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Research Article

Identification and Characterization of *FaFT1*: A Homolog of *FLOWERING LOCUS T* from Strawberry

Hengjiu Lei, Xiao Guo, Yantao Wang, Liping Yao, Shuang Wang and Tianhong Li
Department of Fruit Science, College of Agriculture and Biotechnology, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, People's Republic of China

Abstract: *FLOWERING LOCUS T* (*FT*)-like genes play crucial roles in flowering transition in several plant species. In this study, a homolog of *FT*, designated as *FaFT1*, was isolated and characterized from strawberry. The open reading frame of *FaFT1* was 531 bp, encoding a protein of 176 amino acids. Phylogenetic and sequence analysis showed that the FaFT1 protein contained the conservation of Tyr84 and Gln139, as well as the highly conserved amino acid sequences LGRQTVYAPGWRQN and LYN and that it was a member of the *FT*-like genes of dicots. Subcellular localization analysis revealed that the FaFT1 protein mainly localized in the nuclei of the *Arabidopsis* protoplasts. *FaFT1* was highly expressed in strawberry mature leaves and its expression level decreased under floral induction conditions. Additionally, *FaFT1* expression exhibited diurnal circadian rhythm both under SD and LD conditions. Over expression of *FaFT1* in wild-type *Arabidopsis* caused early flowering. Taken together, these results indicate that *FaFT1* is a putative *FT* homolog in strawberry, acting as a floral promoter in *Arabidopsis*.

Keywords: *Arabidopsis*, *FaFT1*, flowering, strawberry

INTRODUCTION

The transition from vegetative into reproductive phase is a crucial developmental process of flowering plants. The genetic network of flowering has been most extensively studied in the annual model plant *Arabidopsis thaliana* and six genetically pathways have been identified that control flowering: the vernalization, photoperiod, autonomous, ambient temperature, gibberellin and age pathways (Fornara *et al*., 2010). These signaling pathways converge to regulate a small number of floral integrator genes, which include *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*), *LEAFY* (*LFY*) and *FLOWERING LOCUS C* (*FLC*) (Boss *et al*., 2004; Parcy, 2005). Among these genes, *FT* plays a central role in integrating flowering signals from the photoperiodic, vernalization and autonomous pathways (Boss *et al*., 2004).

FT belongs to the FT/TFL subfamily of the phosphatidylethanolamine binding protein (PEBP) family (Kobayashi *et al*., 1999). Recent studies in *Arabidopsis* and rice (*Oryza sativa*) have confirmed that FT protein produced in leaves, is the major component of florigen that moves via the phloem to the shoot apical meristem (Corbesier *et al*., 2007; Jaeger and Wigge, 2007; Mathieu *et al*., 2007; Tamaki *et al*., 2007). In the shoot meristem, FT protein interacts with the b-ZIP transcription factor Flowering Locus D (FD), to promote flowering in *Arabidopsis* by activating several downstream floral meristem genes, such as *APETALA1*(*AP1*), *FRUITFULL*(*FUL*), *SEPALATA3* (*SEP3*) and *SOC1* (Abe *et al*., 2005; Teper-Bamnolker and Samch, 2005; Wigge *et al*., 2005).

The expression of *FT* is directly regulated by the *CONSTANS* (*CO*) and *GIGANTEA* under inductive photoperiod (Suárez-López *et al*., 2001; Sawa and Kay, 2011). CO is directly binds to the *FT* promoter or indirectly acts with other transcriptional factors to regulate *FT* expression (Wenkel *et al*., 2006; Kobayashi and Weigel, 2007). The MADS-box proteins FLC and SHORT VEGETATIVE PHASE (SVP) form a complex that inhibits *FT* expression by binding to the CArG box of *FT* genomic sequence (Li *et al*., 2008). The *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE3* (*SPL3*) can also regulate *FT* expression to control ambient temperature-responsive flowering (Kim *et al*., 2012). Furthermore, *FT* expression is also affected by six miR172-targeted *AP*2-like genes and two TEMPRANILLO proteins (TEM1and TEM2) (Castillejo and Pelaz, 2008; Mathieu *et al*., 2009).

The ectopic expression of *FT* orthologs in *Arabidopsis* and the other plant species have shown that FT-like proteins act as universal flowering promoters (Pin and Nilsson, 2012). Although *FT*-like genes play conserved roles in promoting flowering, their

Corresponding Author: Tianhong Li, Department of Fruit Science, College of Agriculture and Biotechnology, China Agricultural University, 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, China, Tel./Fax: +86 010 62733957

expression pattern differs. For example, the *Malus MdFT1* is mainly expressed in the apical meristem during the floral transition, whereas expression of *MdFT*2 peaks at the reproductive tissues and developing fruits (Kotoda *et al*., 2010). In *Populus*, the *PtFT*1 gene is preponderantly expressed in late winter, *PtFT*2 is expressed in the vegetative growth period (Hsu *et al*., 2011).

Strawberries (*Fragaria* spp.) are members of perennial rosette plants, belonging to the most economically significant berry crops. Cultivated strawberry (*Fragaria×ananassa* Duch.) is a kind of the most popular soft fruit species because of its unique flavor and nutrient values. The proper time of flowering has significant influences on yield, fruit quality and shelf life of strawberry. Most varieties of cultivated strawberry and wild strawberry (*Fragaria vesca* L.) are temperature-dependent Short Day (SD) plants, which flower under decreased photoperiod conditions in autumn (Heide and Sønsteby, 2007). Therefore, the photoperiod plays a key role in the flowering time control of strawberry. In 2012, Koskela *et al*. (2012) used a wild diploid strawberry, *Fragaria ves*ca, to elucidate the photoperiodic flowering in strawberry. The expression and function *FvFT1* were studied in *F. vesca*. However, there were also some other questions need to be study further, which included the ectopic expression in *Arabidopsis* and subcellular localization analysis. Recent studies have shown that there are some differences in *SOC1*-like gene expression between wild diploid and cultivated octoploid strawberry (Lei *et al*., 2013; Mouhu *et al*., 2013).

In this context, a *FT*-like gene, *FaFT1*, was isolated and characterized from cultivated strawberry cv. Camarosa. The sequence information, subcellular localization and expression pattern of *FaFT1* were investigated. We also analyzed the function of *FaFT1* in flowering time using transgenic *Arabidopsis*.

MATERIALS AND METHODS

Plant material: Cultivated strawberry (*Fragaria× ananassa* Duch. cv. Camarosa) runner plants with 3-4 leaves were used for the experiments. The plants were kept in a greenhouse maintained at 22°C under long day (LD) conditions (16 h light/8 h dark) with high pressure sodium lamps (~235 μmol/m^2/s) to prevent floral induction. To monitor changes in gene expression in the leaves during floral induction, the plants were transferred to SD conditions (8 h light/16 h dark) with temperature maintained at 18/15°C (day/night) for six weeks for floral induction, pieces of young fully developed leaves were collected at seven different time points with one-week intervals. Samples of root, stem, leaf, flower bud, flower, stamen, pistil, sepal, petal, receptacle and fruit were collected for tissue-specific expression assays. In diurnal expression analyses, pieces of young fully developed mature leaves were sampled every 2 h after dawn for 24 h as a bulk of 10 plants grown under SD or LD conditions.

Arabidopsis thaliana Columbia-0 (Col-0) was used as the wild-type. The wild-type or transgenic plants were grown at 22±2°C under LD conditions with white fluorescent lights (~150 μmol/m^2/s). For analysis of the flowering time phenotype, plants were grown in soil under LD conditions, flowering time was determined by counting the total number of rosette leaves at the time of floral bolting.

RNA preparation and cDNA cloning: Total RNA was extracted by the pine tree method (Chang *et al*., 1993) and the first-strand cDNA was constructed by oligo (dT15) primer with M-MLV reverse transcriptase (Promega, Madison, USA) according to the manufacturer's instructions. To clone the *FaFT1* gene, the FT protein (GenBank ID: Q9SXZ2) was used for a BLAST search in the strawberry genome GBrowse (http://www.strawberrygenome.org/) and a high homology protein with the gene locus 21535 was found. Then the specific primers for full-length of cDNA cloning were designed for *FaFT1* (forward, 5′-CAG CTA GCT AGC TTG AAG GAT C-3′; reverse, 5′-GCG AAT TAT GAC ATG CAT GTA CT-3′) using the Primer Premier 5.0 program. PCR was performed with *Pfu* DNA polymerase (Promega, Madison, USA) under the following conditions: 95°C for 1 min, followed by 35 cycles at 95°C for 30 s, 58°C for 30 s and 72°C for 1 min. The amplified PCR fragments were cloned into pSIMPLE-19 EcoRV/BAP vector (Takara, Dalian Division, China) and sequenced.

Bioinformatics analysis: The deduced amino acid sequence of *FaFT1* was identified through BLAST searches in GenBank (http://www.ncbi.nlm.nih.gov/BLAST/). The theoretical isoelectric point (pI) and molecular weights were calculated using the Peptide Mass program (http://us.expasy.org/tools/peptide-mass.html). Protein sequences were aligned using Clustal W (Thompson *et al*., 1994) and the alignment edited with BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The phylogenetic tree was constructed with the neighbor-joining method (Saitou and Nei, 1987) using MEGA 5.05 software (Tamura *et al*., 2011). Bootstrap support percentages were calculated from 1000 replications.

Subcellular localization assay: The open reading frame (ORF) of *FaFT1* was amplified with the primer pair: 5′-AAG CTT CAT GCC TAG GGA CAG G-3′ (*Hind*III site underlined) and 5′-GGT ACC GCG ATG ATC TTC TCC T-3′ (*Kpn*I site underlined). The PCR product was cloned into the pE3025-GFP vector to generate pE3025-FaFT1-GFP. For protoplast transient expression assay, the mesophyll protoplasts were isolated from the rosette leaves of four-week-old plants

of wild-type *Arabidopsis*. The transformation of protoplasts was performed as described by Yoo *et al*. (2007). The transformed *Arabidopsis* protoplasts were incubated for 16 h at 22°C in darkness and GFP fluorescence was observed with a NIKON ECLIPSE TE2000-E confocal laser-scanning microscope (Nikon, Tokyo, Japan).

Gene expression assay: Total RNA isolation and cDNA synthesis were performed as described above. The cDNA samples were diluted 1:10 with water, 2 μL of the diluted cDNA was used as a template for quantitative real-time PCR (qRT-PCR) reaction. The qRT-PCR analysis was performed with an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster, USA) using UltraSYBR Mixture (CWBIO, Beijing, China). Reactions were performed by an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 10 s at 95°C and 31 s at 60°C. Each sample represented three biological replicates, each of them included four technical replicates. Data was analyzed using the $2^{-\Delta\Delta CT}$ method as described by Livak and Schmittgen (2001). The primers used in this qRT-PCR were listed below: 5′-CAG ACC AGC AGA GGC TTA TCT T-3′ (forward) and 5′-TTC TGG ATA TTG TAG TCT GCT AGG G-3′ (reverse) for *FaUBI* (Mouhu *et al*., 2009); 5′-GAA GAG TCA TAG GTG ATG TTC TGG A-3′ (forward) and 5′-ACC ATT GTT GAC CTC CTT AGA AGT-3′ (reverse) for *FaFT1*. The *FaUBI* was used as the housekeeping gene.

Plant transformation: The protein-coding region of *FaFT1* cDNA was amplified using the primer pair: 5′-GGA TCC ATG CCT AGG GAC AGG-3′ (*BamH*I site underlined) and 5′-TCT AGA TTA CGA TGA TCT TCT CCT TCC-3′ (*Xba*I site underlined). The PCR product was ligated into the binary plant transformation vector pCBI302-3 under the control of the Cauliflower mosaic virus (CaMV) 35S promoter. The plasmid was transformed into *Agrobacterium tumefaciens* strain GV3101 and then transformed into wild-type *Arabidopsis* plants using the floral dip method (Clough and Bent, 1998). Transgenic seedlings were selected by spraying BASTA (Bayer CropScience, Wolfenbüttel, Germany). Two independent lines of the T_2 generation were randomly chosen for morphological analysis.

RESULTS AND DISCUSSION

Cloning and sequence analysis of *FaFT1*: The ORF of the *FaFT1* gene was 531 bp (GenBank ID: KP184716), encoding a protein of 176 amino acids with an estimated molecular mass of 19.7 kDa and an isoelectric point of 8.3. The FaFT1 protein showed 92, 92, 91, 90 and 89% identity to FTs from *Prunus mume*, *Populus tomentosa*, *Malus domestica*, *Gossypium hirsutum* and *Betula platyphylla*, respectively. The FaFT1 protein had the conserved key amino acid residue Tyr84 (Y) and Gln139 (Q) in the positions corresponding to Tyr85 and Gln140 of *Arabidopsis* FT (Fig. 1). Tyr85/His88 and Gln140/Asp144 are likely to be responsible for the differences in function between FT and TFL1 (Hanzawa *et al*., 2005; Ahn *et al*., 2006). FaFT1 also contained two highly similar sequences to Arabidopsis FT in the 14-amino acid stretch referred to as “segment B” and in the LYN triad in “segment C” (Ahn *et al*., 2006) (Fig. 1). To further evaluate the relationship between members of the *FT* homologous genes, a phylogenetic tree was constructed using their amino acid sequences. The reconstructed tree indicated that the *FT*-like gene family could be separated into dicot and monocot clades, with FaFT1 protein belonging to the dicot clade together with other FT homologs from the Rosaceae (Fig. 2), suggesting that *FaFT1* was a putative ortholog of *FT* in strawberry.

FaFT1 is targeted to the nucleus: For the subcellular localization assay, the coding region of *FaFT1* was fused to GFP to form the fusion gene *FaFT1-GFP* driven by the CaMV 35S promoter in pE3025. The fusion gene plasmid and GFP control plasmid were transformed into *Arabidopsis* mesophyll protoplasts. The fluorescence of FaFT1-GFP was observed exclusively in the nuclei of the *Arabidopsis* protoplasts, whereas, as expected, the GFP protein was detected throughout the cells (Fig. 3). Thus, FaFT1 appeared to be a nucleus-localized protein. This result is consistent with the SFT (SINGLE-FLOWER TRUSS) protein of tomato (Lifschitz *et al*., 2006), but different from MdFT2 of apple (Li *et al*., 2010), which localizes in the membrane. Our results suggested that *FaFT1* might act as a transcriptional factor in the nucleus.

Expression analysis of the *FaFT1* gene: To characterize the expression pattern of *FaFT1*, we first examined the tissue-specific expression of *FaFT1* by qRT-PCR using various tissues. *FaFT1* transcripts were almost undetectable in fruits and roots (Fig. 4), whereas its expression in mature leaves, floral buds, flowers and shoot apices significantly increased, with the highest level in mature leaves. This observation indicated that *FaFT1* might be closely associated with photoperiodic response for flowering and reproductive development in strawberry. Although FT is a mobile signal originating from leaves (Corbesier *et al*., 2007; Tamaki *et al*., 2007), many *FT* homologs are highly expressed in reproductive tissues, such as flower buds and flowers in *Jatropha curcas* (Li *et al*., 2014), flower buds in *F. vesca* (Koskela *et al*., 2012), young ovary of mature flowers in *Cymbidium goeringii* (Xiang *et al*., 2012). Actually, *FT* and its homologs play multiple roles in plant development in addition to flowering time control (Pin and Nilsson, 2012).

To detect the involvement of *FaFT1* in floral transition of strawberry, we monitored the change of *FaFT1* expression in leaves of strawberry during the floral induction. For the experiment, strawberry runner plants were grown under SD conditions for six weeks for floral induction. Interestingly, in our study, *FaFT1*

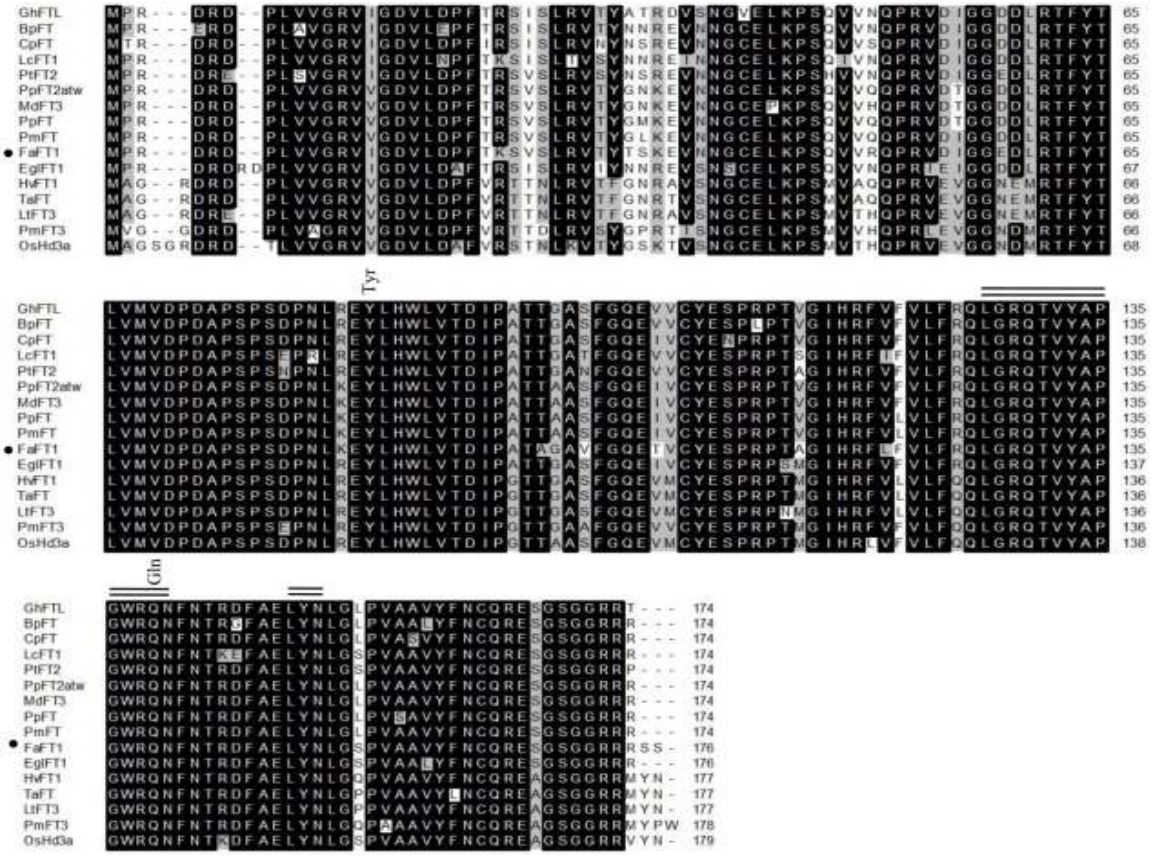

Fig. 1: Amino acid sequences alignment of *FaFT1* and its homologs from other plant species. The proteins were initially aligned using Clustal W and edited with BioEdit. Identical and similar amino acids were presented by black and gray shading, respectively. The conserved residues Tyr and Gln were indicated, the 14-amino-acid stretch (segment B) and the LYN triad (segment C) were marked with double line

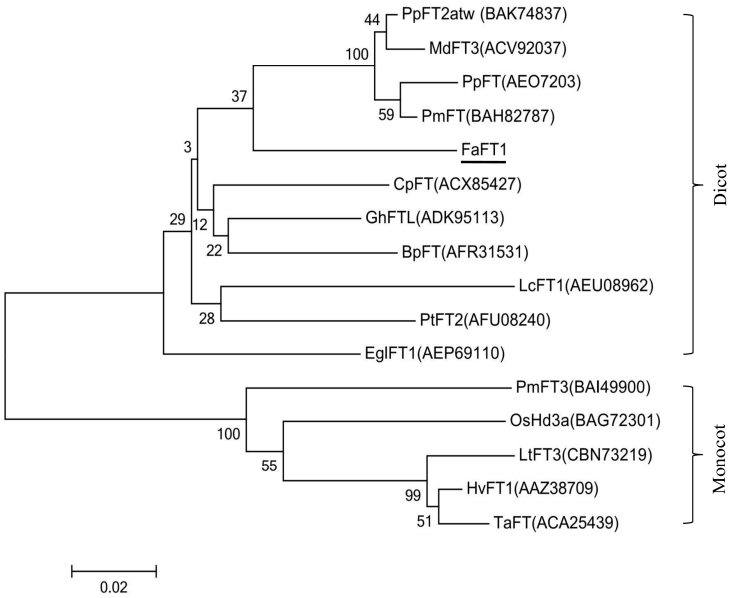

Fig. 2: Phylogenetic analysis of FaFT1 protein and its homologs from various plant species. The proteins were initially aligned using Clustal W and were used for phylogenetic analysis using MEGA version 5.05 software. The phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap replications. Bootstrap percentages were shown at dendrogram branch points

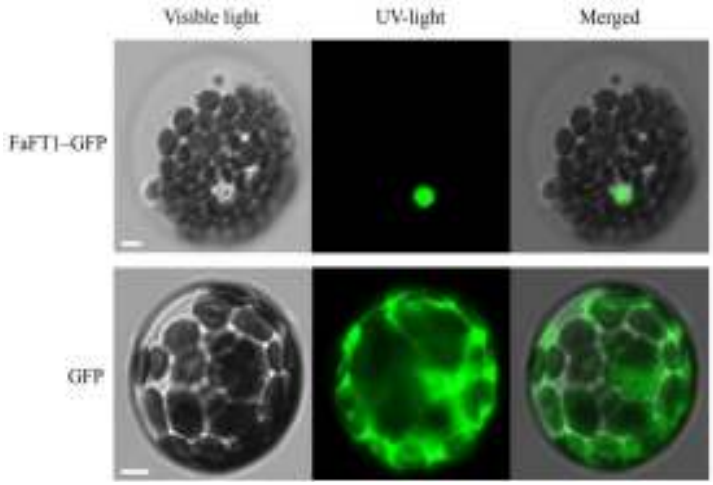

Fig. 3: Subcellular localization of FaFT1. The *Arabidopsis* protoplasts were transformed with the plasmid FaFT1-GFP or with the control GFP. The images were taken with visible light for cell morphology or UV-light for green fluorescence and then merged. Scale bars represent 5 μm

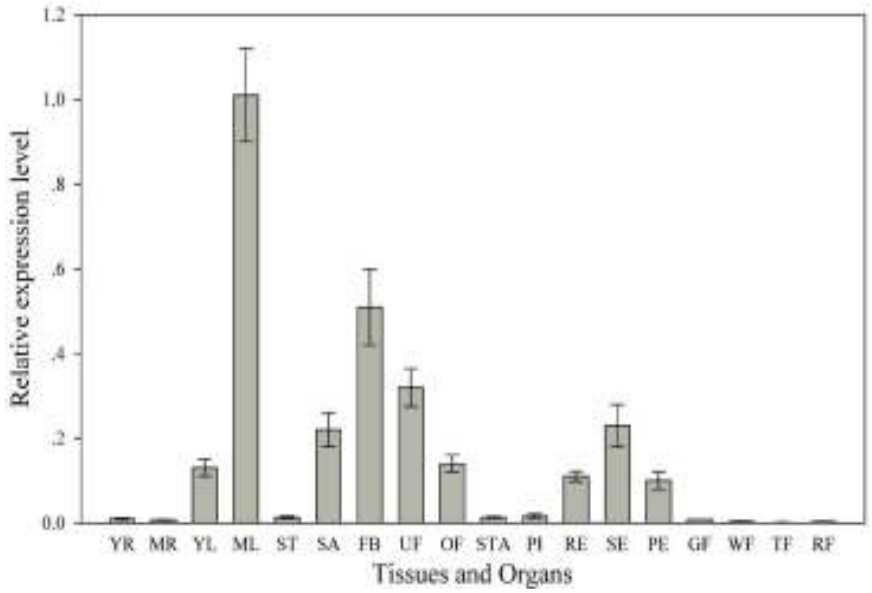

Fig. 4: Expression levels of *FaFT1* in various tissues of strawberry; YR, young root; MR, mature root; YL, young leaf; ML, mature leaf; ST, stem; SA, shoot apex; FB, floral bud; UF, unopened flower; OF, opened flower; STA, stamen; PI, pistil; RE, receptacle; SE, sepal; PE, petal; GF, green fruit; WF, white Fruit; TF, turning fruit; RF, red fruit

expression level decreased under inductive SDs (Fig. 5). This result showed that the *FaFT1* transcripts were negatively correlative with photoperiod requirement for flowering and contrasted to the situation of *GmFT2a*, *GmFT5a* and *CsFTL3* in other SD plants (Kong *et al*., 2010; Oda *et al*., 2012). Although the *FaFT1* expression pattern under SDs was similar to that of *FvFT1* in *F. vesca* (Koskela *et al*., 2012), the *FaFT1* expression decreased gradually in a mild manner during the floral induction.

The diurnal circadian rhythm of gene expression was then analyzed by qRT-PCR for *FaFT1* in leaves under SD and LD conditions. As shown in Fig. 6, *FaFT1* exhibited a diurnal circadian rhythm both under SD and LD conditions. Under LDs, *FaFT1* transcripts increased during the dark phase of the day, peaking around the middle of the dark phase. Interestingly, *FaFT1* also exhibited a trimodal expression pattern in SDs, with peak expression at 3 h before and after dawn, 1 h after dusk (Fig. 6). Although expression level was very low under SDs, *FaFT1* showed a diurnal circadian rhythm. This result was different from the *FvFT1* expression in diploid *F. ves*ca, in which the diurnal rhythm of *FvFT1* expression is present only under LDs (Koskela *et al*., 2012). Taken together, our results revealed that there might be complex regulatory mechanisms to control *FaFT1* expression in the cultivated octoploid strawberry.

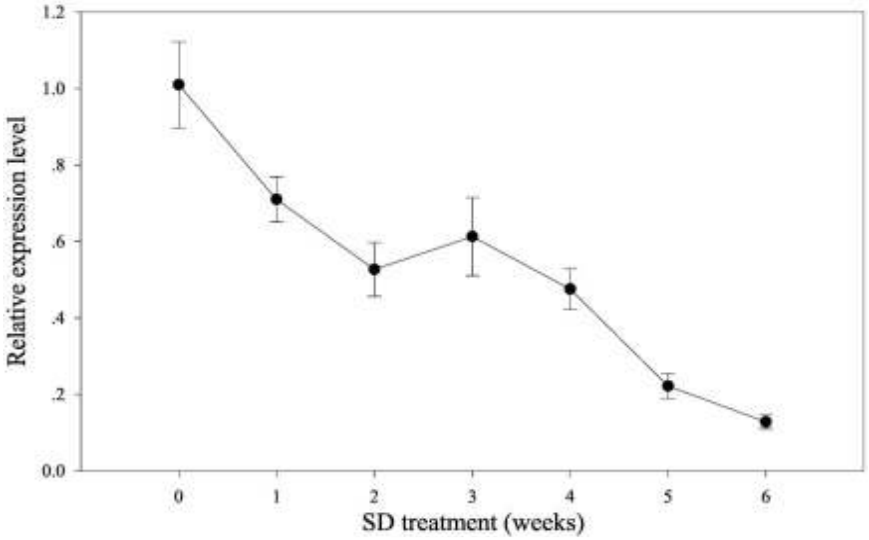

Fig. 5: Expression pattern of *FaFT1* in the shoot apex under floral induction conditions. Each column represented the mean of three replicates. Error bars on each column represented the standard error (S.E.) of three replicates

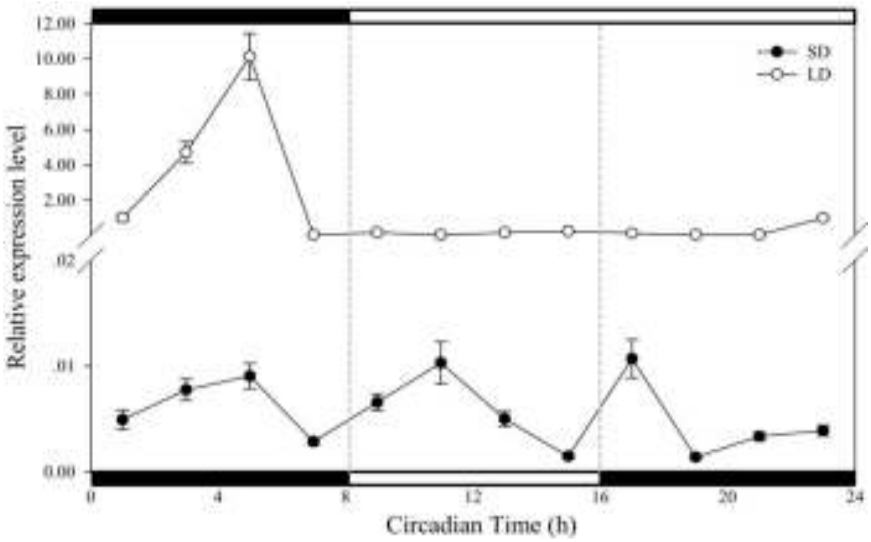

Fig. 6: Diurnal rhythm expression patterns of *FaFT1* under SD and LD conditions. White bars represent light phases and black bars represent dark phases

Fig. 7: Over expression of *FaFT1* in *Arabidopsis* caused early flowering. The wild-type and *35S::FaFT1* plants were grown in soil under LD conditions

Table 1: Number of days to flowering and the number of rosette leaves at flowering of wild-type and 35*S::FaFT1 Arabidopsis* under LD conditions

Plant line	Number of rosette leaves	Days to flowering	n
WT (col)	12.73±0.7	29.87±1.25	15
35*S*::*FaFT1*#L3	10.19±0.62	25.15±1.30	27
35*S*::*FaFT1*#L7	9.38±0.70	24±0.94	26

Over expression of *FaFT1* promotes flowering in *Arabidopsis*: To investigate the function of *FaFT1*, transgenic *Arabidopsis* plants that over expressed *FaFT1* via the CaMV 35S promoter were generated and two independent T_2 lines were selected for flowering time analysis under LD conditions. The transgenic lines flowered earlier than wild-type plants under LD conditions (Fig. 7): Transgenics started bolting with 9.38 or 10.19 rosette leaves compared to 12.73 rosette leaves for wild-type plants. As shown in Table 1, *Arabidopsis* wild-type plants required approximately 30 d to flowering, whereas the transgenic lines only required 24~25 d to flowering. In addition, the transgenic lines showed no obviously morphological changes. These results indicated that *FaFT1* acted in *Arabidopsis* as a flowering activator, promoting the transition from the vegetative phase to the reproductive phase. Further study on the floral-related genes expression are needed to elucidate the mechanism of *FaFT1* that involved in accelerating flowering of transgenic *Arabidopsis*.

CONCLUSION

In summary, a homolog of *FT*, designated as *FaFT1*, was isolated and characterized from strawberry. FaFT1 had high identity to other FT homologs and contained the highly conserved amino acid sequences LGRQTVYAPGWRQN and LYN. FaFT1 protein mainly localized in the nuclei of the *Arabidopsis* protoplasts. *FaFT1* was highly expressed in strawberry mature leaves and its expression level decreased under floral induction conditions. Additionally, *FaFT1* expression exhibited diurnal circadian rhythm both under SD and LD conditions. *FaFT1* acted in *Arabidopsis* as a flowering activator and played a conserved role in regulating floral transition.

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REFERENCES

Abe, M., Y. Kobayashi, S. Yamamoto, Y. Daimon, A. Yamaguchi, Y. Ikeda, H. Ichinoki, M. Notaguchi, K. Goto and T. Araki, 2005. FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science, 309(5737): 1052-1056.

Ahn J.H., D. Miller, V.J. Winter, M.J. Banfield, J.H. Lee, S.Y. Yoo, S.R. Henz, R.L. Brady and D. Weigel, 2006. A divergent external loop confers antagonistic activity on floral regulators FT and TFL1. EMBO J., 25(3): 605-614.

Boss, P.K., R.M. Bastow, J.S. Mylne and C. Dean, 2004. Multiple pathways in the decision to flower: Enabling, promoting and resetting. Plant Cell., 16(Suppl. 1): 18-31.

Castillejo, C. and S. Pelaz, 2008. The balance between CONSTANS and TEMPRANILLO activities determines *FT* expression to trigger flowering. Curr. Biol., 18(17): 1338-1343.

Chang, S., J. Puryear and J. Cairney, 1993. A simple and efficient method for isolating RNA from pine trees. Plant Mol. Biol. Rep., 11(2): 113-116.

Clough, S.J. and A.F. Bent, 1998. Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J., 16(6): 735-743.

Corbesier, L., C. Vincent, S. Jang, F. Fornara, Q. Fan, I. Searle, A. Giakountis, S. Farrona, L. Gissot, C. Turnbull and G. Coupland, 2007. FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. Science, 316(5827): 1030-1033.

Fornara, F., A. de Montaigu and G. Coupland, 2010. Snapshot: Control of flowering in arabidopsis. Cell, 141(13): 550-550.e2.

Hanzawa, Y., T. Money and D. Bradley, 2005. A single amino acid converts a repressor to an activator of flowering. P. Natl. Acad. Sci. USA, 102(21): 7748-7753.

Heide, O.M. and A. Sønsteby, 2007. Interactions of temperature and photoperiod in the control of flowering of latitudinal and altitudinal populations of wild strawberry (*Fragaria vesca*). Physiol. Plant., 130(2): 280-289.

Hsu, C.Y., J.P. Adams, H. Kim, K. No, C. Ma, S.H. Strauss, J. Drnevich, L. Vandervelde, J.D. Ellis, B.M. Rice, N. Wickett, L.E. Gunter, G.A. Tuskan, A.M. Brunner, G.P. Page, A. Barakat, J.E. Carlson, C.W. DePamphilis, D.S. Luthe and C. Yuceer, 2011. *FLOWERING LOCUS T* duplication coordinates reproductive and vegetative growth in perennial poplar. P. Natl. Acad. Sci. USA, 108(26): 10756-10761.

Jaeger, K.E. and P.A. Wigge, 2007. FT protein acts as a long-range signal in *Arabidopsis*. Curr. Biol., 17(12): 1050-1054.

Kim, J.J., J.H. Lee, W. Kim, H.S. Jung, P. Huijser and J.H. Ahn, 2012. The *micro RNA 156-SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3* module regulates ambient temperature-responsive flowering via *FLOWERING LOCUS T* in *Arabidopsis*. Plant Physiol., 159(1): 461-478.

Kobayashi, Y., H. Kaya, K. Goto, M. Iwabuchi and T. Araki, 1999. A pair of related genes with antagonistic roles in mediating flowering signals. Science, 286(5446): 1960-1962.

Kobayashi, Y. and D. Weigel, 2007. Move on up, it's time for change-mobile signals controlling photoperiod-dependent flowering. Genes Dev., 21(19): 2371-2384.

Kong, F., B. Liu, Z. Xia, S. Sato, B.M. Kim, S. Watanabe, T. Yamada, S. Tabata, A. Kanazawa, K. Harada and J. Abe, 2010. Two coordinately regulated homologs of *FLOWERING LOCUS T* are involved in the control of photoperiodic flowering in soybean. Plant Physiol., 154(3): 1220-1231.

Koskela, E.A., K. Mouhu, M.C. Albani, T. Kurokura, M. Rantanen, D.J. Sargent, N.H. Battey, G. Coupland, P. Elomaa and T. Hytönen, 2012. Mutation in *TERMINAL FLOWER1* reverses the photoperiodic requirement for flowering in the wild strawberry *Fragaria vesca*. Plant Physiol., 159(3): 1043-1054.

Kotoda, N., H. Hayashi, M. Suzuki, M. Igarashi, Y. Hatsuyama, S. Kidou, T. Igasaki, M. Nishiguchi, K. Yano, T. Shimizu, S. Takahashi, H. Iwanami, S. Moriya and K. Abe, 2010. Molecular characterization of *FLOWERING LOCUS T*-like genes of apple (*Malus×domestica* Borkh). Plant Cell Physiol., 51(4): 561-575.

Li, C., L. Luo, Q. Fu, L. Ni and Z. Xu, 2014. Isolation and functional characterization of JcFT: A *FLOWERING LOCUS T* (*FT*) homologous gene from the biofuel plant *Jatropha curcas*. BMC Plant Biol., 14: 125.

Li, D., C. Liu, L. Shen, Y. Wu, H. Chen, M. Robertson, C.A. Helliwell, T. Ito, E. Meyerowitz and H. Yu, 2008. A repressor complex governs the integration of flowering signals in *Arabidopsis*. Dev. Cell, 15(1): 110-120.

Li, W.M., Y. Tao, Y.X. Yao, Y.J. Hao and C.X. You, 2010. Ectopic over-expression of two apple *Flowering Locus T* homologues, *MdFT1* and *MdFT2*, reduces juvenile phase in *Arabidopsis*. Biol. Plantarum, 54(4): 639-646.

Lifschitz, E., T. Eviatar, A. Rozman, A. Shalit, A. Goldshmidt, Z. Amsellem, J.P. Alvarez and Y, Eshed, 2006. The tomato *FT* ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. P. Natl. Acad. Sci. USA, 103(16): 6398-6403.

Livak, K.J. and T.D. Schmittgen, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. Methods, 25(4): 402-408.

Mathieu, J., N. Warthmann, F. Küttner and M. Schmid, 2007. Export of FT protein from phloem companion cells is sufficient for floral induction in *Arabidopsis*. Curr. Biol., 17(12): 1055-1060.

Mathieu, J., L.J. Yant, F. Mürdter, F. Küttner and M. Schmid, 2009. Repression of flowering by the miR172 target SMZ. PLoS Biol., 7(7): e1000148.

Mouhu, K., T. Hytönen, K. Folta, M. Rantanen, L. Paulin, P. Auvinen and P. Elomaa, 2009. Identification of flowering genes in strawberry, a perennial SD plant. BMC Plant Biol., 9: 122.

Mouhu, K., T. Kurokura, E.A. Koskela, V.A. Albert, P. Elomaa and T. Hytönen, 2013. The *Fragaria vesca* homolog of SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 represses flowering and promotes vegetative growth. Plant Cell, 25(9): 3296-3310.

Oda, A., T. Narumi, T. Li, T. Kando, Y. Higuchi, K. Sumitomo, S. Fukai and T. Hisamatsu, 2012. CsFTL3: A chrysanthemum *FLOWERING LOCUS T*-like gene, is a key regulator of photoperiodic flowering in chrysanthemums. J. Exp. Bot., 63(3): 1461-1477.

Parcy, F., 2005. Flowering: A time for integration. Int. J. Dev. Biol., 49: 585-593.

Pin, P. and O. Nilsson, 2012. The multifaceted roles of *FLOWERING LOCUS T* in plant development. Plant Cell Environ., 35(10): 1742-1755.

Saitou, N. and M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4(4): 406-425.

Sawa, M. and S.A. Kay, 2011. GIGANTEA directly activates *Flowering Locus T* in *Arabidopsis thaliana*. P. Natl. Acad. Sci. USA, 108(28): 11698-11703.

Suárez-López, P., K. Wheatley, F. Robson, H. Onouchi, F. Valverde and G. Coupland, 2001. *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature, 410(6832): 1116-1120.

Tamaki, S., S. Matsuo, H.L. Wong, S. Yokoi and K. Shimamoto, 2007. Hd3a protein is a mobile flowering signal in rice. Science, 316 (5827): 1033-1036.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol. Biol. Evol., 28(10): 2731-2739.

Teper-Bamnolker, P. and A. Samach, 2005. The flowering integrator FT regulates *SEPALLATA3* and *FRUITFULL* accumulation in *Arabidopsis* leaves. Plant Cell, 17(10): 2661-2675.

Thompson, J.D., D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res., 22(22): 4673-4680.

Wenkel, S., F. Turck, K. Singer, L. Gissot, J. Le Gourrierec, A. Samach and G. Coupland, 2006. *CONSTANS* and the *CCAAT* box binding complex share a functionally important domain and interact to regulate flowering of *Arabidopsis*. Plant Cell, 18(11): 2971-2984.

Wigge, P.A., M.C. Kim, K.E. Jaeger, W. Busch, M. Schmid, J.U. Lohmann and D. Weigel, 2005. Integration of spatial and temporal information during floral induction in *Arabidopsis*. Science, 309(5737): 1056-1059.

Xiang, L., X. Li, D. Qin, F. Guo, C. Wu, L. Miao and C. Sun, 2012. Functional analysis of *FLOWERING LOCUS T* orthologs from spring orchid (*Cymbidium goeringii* Rchb. f.) that regulates the vegetative to reproductive transition. Plant Physiol. Biochem., 58: 98-105.

Yoo, S.D., Y.H. Cho and J. Sheen, 2007. *Arabidopsis* mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nat. Protoc., 2(7): 1565-1572.