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Functional divergence of MFT paralogues in flowering promotion and organ development in Platanus acerifolia

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Abstract

Two *MFT*-like genes, *PlacMFT1* and *PlacMFT2*, were isolated and characterized from London plane tree (*Platanus acerifolia*). Both genes possess the conserved four-exon/three-intron structure characteristic of the PEBP family. Phylogenetic analysis placed them into two distinct clades, MFT1 and MFT2, indicating an ancient duplication event. qRT-PCR revealed that *PlacMFT1* was broadly expressed in vegetative and reproductive tissues, with high transcript levels in stems, whereas *PlacMFT2* exhibited strong fruit-specific expression and complementary patterns in juvenile organs. Ectopic overexpression of either gene in *Arabidopsis* under the 35S promoter accelerated flowering, confirming their conserved function in promoting floral transition. However, *35S:PlacMFT1* transgenic lines displayed pleiotropic developmental abnormalities, including fused flowers, malformed floral organs, elongated pedicels with shortened siliques, and curled rosette leaves, while *35S:PlacMFT2* lines showed no obvious morphological alterations. These results indicate that the two *MFT* paralogs have undergone subfunctionalization through spatiotemporal expression divergence, with *PlacMFT1* acquiring additional roles in organ development. This study provides insights into the functional evolution of *MFT* genes in basal core eudicots and establishes a molecular foundation for breeding low-fruit cultivars of London plane trees.

Introduction

London plane tree (*Platanus acerifolia* Willd.) originates from interspecific hybridization between *Platanus orientalis* L. and *Platanus occidentalis* L. Taxonomically, this species falls into Platanaceae under Proteales, representing a primitive lineage of core eudicots as documented in APG IV classification (Bi, et al. 2016). Valued for its outstanding ornamental and ecological adaptability, it has been extensively cultivated as urban landscaping vegetation and is well-known as the premier street tree worldwide. Nevertheless, mature plane trees generate massive reproductive organs each year: abundant pollen released from inflorescences in spring, alongside seed trichomes shed from ripening fruits in early summer. These dispersed biological debris severely disturb urban ecological environments and pose potential hazards to residents' physical well-being, constituting a prominent environmental issue demanding effective solutions across Chinese cities (Yan, et al. 2024). Developing sterile cultivars that lack flowers or mature fruits is therefore a critical breeding target for this tree species. Under such context, dissecting the molecular regulatory pathways governing floral transition and reproductive development in plane trees carries substantial theoretical and practical significance.

Previous studies have demonstrated that genes belonging to the phosphatidylethanolamine-binding protein (PEBP) family exert vital functions in regulating floral transition in plants (Wickland and Hanzawa 2015). The six members of the PEBP gene family in *Arabidopsis thaliana* include *FLOWERING LOCUS T*(*FT*)、*TERMINAL FLOWER 1*（*TFL1*）、*TWIN SISTER OF FT*（*TSF*）、*BROTHER OF FT AND FT*（*BFT*）、*ARABIDOPSIS CENTRORADIALIS HOMOLOG*（*ATC*）、*MOTHER OF FT AND TFL1*（*MFT*）(Ahn, et al. 2006; Khosa, et al. 2021; Wang, et al. 2015; Yamaguchi, et al. 2005; Yoo, et al. 2010; Yoo, et al. 2004)。Among them, *FT* and *TFL1* are two key genes involved in flowering induction. They share approximately 60%

amino acid sequence identity yet exert opposite functions: *FT* promotes the transition from vegetative growth to reproductive development, whereas *TFL1* represses floral transition (Ahn, et al. 2006; Hanzawa, et al. 2005). *TSF* exhibits the highest homology to *FT*; it can be induced by *CO* and interacts with *FD*, and its overexpression accelerates flowering (Jang, et al. 2009). *BFT* also possesses high sequence homology with *FT* and displays a circadian rhythm similar to *FT*, but it functions like *TFL1* and acts as a flowering repressor (Yoo, et al. 2010). *ATC* inhibits flowering under short-day conditions and, like *FT*, is capable of moving from vascular tissues to the shoot apex (Huang, et al. 2012).

As a PEBP member, *MFT* shares high homology with both *FT* and *TFL1*. It participates in floral induction and may be functionally redundant with *FT* (Yoo, et al. 2004). Furthermore, *MFT* is a key seed germination regulator in response to exogenous abscisic acid (ABA) by directly repressing the transcription of *ABA-INSENSITIVE5* (*ABI5*) in *Arabidopsis thaliana* (Xi, et al. 2010). Accumulating evidence indicates that *MFT* functions as a critical suppressor of seed germination under shade conditions through modulating abscisic acid (ABA) and gibberellic acid (GA) signaling cascades (Bi, et al. 2016; Vaistij, et al. 2018).

Even though *MFT* is capable of directly repressing downstream targets such as *ABI5*, it lacks a DNA-binding domain and thus needs to form protein complexes by interacting with other factors to directly regulate the expression of target genes (Collani and Neumann 2019; Khosa, et al. 2021; Zhu, et al. 2021). Recent research has demonstrated physical interactions between MFT and two key transcription factors, OsbZIP66 and OsMYB26, which confer improved drought tolerance in rice (Chen, et al. 2021). These findings broaden our knowledge of *MFT* biological functions beyond its established role in seed-germination control, and offer mechanistic insights into how *MFT* mediates the transcription of downstream target genes.

Homologous *MFT* proteins exhibit highly diversified biological functions across different plant taxa, indicating profound functional divergence during plant evolution. In the fern *Adiantum capillus-veneris*, *AcMFT* was shown to accelerate fioral transition and could partially rescue the late-flowering phenotype of *Arabidopsis ft-1* mutant (Hou and Yang 2016). Nevertheless, heterologous overexpression of *Dendrobium nobile DnMFT* in *Arabidopsis* led to slightly late-flowering (Li, et al. 2012), and a similar effect was observed for *Hevea brasiliensis HbMFT1* (Bi, et al. 2016). Additionally, soybean *GmMFT* participates in the regulation of the seed oil and protein content (Cai, et al. 2023).

Although the PEBP family has been widely characterized across various plant species, the evolutionary features and biological roles of *MFT* paralogs remain poorly elucidated in basal core eudicots such as London plane tree (*Platanus acerifolia*). Up to now, little research has uncovered the subfunctionalization of duplicated *MFT* homologs and their regulatory roles in floral transition and fruit development within perennial woody basal eudicots. In the present work, two *MFT*-like genes, namely *PlacMFT1* and *PlacMFT2*, were isolated and systematically identified from London plane tree. This study intends to clarify the functional divergence of *MFT* copies in *Platanus* and lay a molecular foundation for genetic improvement to breed low-fruit cultivars. We analyzed their gene structural characteristics, phylogenetic

relationships, and spatiotemporal expression profiles in juvenile and adult plane tree tissues. Furthermore, heterologous overexpression in *Arabidopsis* was performed to dissect the distinct biological functions of the two *MFT* homologs. The obtained findings offer critical insights into the functional evolution of *MFT*-like genes in basal core eudicots and supply theoretical guidance for developing sterile, fruitless plane tree varieties.

Material and methods

Plant materials

Plant samples were collected from juvenile (2-year-old) and mature (more than 30-year-old) *Platanus acerifolia* trees. Floral and fruit developmental processes of plane trees span two growing seasons and require an extended period to complete. To comprehensively characterize the expression patterns of target genes, tissue sampling was implemented every half month or every month across distinct developmental stages.

Samples harvested from mature trees included stems (S), young leaves (YL), mature leaves (ML), apical buds (AB) and axillary buds (SB) collected in April and May, vegetative buds (VB), mixed buds containing both floral and vegetative primordia (MB), vegetative parts separated from mixed buds (MB-V), floral parts separated from mixed buds (MB-F), and developing fruits (F) sampled from June to September (at this stage, male and female inflorescences have initiated differentiation but cannot be clearly distinguished). Male inflorescences (MF), female inflorescences (FF) and fruits (F) at successive developmental stages were collected from October to the following April. Male inflorescences collected in mid-March and April were further dissected into anthers (MF-A) and pedicels (MF-P).

For juvenile materials, roots (JR), stems (JS), young leaves (JYL), mature leaves (JML) and axillary buds (JSB) were sampled from 2-year-old plane tree saplings in June. All tissue samples were collected from three independent individual trees and immediately frozen in liquid nitrogen for subsequent storage.

Cloning of Platanus acerifolia MFT-like genes

Total RNA was extracted from plane tree tissues using the CTAB method described by Li et al. (Li, et al. 2008). A total of 2 μg RNA was reverse-transcribed with the PrimeScript™ RT reagent kit (Takara, Japan). Genomic DNA was isolated via the same CTAB protocol. Purified RNA and DNA samples were preserved long-term at − 80°C.

Specific primer pairs were designed to amplify the full-length cDNA and corresponding genomic DNA sequences of *PlacMFT1* and *PlacMFT2*, based on the *Platanus acerifolia* genome dataset from CoGe (gene ID: 67387) and transcriptomic data. All primer sequences are listed in Supplementary Table 1. Amplified fragments were ligated into the pMD18-T vector (Takara, Japan), and 4–5 positive single colonies were randomly selected for Sanger sequencing.

Sequence alignment and phylogenetic analysis

Sequence alignment was performed using Vector NTI version 11.5 (Invitrogen, USA). MFT-like protein sequences of multiple plant species were retrieved from the NCBI database (Supplementary Table 2) for phylogenetic reconstruction. Full-length amino acid sequences were first aligned using the MUSCLE algorithm with default parameters implemented in MEGA X (Kumar, et al. 2018), followed by manual refinement according to previously published references. A neighbor-joining (NJ) phylogenetic tree was constructed in MEGA X with 1000 bootstrap replicates.

Gene expression analysis via quantitative real-time RT-PCR

Gene expression analysis via quantitative real-time RT-PCR
qRT-PCR was conducted to quantify the transcript abundance of two *MFT*-like paralogs across diverse tissues and developmental stages of *Platanus acerifolia*. Gene-specific primers targeting the divergent C-terminal or 3’ UTR of each gene were designed with Primer Premier 6, generating amplicons ranging from 90 to 300 bp (Supplementary Table 1). The size of each PCR product was verified by agarose gel electrophoresis, and primer specificity was validated using plasmid templates.

Quantitative amplification was carried out on an ABI Prism 7500 Sequence Detection System (Applied Biosystems, USA) with SYBR Premix Ex Taq (Takara, Japan). Each 10 μL reaction system contained 1.0 μL reverse transcription template, 5 μL 2× SYBR Green Master Mix, 0.2 μL forward primer (10 μmol L^{-1}), 0.2 μL reverse primer (10 μmol L^{-1}), and nuclease-free water to a final volume of 10 μL. Three technical replicates were set for each reaction, and data were presented as mean ± standard error. Amplification efficiency of each primer pair was calculated from standard curves generated by serially diluted cDNA templates. Raw fluorescence data were exported to Microsoft Excel via Sequence Detector Version 1.3.1 (PE Applied Biosystems). Relative gene expression levels were calculated using REST-MCS v2 (Multiple Condition Solver), with the *Platanus acerifolia triose phosphate isomerase* gene (*TPI*) selected as the internal reference (Zhang, et al. 2023).

Vector construction

The full-length coding sequence of *PlacMFT1* released from pMD18-T was double-digested with restriction endonucleases SalI and BamHI, while the coding fragment of *PlacMFT2* was excised using SalI and XbaI. Purified target fragments were separately inserted into the binary vector P2300 harboring the CaMV 35S promoter and Nos 3’ terminator, yielding overexpression constructs *35S:PlacMFT1* and *35S:PlacMFT2*. All recombinant plasmids were verified by PCR amplification and restriction digestion. Confirmed constructs were transformed into Agrobacterium tumefaciens strain GV3101 via electroporation (Mahmood, et al. 2008).

Plant transformation and phenotype characterization

The two *MFT* overexpression vectors were introduced into wild-type *Arabidopsis thaliana* ecotype Columbia-0 (Col-0) using the floral dip method (Clough and Bent 1998). T_1seeds harvested from transformed plants were screened on solid MS medium supplemented with 50 μg mL^{-1} kanamycin and 50 μg mL^{-1} cefotaxime, and putative positive seedlings were phenotypically observed. Transgenic lines showing a 3:1 segregation ratio in kanamycin resistance were selected via chi-square test and cultivated in growth chambers at 22°C under long-day conditions (16 h light / 8 h dark). For each transgenic line, 16 individual plants were scored for flowering time and floral morphological traits.

Results

Isolation and phylogenetic analysis of MFT-like genes from Platanus acerifolia

Full-length cDNA and genomic DNA sequences of *PlacMFT1* and *PlacMFT2* were obtained from the CoGe genome database (gene ID: 67387) and transcriptomic resources. The predicted proteins encoded by *PlacMFT1* and *PlacMFT2* consist of 176 and 172 amino acids, respectively. Both genes share a conserved gene structure with four exons and three introns, exhibiting 63.5% nucleotide identity and 65.9% amino acid identity (Fig. 1). The third intron of *PlacMFT2* is remarkably long, leading to a total genomic length of 1640 bp, whereas the full genomic sequence of *PlacMFT1* is only 789 bp (Fig. 1a).

Multiple sequence alignment of FT/TFL1 family proteins from *Platanus acerifolia*, *Arabidopsis thaliana*, *Vitis vinifera* and *Nelumbo nucifera* revealed that residues His88, Ser142 and Asp144 are functionally critical sites determining the antagonistic activities of *FT* and *TFL1*. In addition, the amino acid at position 156 serves as a diagnostic residue to distinguish *PlacMFT1* and *PlacMFT2* (Fig. 1b).

Phylogenetic reconstruction indicated that *MFT* homologs exist in basal land plant lineages including mosses and lycophytes, which evolved earlier than gymnosperms (Fig. 2). Angiosperm MFT proteins split into two distinct clades, MFT1 and MFT2. PlacMFT1 was clustered within the MFT1 clade, closely related to NnuFTL11, NnuFTL12 from sacred lotus and CkMFT from *Cinnamomum kanehirae*. By contrast, PlacMFT2 fell into the MFT2 subclade and grouped together with lotus NnuFTL6 and NnuFTL1 (Fig. 2).

Tissue-specific expression patterns of MFT-like genes in London Plane

qRT-PCR profiling with *TPI* as the internal reference was performed to dissect divergent expression patterns of the two *MFT* paralogs across vegetative and reproductive tissues of juvenile and mature plane trees.

Significant differentiation was detected between the expression profiles of *PlacMFT1* and *PlacMFT2*. *PlacMFT1* exhibited broad expression across nearly all tested vegetative and reproductive tissues, with undetectable transcripts only in juvenile roots and juvenile axillary buds; its expression peaked in vegetative stems. Transcripts of *PlacMFT1* were also detected in male inflorescences, female inflorescences and fruits, with generally higher accumulation in fruits (Fig. 3).

PlacMFT2 displayed strong fruit-specific expression: its transcript level increased sharply in August fruits and exceeded that of *PlacMFT1*, even reaching a higher abundance than the internal reference gene *TPI* (Fig. 3). The two paralogs showed complementary expression in juvenile vegetative tissues: *PlacMFT2* was specifically expressed in juvenile roots and juvenile axillary buds where *PlacMFT1* was silent, while almost no signal of *PlacMFT2* was detected in juvenile stems and juvenile leaves (Fig. 3).

Ectopic overexpression of PlacMFT-like genes in Arabidopsis thaliana

To characterize the biological functions of *PlacMFT1* and *PlacMFT2* in floral induction and floral fate determination, the two genes were ectopically expressed in *Arabidopsis* driven by the CaMV 35S constitutive promoter. A total of 56 independent T_1 lines of *35S:PlacMFT1* and 17 independent T_1 lines of *35S:PlacMFT2* were generated. Based on phenotypic variation in T_1 generations, 3–5 T_2 lines with a 3:1 kanamycin segregation ratio were selected to record flowering time and floral morphology.

Both *35S:PlacMFT1* and *35S:PlacMFT2* transgenic *Arabidopsis* lines exhibited an early-flowering phenotype (Fig. 4a, b; Table 1), demonstrating conserved roles of the two PlacMFT proteins in promoting the vegetative-to-reproductive transition. Nevertheless, obvious functional divergence was observed between the two paralogs. Only *35S:PlacMFT1* lines displayed severe developmental defects in reproductive organs and vegetative leaves, including fused multiple flowers (Fig. 4c), disordered and malformed floral organ architecture (Fig. 4d), elongated pedicels coupled with short siliques (Fig. 4e), and curled rosette leaves (Fig. 4f). No significant morphological differences in flowers, fruits or leaves were observed between *35S:PlacMFT2* transgenic plants and wild-type Col-0.

a, **b** Wild-type (left) and *35S: PlacMFT1*, *35S: PlacMFT2* (middle and right) transgenic plants respectively. **c**, **d**, **e**, **f** *35S: PlacMFT1* transgenic plants showed large fused terminal flowers (**c**), abnormal floral oragan morphological characteristics (**d**), prolonged fruit pedicels and short fruits (**e**) and curled leaves (**f**). Bars: 10mm (**a**-**f**), 1mm (**g**-**i**).

Table 1
Phenotypic statistics of the transgenic Arabidopsis plants overexpressing MFT -like genes from London plane

Lines	rosette leaves	cauline leaves	bolting time	anthesis time
Col-0	11.83 ± 1.73	2.57 ± 0.76	29.14 ± 1.89	32.92 ± 1.52
35S:PlacMFT1				
MFT1-7	5.76 ± 0.66*	2.29 ± 0.47	23.12 ± 2.18*	26.53 ± 2.40*
MFT1-11	10.27 ± 1.10*	1.82 ± 0.75*	30.55 ± 2.38	33.55 ± 2.38
MFT1-16	9.82 ± 1.40*	2.09 ± 1.04	30.64 ± 1.12	33.00 ± 1.26
MFT1-18	4.44 ± 0.73*	3.44 ± 0.51*	19.94 ± 2.11*	23.31 ± 2.27*
MFT1-27	2.17 ± 0.38*	3.83 ± 0.38*	15.00 ± 0.00*	18.44 ± 0.51*
35S:PlacMFT2				
MFT2-10	11.80 ± 2.04	2.67 ± 1.05	30.67 ± 3.22	33.80 ± 3.17
MFT2-17	11.31 ± 1.49	2.69 ± 0.60	30.19 ± 1.38	33.25 ± 1.39
MFT2-14	5.07 ± 1.14*	2.07 ± 0.73	26.64 ± 2.06*	29.93 ± 1.86*

T_1 transgenic lines whose progeny fitted a 3:1 segregation ratio of kanamycin resistance were chosen to record flowering time and floral phenotype. Values are mean ± SD (n = 16). Symbols indicated significantly difference in comparison with Col-0 (*P < 0.05)

Disscussion

Phylogenetic analysis has shown that the FT/TFL1 gene family in higher plants can be divided into three major clades: *MFT*-like, *FT*-like and *TFL1*-like. In basal land plants such as mosses and ferns, which occupy evolutionarily primitive positions, only *MFT*-like homologs are present, and no *FT*-like or *TFL1*-like members have been identified, confirming that *MFT*-like genes represent the ancestral gene group of the FT/TFL1 family (Hedman, et al. 2009; Karlgren, et al. 2011; Zhang, et al. 2023). During plant evolution, the ancestral *MFT*-like gene underwent duplication, giving rise to the primitive MFT lineage and the common FT/TFL1 lineage. With the origin and radiation of angiosperms, the FT/TFL1 lineage underwent further gene duplication and functional diversification, ultimately forming the functionally opposing *FT*-like flowering-promoting branch and TFL1-like flowering-repressing branch (Hedman, et al. 2009; Karlgren, et al. 2013; Karlgren, et al. 2011). Gymnosperms also possess all three classes of *FT/TFL1* homologs; however, both their FT and TFL1 proteins exhibit TFL1-like flowering-repression functions. The functional divergence between *FT* (promotion) and *TFL1* (repression) occurred after the separation of gymnosperms and angiosperms (Karlgren, et al. 2013; Liu, et al. 2016).

In this study, two *MFT* homologs, *PlacMFT1* and *PlacMFT2*, were isolated from London plane tree (*Platanus acerifolia*). As shown in the phylogenetic tree (Fig. 2), the vast majority of angiosperms retain

two *MFT* copies, which cluster into two independent evolutionary clades, *MFT1* and *MFT2*. This indicates that a critical duplication event of the *MFT* gene occurred in the common ancestor of extant angiosperms, laying the genetic foundation for subsequent subfunctionalization between the two copies. However, the evolutionary patterns in gymnosperms and monocots differ markedly. Species such as *Ginkgo biloba*, *Picea abies* and *Oryza sativa* also contain two *MFT* homologs, but their two copies cluster together on the phylogenetic tree, suggesting that *MFT* duplications in gymnosperms and monocots occurred independently as species-specific events, distinct from the ancient duplication that occurred in the angiosperm ancestor (Hedman, et al. 2009)

Traditional taxonomy previously placed sacred lotus and water lilies together in the Nymphaeaceae based on morphological characteristics, regarding them as primitive angiosperm groups. However, the APG IV molecular phylogenetic system confirmed that sacred lotus is most closely related to the Platanaceae family, with both belonging to the basal core eudicots of the order Proteales, forming an independent evolutionary lineage together with Sabiaceae and Proteaceae; hence sacred lotus is now placed in its own family, Nelumbonaceae (Group TAP, et al. 2016). The genome of *Nelumbo nucifera* experienced a recent independent whole-genome duplication event, which expanded its *MFT* copy number to four—exactly twice the number found in London plane tree. This difference is also directly reflected in the phylogenetic clustering (Fig. 2), where the four *MFT* genes of lotus are distributed between the MFT1 and MFT2 clades, in contrast to the two-copy pattern observed in London plane tree (Ming, et al. 2013; Wang, et al. 2013)

It is generally believed that there is an important link between gene expression patterns and their functions. Compared with changes in coding sequences, alterations in expression patterns of different members of the same gene family or of orthologous genes across different species may play a more critical role in functional divergence. *Arabidopsis thaliana* contains only a single *MFT* gene, which has been shown to be regulated by ABA and GA signaling pathways and specifically expressed in the radicle-hypocotyl transition zone, functioning in the regulation of seed germination (Xi et al 2010). Unlike the annual herb *Arabidopsis*, the perennial woody London plane tree shows high expression of both *PlacMFT1* and *PlacMFT2* in fruit tissues, and their expression patterns exhibit significant spatiotemporal divergence. *PlacMFT1* displays constitutive and broad expression: transcripts are detectable in almost all juvenile tissues except roots, in various adult vegetative organs, in male and female inflorescences, and in developing fruits, with the highest expression in stems, suggesting multiple functions in vegetative growth maintenance, floral organ development, and fruit development (Fig. 3). *PlacMFT2* exhibits strong fruit-tissue specificity, with transcript levels sharply upregulated in August developing fruits, reaching abundances even higher than the internal reference gene *TPI*, indicating a predominant and specialized role in fruit development (Fig. 3). In juvenile vegetative tissues, the two genes show complementary expression: *PlacMFT2* is specifically expressed in juvenile roots and juvenile axillary buds where *PlacMFT1* is silent, whereas almost no *PlacMFT2* signal is detected in juvenile stems and leaves (Fig. 3). Thus, the two *MFT* copies in London plane tree have achieved functional partitioning through spatiotemporal expression divergence, accomplishing the division of functions from the ancestral single-copy MFT gene, which is a typical subfunctionalization evolutionary pattern. In the

biofuel plant *Jatropha curcas*, two *MFT*-like genes also show highest expression in fruits, yet their expression patterns are not identical: *JcMFT1* has relatively high expression in seeds and roots of adult plants, whereas *JcMFT2* is not expressed in seeds but has higher expression in roots of seedlings (Li, et al. 2015)

Heterologous overexpression in *Arabidopsis* further confirmed that *PlacMFT1* and *PlacMFT2* share a conserved flowering-promoting function while exhibiting significant functional divergence. Constitutive 35S-driven overexpression of either gene in *Arabidopsis* resulted in early flowering in all transgenic lines, indicating that the plane tree MFT proteins retain the conserved function of promoting the transition from vegetative to reproductive growth that is characteristic of land plant MFT proteins (Fig. 4). Meanwhile, in the heterologous *Arabidopsis* overexpression system, we observed obvious phenotypic differences between the two types of transgenic lines: *35S:PlacMFT1* transgenic lines exhibited various organ developmental abnormalities, including fused flowers, disordered floral organ architecture, elongated pedicels with shortened siliques, and curled rosette leaves; in contrast, *35S:PlacMFT2* transgenic lines showed no significant differences in vegetative organs, flowers or fruits compared with wild-type Col-0 (Fig. 4). These phenotypic differences suggest that the two *MFT* homologs in London plane tree have undergone functional divergence in organ development regulation, and *PlacMFT1* may have acquired additional developmental regulatory functions. This further illustrates that *MFT* genes have gained new functions during plant evolution, as exemplified by the role of soybean *GmMFT* in regulating seed oil and protein content (Cai, et al. 2023)and rice *OsMFT1* in modulating seed germination under salt stress (Lu, et al. 2023). Recent studies have also unraveled a fourth clade in the PEBP gene family in plants, termed SFT, which is closely related to the MFT clade but sister to the branch point of the FT/TFL subfamilies, suggesting a long-standing evolutionary divergence (Burton, et al. 2025).

In summary, through systematic identification and functional analysis of two *MFT* homologs from London plane tree, this study reveals the duplication and subfunctionalization evolutionary pattern of *MFT* genes in basal core eudicots. The two *PlacMFT* copies have achieved functional partitioning through spatiotemporal expression divergence while retaining the ancestral MFT function of promoting floral transition. This finding not only provides important evidence for understanding the early functional diversification of the *MFT* gene family during angiosperm evolution, but also lays a theoretical foundation for breeding low-fruit or fruitless plane tree cultivars via molecular breeding approaches.

Declarations

Declarations

Ethical approval

Not applicable.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author Contribution

S.Z. conceived and designed the research, performed the experiments, analyzed data and wrote the original manuscript. Y.T. carried out experiments and performed data analysis. J.J. conducted experiments and curated experimental data. J.W. provided experimental materials and validated the results. C.N. and X.Z. participated in experimental work. G.L. designed the study, supervised the whole research, and revised the manuscript. All authors have read and approved the final manuscript.

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Data Availability

The raw data generated and analysed during the current study are not publicly available due to restrictions on participant privacy, but are available from the corresponding author on reasonable request.

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Figures

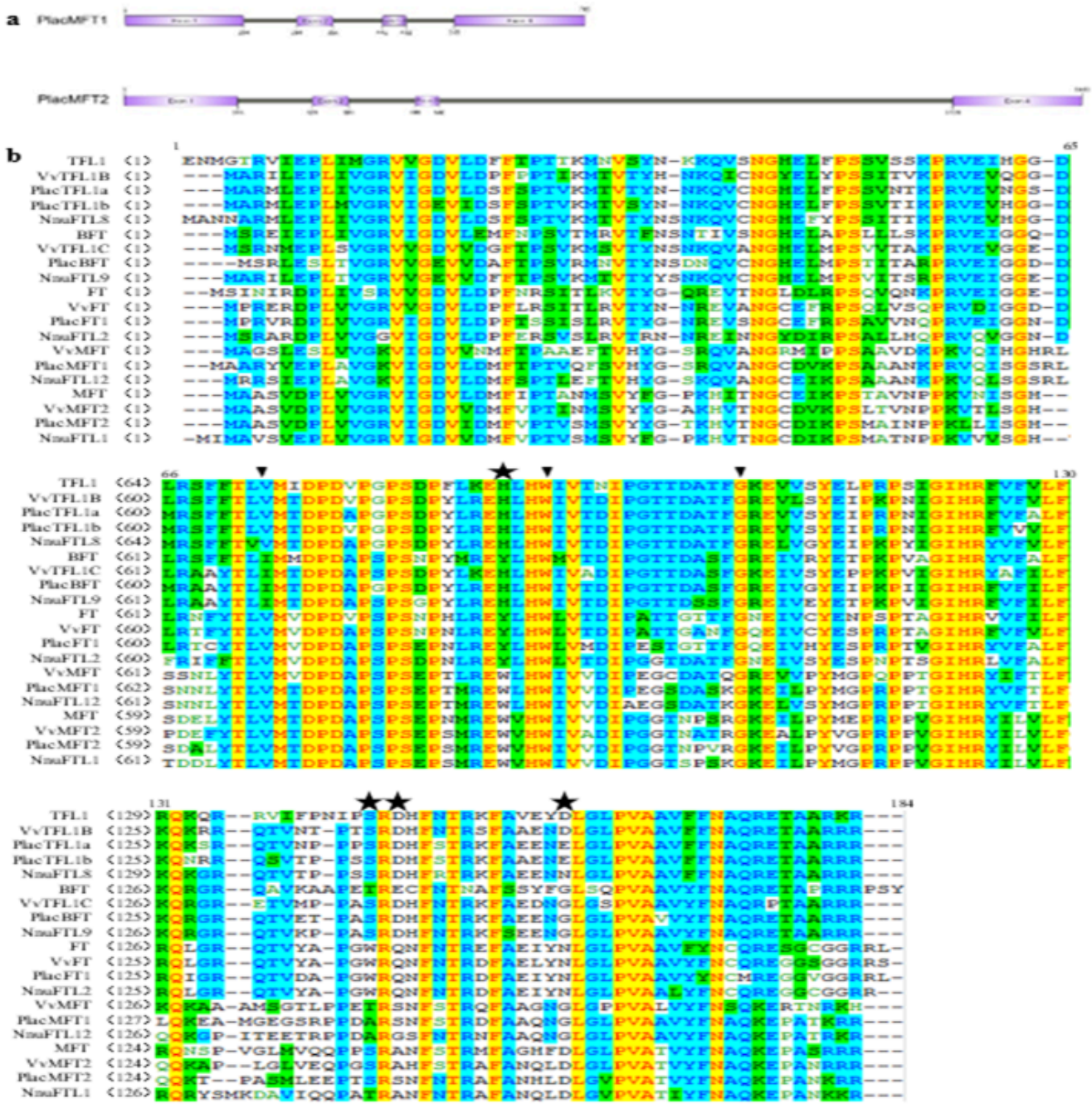


Figure 1

Gene structure and sequence alignment of *PlacMFT1/2* genes. **a** Comparison of structure of the two *MFT*-like gene sequences from London plane. **b** Alignment of the deduced amino acid sequences of the products of FT/TFL1 family in *Platanus acerifolia*, *Arabidopsis thaliana*, *Vitis vinifera* and *Nelumo 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

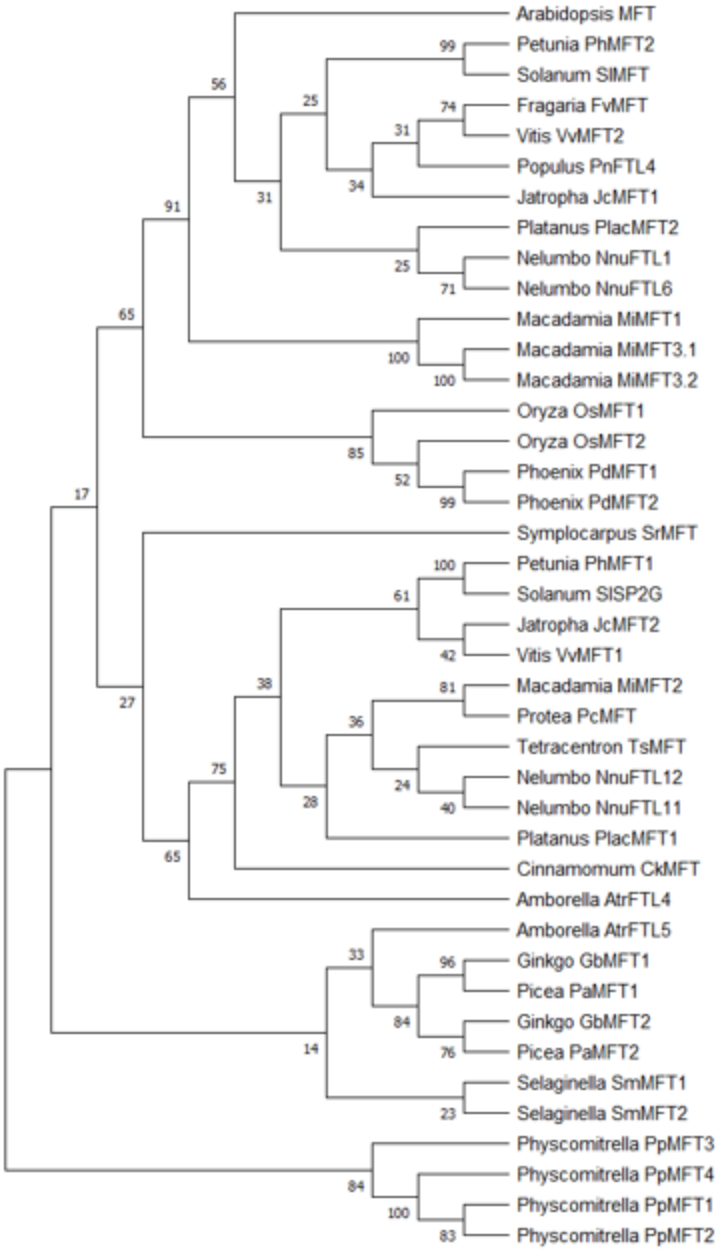


Figure 2

Phylogenetic analysis of *MFT*-like genes. The tree was generated with MEGA X software, using the Neighbor-Joining (NJ) method and 1000 bootstrap replicates.

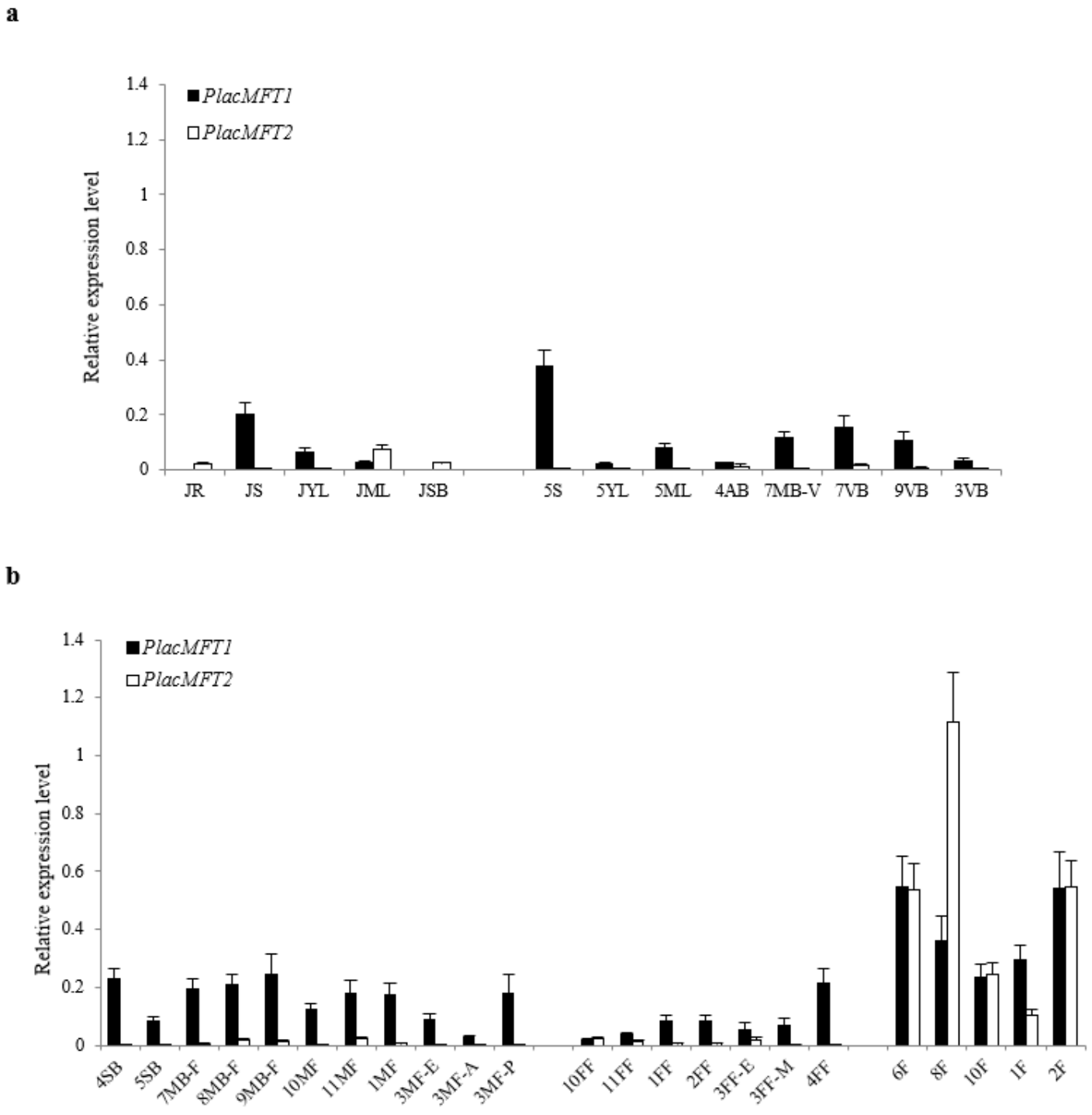


Figure 3

Expression profile of *MFT*-like genes in London plane**.** 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; VB, vegetative subpetiolar buds; SB, subpetiolar buds; MB, mixed flower buds; MB-V, vegetative tissues in 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.

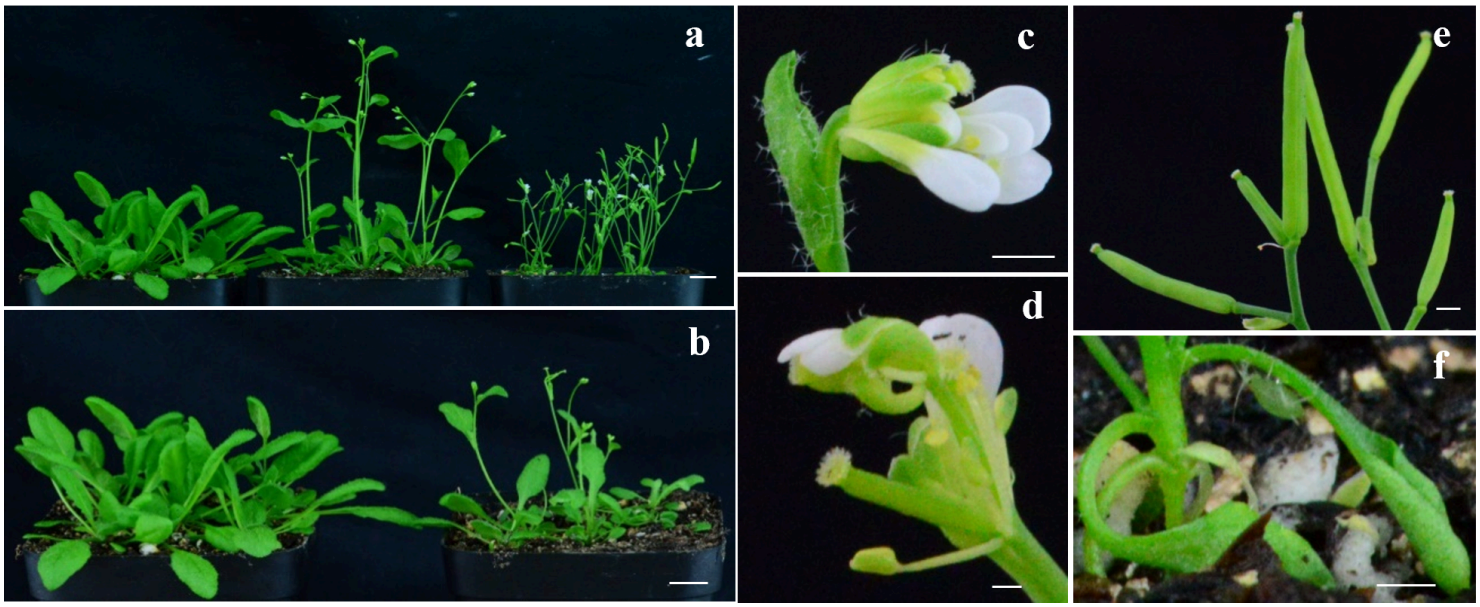


Figure 4

Phenotype analysis of transgenic *Arabidopsis* plants ectopically expressing *PlacMFT1/2* genes

Supplementary Files

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

- TableS1.Listofprimersusedinthisstudy.docx
- TableS2Accessionofgenesusedinphylogenetictree.docx