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Research article

Expression profiles of five *FT*-like genes and functional analysis of *PhFT-1* in a *Phalaenopsis* hybrid

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ABSTRACT

Background: Phalaenopsis is an important ornamental flowering plant that belongs to the Orchidaceae family and is cultivated worldwide. *Phalaenopsis* has a long juvenile phase; therefore, it is important to understand the genetic elements regulating the transition from vegetative phase to reproductive phase. In this study, *FLOWERING LOCUS T* (*FT*) homologs in *Phalaenopsis* were cloned, and their effects on flowering were analyzed.
Results: A total of five *FT*-like genes were identified in *Phalaenopsis*. Phylogenetic and expression analyses of these five *FT*-like genes indicated that some of these genes might participate in the regulation of flowering. A novel *FT*-like gene, *PhFT-1*, distantly related to previously reported *FT* genes in *Arabidopsis* and other dicot crops, was also found to be a positive regulator of flowering as heterologous expression of *PhFT-1* in *Arabidopsis* causes an early flowering phenotype.
Conclusions: Five *FT* homologous genes from *Phalaenopsis* orchid were identified, and *PhFT-1* positively regulates flowering.

1. Introduction

Floral transition is a critical developmental phase in flowering plants that is affected by various seasonal and development cues, such as light (e.g., photoperiod), temperature (e.g., vernalization), and vegetative growth condition (e.g., the autonomous flowering pathway) through a complex flowering regulatory network [1,2].

FLOWERING LOCUS T/TERMINAL FLOWER 1 (*FT/TFL1*) gene family comprises a critical regulatory node of flowering pathways. Despite the fact that FT and TFL1 share high amino acid identity (~60%), changes in critical amino acid sites lead to opposite functions of these proteins. *FT* encodes a small flowering-related protein with homology to phosphatidylethanolamine-binding protein (PEBP) [3]. *Arabidopsis FT* plays a central role in the promotion of flowering, and its transcripts are concentrated in leaves. The FT protein is transported from leaves to shoot apical meristem (SAM) through the phloem with physical interaction with the FD protein [4,5]. *FT*-like genes have been identified in various plant species, including dicot plant species such as tomato (*Solanum lycopersicum*) and rose (*Rosa* spp.) and monocot species such as rice (*Oryza sativa*) and maize (*Zea mays*) [6,7,8,9]. However, these *FT* orthologs from different plant species transcriptionally responded differently to external environmental factors and had varied functions in the regulation of flowering [10]. For example, overexpression of *SINGLE-FLOWER TRUSS* (*SFT*), a tomato ortholog of the *FT* gene, in tomato and tobacco can promote early flowering. The mutation of *SFT* produces a late flowering phenotype and affects inflorescence structure and floral development [6]. In wild and cultivated roses, different alleles of *RoFT* showed that these *RoFTs* regulate flowering in distinct ways and are not only involved in flowering transition but also in flower development [7]. In rice, two *FT* orthologs, *Heading date 3a* (*Hd3a*) and *RICE FLOWERING LOCUS T 1* (*RFT1*), are both involved in regulating floral transition [8]. The maize *FT* orthologous gene, *CENTRORADIALIS* 8 (*ZCN8*), is highly expressed in the leaf phloem and its protein can be transported to SAM through phloem. Through ectopic expression, *ZCN8* driven by a SAM-specific promoter can promote early flowering in maize, and conversely, small RNA-induced *ZCN8* silencing leads to a late flowering phenotype [9].

However, there are more than one *FT*-like genes with diversified and even antagonistic functions in the regulation of flowering in most plant species. For example, in onion (*Allium cepa*), six *FT*-like genes have been identified, and their functions are different: upregulation of *AcFT2* promotes early flowering and *AcFT1* boosts bulb formation, while

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AcFT4 restrains the expression of *AcFT1* and prevents bulbing in transgenic onions [11]. In pea (*Pisum sativum*), five *FT*-like genes with different expression patterns were isolated, and the flowering induction in pea is likely caused by the interaction effects of several *FT* members [12]. Fifteen *FT*-like genes have been identified in maize, and most of them have distinct expression patterns, suggesting that they may have different functions [13]. In tobacco (*Nicotiana tabacum*), four novel *FT*-like genes were isolated. Transgenic analysis shows that NtFT1, NtFT2, and NtFT3 proteins are floral inhibitors, whereas only NtFT4 is a floral inducer [14].

Phalaenopsis, also known as the moth orchid, is the most popular orchid flower cultivated worldwide. It takes about 15–18 months for *Phalaenopsis* to start flowering. In production, sophisticated flowering regulation of *Phalaenopsis* is the key to guarantee a regular supply of *Phalaenopsis*. Molecular biological studies on the flowering regulation of orchid plants are underway, and *FT* homologous genes have been isolated and cloned in several orchid species including *Oncidium* [15], *Dendrobium* [16], *Cymbidium goeringii* [17,18], *Cymbidium faberi* [19], and *Phalaenopsis* [20,21]. So far, one *FT* orthologous gene (*PaFT1*) has been cloned from *Phalaenopsis aphrodite*, which positively regulates flowering [20,21]. However, there are more than one *FT*-like genes in *Phalaenopsis*, and their functions, synergistic or antagonistic to *PaFT1*, are yet to be characterized.

The objective of this study was to identify *FT* homologs in *Phalaenopsis* genome and to understand their expression patterns and potential role in flowering regulation. In addition to the one (*FT-3*) previously characterized [21], the other four (*FT-1*, *FT-2*, *FT-4*, and *FT-5*) are novel genes without functional annotation. The expression patterns of these genes were analyzed, and overexpression of one gene (*PhFT-1*) in *Arabidopsis* led to early flowering.

2. Materials and methods

2.1. Plant material and growth conditions

In this study, *Phalaenopsis* hybrid (*Doritaenopsis* Tailin Red Angel 'V31') plants were grown in the greenhouse of Shanghai Academy of Agricultural Sciences. Samples of flower buds at different stages, separated floral organs (sepals, petals, lips, and columns), roots, and leaves were collected and dissected. The samples were frozen in liquid nitrogen immediately and stored at −80°C for RNA extraction. Wild-type *Arabidopsis* (Ecotype *Columbia*-0) seeds were used to obtain transgenic plants. *Arabidopsis* seeds were sown on MS medium and transplanted into the matrix in a growth chamber under 22/20°C (day/night) with a 16-h photoperiod. For *Arabidopsis* flowering time measurement, 15 plants *per* transgenic line were counted for flowering days after transplantation, and the total number of leaves was recorded when their first flowers were at anthesis.

2.2. Bioinformatics analysis

According to the *Phalaenopsis equestris* Genome Database (http://orchidbase.itps.ncku.edu.tw/est/home2012.aspx), five *FT*-like genes were identified using FT proteins conservative sequence as the BLAST query. The complete amino acid sequences of different FT homologs were obtained from GenBank (http://www.ncbi.nlm.nih.gov/). In addition, the multiple amino acid sequence alignment of *PeFTs* from *P. equestris* with other FT homologs was performed using the DNAMAN software. Phylogenetic trees based on the complete sequences were constructed using MEGA 4.0 with the neighbor joining method, and bootstrap values were derived from 1000 replicates [22]. The conserved motifs of the five *Phalaenopsis* FT proteins were predicted by MEME, and the logos were obtained through the online tool (http://meme-suite.org/).

Table 1
Primers used in this study.

Primers for qRT-PCR and semi-quantitative PCR	
PhFT-1-F	5′-AGCCAGACTCACGTGAAAAC-3′
PhFT-1-R	5′-CATGGCTGCTTAGTAGAGGA-3′
PhFT-2-F	5′-CAGCTTGGCAGAAACACAG-3′
PhFT-2-R	5′-TGACAGTTGAAATAGGCGAG-3′
PhFT-3-F	5′-CATTCGGCAGAGAAATAGTGT-3′
PhFT-3-R	5′-TGGCGAACCGAGATTATTGAG-3′
PhFT-4-F	5′-TGTCGAGGGACACTCTAGTTG-3′
PhFT-4-R	5′- TATCCTTCTATCTCCACGCG-3′
PhFT-5-F	5′-TGGAAGGGATCCACTTGTGC-3′
PhFT-5-R	5′-TCTTCACCATCAACCTCTACC-3′
PhGHPDH-F	5′-AGACTTGAGAAGAAGGCCAC-3′
PhGHPDH-R	5′-AAGTCGGTTTGACACAACATC-3′
AtUBQ10-F	5′-GATCTTTGCCGGAAAACAATTGGAGGATGGT-3′
AtUBQ10-R	5′-CGACTTGTCATTAGAAAGAAAGAGATAACAGG-3′
Primers for cloning	
PhFT-1-clone-F	5′-GAGAGATGTCAGGGAGTTCTTCA-3′
PhFT-1-clone-R	5′-CATGGCTGCTTAGTAGAGGA-3′

2.3. qRT-PCR and Semi-quantitative RT-PCR analysis

Total RNA was extracted from different *Phalaenopsis* hybrid 'V31' tissues or *Arabidopsis* leaves using RNA extraction kit (EASYspin, China), and cDNA was synthesized using a PrimeScript® RT reagent kit with gDNA Eraser (Takara, Japan). A LightCycler® 480II Real-Time PCR System (Roche, Switzerland) and SYBR Premix EX Taq Kit (Takara, Japan) were used for qRT-PCR experiments. Each qRT-PCR assay was repeated three times. The *Phalaenopsis* housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and the *Arabidopsis AtUBQ10* gene were used as internal references to normalize the expression data. The gene expression data were evaluated using the $2^{-\Delta\Delta CT}$ method [23], and the standard deviation was calculated from three biological replicates. The gene specific primers are listed in Table 1.

2.4. Cloning of PhFT-1 gene from Phalaenopsis hybrid

Total RNA was isolated from the young flower buds of *Phalaenopsis* 'V31' using a RNA extraction kit (EASYspin, China) with gDNA removed by DNase I. The RNA was reverse transcribed into cDNAs using M-MLV (Promega, USA). The cDNAs were used as templates to amplify *PhFT-1* coding sequences. PCR was performed using TransTaq DNA Polymerase High Fidelity (TransGen, China), and the thermal cycling parameters were as follows: 94°C, 2 min; 35 cycles of 94°C, 30 s, 54°C, 30 s, and 72°C, 40 s; followed by an extension step of 72°C, 10 min. The amplified PCR product was cloned into pUCM-T vector (Sangon, China) and sequenced. The primers used for cloning are listed in Table 1.

2.5. Heterologous expression of PhFT-1 in Arabidopsis

The full-length coding sequence of *PhFT-1* was cloned into pCAMBIA1300 vector after a 35S promoter using *Sac*I and *BamH*I sites. The recombinant plasmids were then introduced into *Agrobacterium tumefaciens* 'GV3101' by the freezing and thawing method and

Table 2
Protein identity of FT in *Phalaenopsis* and *Arabidopsis*.

Gene name	*PeFT-1*	*PeFT-2*	*PeFT-3*	*PeFT-4*	*PeFT-5*
AtFT	57%	59%	72%	67%	55%
PeFT-1		64%	62%	51%	43%
PeFT-2			60%	56%	45%
PeFT-3				65%	52%
PeFT-4					56%

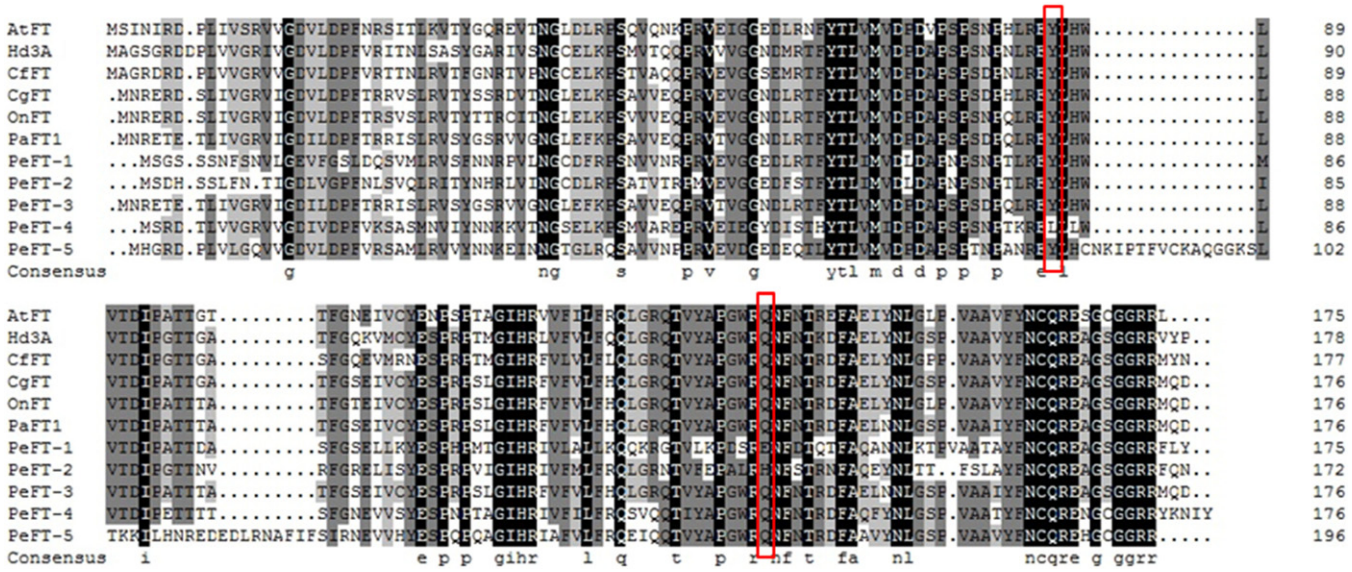

Fig. 1. Multiple amino acid sequence alignment of PeFT1-5 from *Phalaenopsis* with other FT homologs. AtFT (accession. no. AB027504), *Arabidopsis thaliana*; Hd3a (accession no. AB838411), *Oryza sativa*; CfFT (accession no. KC138734), *Cymbidium faberi*; CgFT (accession no. HM106985), *Cymbidium goeringii*; OnFT (accession no. KJ909968), *Oncidium* hybrid cultivar; PaFT1 (accession no. KC138805), *Phalaenopsis aphrodite*. Identical and similar amino acids are shaded in black and gray, respectively. Key amino acid residues of FT-like proteins are marked by red box.

transformed into wild-type (*Col*-0) *Arabidopsis* through the floral dip method [24]. The transgenic plants were screened on MS medium with 30 mg/L hygromycin.

2.6. Statistical analysis

Unpaired Student's *t*-test was used for the data analysis. *P* value of <0.01 was considered significant.

3. Results

3.1. Identification of FT-like genes in Phalaenopsis and bioinformatics analysis

To identify *FT*-like genes from *Phalaenopsis*, we performed BLAST search against *P. equestris* genome database on the basis of the conserved amino acid sequence of FT proteins. Five *FT*-like genes were identified and named *PeFT-1*(PEQU_33459), *PeFT-2*(PEQU_08401),

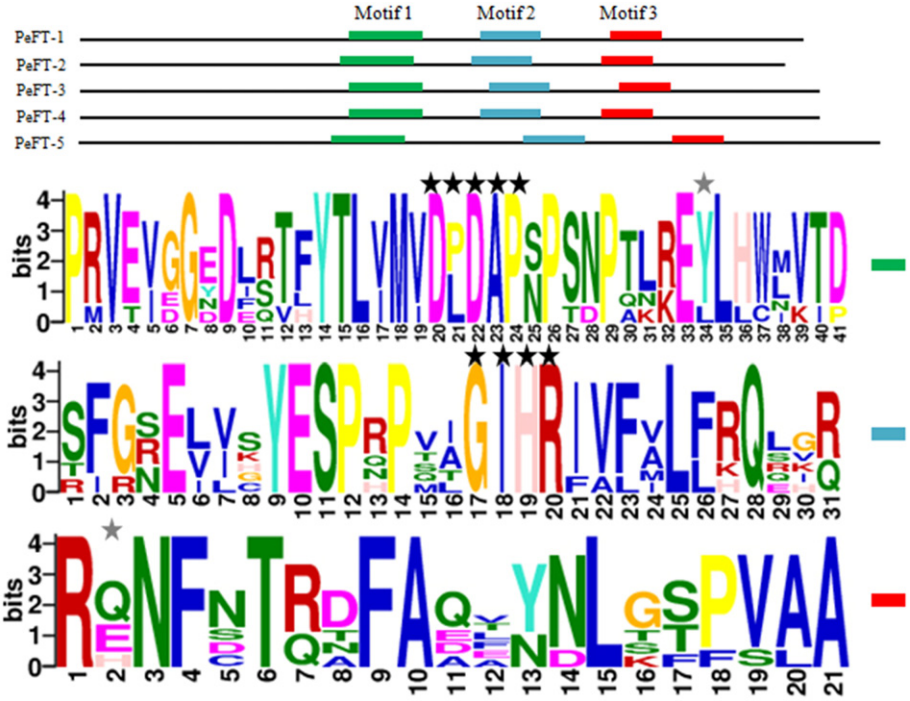

Fig. 2. Sequence conservation analysis of the predicted motifs in the five *Phalaenopsis* FT proteins. Green, motif 1; blue, motif 2; red, motif 3. The sequence logos of three motifs are based on full-length alignments of all the five Phalaenopsis FT proteins. The overall height of each stack shows the conservation of the sequence at that position, and the size of letters within each stack indicates the relative frequency of the corresponding amino acid. Gray star indicates key amino acid residues of FT-like proteins; black star indicates conserved motif D-P-D-X-P and G-X-H-R of PEBP family.

PeFT-3(PEQU_19304), *PeFT-4* (PEQU_16731), and *PeFT-5*(PEQU_15502). Among them, the *PeFT-3* gene is the flowering gene *PaFT1,* which has been reported previously [21]. In comparison with the *Arabidopsis* FT (AtFT), the highest identity is with PeFT-3 (72%) and the least with PeFT-5 (55%) (Table 2).

Previous studies suggested that FT belongs to the PEBP family, which contains two key amino acid (AA) residues at the AA_{85} (Tyr, Y) and AA_{140} (Gln, Q) position in *Arabidopsis* [25,26]. Protein alignment of FT homologs from *Arabidopsis* (AtFT), *Oryza sativa* (Hd3a), *Cymbidium faberi* (CfFT), *Cymbidium goeringii* (CgFT), *Oncidium* hybrid cultivar (OnFT), and *Phalaenopsis* (PeFTs) was performed using the DNAMAN software (Fig. 1). Protein identity varied from 43 to 65% between these *PeFT*-like genes in *Phalaenopsis* (Table 2). Additionally, the key amino acid residues of the vast majority of *PeFT*-like genes are highly conserved, except that Gln at AA_{140} is replaced by Glu (E) and His (H) in *PeFT-1* and *PeFT-2*, respectively, and Tyr at AA_{85} is replaced by Leu (L) in *PeFT-4* (Fig. 1). Furthermore, conserved protein patterns of these FT-like proteins were analyzed and compared using MEME motif with the statistical significance of the predicted motif determined at E-value <0.05. Three potential motifs were predicted, among which motif 1 (E-value = $3.2e^{-100}$) contains a conserved motif D-P-D-X-P and the key 85 amino acid residues (Y), motif 2 (E-value = $5.5e^{-32}$) contains a conserved motif G-X-H-R, and motif 3 (E-value = $8.3e^{-55}$) contains the key 140 amino acid residues (Q) (Fig. 2).

Phylogenetic tree of the deduced FT proteins from six species was constructed to analyze the genetic distance between them. The phylogenetic analysis showed that PeFT-3 was clustered with the CgFT and OnFT genes and closely related to FT-like homologs in monocots (Fig. 3). PeFT-4 and PeFT-5 as well as PeFT-1 and PeFT-2 formed two separate subclades distantly related to the rest FTs (Fig. 3), implying that these FT-like proteins in *Phalaenopsis* may have diversified functions related to flowering regulation as compared with the rest FT orthologs clustered together with PaFT-3. To test this hypothesis, we first profiled their expression patterns in different organs and at different floral developmental stages.

3.2. Expression patterns of FT-like genes in Phalaenopsis hybrid

The expression pattern of *FT*-like genes in *Phalaenopsis* was studied in various tissues of *Phalaenopsis* hybrid 'V31' by qRT-PCR analysis. Flower buds of *Phalaenopsis* were divided into six stages (F1–F6) according to the size of flower buds (Fig. 4A). In addition, the flower

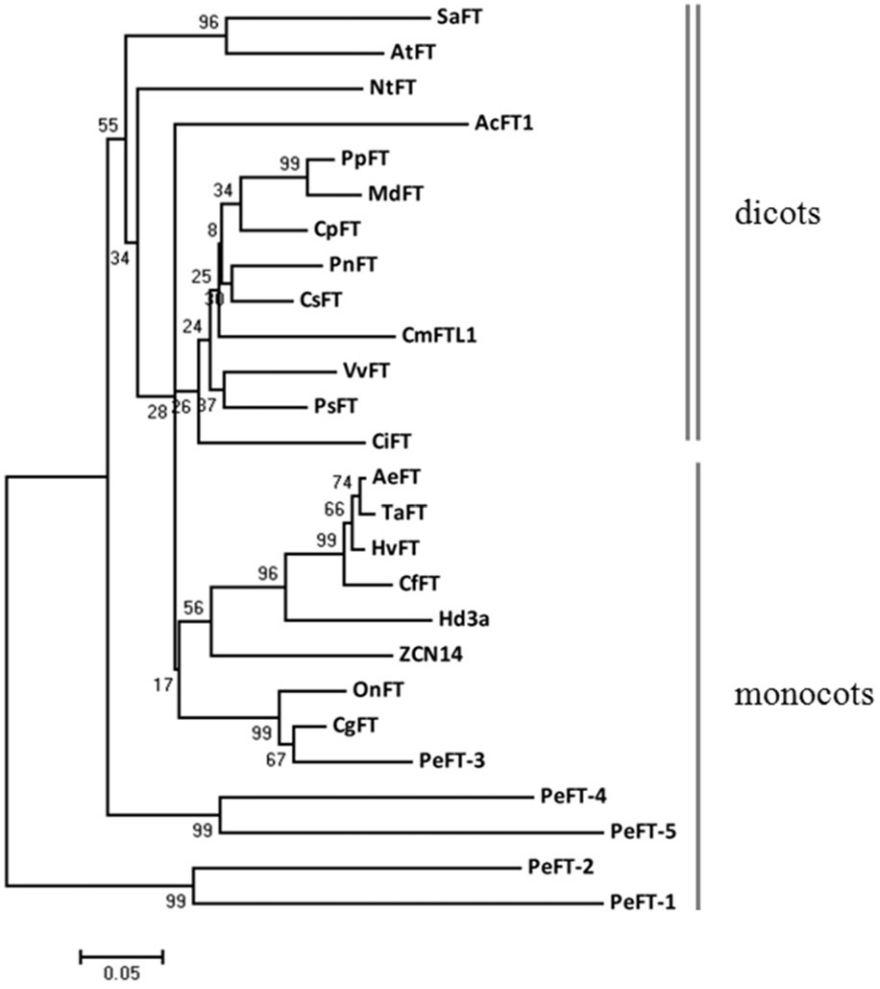

Fig. 3. Phylogenetic relationship of orthologs of FT proteins from various plant species. The complete amino acid sequences of PeFT1-5 and other FT homologous proteins were aligned by Clustalx1.83, and the neighbor-joining tree was constructed using MEGA 4.0 with 1000 bootstrap replications. The scale bar represents a genetic distance of 0.05, and numbers on the tree indicate bootstrap values. The double vertical lines and single vertical line indicate homologs of FT genes from dicotyledon species and monocotyledon species, respectively. AeFT (accession. no. ABI34864), *Aegilops tauschii*; TaFT (accession. no. ACA25439), *Triticum aestivum*; HvFT (accession. no. ABI55201), *Hordeum vulgare*; CfFT (accession no. KC138734), *Cymbidium faberi*; Hd3a (accession no. AB838411), *Oryza sativa*; ZCN14 (accession no. ABX11016), *Zea mays*; OnFT (accession no. KJ909968), *Oncidium* hybrid cultivar; CgFT (accession no. HM106985), *Cymbidium goeringii*; CiFT (accession no. BAA77836), *Citrus unshiu*; CmFTL1 (accession no. ABR20498), *Cucurbita moschata*; CsFT (accession no. AGD93126), *Camellia sinensis*; PnFT (accession no. BAG12904), *Populus nigra*; PsFT (accession no. AKS43551), *Paeonia suffruticosa*; VvFT (accession no. ABL98120), *Vitis vinifera*; CpFT (accession no. ACX85427), *Carica papaya*; MdFT (accession no. ACL98164), *Malus domestica*; PpFT (accession no. ACH73165), *Prunus persica*; AcFT1 (accession no. AFQ00669), *Allium cepa*; NtFT (accession no. AGJ83935), *Nicotiana tabacum*; AtFT (accession no. AAF03936), *Arabidopsis thaliana*; SaFT (accession no. ACM69284), *Sinapis alba.*

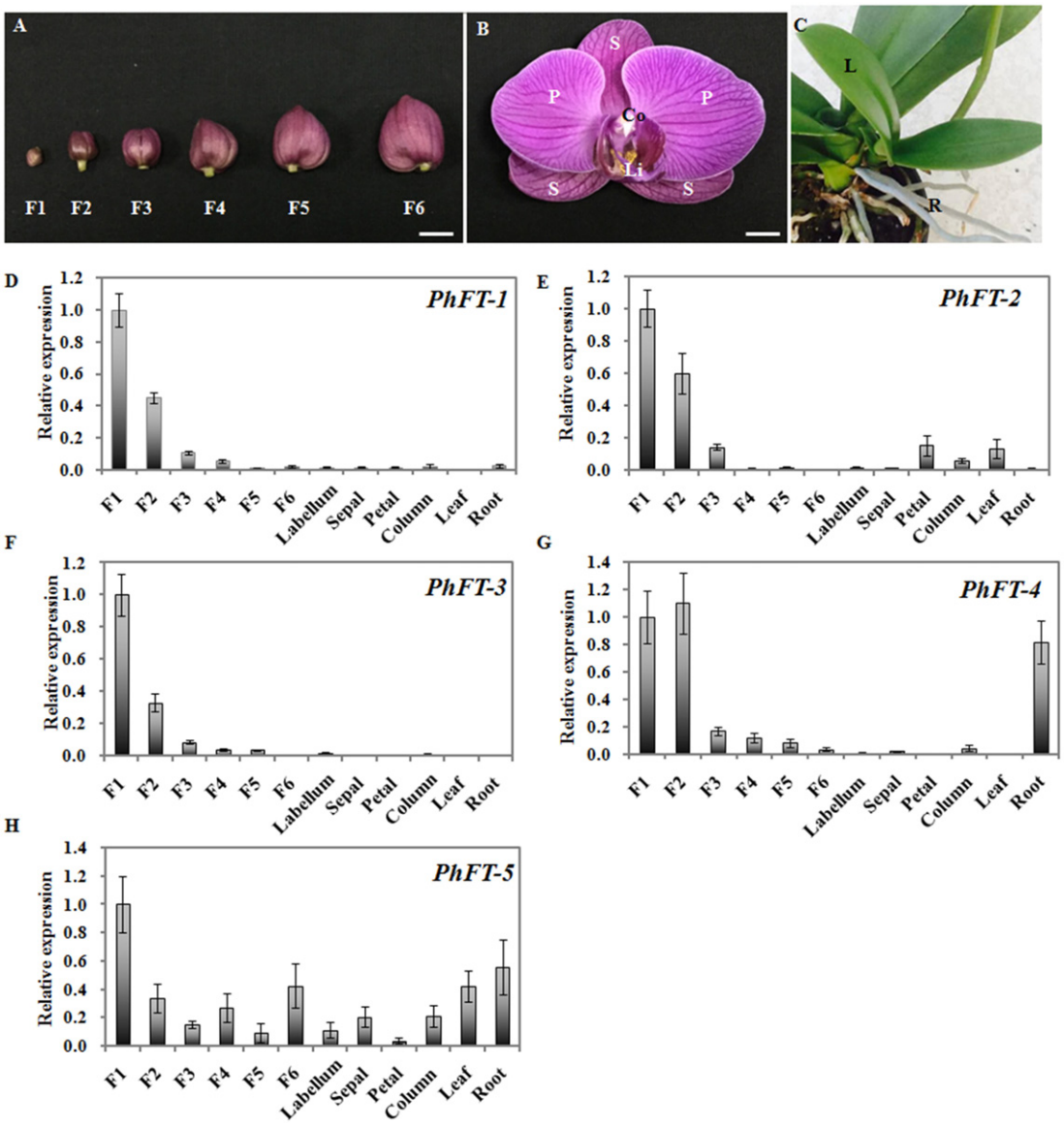

Fig. 4. Expression patterns of *FT*-like genes at different developmental stages of flower buds and different flower organs in *Phalaenopsis* hybrid. A. Different size flower buds. F1 bud size is less than 5 mm, F2 bud size is 6–7 mm, F3 bud size is 9–10 mm, F4 bud size is 12–13 mm, F5 bud size is 15–16 mm, and F6 bud size is 18–19 mm. B. Different flower organs include sepal (S), petal (P), lip (Li), and column (C). C. Vegetative tissues include leaf (L) and root (R). Bar = 1 cm. D. *PhFT-1*; E. *PhFT-2*; F. *PhFT-3*; G. *PhFT-4*; H. *PhFT-5*. The amounts of *PhGAPDH* transcripts were used as an internal control. The primers used in this study are listed in Table 1. At least three biological replicates were performed for RNA extraction in expressional analyses.

organs were dissected into four parts (sepals, petals, lip, and column) from opening flowers (Fig. 4B). Total RNA was extracted from flower buds at different stages (Fig. 4A), separated flower organs (Fig. 4B), young leaves, and roots (Fig. 4C). The result showed that all *FT*-like genes were strongly expressed in flower buds, especially the young flower bud (Fig. 4D–H). *PhFT-3* showed a similar expression pattern as *PhFT-1* and *PhFT-2*, which had the highest expression level in young flower buds (F1) that decreased gradually during the floral development. Transcripts of these three genes were markedly low in other tissues (Fig. 4D–F). The expression pattern of *PhFT-3* was consistent with previous studies [21]. Transcripts of *PhFT-4* were specifically abundant both in young flower buds (F1 and F2 stages) and roots (Fig. 4G). *PhFT-5* mRNA was detected in various tissues (Fig. 4H). However, the expression level of *PhFT-5* was higher in the young leave than other *PhFTs* (Fig. 4H), suggesting that *PhFT-5* might function in a similar manner as its *Arabidopsis* homolog in regulating the flowering of *Phalaenopsis*. Generally, transcripts of all five *PhFT*-like genes were substantially abundant in the young flower buds, indicating that they might be involved in the regulation of flowering in *Phalaenopsis*.

3.3. Isolation of the PhFT-1 gene

To further investigate the function of *FT*-like genes in *Phalaenopsis*, we isolated and cloned the novel *Phalaenopsis* orchid flowering gene *PhFT-1* from young flower buds (F1) of *Phalaenopsis* 'V31'. The open reading frame sequence was 528 bp in length, encoding 175 amino acids. The deduced amino acid sequence of PhFT-1 contained the key amino acid residue of Tyr85 and one conserved motif G-X-H-R (Fig. 5A). BLASTp search showed that the deduced amino acid sequence of PhFT-1 had 62% identity (E-value = $3e^{-74}$) with CgFT (accession no. HM106985), 60% identity (E-value = $6e^{-73}$) with CfFT (accession no. KC138734), and 62% identity (E-value = $2e^{-74}$) with OnFT (accession no. KJ909968). The analysis of conserved domain

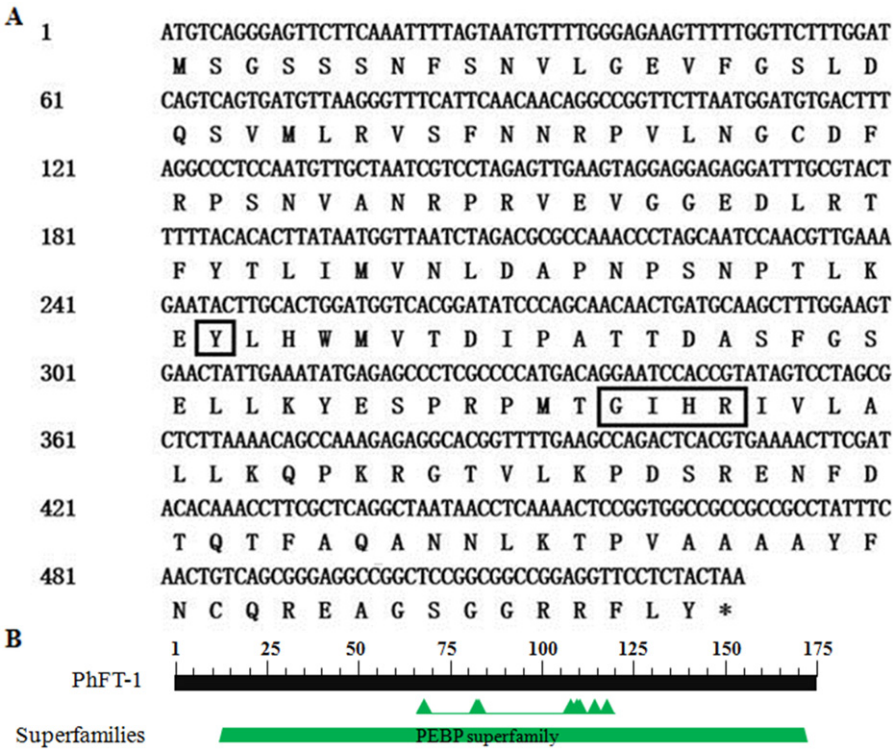

Fig. 5. Full-length coding sequence and deduced amino acid sequence of *PhFT-1*. A. The stop codon (TAA) is marked with an asterisk. Numbers corresponding to the nucleotide sequence are indicated on the left. The key amino acid residues and the conserved motif G-X-H-R are marked with a square. B. The prediction of conserved domain of *PhFT-1*.

based on an NCBI online tool Conserved Domain Search Service (CD Search) (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) suggested that PhFT-1 belongs to the PEBP superfamily (Fig. 5B).

3.4. Heterologous expression of PhFT-1 in Arabidopsis affects the flowering time

To investigate the possible function of *PhFT-1* in flowering control, transgenic *Arabidopsis* lines overexpressing *PhFT-1* under the cauliflower mosaic virus (CaMV) 35S promoter were generated. A total of 12 T2 hygromycin-resistant transgenic plants were obtained and used for further analysis. Overexpression of *PhFT-1* led to a precocious flowering phenotype compared to the wild type (WT) (Fig. 6A). Flowering days were calculated from the day plants were transplanted to the substrate, and the rosette leaf numbers were recorded when the first flower opened in the transgenic lines and WT plants. The average flowering time was shorter in the transgenic lines than in the WT plants. In addition, the numbers of rosette leaves ranged from 14.0 ± 1.3 in WT to 9.0 ± 1.7 in transgenic line #27 (Fig. 6B). Semi-quantitative PCR analysis was performed to confirm the transcript level of *PhFT-1* in the overexpression lines and WT plants. The result showed that the transcript accumulation of *PhFT-1* in 12 transgenic lines was distinct and consistent with the variance of flowering time in these transgenic *Arabidopsis* (Fig. 6C). These results suggested that *PhFT-1* played a positive role in the regulation of flowering.

4. Discussion

4.1. Characterization of the Phalaenopsis FT-like gene family

In plant development, the florigen *FT* promotes the transition to reproductive development and flowering, which has been studied extensively [10]. Previous studies showed that a single amino acid determines the antagonistic activity on floral regulators FT and TFL1. The Tyr85/His88 and Gln140/Asp144 residues in the FT/TFL1 proteins, which form a hydrogen bond in TFL1, but not FT, are likely the most critical residues for distinguishing FT and TFL1 activity [25,26]. Although one *FT* orthologous gene, *PaFT1* has been cloned and was found to be upregulated during flowering induction and involved in the regulation of flowering [21], the function of the *FT*-like genes family in *Phalaenopsis* remains elusive.

In this study, five *FT*-like genes from *Phalaenopsis* and the key amino acid residues of the five *PeFT* genes were identified: the AA85 residue of most *PeFT*-like genes is Y, except *PeFT-4*, and the AA140 residue is Q in most *FT*-like genes, except *PeFT-1* and *PeFT-2*, implying that the functions of these *FT*-like genes may be largely conserved. The results of the prediction on the conserved motifs of all *PeFT* genes show that these genes belong to the PEBP family, which not only includes the key amino acid residues but also two conserved motifs D-P-D-X-P and G-X-H-R [27]. Through phylogenetic analysis, we further divided these five *PeFT* genes into three subclades. The genetic distance of *PeFT-3* is closer to the other FT homologs than that of the remaining four *PeFT* genes (*PeFT-1*, *PeFT-2*, *PeFT-4*, and *PeFT-5*) (Fig. 3). Nevertheless, the functional characterization of all of the remaining *FT*-like genes is unknown.

4.2. Expression pattern of FT-like genes in Phalaenopsis

Florigen, a long-distance signal hormone, can be transported through the phloem vascular system from leaves to the shoot meristem. In *Arabidopsis*, the *FT* transcripts are accumulated in leaves in response to photoperiod, but FT protein is expressed specifically in phloem cells moving to the floral meristem and acts as a florigen signal that induces flowering [5]. Many studies have found that the transcripts of *FT* orthologs were accumulated extensively in the leaves

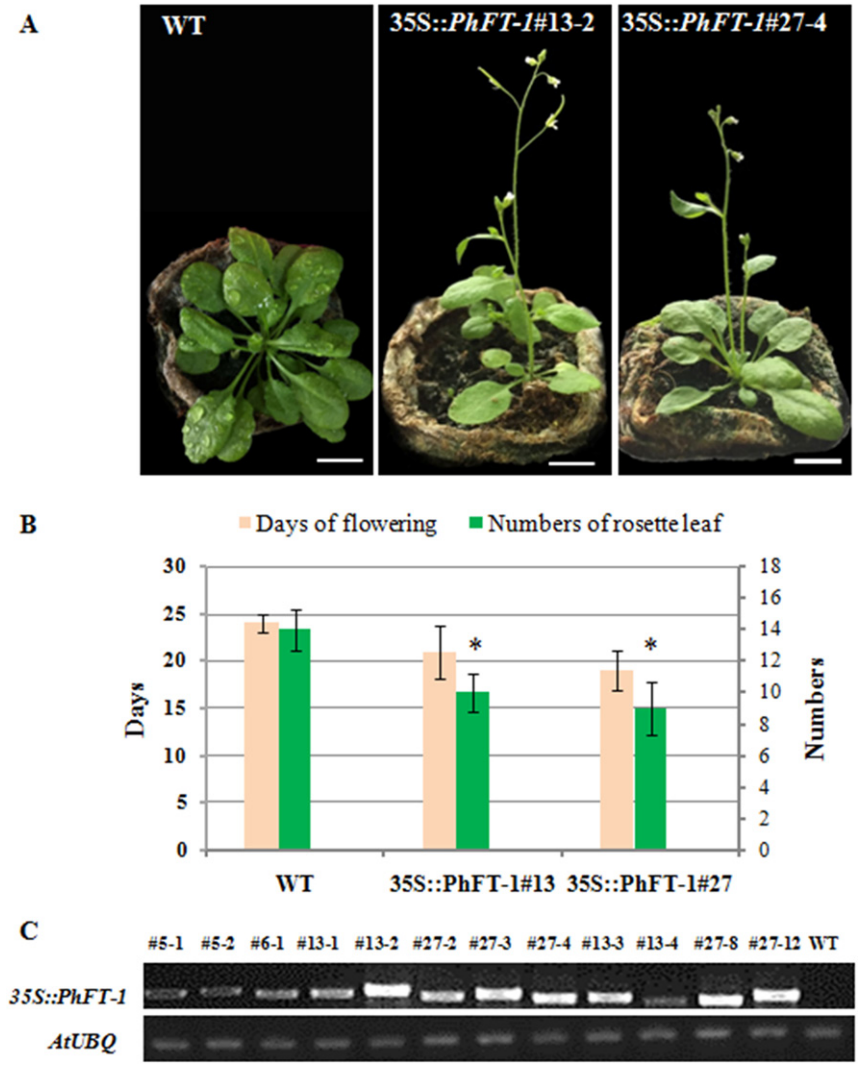

Fig. 6. Heterologous expression of *PhFT-1* in wild-type *Arabidopsis*. A. The flowering phenotype comparison between *35S::PhFT-1* transgenic *Arabidopsis* and wild-type *Arabidopsis* (ecotype *Col*-0). Left, wild-type *Arabidopsis* (24 d from transplanting to soil); middle, *35S::PhFT-1*#13-2; right, *35S::PhFT-1*#27-4. Bar = 1 cm. B. Statistics of days of flowering and numbers of rosette leaf in transgenic *35S::PhFT-1 Arabidopsis* and wild *Arabidopsis* (WT). Fifteen transgenic plants were counted in each transgenic line. Error bars indicate the standard deviation. Asterisks indicate significant difference between transgenic lines and WT (unpaired test, $P < 0.05$). C. The detection of transcription level of *PhFT-1* in four transgenic *Arabidopsis* lines and wild *Arabidopsis* (WT). The *AtUBQ* gene in *Arabidopsis* was used as an internal control.

of plants, such as maize [28], soybean [29], sunflower (*Helianthus annuus*) [30], tobacco [14], and tomato [6,31]. However, in some species, *FT*-like genes were mainly expressed in the flower buds but not restricted into the leaves. For example, two apple (*Malus domestica* Borkh.) *FT*-like genes, *MdFT1* and *MdFT2*, are specifically expressed in the apical buds and floral organs, respectively [32]. In rose, a flowering gene, *RoFT,* was specifically expressed in the floral buds [33]. Additionally, the cucumber (*Cucumis sativus* L.) *CsFT* gene is highly expressed in male and female flowers, which is almost undetectable in the leaves [34]. In *Phalaenopsis* hybrid, the expression pattern of five *FT*-like genes was coincident, which was mainly detected in the flower buds (Fig. 4D–H). Further, the transcript abundance of these five *PhFTs* was stronger in young buds than that in old buds (Fig. 4D–H). In addition, the *PhFT-4* was highly expressed in the root, which may be involved in the regulation of root development (Fig. 4G). Furthermore, the transcripts of *PhFT-5* also increased significantly in the leaf, which is consistent with the fact that the transcripts of *AtFT* are accumulated in the leaves and act as a florigen signal that induces flowering (Fig. 4H). These results suggested that the variation in the expression pattern of these *FT*-like genes in different species may lead to functional divergence in the *FT*-like homologs.

4.3. PhFT-1 regulates flowering transition and controls flowering time

The florigen FT promotes reproduction transition in various ways. Earlier studies on the *Arabidopsis* FT found that FT facilitated the transition to flowering in response to the photoperiodic input induction [35]. Recently, other environmental factors were also reported to be involved in modulating *FT* expression [10,36]. Wickland and Hanzawa [10] reviewed the diverse functions and regulatory inputs of the *FT*-like gene in 49 plant species and found that these FT orthologs regulate flowering in response to various factors such as vernalization, warm or cool temperature, salicylic acid, nutrient stress, far-red and blue light, and long day (LD) or short day (SD) photoperiod [10]. In some orchids, the homologs of *FT*-like genes have also been reported to be involved in the flowering transition. Heterologous expression of *OnFT* in *Arabidopsis* could generate the phenotype of early flowering and partially complement the late

flowering defect of *ft-1* mutant [15]. In *Cymbidium goeringii*, the *FT* homolog *CgFT* regulated the vegetative to reproductive transition in flowering, similar to its *Arabidopsis* ortholog [17,18]. Two *FT*-like genes have been isolated and cloned from *P. aphrodite* subsp. *formosana* and *P.* hybrid 'Fortune Saltzman' [20,21]. By sequence comparison, it is found that the two *FT*-like genes are the same gene (*PeFT-3*). In addition, expression analysis indicated that the *FT*-like gene from *Phalaenopsis* responded to the low temperature treatment but was not controlled by photoperiod. Furthermore, the transgenic results showed that this *FT*-like gene could promote early flowering in *Arabidopsis* and rice. The expression of *FT* in *Phalaenopsis* was regulated by temperature control but not light; one reason might be that *Phalaenopsis* is a day neutral plant and is not sensitive to photoperiod regulation. The other reason might be that *FT*-like genes have formed their own unique expression regulation mechanism in the evolutionary process of Orchidaceae plants.

A novel *FT*-like gene, *PhFT-1,* was isolated from *Phalaenopsis* 'V31'. The expression analysis of *PhFT-1* and reported *PhFT-3* showed that they had a similar expression pattern as the highest expression level in the young flower buds (Fig. 4D and F). Moreover, overexpression of *PhFT-1* in transgenic *Arabidopsis* caused early flowering (Fig. 6). However, compared with the strong or weak effects on the transgenic phenotype of flowering, *PhFT-1* affected the level of early flowering, which was weaker than *PhFT-3*. The number of rosette leaves was significantly different between *35S::PhFT-1* transgenic lines and the WT (Fig. 6B). These results suggested that the transcripts of *PhFT-1* might be involved in the floral transition but not in flowering time in *Phalaenopsis*. Similar findings were also reported with six *FT*-like genes in onion. Among the six onion *FT*-like genes, *AcFT2* mainly promoted flowering by responding to the vernalization and *AcFT1* and *AcFT4* regulated bulb formation in an antagonistic manner [11]. Therefore, different *Phalaenopsis FT*-like genes might regulate various development processes, and the functions of the remaining *FT*-like genes need to be further studied.

5. Conclusions

Five *FT*-like genes were identified from *Phalaenopsis,* which shared conserved FT protein motifs and key amino acid residues. Phylogenetic tree analysis showed that four of these *FT*-like genes were grouped separately from the previously reported *PaFT1* gene. Expression profiles showed that these five genes were mainly expressed in the young flower buds. One of these *FT* genes, designated as *PhFT*-1, caused an early flowering phenotype in *Arabidopsis* when over-expressed. This is the first study to comprehensively analyze the expression characteristics of all five *FT*-like genes in *Phalaenopsis* and provided helpful information for understanding the functions of *PhFT*-1 in regulating *Phalaenopsis* flowering time.

Conflict of interest

The authors declare that they have no conflict of interest.

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Supplementary material

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