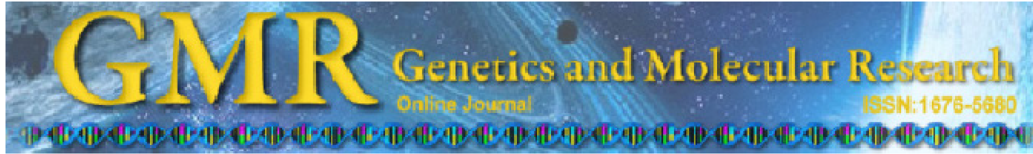

Molecular characterization and functional analysis of a *Flowering locus T* homolog gene from a *Phalaenopsis* orchid

D.M. Li, F.B. Lǚ, G.F. Zhu, Y.B. Sun, H.L. Liu, J.W. Liu and Z. Wang

Guangdong Key Laboratory of Ornamental Plant Germplasm Innovation and Utilization, Environmental Horticulture Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou, China

Corresponding authors: D.M. Li / G.F. Zhu
E-mail: biology.li2008@163.com / genfazhu@163.com

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ABSTRACT. Warm day and cool night conditions significantly induce reproductive spike formation in *Phalaenopsis* plants; hence, determining the flowering mechanism regulating the reproductive transition is important. *Flowering locus T* (*FT*) plays important roles in flowering induction in several plants. To explore spike induction by warm days and cool nights in *Phalaenopsis* orchids, we isolated the *FT* (*PhFT*) from *Phalaenopsis* hybrid Fortune Saltzman. The cDNA of *PhFT* was 809-bp long and contained a 531-bp open reading frame encoding a putative protein of 176 amino acids, a 58-bp 5'-untranslated region (UTR), and a 220-bp 3'-UTR. The predicted molecular mass of PhFT was 19.80 kDa, with an isoelectric point of 8.68. The PhFT was predicted to possess the conserved functional regions of the phosphatidylethanolamine-binding protein superfamily. Nucleotide sequence data indicated that *PhFT* contained 3 introns and 4 exons. Sequence alignment and phylogenetic analyses of PhFT revealed high homology to the FT proteins of *Cymbidium goeringii* and *Oncidium* Gower Ramsey. Quantitative real-time polymerase chain reaction analysis indicated that *PhFT* mRNA

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was expressed in roots, apical leaves, mature leaves, and flowers. In flowers, *PhFT* was expressed more in developing floral buds than in mature flowers and was predominantly expressed in ovaries and petals. Ectopic expression of *PhFT* in *Arabidopsis ft-1* mutants showed novel early-flowering phenotypes that lost their siliques. Our results indicated that the ectopic expression of *PhFT* could partially complement the late flowering defect in transgenic *Arabidopsis ft-1* mutants. Our findings suggest that *PhFT* is a putative *FT* homolog in *Phalaenopsis* plants that regulates flowering transition.

Key words: *Flowering locus T*; Flowering transition; Gene expression; *Phalaenopsis* orchids; Warm day and cool night conditions

INTRODUCTION

Floral transition is crucial for successful reproduction in flowering plants. The transition is controlled by both internal and environmental signals. For example, *Arabidopsis thaliana* contains at least five flowering pathways that are responsive to these signals, including the photoperiod, vernalization, gibberellin, autonomous, and aging pathways (Srikanth and Schimid, 2011). Therefore, a complex flowering regulatory network has been determined and is known to include many known genes such as *FT* (*Flowering locus T*), *SOC1* (*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1*), *AP1* (*APETALA1*), and *LFY* (*LEAFY*) (Abe et al., 2005; Corbesier et al., 2007; Lee et al., 2008; Lee and Lee, 2010). Of these genes, the *FT* gene of some plants plays a pivotal role in the induction of phase transition and is proposed to encode a florigen (Corbesier et al., 2007; Lin et al., 2007; Tamaki et al., 2007).

FT belongs to a small protein family whose members show homology to the mammalian phosphatidylethanolamine-binding protein (PEBP; Kardailsky et al., 1999; Kobayashi et al., 1999). The FT-like proteins that promote flowering have been confirmed in a series of dicotyledonous and monocotyledonous plants, such as sugar beet (Pin et al., 2010), sunflower (Blackman et al., 2010), pea (Hecht et al., 2011), soybean (Sun et al., 2011), medicago (Laurie et al., 2011), lettuce (Fukuda et al., 2011), chrysanthemum (Oda et al., 2012), rice (Kojima et al., 2002), corn (Meng et al., 2011), and orchids such as *Oncidium* Gower Ramsey (Hou and Yang, 2009) and Chinese *Cymbidium* (Huang et al., 2012; Xiang et al., 2012). More recently, an antiflorigenic effect of *FT*-like genes on flowering was identified in several plant species. In sunflower, a frameshift mutation allele in *Helianthus annuus FT1* (*HaFT1*) was found to delay flowering through interference with the action of *HaFT4* (Blackman et al., 2010). In sugar beet *Beta vulgaris*, *FT1* (*BvFT1*) represses flowering (Pin et al., 2010). In tobacco *Nicotiana tabacum*, FT1, FT2, and FT3 proteins act as floral inhibitors (Harig et al., 2012). Furthermore, *FT* regulates stomatal opening and modulates lateral shoot outgrowth in *A. thaliana* (Kinoshita et al., 2011; Hiraoka et al., 2013) and affects the architecture but not floral organogenesis in cotton (McGarry et al., 2013).

Flowering time has an important impact on economic benefits; therefore, understanding how flowering is regulated in ornamental crops such as *Phalaenopsis* orchids is necessary. *Phalaenopsis* orchids are epiphytic plants that show crassulacean acid metabolism and are widely cultivated worldwide (Endo and Ikusima, 1989; Guo and Lee, 2006). Researchers

have been attempting to reveal how environmental conditions, such as temperature, regulate flowering (Sakanishi et al., 1980; Chen et al., 1994, 2008) in order to achieve stable year-round flower production. They found that the average daily temperatures of 25°-30°C were required to promote leaf development and inhibit flower initiation during greenhouse production (Sakanishi et al., 1980; Chen et al., 1994), and that fluctuating warm day (28°C) and cool night (20°C) conditions significantly induced the formation of spikes in *Phalaenopsis* plants (Chen et al., 2008). Furthermore, the effects of warm day and cool night conditions on the photosynthetic efficiency, metabolic pools, and physiology of *Phalaenopsis* plants have been studied (Chen et al., 2008; Pollet et al., 2011a,b). Despite the efforts of researchers to understand the mechanisms underlying the effects of warm day and cool night conditions on the induction of flowering in *Phalaenopsis* orchids, very little is known about the genetic basis of flowering transition and development of floral buds induced by warm days and cool nights.

In a previous study, we obtained one full-length cDNA [JK720571, 809 bp, containing the 5'-untranslated region (UTR) and 3'-UTR] from a suppression subtractive hybridization cDNA library obtained from the spikes of *Phalaenopsis* hybrid Fortune Saltzman subjected to warm day and cool night conditions (Li et al., 2014). The cDNA sequence was highly homologous to the FT proteins of *Cymbidium goeringii* (92%), *Cymbidium faberi* (91%), and *Oncidium* Gower Ramsey (90%) by BLASTx search. This cDNA was designated as *PhFT*. In this study, the full-length *PhFT* cDNA and the corresponding genomic DNA region were characterized, and the corresponding putative protein was analyzed using various bioinformatic tools and methods. Transcript levels of the *PhFT* gene in different developing floral buds and flowers induced by warm days and cool nights and in the vegetative leaves and roots under warm daily temperature conditions were tested using quantitative real-time PCR. Finally, the ectopic expression of the *PhFT* gene that promoted flowering in *Arabidopsis ft-1* mutation lines was investigated. To our knowledge, this is the first study to report the functional characterization of the *PhFT* gene in *Phalaenopsis* orchids. The present study might contribute to an understanding of the molecular function of *PhFT* and its possible function in controlling flowering time in *Phalaenopsis* plants.

MATERIAL AND METHODS

Plant materials, growth conditions, and treatments

In August 2010, mature, non-flowering plants with at least 5 fully developed leaves of *Phalaenopsis* hybrid Fortune Saltzman were grown in plastic pots (diameter = 6.35 cm) filled with sphagnum moss in a greenhouse at the Environmental Horticulture Research Institute, Guangdong Academy of Agricultural Sciences, under natural light and controlled temperature conditions. Before the start of the experiment, the plants were placed in a greenhouse for at least 2 weeks with day [relative humidity (RH) = 70-95%] and night (RH = 100%) temperature regime of 28° ± 2°C. Plants in the untreated group were subsequently grown at day (28° ± 2°C; RH = 70-95%) and night (26° ± 1.5°C; RH = 100%) conditions to maintain the vegetative phase. Warm day and cool night treatment was carried out at day (28° ± 2°C; RH = 70-95%) and night (21° ± 1.5°C; RH = 100%) conditions for 1.5 months to complete the spike transition. In the untreated group, the apical vegetative leaves, first mature leaves from the top, and roots were collected. In the treated group, floral buds at different developing stages,

mature flowers, and different flower organs were collected. All samples collected were washed with double-distilled water and immediately frozen in liquid nitrogen. Frozen samples were stored at -80°C until RNA extraction.

RNA extraction

Total RNA was extracted using the RNeasy plant mini kit (Qiagen, Germany) according to manufacturer protocols. Total RNA was then treated by RNase-free DNase I (TaKaRa, Japan) to remove DNA contamination. The total RNA yield and quality were determined spectrophotometrically (Nanodrop ND-2000C; Thermo, USA). The integrity of the total RNA was checked by running samples on 1.2% denaturing agarose gels.

DNA preparation and amplification of the coding sequence of *PhFT*

Genomic DNA was extracted from mature flowers of *Phalaenopsis* hybrid Fortune Saltzman as described previously (Li et al., 2007). The gene-specific primers (*PhFT*-F1 and *PhFT*-R1; Table 1) were used for the amplification of *PhFT* from the isolated genomic DNA. The PCR system (25 μL) contained 12.5 μL 2X GC buffer I, 4.0 μL 2.5 mM of each dNTP, 0.25 μL 5 U/μL *LA* Taq DNA polymerase (TaKaRa), 1.0 μL 10 μM of each gene-specific primer, 1.0 μL 30 ng/μL genomic DNA, and 5.25 μL distilled water. Reactions were performed using a Bio-Rad T100™ thermal cycler with the following amplification protocol: 4 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 58°C, 2 min at 72°C, and a final step of 7 min at 72°C. PCR products were gel purified, sub-cloned, and sequenced (Invitrogen Biotech).

Table 1. Primers used in this study.

Primer codes	Sequences (5'→3')
PhFT genomic PCR	
PhFT-F1	ATGAATAGAGAGACGGACGC
PhFT-R1	TCAATCTTGCATCCTTCTTCCAC
PhFT real-time PCR	
PhFT-F2	ACCGCTTTGTCTTCGTGCTG
PhFT-R2	CGACTGGCGAACCGAGATTA
Phactin real-time PCR	
Phactin-F	CAGTGTTTGGATTGGAGGTT
Phactin-R	TCTCGGGTTCCATTTCCATC
PhFT ectopic expression	
PhFT-F3	<u>CCATGG</u>ATGAATAGAGAGACGGACGC*
PhFT-R3	<u>AGATCT</u>ATCTTGCATCCTTCTTCCAC**

*Underlined added *Nco*I restriction site. **Underlined added *Bgl*II restriction site.

Bioinformatic analysis

DNA sequence data were analyzed using BLAST (http://www.ncbi.nlm.nih.gov/blast; Altschul et al., 1997), GENSCAN (Burge and Karlin, 1997), and Compute p*I*/Mw tool (http://www.expasy.ch/tools/pi_tool.html). Multiple alignments were analyzed using the ClustalX software version 1.81 (Thompson et al., 1997). A phylogenetic tree was constructed using the neighbor-joining method (1000 bootstrap replicates) by using the MEGA program version 4 (Tamura et al., 2007).

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Analysis of the *PhFT* transcript in *Phalaenopsis* orchid

Real-time PCR was used to analyze the expression levels of *PhFT* in different tissues and organs of *Phalaenopsis* hybrid Fortune Saltzman. Total RNA (1.0 µg) of each sample was synthesized using the PrimeScript™ 1st strand cDNA synthesis kit (TaKaRa) according to the manufacturer protocol. Primers used for real-time PCR are shown in Table 1. Real-time PCR was carried out using the SYBR Green I kit (TaKaRa) in a final volume of 20 µL that contained 0.5 µL 10 µM forward primer, 0.5 µL 10 µM reverse primer, 10 µL 2X SYBR green premix, 2.0 µL diluted first-strand cDNA, and 7.0 µL sterile distilled water. The reactions were performed using the Light Cycler 480 real-time PCR system (Roche Diagnostics, USA) using the following program: preheating at 95°C for 30 s, followed by 40 cycles of 5 s at 95°C, 15 s at 58°C, and 30 s at 72°C. The levels of gene expression were analyzed using the Light Cycler 480 software (Roche Diagnostics) and normalized with the results for *actin* (AY134752). Real-time PCR was performed in three replicates for each sample, and data are reported as means ± standard deviation (N = 3).

Transformation vector construction and transgenic plant analysis

For *Arabidopsis* transformation, wild-type (Landsberg erecta background, Ler) and *ft-1* mutation lines of the Ler plants were obtained from the *Arabidopsis* Biological Resource Center (Ohio State University, Columbia, OH, USA). The protein-coding region of *PhFT* cDNA was PCR-amplified using primers containing *Nco*I and *Bgl*II restriction sites (Table 1). The amplified product was cloned into the binary vector pCAMBIA1303 (CAMBIA, Canberra, Australia) under the control of the CaMV 35S promoter. *Arabidopsis* plants were transformed using the improved floral dipping method (Logemann et al., 2006). Hygromycin-resistant transformants were grown at 22°C under long-day (LD) conditions (16-h light/8-h dark). The T1 generation was analyzed morphologically. Analysis of variance was performed using the SPSS software version 16.0.

RESULTS

Characterization of the cDNA sequence of *PhFT*

We obtained one full-length cDNA (JK720571, 809 bp) from a suppression subtractive hybridization cDNA library of *Phalaenopsis* hybrid Fortune Saltzman spikes induced by warm days and cool nights in a previous study (Li et al., 2014). BLASTx search showed that the cDNA sequence was highly homologous to the FT proteins of *C. goeringii* (accession No. ADI58462.1; 92%), *C. faberi* (ADW76861.1; 91%), and *Oncidium* Gower Ramsey (ACC59806.1; 90%). It was designated as *PhFT*. The cDNA sequence region from 59 to 589 nucleotides corresponded to an open reading frame encoding a polypeptide of 176 amino acids, with a 58-bp 5'-UTR upstream of the start codon ATG and a 220-bp 3'-UTR downstream of the stop codon TGA (Figure 1). The 3'-UTR region contained a putative polyadenylation signal AATAA at a position 97 bp downstream from the stop codon (Figure 1).

```
1     GACACACACTTCTCTCACTTCAAGCCCAGGACTTCAACATTATCTAAAGAACATGGATAT
                                                               M
61    GAATAGAGAGACGGACGCATTAATTGTTGGAAGAGTGATAGGAGATATACTTGATCCCTT
       N  R  E  T  D  A  L  I  V  G  R  V  I  G  D  I  L  D  P  F
121   CACAAGAAGGGTTTCTCTCAGAGTTAGTTACGGTTCAAGAGTTGTCAGTAATGGCATAGA
       T  R  R  V  S  L  R  V  S  Y  G  S  R  V  V  S  N  G  I  E
181   ATTTAAGCCCTCGGCAGTAGTGGAGCAGCCGAGAGTTAAAGTTGGTGGGAATGATCTCAG
       F  K  P  S  A  V  V  E  Q  P  R  V  K  V  G  G  N  D  L  R
241   GACTTTCTACACTCTTGTCATGGTAGATCCAGATGCTCCAAGTCCAAGTGATCCTCAACT
       T  F  Y  T  L  V  M  V  D  P  D  A  P  S  P  S  D  P  Q  L
301   TAGAGAATACTTACACTGGTTAGTCACGGATATCCCTGCAACAACAACAGCAACATTCGG
       R  E  Y  L  H  W  L  V  T  D  I  P  A  T  T  T  A  T  F  G
361   CAGAGAAATAGTGTGCTATGAGAGCCCACGCCCAAGTCTAGGCATACACCGCTTTGTCTT
       R  E  I  V  C  Y  E  S  P  R  P  S  L  G  I  H  R  F  V  F
421   CGTGCTGTTTCATCAACTAGGCCGACAGACAGTTTACGCCCCTGGCTGGCGTCAAAATTT
       V  L  F  H  Q  L  G  R  Q  T  V  Y  A  P  G  W  R  Q  N  F
481   CAACACCCGTGATTTTGCCGAGCTCAATAATCTCGGTTCGCCAGTCGCAGCCGTCTACTT
       N  T  R  D  F  A  E  L  N  N  L  G  S  P  V  A  A  V  Y  F
541   TAACTGCCAAAGAGAGGCCGGCTCCGGTGGAAGAAGGATGCAAGATTGATGAATTTCCAT
       N  C  Q  R  E  A  G  S  G  G  R  R  M  Q  D  *
601   GTGTTTGTGCTTCTACCATTAGTTATTCTCTCACAACCGCTTATCTGTTCAAGTATTTTG
661   CTATAATGTCTTCTATGTCGGATGATAATAAGCTAATAGTATAGGTCATATCAATGATTT
721   ATGCATGATGTTTTGATTTCCAAGGTTGCTTTCAGTAACTTATTTATAATATCTTAGTTC
781   TAGCCAAAAAAAAAAAAAAAAAAAAAAAA
```

Figure 1. Full-length cDNA sequence and deduced amino acid sequence of *PhFT*. The start codon (ATG) is boxed and the stop codon (TGA) is marked with an asterisk. Numbers corresponding to the nucleotide sequence are indicated on the left.

Analysis of the genomic *PhFT* sequence

The genomic DNA sequence of *PhFT* was 2055 bp in length from the start codon to the stop codon. It was submitted to GenBank under the accession No. JX162558. The exon/intron boundaries of the genomic sequence were determined by performing GENSCAN analysis, and the genomic sequence was aligned with the corresponding cDNA sequence. The genomic DNA of *PhFT* contained 3 introns and 4 exons (Figure 2). The sizes of the introns and exons were 128, 1312, and 84 bp and 198, 62, 41, and 230 bp, respectively (Figure 2). The first, second, and third introns had T/GT and AG/G splice site sequences, G/GT and AG/G splice site sequences, and G/GT and AG/G splice site sequences, respectively. All sequences of the introns followed the "GU-AG rule" for *cis*-splicing (Blumenthal and Steward, 1997).

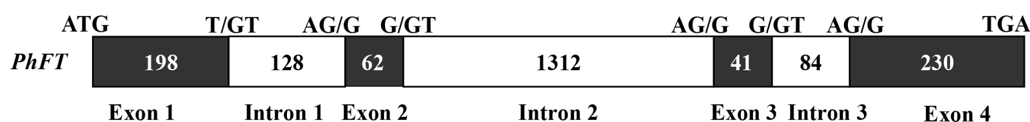

Figure 2. Genomic structure of the *PhFT* gene. Black boxes indicate exons, and open boxes represent introns. Exons and introns are drawn schematically to indicate their relative positions and sizes.

Analysis of the deduced amino acid sequence of *PhFT*

The protein coded by *PhFT* had a calculated molecular mass of 19.80 kDa and a theoretical isoelectric point of 8.68. BLASTp comparison of the deduced amino acid sequence of PhFT with that of other FT proteins revealed that PhFT had 92% sequence identity (Expect = 5e-116) with *C. goeringii* FT (ADI58462.1), 91% sequence identity (Expect = 1e-115) with *C. faberi* FT (ADW76861.1), and 90% sequence identity (Expect = 1e-114) with *Oncidium* Gower Ramsey FT (ACC59806.1). BLASTp also suggested that PhFT contained a PEBP superfamily domain, containing 25 to 163 amino acid sites (Figure 3).

The predicted protein of *PhFT* was further aligned with some other related FT proteins, including *C. goeringii* (ADI58462.1), *Oncidium* Gower Ramsey (ACC59806.1), *Gossypium hirsutum* (ADK95113.1), *Pyrus pyrifolia* (BAJ11577.3), *Oryza rufipogon* (BAG72301.1), *Prunus mume* (BAH82787.1), and *Hordeum vulgare* subsp *vulgare* (AAZ38709.1) by using Clustal X. The analysis results suggested that PhFT had high identities with these FT proteins (Figure 3).

Figure 3. Multiple alignment of the deduced amino acid sequence of *PhFT* with other reported FT proteins. Alignment of the amino acid sequences of *PhFT* and related FT proteins from *Cymbidium goeringii* (ADI58462.1), *Oncidium* Gower Ramsey (ACC59806.1), *Gossypium hirsutum* (ADK95113.1), *Pyrus pyrifolia* (BAJ11577.3), *Oryza rufipogon* (BAG72301.1), *Prunus mume* (BAH82787.1), and *Hordeum vulgare* subsp *vulgare* (AAZ38709.1). The numbers on the right side indicate the amino acid positions in the sequence. (-), (*), (:), and (.) indicate gaps, identical amino acid residues, conserved substitutions, and semi-conserved substitutions in the aligned sequences, respectively.

Phylogenetic analysis

The phylogenetic relationship of PhFT with FT proteins from other species was determined by aligning the sequences of various FT proteins from other plant species and constructing a phylogenetic tree using the neighbor-joining method (Figure 4). The PhFT protein, *C. goeringii* (ADI58462.1), *C. faberi* (ADW76861.1), and *Oncidium* Gower Ramsey (ACC59806.1) clustered into the same subgroup (Figure 4). The dendrogram obtained revealed that PhFT was closely related to FT proteins belonging to the genus *Oncidium* and *Cymbidium*.

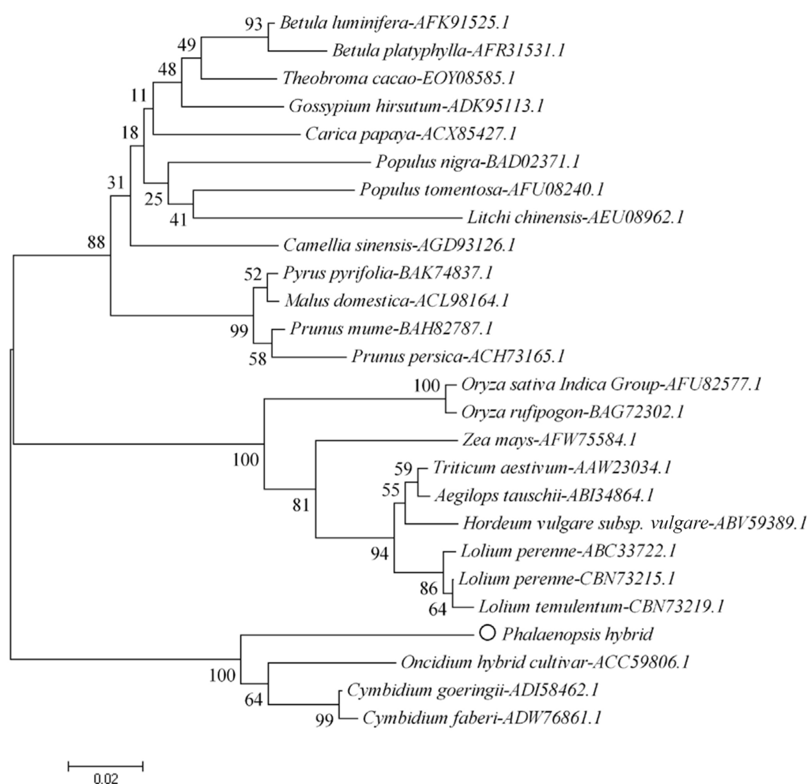

Figure 4. Phylogenetic analyses of orthologs of Flowering locus T (FT) proteins from various plant species. Numbers on the branches represent bootstrap support for 1000 replicates. The scale bar represents a genetic distance of 0.02, and numbers on the tree indicate bootstrap values. The circle indicates the FT protein of *Phalaenopsis* hybrid Fortune Saltzman. FT proteins of various plant species in the tree are indicated by species names followed by GenBank accession numbers.

Gene expression analysis of *PhFT*

PhFT mRNA was detected in floral buds at different development stages, mature flowers, leaves, and roots. Among these samples tested, *PhFT* showed the highest expression in roots and the least expression in mature leaves at the vegetative phase. In flowers, *PhFT* was expressed more in young floral buds than in mature flowers and was markedly expressed in the ovaries and petals (Figure 5).

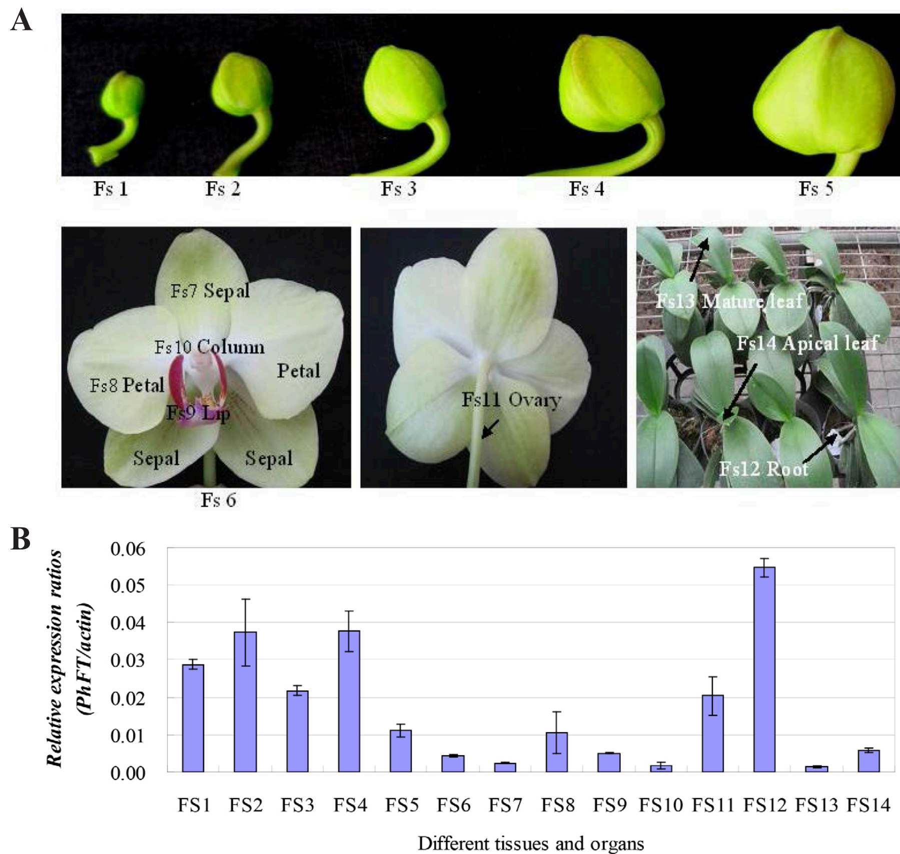

Figure 5. Tissues and detection of expression of *PhFT* by real-time polymerase chain reaction (PCR) in *Phalaenopsis* hybrid Fortune Saltzman. **A.** Floral buds of *Phalaenopsis* hybrid Fortune Saltzman at different developmental stages, a mature flower of *Phalaenopsis* hybrid Fortune Saltzman consisting of three sepals, two petals, a lip, a column and an ovary, apical leaves, mature leaves, and roots. **B.** Detection of expression of *PhFT* by real-time PCR.

Functional complementation of *PhFT* in *Arabidopsis*

Whether *PhFT* could compensate for the *FT* function in *Arabidopsis* was confirmed by analyzing the ectopic expression of *PhFT* in *Arabidopsis ft-1* mutants. A representative *ft-1 35S:PhFT* line grown in LD flowered earlier than the untransformed *ft-1* mutants (Figure 6A). The average flowering transition time for these *ft-1 35S:PhFT* transgenic plants was about 37 days after sowing until when about 11 leaves were produced (Figure 6B). The flowering transition time of *ft-1* mutants was about 48 days, and more than 24 leaves were produced (Figure 6B). However, the average flowering transition time for these *ft-1 35S:PhFT* transgenic plants was still about 13 days later than that of the wild-type Ler plants (Figure 6B). Unlike the wild-type Ler plants, *ft-1 35S:PhFT* transgenic lines kept losing siliques after flowering (Figure 6C). These results indicated that, although the function of *PhFT* was similar to that of *Arabidopsis FT*, it was not able to completely complement the late flowering defect in *Arabidopsis ft-1* mutants.

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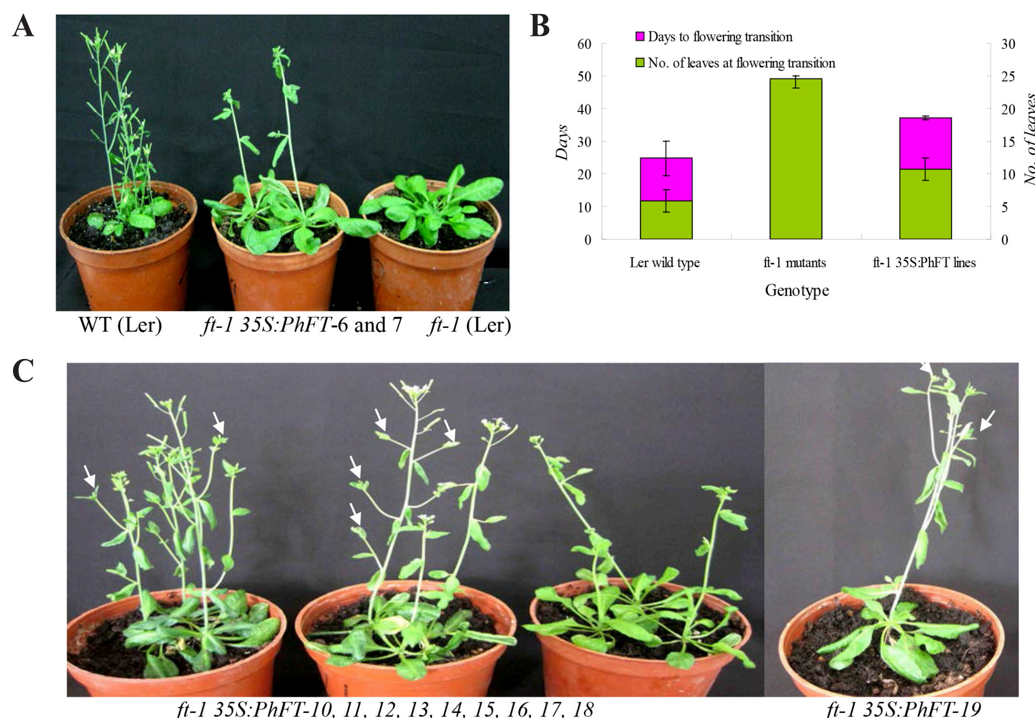

Figure 6. Phenotype analysis of transgenic *Arabidopsis* plants with *PhFT*. **A.** Representative plants grown in long-day (LD) conditions for 42 days. Flowering occurred in all lines, but not in the untransformed *ft-1* mutants. WT = wild type. **B.** Days of flowering transition and leaf numbers at flowering transition of Ler, *ft-1* mutants, and *ft-1 35S:PhFT* lines of *Arabidopsis* plants. Numbers of Ler, *ft-1* mutants, and *ft-1 35S:PhFT* plants were 21, 13, and 40, respectively. **C.** Early flowering but silique lost in *ft-1 35S:PhFT* lines of *Arabidopsis* plants.

DISCUSSION

The reported *FT* genes have been characterized in many plant species; however, the *FT* from *Phalaenopsis* orchids has not yet been characterized. In this study, the role of *FT* in regulating the transition from vegetative to reproductive growth in *Phalaenopsis* orchids was investigated by molecularly characterizing and functionally analyzing the ortholog of the *FT* of *Phalaenopsis* hybrid Fortune Saltzman (*PhFT*). Similar to the *FT* genes from other plant species, the *PhFT* contained 3 introns and 4 exons (Figure 2). Although the sizes of *FT* introns vary across different plant species, the lengths of the second (62 bp) and third (41 bp) exons seem to be conserved across different plant species (Fukuda et al., 2011; Harig et al., 2012). PhFT had a highly conserved amino acid sequence from the N-terminal to C-terminal, which was concomitant with the FT proteins of *C. goeringii*, *Oncidium* Gower Ramsey, *G. hirsutum*, *P. pyrifolia*, *O. rufipogon*, *P. mume*, and *H. vulgare* subsp *vulgare* (Figure 3). Phylogenetic analysis showed that PhFT was closely related to the FT proteins of species belonging to the genus *Oncidium* and *Cymbidium* (Figure 4). Moreover, the FT proteins from *Oncidium* and *Cymbidium* species were reported to regulate the vegetative to reproductive phase transition and flowering initiation (Hou and Yang, 2009; Huang et al., 2012; Xiang et al., 2012). Thus,

these data suggest that *PhFT* is potentially an *FT* ortholog that regulates flowering transition and initiation in *Phalaenopsis* hybrid Fortune Saltzman.

Our data indicate that the expression patterns of *PhFT* vary across different tissues and organs. In the vegetative phase, the transcript levels of *PhFT* were higher in the apical leaves than in the mature ones. This result was different from that obtained in lettuce. In lettuce, the transcripts of *FT* were the most abundant in the largest leaves (Fukuda et al., 2011). More interestingly, the transcripts of *PhFT* in the roots under vegetative phase were the highest than in the leaves and flowers tested. The root is an essential organ for *Phalaenopsis* orchids and is involved in absorbing water, providing mineral nutrition, and supplying compounds for leaf, spike, and floral bud development. These results suggest that roots might play an important role before flowering transition in *Phalaenopsis* hybrid Fortune Saltzman and might be involved in the flowering transition and development signaling pathway. Compared with that in the apical leaves under warm daily temperatures, the expression of *PhFT* was obviously higher under warm day and cool night conditions; such conditions induced 0-3-mm long spikes and gradually reduced during spike development (Li et al., 2014). The mRNA expression of *PhFT* during flowering was remarkably higher in the flower buds at the different developmental stages (Figure 5), similar to observations for *FT* in *Oncidium* Gower Ramsey (*OnFT*) (Hou and Yang, 2009). However, the expression patterns of *PhFT* fluctuated at the early stages (FS1-FS4), and then gradually decreased at later stages (FS4-FS6; Figure 5). This pattern was different from that of expression of *FT* orthologs in *Oncidium* Gower Ramsey and *Arabidopsis*. In *Oncidium* Gower Ramsey, the expression of *OnFT* was significantly higher in young F1 flower buds and gradually decreased during flower maturation (Hou and Yang, 2009). In *Arabidopsis*, the expression of *FT* gradually increased during flower maturation (Kobayashi et al., 1999).

The function of *PhFT* in flower transition was revealed by conducting functional complementation analysis using transgenic *A. thaliana*. Compared with the *Arabidopsis ft-1* mutants, the early-flowering phenotype and production of less rosette leaves were observed in *ft-1 35S:PhFT* transgenic plants (Figure 6A and B). This was similar to *Arabidopsis* and other plant species that ectopically express *FT* orthologs (Kardailsky et al., 1999; Kobayashi et al., 1999; Hou and Yang, 2009; Fukuda et al., 2011; Hecht et al., 2011; Laurie et al., 2011; Sun et al., 2011; Huang et al., 2012; Xiang et al., 2012). In addition, the *ft-1 35S:PhFT* transgenic plants lost siliques after flowering (Figure 6C). This was different from the observation in *Oncidium* Gower Ramsey expressing *OnFT*. Ectopic expression of *OnFT* in transgenic *Arabidopsis* plants showed novel phenotypes by losing the indeterminacy of inflorescence (Hou and Yang, 2009). Our data suggest that *PhFT* might be related with the ability of producing seeds in *Phalaenopsis* orchids.

In conclusion, the full-length *FT* gene, *PhFT*, was characterized from *Phalaenopsis* hybrid Fortune Saltzman in this study. The protein encoded by *PhFT* shared a conserved PEBP superfamily domain with the corresponding proteins from other plant species. Gene expression data indicated that *PhFT* was involved in a wide range of developmental processes, including vegetative growth and spike and floral bud development. Furthermore, the ectopic expression of *PhFT* could partially complement the late flowering defect in transgenic *Arabidopsis ft-1* mutants. Our results indicated that *PhFT* is a putative *FT* homolog in *Phalaenopsis* plants that regulates flowering transition and controls flowering time. Future studies are required to elucidate the mechanism of *PhFT* mRNA or protein transportation in *Phalaenopsis* plants.

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