10⁻⁵ of each culture were spotted on the selected SD -Leu/-Trp/-His/-Ade plates with X-α-gals

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [supplementarymaterials.docx](#)