Plant Cell Tiss Organ Cult (2012) 111:143–151
DOI 10.1007/s11240-012-0178-x

ORIGINAL PAPER

Functional characterization of *FT* and *MFT* ortholog genes in orchid (*Dendrobium nobile* Lindl) that regulate the vegetative to reproductive transition in *Arabidopsis*

Ruihong Li · Aike Wang · Shulan Sun · Shan Liang · Xiaojing Wang · Qingsheng Ye · Hongqing Li

Received: 11 January 2012 / Accepted: 8 May 2012 / Published online: 23 June 2012

Abstract Genes belonging to the phosphoethanolamine binding protein (PEBP) superfamily play important roles in controlling the switch between vegetative and reproductive growth in higher plants. Here we reported the isolation of two genes from the PEBP superfamily in *Dendrobium nobile* Lindl homologous to *FLOWERING LOCUS T* (*FT*) and *MOTHER OF FT* (*MFT*). These two genes, designated as *DnFT* and *DnMFT*, were predominantly expressed in the auxiliary buds and leaves of the plant. In auxiliary buds, *DnFT* was expressed at a higher level in young buds than in mature buds, while mature leaves expressed more *DnFT* than young ones. Low temperature treatment led to an increased expression of *DnFT* in leaves, but a decreased expression in buds. In contrast, the expression of *DnMFT* increased in buds and decreased in leaves during flower bud development and was not influenced by low temperature treatment. Ectopic expression of *DnFT* in *Arabidopsis* plants showed an early-flowering phenotype and inflorescence indeterminacy loss. Over-expression of *DnMFT* in *Arabidopsis* led to slightly late-flowering. Our results revealed that *DnFT* functions as a floral inducer in *D. nobile* Lindl by regulating flower transition in a similar way to its ortholog in *Arabidopsis*. Early flowering, induced by a low temperature treatment, was probably due to the activation of *DnFT* transcription in *D. nobile* Lindl.

R. Li · A. Wang · S. Sun · S. Liang · X. Wang · Q. Ye · H. Li (✉)
College of Life Science, South China Normal University, Guangzhou, China
e-mail: hqli@scnu.edu.cn

R. Li
e-mail: lrhnet@163.com

R. Li
South China Botanical Garden, Guangzhou, China

Keywords Flowering time · *MOTHER OF FT* (*MFT*) · *FLOWERING LOCUS T* (*FT*) · *Dendrobium nobile* Lindl

Abbreviations

CaMV	Cauliflower mosaic virus
RACE	Rapid amplified cDNA ends

Introduction

The developmental transition from the vegetative to reproductive phase is a major developmental stage in the plant life cycle and critical to reproductive success. In higher plants, four major pathways: photoperiod, vernalization, hormone and autonomous regulation act to promote flowering (Levy and Dean 1998; Simpson et al. 1999; Shen et al. 2012). These multiple pathways form a regulatory network that integrates the developmental state of plants with responses to environmental signals in order to control flowering time (Kardailsky et al. 1999; He and Amasino 2005; Baurle and Dean 2006). These signals are transmitted via integrator genes, such as *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVER-EXPRESSION OF CONSTANS1* (*SOC1*) to the floral meristem identity genes *APETALA1* (*AP1*) and *LEAFY* (*LFY*) at the apical meristem. Of these two genes, *FT*, a well known floral integrator gene plays an important role in controlling flowering time (Bradley et al. 1997; Boss et al. 2004).

FT encodes for a small protein similar to human phosphoethanolamine binding protein (PEBP) and Raf kinase inhibitor protein (RKIP) which belongs to PEBP superfamily. The plant PEBP genes group into six *FT*-like sub-families in *Arabidopsis* including *FT*, *TWIN SISTER OF FT* (*TSF*), *BROTHER OF FT* (*BFT*), *ARABIDOPSIS*

THALIANA RELATIVE OF CENTRORADIALIS (*ATC*), *MOTHER OF FT* (*MFT*) and *TERMINAL FLOWER1* (*TFL1*) (Bradley et al. 1997; Mimida et al. 2001; Yoo et al. 2004; Yamaguchi et al. 2005; Franziska et al. 2008; Hedman et al. 2009; Mohamed et al. 2010). Thus, the six genes comprise a small gene family (herein referred to as the *FT/TFL1* gene family) in the *Arabidopsis* genome. It is likely that the members of the family are important regulators for flowering time because constitutive expression of some of these members modulated flowering time (Mimida et al. 2001). *FT* is a floral promoter and acts in the photoperiod-dependent pathway (Kardailsky et al. 1999). *FT* is regulated by *CONSTANS* (*CO*). Expression of *CO* is upregulated, which in turn induces expression of *FT* (An et al. 2004). Ectopic expression of either *CO* or *FT* causes early flowering. Recent report suggest that FT protein is the mobile flower-inducing signal that moves from an induced leaf through phloem to the shoot apex (Jager and Wigge 2007; Lin et al. 2007; Tamaki et al. 2007). FT protein is produced within the phloem of leaves and acted in the shoot apex where it interacts with the bZIP transcription factor FLOWERING LOCUS D (FD) to form a FT/FD heterodimer, that promotes the transition to flowering by activating suppression of over-expression of *SOC1* and *AP1* (Abe et al. 2005; Wigge et al. 2005). In *Arabidopsis*, FT is not only an output of the photoperiodic pathway, but also of the autonomous and vernalization pathways. The effect of vernalization is downregulation of *FLOWERING LOCUS C* (*FLC*), thus allowing *FT* to be expressed in the leaves and increasing the ability of the shoot apex to respond to *FT* (Michaels et al. 2005; Searle et al. 2006; Zeevarrt 2008).

TFL1 has an antagonistic regulatory effect on *FT* which in turns affects flowering. Mutant plants where *TFL1* has ceased to function resulted in early flowering independently of day length led to the germination of a terminal flower, similar to *FT*-over-expressing plants (Bradley et al. 1997). MFT sequence is almost equally similar to both FT and TFL1, making it difficult to predict MFT function. Enaction of *MFT* was unclear, there were no obvious phenotypic changes in the *MFT-1* mutant, but over-expression of *MFT-1* slightly accelerated flowering time in *Arabidopsis*, suggesting it might act redundantly to control flowering time (Yoo et al. 2004).

Dendrobium nobile Lindl is an economically important orchid in flower markets globally with a high ornamental and medicinal value. Little research has been reported on the functions of flowering time genes of orchids (Hou and Yang 2009). In this study, we presented the isolation of the *DnFT* and *DnMFT* genes and their expression in *D. nobile* Lindl and, their role in transgenic *Arabidopsis* plants were also studied. The results showed that ectopic expression of *DnFT* led to early flowering in *Arabidopsis* through inducing the expressions of *AP1* and *LFY*. Over-expression of *DnMFT* in transgenic *Arabidopsis* plants gave rise to a slightly late-flowering phenotype.

Materials and methods

Plant materials and growth conditions

The orchid *D. nobile* Lindl, was grown in greenhouses at the Orchid Research Center at the South China Normal University. Plants with finished floral bud differentiation were selected. The selected orchid plants were transferred to a growth chamber under a low-temperature environment (12 ± 2 °C during the day, 9 ± 1 °C at night) for 40 days. The buds and leaves were collected on days: 0, 5, 10, 20, 30 and 40 under cold treatment for RNA isolation. Meanwhile, the buds and leaves without cold treatment were selected as a control.

The *Arabidopsis* (ecotype Columbia) seedlings were grown in a greenhouse under long days (LD) conditions (16 h light/8 h dark at 22 °C) for future transformation.

Cloning of *DnFT* and *DnMFT* genes in *D. nobile* Lindl

DNA fragments of *DnFT* and *DnMFT* genes were obtained from a previously constructed cDNA library by sequencing (unpublished). The 3′ and 5′ end of these genes were then isolated with a 3′ and 5′RACE cDNA Amplification Kit (TaKaRa, Japan). The gene-specific primers for 3′ and 5′RACE of *DnFT* were 3′RACE outer: 5′-TCCAGATG CTCCAAGTCCAA-3′, 3′RACE inner 5′-CAACGACCA GCGCGACATTT-3′; 5′RACE outer: 5′-AAATGTCGCG CTGGTCGTTG-3′, 5′RACE inner: 5′-TTGGACTTGGA GCATCTGGA-3′, respectively. Gene-specific primers for 3′ and 5′RACE of *DnMFT* were 3′RACE outer: 5′-ATC AATGGCGGTCAACCCAC-3′, 3′RACE inner: 5′-GTGA GCCCAGAATGAGGGAA-3′; 5′RACE outer: 5′-TTCCC TCATTCTGGGCTCAC-3′, 5′RACE inner: 5′-GTGGGTT GACCGCCATTGAT-3′, respectively. Full-length cDNA for *DnFT* was obtained by PCR amplification using the forward primer: 5′-AAATCTAGAATGAGTAGAGAGAG AGACCC-3′ and reverse primer: 5′-AAAGGATCCCAGT CTTGCATTCTTCTTCCG-3′. The cDNA for *DnMFT* was obtained by PCR amplification using the forward primer: 5′-AAATCTAGAATGGCCTCAACCTTTGTGGA-3′ and the reverse primer: 5′-AAAGGATCCACTAGCGGCGG CGGTTGTT-3′. The forward and reverse primers for *DnFT* and *DnMFT* contained a *Bam*HI(GGATCC) and a *Xba*I(TCTAGA) recognition site respectively to facilitate the cDNAs subsequent cloning.

Sequence analysis

Sequences of PEBP proteins were downloaded from GenBank for comparison. The alignment was performed using programs from DNAssist Package version 2 and CLUSTAL W. (http://www-igbmc.u-strasbg.fr/BioInfo/ClustalX). Sequence relatedness was analyzed using CLUSTAL X following the neighbor-joining method (Goloveshkina et al. 2012).

Quantitative real-time RT-PCR analysis

Quantitative real-time RT-PCR was carried out using SYBR Premix Ex Taq Kit (TaKaRa, Shiga, Japan). The amplification conditions were 95 °C for 30 s, followed by 40 cycles of amplification (95 °C for 5 s, 60 °C for 20 s) with plate reading after each cycle. Primer pairs used for quantitative real-time RT-PCR detection were as follows: *DnFT*: forward primer (5′-GCCAAGCCTAGGCATACA TCGC-3′) and reverse primer (5′-CAGTCTTGCATTCTT CTTCCGCC-3′); *DnMFT*: forward primer (5′-ACCGCT GGGCATCCACCG-3′) and reverse primer (5′-GGTTGT TGGGCTCTCGCTGG-3′). UBQ (Ubiquitin) was used for normalization with forward primer (5′-CGCCGTCAACCT CATTCCAT-3′) and reverse primer (5′-GTGAGGTAGC GACCGTGGC-3′). All the experiments were performed in triplicates. Semi-quantitative RT-PCR was carried out to detect *AP1* and *LFY* RNA in the transformed plant. *AP1*: forward primer (5′-ATGGGAAGGGGTAGGGTTCAAT TG-3′) and reverse primer (5′-ATGCTGTTTTGCTCCTG TATGG-3′); *LFY*: forward primer: 5′-GCTAAAGACC GTGGCGAA-3′ and reverse primer: 5′-GCATCCACCA CGTCCAGA-3′.

Plant transformation and transgenic plant analysis

*Xba*I and *Bam*HI fragments containing the full length cDNA for *DnFT* and *DnMFT* were cloned into the binary vector pCAMBIA1390 under the control of the CaMV35S promoter. *Arabidopsis* columbia plants were transformed by a floral dip method as described earlier (Clough and Bent 1998). Putative transformants that survived in a medium containing hygromycin (30 mg L^{-1}) were further selected by RT-PCR analyses. The hygromycin-resistant seeds obtained from the T_1, T_2 or T_3 generations (primary transformants were defined as T_1) were sown on agar plates followed by stratification at 4 °C for 3 days and then grown in a growth chamber controlled at 22 °C under LD.

Southern blot analysis

For Southern blot analysis, 10 μg of genomic DNA per sample was digested with *Hin*dIII restriction enzyme at 37 °C overnight, separated on a 0.8 %(w/v) agarose gel, and transferred to Hybond N+ membranes. A digoxigenin (DIG) labeled *DnFT* and *DnMFT* cDNA fragment was synthesised by PCR and used as the probe. Prehybridization, washing, and chemiluminescent detection of the blots was performed according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). The primers used for amplification of the *DnFT* fragment were forward 5′-AAATCTAGAATGAGTAGAGAGAGAGAC CC-3′, reverse 5′-AAAGGATCCCAGTCTTGCATTCTT CTTCCG-3′; and *DnMFT* fragment were forward 5′-A AATCTAGAATGGCCTCAACCTTTGTGGA-3′, reverse 5′-AAAGGATCCACTAGCGGCGGCGGTTGTT-3′.

Results

Protein sequence analysis of DnFT and DnMFT

Two initial fragments from the cDNA library that exhibited sequence similarities to other PEBP/RKIP genes ($E \leq e-19$) were selected, and 5′ and 3′RACE were used to isolate their full-length cDNAs. *DnFT* cDNA encoded for a-176-amino acid protein that showed 94, 91, 83, 81 and 74 % identity to *Cymbidium goeringii* CgFT, *Oncidium* Gower Ramsey OnFT, *Populus nigra* PnFT, rice Hd3a and *Arabidopsis* FT, respectively (Fig. 1). The conserved key amino acid residue Tyr in FT homologs was indentified at position 84 of the DnFT protein (Fig. 1), DnFT also contained two highly similar sequence to *Arabidopsis* FT in the 14-AA stretch referred as "segment B" and in the LYN triad in "segment C" (Ahn et al. 2006) (Fig. 1). The cDNA sequence of *DnMFT* encoded for a-173-amino acid protein that showed 67, 64, 61 % identity to *Citrus unshiu* CuMFT, *Hordeum vulgare* HvMFT and *Arabidopsis thaliana* At-MFT, respectively. DnFT and DnMFT showed 50.9 % sequence similarity.

To investigate the phylogenetic relationships of PEBP proteins, a phylogenetic tree was constructed with several other FT/MFT orthologs (Fig. 2). The results revealed that DnFT had a close relation to OnFT in *Oncidium* Gower Ramsey, while DnMFT was closely related to HvMFT in *Hordeum vulgare*.

Expression pattern of *DnFT* and *DnMFT*

To determine whether the transcriptional regulation of *DnFT* and *DnMFT* was involved in floral transition, quantitative RT-PCR was performed at different developmental stages of the buds and leaves. At the same time, the expression of the two genes was investigated under a low temperature (10 °C for 40 days) treatment that mimics the vernalization which induced flowering in *D. nobile* Lindl.

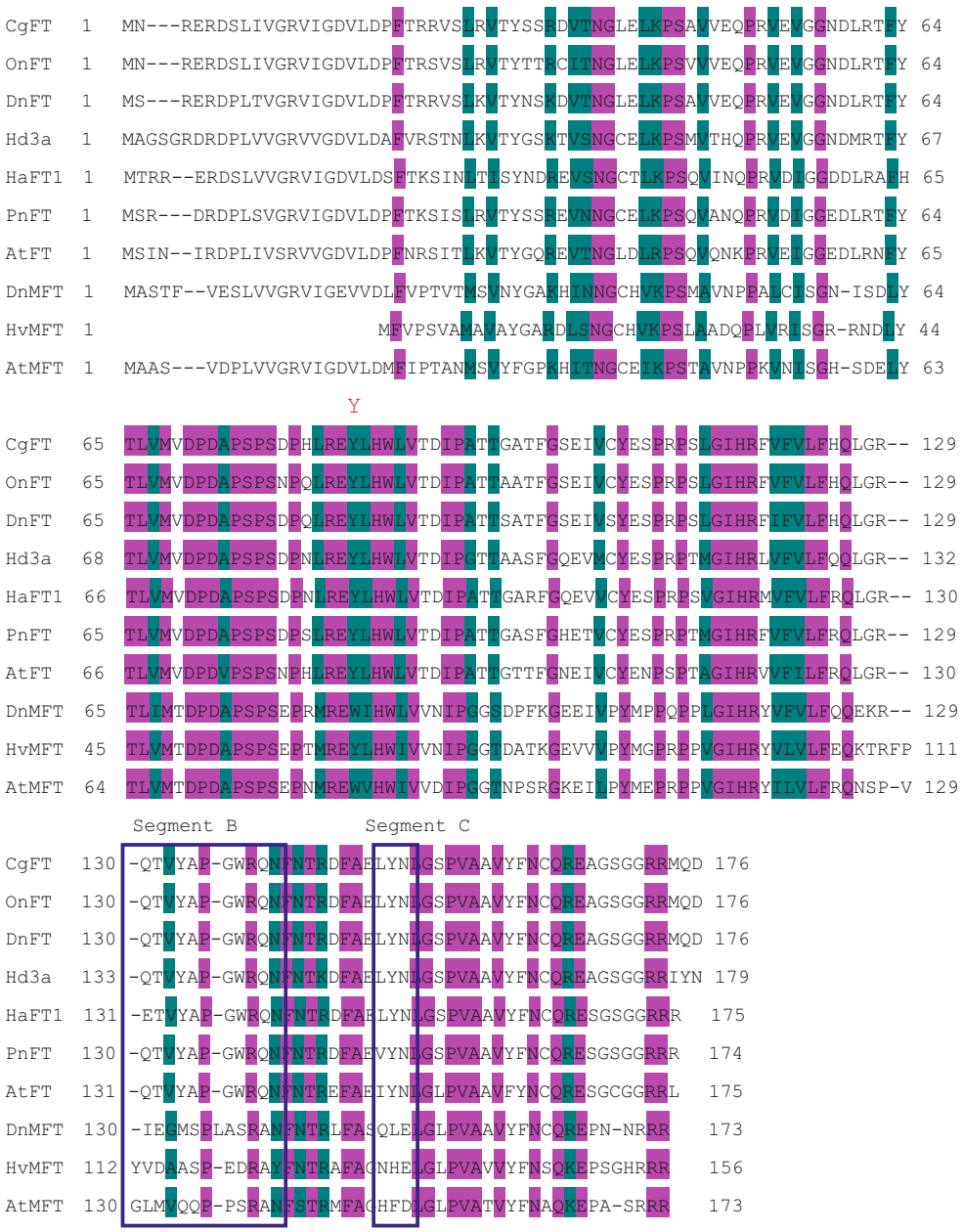

Fig. 1 Alignment of amino acid sequences of DnFT and DnMFT with other closely related PEBP protein. *Shaded in purple* are amino acid positions identical in all sequences. *Shaded in green* are amino acid positions identical in two or three sequences. *Blue squares* indicate the 14-amino-acid-stretch (segment B) and the LYN triad in segment C, and a highly conserved amino acid Tyr among FT orthologs are indicated. AtMFT (*Arabidopsis thaliana*), AtFT (*Arabidopsis thaliana*),CgFT (*Cymbidium goeringii*), DnFT (*Dendrobium nobile*), DnMFT (*Dendrobium nobile*), HaFT1 (*Helianthus annuus*), Hd3a (*Oryza Sativa*), HvMFT (*Hordeum vulgare*), OnFT (*Oncidium Gower Ramsey*), PnFT (*Populus nigra*). (Color figure online)

The two genes transcripts were detectable both in buds and leaves under field conditions (Fig. 3). The expression of *DnFT* decreased in buds during flower bud development (Fig. 3b), while its transcript level increased in leaves (Fig. 3a). Under low temperature treatment, the *DnFT* transcript in buds accumulated to a lesser degree than under normal conditions (Fig. 3b), while higher *DnFT* expression was detected in the leaves compared to that found under normal conditions (Fig. 3a). The expression of *DnMFT* increased in buds and decreased in leaves during flower bud development compared to normal conditions and its transcription did not change significantly under cold treatment (Fig. 3c, d).

Constitutive expression of *DnFT* led to early flowering in *Arabidopsis*

To test whether *DnFT* was involved in the regulation of flowering time, *DnFT* cDNA driven by a CaMV35S promoter was transformed into wild-type *Arabidopsis* columbia plants. After antibiotic selection, transgenic seedlings were used to perform a RT-PCR experiment to check if *DnFT* was overexpressed in those plants (data not shown). Thirty independent 35S::*DnFT* transgenic Arabidopsis T_1 plants were obtained. Three lines of 35S::*DnFT* in the T_3 generation were grown under LD conditions and their phenotypes were examined. In addition, we performed

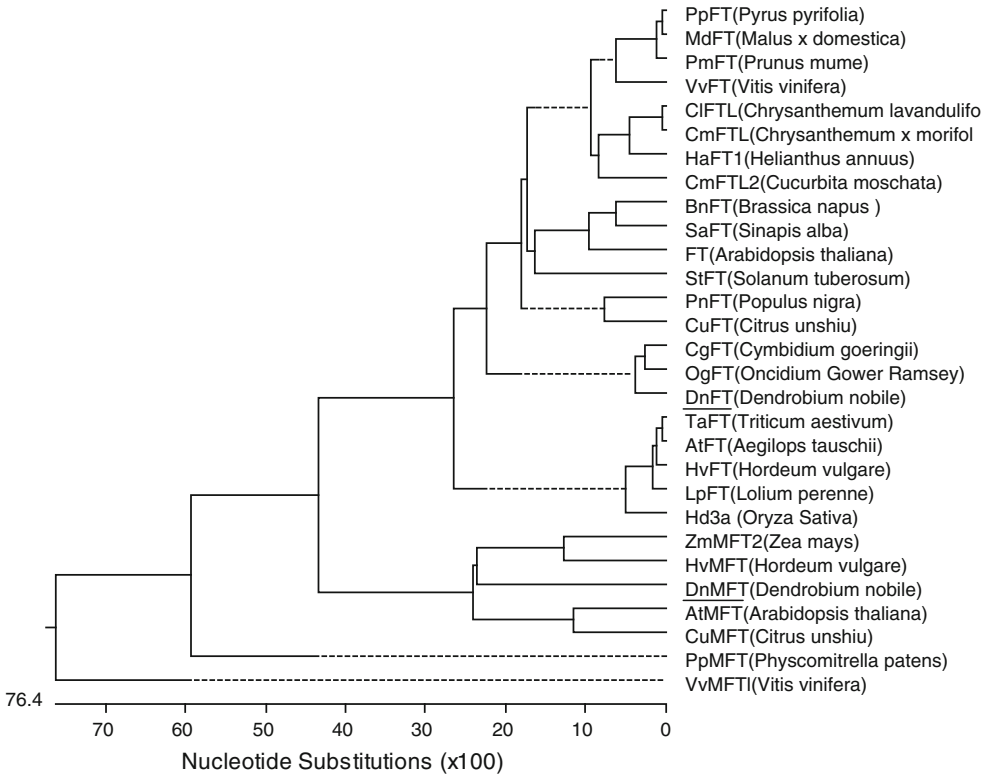

Fig. 2 Phylogenetic relationships among selected PMBP proteins. The phylogenetic tree was constructed based on the full sequences of FT/MFT proteins by the maximum likelihood method using the CLUSTAL X software. In the scale, the *bar* 0.1 is equal to 10 % sequence divergence

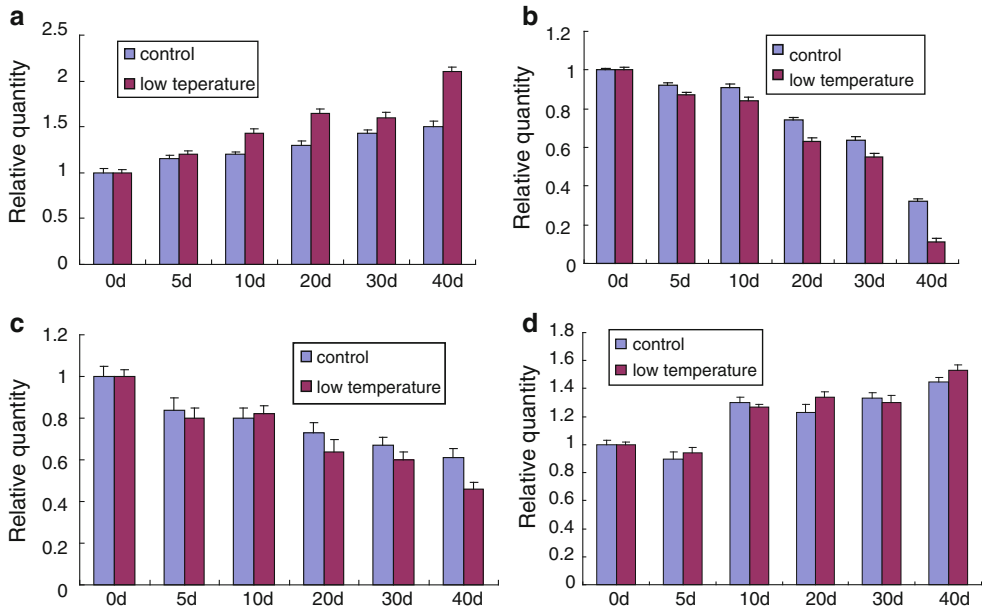

Fig. 3 Detection of expression of *DnFT* and *DnMFT* by real-time PCR. **a** Quantification of the expression of *DnFT* during leaf development. **b** Quantification of the expression of *DnFT* during bud development. **c** Quantification of the expression of *DnMFT* during leaf development. **d** Quantification of the expression of *DnMFT* during bud development

Southern-blot with the DNA extracted from the 3 lines. As we used *Hin*dIII for DNA restriction and there was only one *Hin*dIII site in the T-DNA region, the number of hybridization band(s) represented transgene copies. Thus we found that all 3 lines of 35S::*DnFT* transgenic plants had single copy T-DNA integration (Fig. 4a).

Transgenic plants flowered (derived from *35S::DnFT-3*) 14.44 ± 0.73 days after sowing by producing 4.67 ± 0.5

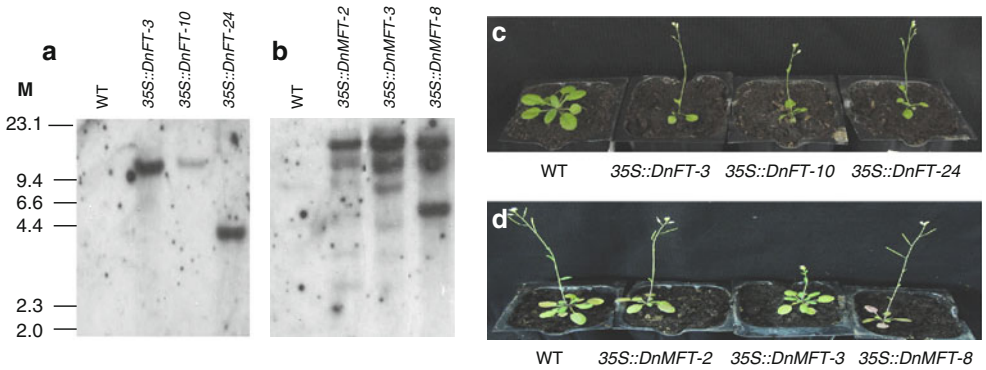

Fig. 4 Southern-blot analysis of the transgene integration and phenotypes of transgenic *Arabidopsis* ectopically expressed *DnFT* and *DnMFT* under long-day conditions. **a** Southern-blot with DnFT probe for *DnFT* transgenic plants, **b** Southern-blot with DnMFT probe for *DnMFT* transgenic plants, **c** 25-day-old wild plant (*right*), *35S::DnFT-3*, *35S::DnFT-10* and *35S::DnFT-24* **d** 29-day-old wild plant (*right*), *35S::DnMFT-2*, *35S::DnMFT-3* and *35S::DnMFT-8*

Table 1 Comparison of flowering time, leaf number of wild-type and transgenic *Arabidopsis* plants and ectopic expression of *DnFT* and *DnMFT*

Genotype	No. of plants	Days of flowering	Rosette leaf
A			
WT	22	24.00 ± 1.38	10.50 ± 1.26
35S::DnFT-3	9	14.44 ± 0.73	4.67 ± 0.50
35S::DnFT-10	8	17.00 ± 1.07	6.50 ± 1.07
35S::DnFT-24	9	13.11 ± 0.78	5.33 ± 0.71
B			
WT	8	26.25 ± 1.04	10.63 ± 0.74
35S::DnMFT-2	8	27.63 ± 1.41	11.25 ± 1.04
35S::DnMFT-3	8	29.50 ± 1.31	11.38 ± 0.52
35S::DnMFT-8	8	28.13 ± 1.46	10.75 ± 0.89

Values are mean ± SE of the results from average independent lines of transgenic plants

rosette leaves; transgenic plants (*35S::DnFT-10*) flowered at 17 ± 1.07 days after sowing by producing 6.5 ± 1.07 rosette leaves; transgenic plants (*35S::DnFT-24*) flowered at 13.11 ± 0.78 days after sowing by producing 5.33 ± 0.71 rosette leaves (Table 1) while the flowering times of wild-type plants were about 24 ± 1.38 days, producing 10.5 ± 1.26 rosette leaves (Table 1). In contrast to wild-type plants, the inflorescence was terminated by two or three flowers being produced at the end of the inflorescence in the 35S::*DnFT* plants (Fig. 4c). These results revealed that constitutive expression of *DnFT* accelerated phase transition in *Arabidopsis*. To explore whether the early flowering phenotype was correlated with *DnFT* expression in 35S::*DnFT* transgenic plants, RT-PCR analysis was performed. As shown in Fig. 5, higher *DnFT* expression was observed in the 35S::*DnFT* transgenic

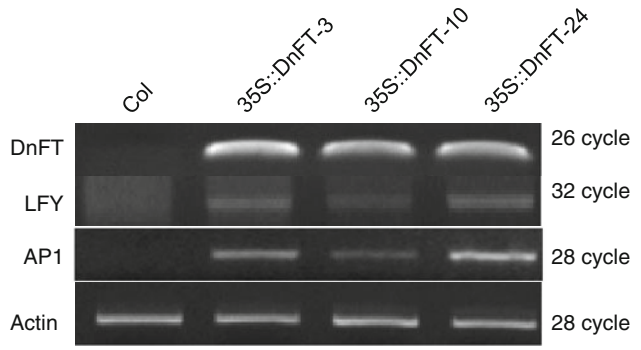

Fig. 5 Ectopically express *DnFT* activates downstream genes *AP1* and *LFY* in *Arabidopsis*. Three independent transgenic lines were used for the analysis

plants. Further analysis indicated that the promotion of flowering time in 35S::*DnFT* transgenic plants was also correlated with significant up-regulation of the flower meristem identity gene *AP1* and *LFY* in transgenic plants (Fig. 5).

Constitutive expression of *DnMFT* delayed flowering in Arabidopsis

To explore whether *DnMFT* was also able to regulate flowering transition, *DnMFT* driven by the CaMV35S promoter was transformed into wild-type *Arabidopsis*. Sixteen independent 35S::*DnMFT* transgenic T_1 plants were obtained. Among them, three lines of 35S::*DnMFT* in the T_3 generation were selected and grown under LD conditions. Southern-blot analysis of the 3 transgene lines revealed that the number of T-DNA copies in each line were between 2 and 3 (Fig. 4b). In these transgenic lines, the flowering time was delayed by 1–2 days compared to the wild-type. 35S::*DnMFT-2* plants flowered at 11.25 ± 1.04 rosette leaves, 35S::*DnMFT-3* plants

flowered at 11.38 ± 0.52 rosette leaves and 35S::*DnMFT-8* plants flowered at 10.75 ± 0.89 rosette leaves. In contrast, wild-type plants flowered at 10.63 ± 0.74 rosette leaves (Fig. 4d; Table 1). Over-expression of *DnMFT* in transgenic *Arabidopsis* plants gave rise to a slightly late-flowering phenotype.

Discussion

FT plays an important roles in floral induction, and its functions are conserved across different plant species. There are varying numbers of *FT* homologs present in different plant species however. The number of *FT* homologs varies from two in *Arabidopsis* (*FT* and *TSF*) to thirteen in rice (Izawa et al. 2002) and fifteen in maize (Danilevskaya et al. 2008). Most of *FT* functions remain undetermined.

In this study, a *FT* and a *MFT* homolog were identified in the orchid *D. nobile* Lindl and their expression and function in regulating the transition from vegetative to reproductive stage was investigated. These results showed that the expression of *DnFT* in leaves increased during plant growth and over-expression of *DnFT* in *Arabidopsis* produced an early flowering phenotype. These results indicated that DnFT may act as a promoter of flowering in *D. nobile* Lindl.

D. nobile Lindl is a biennial plant, its flowering requires a winter vernalization stage. In *Arabidopsis*, vernalization promotes flowering by suppressing *FLC* activity (Chanvivattana et al. 2004). *FT* is not only the output of the photoperiodic pathway, but also of the autonomous and vernalization pathways. In cold-requiring accessions of *Arabidopsis*, the MADS-box transcriptional regulator *FLC* represses expression of *FT* in the leaf and *FD* and *SOC1* in the shoot apex (Searle et al. 2006). *FT* was specifically induced by *CO* in phloem cells (Takada and Goto 2003; An et al. 2004). In rice, a very low accumulation of *Hd3a* mRNA was detected in the leaf blade tissue and sheath (Tamaki et al. 2007; Wu et al. 2008). The *DnFT* was expressed at a low level in young leaves and increased during leaf development. After 40 days cold treatment, the expression level was higher than in the control (Fig. 3a). The effect of vernalization might be to down-regulate the *FLC* in *D. nobile* Lindl, thus allowing *DnFT* to be expressed in the leaves and increasing the ability of the shoot apex to respond to *DnFT*. The *DnFT* transcription in the axillary buds was also examined and it was observed that *DnFT* transcription was higher in the early stage of axillary bud development but then gradually decreased after the 40 day point (Fig. 3b). A similar expression pattern for *OnFT* was also reported in *Oncidium* Gower Ramsey (Hou and Yang 2009). This was different from that in some plant species where *FT* expression gradually increased during flower maturation (Kobayashi et al. 1999). Despite the conserved functions of *FT* homologs, their expression may therefore be controlled by different systems in different species. These differences were thought to be important in the variation of flowering time and in the distinctive morphological characteristics among plant species (Andersen et al. 2004). The results from this study indicated that the DnFT produced in a leaf might act as florigen in *D. nobile* Lindl, while the high expression of *DnFT* in the axillary buds during the early vegetative stage was not sufficient to induce transition in *D. nobile* Lindl.

In contrast to *DnFT*, *DnMFT* had a different tissue-type expression pattern. The expression of *DnMFT* increased in buds and decreased in leaves during flower bud development. Their different expression patterns suggested functional diversification. The higher expression of *DnMFT* in developing buds suggested that it might maintain inflorescence meristem identity. The expression of *DnMFT* was not regulated by temperature.

The function of *DnFT* in flower transition was also revealed by functional analysis. The early flowering phenotype and the loss of inflorescence indeterminacy observed in 35S::*DnFT* transgenic *Arabidopsis* (Fig. 4c) suggested that *DnFT* was potentially a *FT* ortholog,that regulated flower transition in *D. nobile* Lindl as in other plant species (Kardailsky et al. 1999; Kobayashi et al. 1999; Lifschitz et al. 2006; Hayama et al. 2007; Lgasaki et al. 2008; Komiya et al. 2008; Takahashi et al. 2009). The expression of *AP1* and *LFY*, a marker for floral initiation and the downstream gene of *FT*, was up-regulated in 35S::*DnFT* transgenic *Arabidopsis* plants (Fig. 5). This indicated that constitutive expression of *DnFT* acted similarly to *Arabidopsis FT* in regulating flower transition by activating *AP1* and *LFY*.

The *DnMFT* sequence had almost same homologous to *FT* and *TFL1*. Although the sequences of *FT/TFL1* gene family members were homologous, their effects on flowering time were different. *FT* over-expression caused early flowering while ectopic expression of *TFL1* showed the opposite phenotype (Kardailsky et al. 1999). In this study, the role of *DnMFT* in *Arabidopsis* was also examined. Constitutive expression of *DnMFT* delayed phase transition in *Arabidopsis* and the transgenic plants flowered 2–3 days later than wild-type plants. Compared with the phenotypes in *Arabidopsis* that ectopically express *TFL1* orthologs, the 35S::*DnMFT* transgenic plants did not have more rosette leaves (Mimida et al. 2001; Hou and Yang 2009). Furthermore, over-expression of *MFT* and *PopMFT* in *Arabidopsis* caused slightly early flowering (Yoo et al. 2004; Franziska et al. 2008). These results indicated that the role of *DnMFT* in regulation of flowering time was slightly different from its homologs in other plant species.

Conclusion

In summary, this paper described two PEPB genes, *DnFT* and *DnMFT* of *D. nobile* Lindl, and elucidated the function of each gene by transcription analysis and over-expression of the genes in *Arabidopsis*. These results suggested that *DnFT* is potentially a *FT* ortholog that regulated flower transition in *D. nobile* Lindl. *DnMFT* can influence flowering time but functions slightly differently from the homologs of other plants. Further studies will be undertaken in orchids to characterize more PEPB genes and to utilize these genes to control flowering time in orchids.

Acknowledgments We thank Dr. R.A. Jefferson, CAMBIA, Canberra, Australia, for kindly providing pCAMBIA vectors. This research was supported by the Joint Funds of NSFC-Guangdong (Grant No.: U0731006) the Funds of NSFC (Grant No.: 31101579) and the Funds of NSFGD (Grant No.: 2011A020102007).

References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator *FT* at the shoot apex. Science 309:1052–1056

Ahn JH, Millar D, Winter VJ, Banfield MJ, Lee JH, Yoo SY, Henz SR, Brady RL, Weigel D (2006) A divergent external loop confers antagonistic activity on floral regulators *FT* and *TFL1*. EMBO J 25:605–614

An H, Roussot C, Suarez-Lopez P, Corbesier L, Vincent C, Pineiro M, Hepworth S, Mouradov A, Justin S, Turnbull C, Coupland G (2004) CONSTANTS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development 131:3615–3626

Andersen CH, Jensen CS, Petersen K (2004) Similar genetic switch systems might integrate the floral inductive pathways in dicots and monocots. Trends Plant Sci 9:105–107

Baurle I, Dean C (2006) The timing of developmental transitions in plants. Cell 125:655–664

Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. Plant Cell 16 Suppl:S18–S31

Bradley D, Ratcliffe O, Vincent C, Carpenter R, Coen E (1997) Inflorescence commitment and architecture in Arabidopsis. Science 275:80–83

Chanvivattana Y, Bishopp A, Schubert D, Stock C, Moon YH, Sung ZR, Goodrich J (2004) Interaction of polycomb-group proteins controlling flowering in Arabidopsis. Development 131:5263–5276

Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J 16(6):735–743

Danilevskaya ON, Meng X, Hou Z, Ananiev EV, Simmons CR (2008) A genomic and expression compendium of the expanded *PEBP* gene family from maize. Plant Physiol 146:250–264

Franziska T, Fabio F, George C (2008) Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu Rev Plant Biol 59:573–594

Goloveshkina EN, Shchennikova AV, Kamionskaya AM, Skryabin KG, Shulga OA (2012) Influence of ectopic expression of Asteraceae MADS box genes on plant ontogeny in tobacco. Plant Cell Tiss Organ Cult 109:61–71

Hayama R, Agashe B, Luley E, King R, Coupland G (2007) A circadian rhythm set by dusk determines the expression of FT homologs and the short-day photoperiodic flowering response in Pharbitis. Plant Cell 19:2988–3000

He Y, Amasino RM (2005) Role of chromatin modification in flowering-time control. Trends Plant Sci 10:30–35

Hedman H, Kallman T, Lagercrantz U (2009) Early evolution of the MFT-like gene family in plants. Plant Mol Biol 70:359–369

Hou CJ, Yang CH (2009) Function analysis of *FT* and *TFL1* orthologs from orchid (*Oncidium* Gower Ramsey) that regulate the vegetative to reproductive transition. Plant Cell Physiol 50(8):1544–1557

Izawa T, Oikawa T, Sugiyama N, Tanisaka T, Yano M, Shimamoto K (2002) Phytochrome mediates the external light signal to repress *FT* orthologs in photoperiodic flowering of rice. Genes Dev 16:2006–2020

Jager KE, Wigge PA (2007) FT protein acts as a long-range signal in *Arabidopsis*. Curr Biol 17:1050–1054

Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer FT. Science 286:1962–1965

Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science 286:1960–1962

Komiya R, Ikegami A, Tamaki S, Yokoi S, Shimamoto K (2008) *Hd3a* and *RFT1* are essential for flowering in rice. Development 135:767–774

Levy YY, Dean C (1998) The transition of flowering. Plant Cell 10:1973–1989

Lgasaki T, Watanabe Y, Nishiguchi M, Kotoda N (2008) *The FLOWERING LOCUS T/TERMINAL FLOWER1* family in Lombardy poplar. Plant Cell Physiol 49:291–300

Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshimidt A, Amesellem Z, Alvarez JP, Eshed Y (2006) The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proc Natl Acad Sci USA 15:6398–6430

Lin MK, Belanger H, Lee YJ, Varkonyi-Gasic E, Taoka KI, Miura E, Xoconostle-Cázares B, Gendlere K, Jorgensene RA, Phinneyc B, Loughb TJ, Lucasa WJ (2007) FLOWERING LOCUS T protein may act as the long distance florigenic signal in the cucurbits. Plant Cell 19:1488–1506

Michaels SD, Himelblau E, Kim SY, Schomburg FM, Amasino RM (2005) Integration of flowering signals in winter-annual Arabidopsis. Plant Physiol 137:149–156

Mimida N, Goto K, Kobayashi Y, Araki T, Ahn JH, Weigel D, Murata M, Motoyoshi F, Sakamoto W (2001) Functional divergence of the TFL1-like gene family in Arabidopsis revealed by characterization of a novel homologue. Genes Cells 6:327–336

Mohamed R, Wang CT, Ma C, Shevchenko O, Dye SJ, Puzey JR, Etherington E, Sheng XY, Meilan R, Strauss SH, Brunne AM (2010) Populus CEN/TFL1 regulates first onset of flowering, axillary meristem identity and dormancy release in Populus. Plant J 62:674–688

Searle I, He Y, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G (2006) The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis*. Genes Dev 20:898–912

Shen LL, Chen Y, Su Xh, Zhang SG, Pan HX, Huang MR (2012) Two FT orthologs from *Populus simonii* Carrie're induce early flowering in Arabidopsis and poplar trees. Plant Cell Tiss Organ Cult 108:371–379

Simpson GG, Gendall AR, Dean C (1999) When to switch to flowering. Annu Rev Cell Biol 8:483–486

Takada S, Goto K (2003) TERMINAL FLOWER2, an Arabidopsis homolog of HETEROCHROMATIN PROTEIN1, counteracts the activation of FLOWERING LOCUS T by CONSTANTS in the vascular tissue of leaves to regulate flowering time. Plant Cell 15:2856–2865

Takahashi Y, Teshima KM, Yokoi S, Innan H, Shimamoto K (2009) Variations in Hd1 proteins, Hd3a promoters, and Ehd1 expression levels contribute to diversity of flowering time in cultivated rice. Proc Natl Acad Sci USA 106:4555–4560

Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K (2007) Hd3a protein is a mobile flowering signal in rice. Science 316: 1033–1036

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. Science 309:1056–1059

Wu C, You C, Li C, Long T, Chen G, Byrne ME, Zhang GH (2008) RID1, encoding Cys2/His2-type zinc finger transcription factor, acts as a master switch from vegetative to floral development in rice. Proc Natl Acad Sci USA 35:12915–12920

Yamaguchi A, Kobayashi Y, Goto K, Abe M, Araki T (2005) TWIN SISTER OF FT (TSF) acts as a floral pathway integrator redundantly with FT. Plant Cell Physiol 46:1175–1189

Yoo SY, Kardailsky I, Lee JS, Weigel D, Ahn JH (2004) Acceleration of flowering by overexpression of MFT (MOTHER OF FT AND TFL1). Mol Cells 17:95–101

Zeevarrt JAD (2008) Leaf-produced floral signals. Curr Opin Plant Biol 11:541–547