



Expression Profile of *FaFT1* and Its Ectopic Expression in *Arabidopsis* Demonstrate Its Function in the Reproductive Development of *Fragaria × ananassa*

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Abstract

FT homologs are crucial for the flowering process, playing a vital role as ‘florigens’ in plants. In this study, we isolated and characterized an *FT* homolog, *FaFT1*, from cultivated strawberry. Nucleotide sequence analysis revealed a 531-bp open reading frame in *FaFT1*, encoding a putative protein with typical DPDxP and GxHR motifs belonging to the PEBP family proteins. For vegetative tissues or organs, qRT-PCR revealed that *FaFT1* was primarily expressed in leaves. Notably high expression levels were detected in flowers and fruits, including the pith and cortex of the receptacle. Analysis of potential putative *cis*-acting regulatory elements (*CREs*) in this gene promoter indicated that many of them are associated with plant hormonal responses and abiotic stress responses. Expression detection confirmed that GA₃ treatment enhanced the expression of *FaFT1*. When ectopically expressed in *Arabidopsis*, *FaFT1* could promote the flowering process under SD conditions. These results suggested the possible role of *FaFT1* in the regulation of reproductive development in cultivated strawberry.

Keywords Strawberry · *FT* homolog · Flowering · Fruit · Development

Introduction

Flowering is regulated by different cues, such as hereditary characteristics or plant hormone levels, the endogenous factors, temperature or day length, the environmental conditions. There are six defined distinct genetic pathways involved in flowering induction, including the autonomous, photoperiod, vernalization, gibberellin, thermosensory, and

age pathways (Srikanth and Schmid 2011). In *Arabidopsis*, it has been reported that more than 300 genes are implicated in flowering-time regulation (Bouché et al. 2016). Such signaling pathways responding to endogenous or environmental signals converge on key regulators, such as *FLOWERING LOCUS T (FT)*, which then activate other floral homeotic genes. FT proteins contain the phosphatidylethanolamine-binding (PEBP) domain (Pin and Nilsson 2012; Putterill and Varkonyi-Gasic 2016) and belong to the PEBP protein family. In the LD (long-day) plant *Arabidopsis*, FT is induced by photoperiod and other activators in leaves and then moves from leaves to the shoot apex through the phloem (Krzymuski et al. 2015). In the shoot apex, together with the bZIP transcriptional factor FD (*FLOWERING LOCUS D*), FT promotes the activation of other key flowering regulation genes, such as *LEAFY (LFY)* and *APETALA1 (API)*, to induce flowering (Abe et al. 2005; Pin and Nilsson 2012; Song et al. 2013).

The functions of FT-like proteins in flowering regulation are largely conserved in different photoperiodic sensitive plants. In rice, which flowers earlier in SD (short-day) conditions, the flowering process is also induced by two FT homologs, *HEADING DATE 3a (Hd3a)* and *Rice FT 1*

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(RFT1), under SD or LD conditions, respectively (Komiya et al. 2008). However, there are also other cases. In sugar beets, two FT homologs, BvFT1 and BvFT2, play antagonistic roles in flowering regulation by acting as inhibitor or activator, respectively (Pin et al. 2010). Similar results were also found in poplar (Hsu et al. 2011) and soybean (Zhai et al. 2014). Considering the importance of flowering regulation in crop production, the function of FT homologs in different economically significant crops needs to be verified separately.

Cultivated strawberry (*Fragaria* × *ananassa*) is one of the most economically important berry crops worldwide. Most cultivars of strawberry are Junebearing SD plants, which means that they are induced to flower in a decreased photoperiod and lower temperature in autumn (Stewart and Folta 2010). There are also some strawberry cultivars that flower perpetually and do not require SDs or low-temperature for flowering, known as Everbearing types (EB). Flowering habits are an important agricultural trait for most crops, and different flowering times or flower numbers lead to varied marketing times or yields in crops. Understanding the molecular regulation mechanism controlling strawberry flowering is of crucial importance for the breeding or production of this important berry crop.

The FT homolog of *Fragaria* was first reported in *Fragaria vesca*, the diploid wild strawberry ($2n=2x=14$). The expression of *FvFT1* is detected in leaf tissues exclusively under LDs (Koskela et al. 2012). Further studies found that *FvFT1* could promote the expression of *FvSOC1*, which then activates the expression of *FvTFL1*, the floral inhibitor in *F. vesca* (Rantanen et al. 2014). It seems that the expression of *FvFT1* correlates negatively with flowering in SD genotypes of *F. vesca*. However, knockdown of *FvFT1* in perpetual flowering *F. vesca* accession H4 by RNAi produced a clearly late flowering phenotype under LD conditions (Koskela et al. 2012), which implies that *FvFT1* functions as the floral promoter at least when the function of *TFL1* is absent.

Nakajima et al. (2014) first reported the *Fragaria ananassa* FT homologue *FaFT1* (AB840273) which was isolated from cultivated strawberry cv. Tochimoto. It was highly expressed in leaves under non-inductive long-day and high-temperature (LDHT) condition, but not expressed in leaves under inductive short-day and low-temperature condition (SDLD). Further study by Koembuoy et al. (2017) revealed that ectopic expression of this gene in *Arabidopsis* couldn't cause an early flowering phenotype.

Another two strawberry FT homologues, *FaFT1* (KP184716) and *FaFT2* (LC017713), were reported by Lei et al. (2015) and Nakano et al. (2015) individually in the cultivated strawberry cv. Camarosa and cv. Nyoho, respectively. Although *FaFT1* (KP184716) exhibited a diurnal circadian rhythm both under SD and LD conditions, its expression level under SD is lower. While ectopic expression of *FaFT1*

(KP184716) in *Arabidopsis* caused an early flowering phenotype in LD. *FaFT1* (LC017713) was expressed with a much higher level in the leaves under long-day condition than in SD condition. Wang et al. (2017) also reported a FT homologue of cultivated strawberry, *FaFT* (FR729042). Ectopic expression of this gene in tobacco produced a moderately late flowering time.

Considering the crucial role played by FT homologs in the flowering regulation process in plants and the inconsistent results about *FaFTs* in different reports, detailed information about the FT homolog in cultivated strawberry, the octoploid species ($2n=8x=56$) of *Fragaria* is needed.

In this work, *FaFT1* (GenBank: JQ364958), an FT homolog of cultivated strawberry cv. Huaji was isolated and characterized. Based on the promoter information analysis, its expression in response to gibberellin was further investigated. Finally, expression profile and ectopic expression in *Arabidopsis* indicated its possible role not only in flowering time but also in fruit development. Our data provide more insights about the molecular information of FT homologs in cultivated strawberry.

Materials and Methods

Plant Materials and Nucleic Acid Extraction

F. ananassa cv. Huaji, a Junebearing cultivar, was used in this study. Strawberry plants were grown under natural conditions in a solar greenhouse at Shenyang Agricultural University. The plants were planted in September and blossomed and set fruits in December to April of the next year. The temperature in the greenhouse in this period was approximately 2–10 °C at night and 10–30 °C at daytime. To keep the solar greenhouse inside warmer, the blanket was also used at night in addition to the PVC plastic sheet at the top of the solar greenhouse from November to March. The average sunshine time in this period was approximately 9 h per day, which is an SD condition for strawberry. After March, the blanket was no longer used and it turns to LD conditions after March.

Tissues and flower organs, including leaf, stolon stem, stolon bud, flower, fruit and separated flower organs, were separated individually from the plants and then immediately frozen in liquid nitrogen for RNA extraction. Flowers were collected at 3–5 days after anthesis, and young leaves were approximately 80–100 mm in length. Total RNA of the relative tissues or organs was extracted by the modified CTAB method as described (Chang et al. 2007). DNA was extracted from young leaves. All the tissues or organs used for expression detection were collected on April 20, 2019 at 9:00 AM (4 h after dawn), which is an LD condition for strawberry with approximately 13 h of sunshine per day.

Wild-type *Arabidopsis* of Columbia (*Col*) was used in the ectopic expression analysis.

Cloning of the *FaFT1* Gene

Gene-specific primers *FaFTfwd* and *FaFTrev* were designed based on the reported diploid strawberry (*F. vesca*) FT homolog *FvFT1* (JN172098). Primer information and the PCR program are listed in Table 1. Leaf cDNA and DNA were used for cDNA fragment and gDNA fragment amplification, respectively. LA Taq (TaKaRa) was used in the PCR. First-strand cDNA was synthesized using a PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa) following the instruction manual.

The amplified fragments were cloned into the pGEM-T easy vector (Promega) individually and then sequenced with the Sanger method.

Isolation of the Promoter Sequence

Compared to diploid strawberry, genomic information of octoploid strawberry is insufficient. A forward primer PFF (Table 1) was designed based on the *FvFT1* promoter detected in the diploid strawberry genome data at GDR (Genome Database for Rosaceae) website: <https://www.rosaceae.org/species/fragaria/all>. The reverse primer PFR was designed based on the *FaFT1* DNA sequence. PCR was performed using leaf DNA as the template.

Bioinformatic Analysis

The amino acid sequences were aligned with Clustal W. A phylogenetic tree was generated using the neighbor-joining method. Boot strap values were derived from 1000 replicate runs. The phylogenetic tree was constructed by MEGA 7.0 software (Kumar et al. 2016).

Cis-acting regulatory elements (*CREs*) distributed in the putative *FaFT1* promoter were analyzed by PlantCARE online software (Lescot et al. 2002): <http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>.

Expression Detection

Vegetative tissues/organs, such as leaf, stolon stem and stolon bud, and reproductive tissues/organs, such as flower, inflorescence stem and fruits, were used to detect the gene expression profile. Four whorls of flower organs were also included in the expression detection. For expression detection in fruit tissues, green fruit without seeds was cut in half and the pith and cortex were separated individually for RNA extraction (Fig. S1).

To investigate the regulatory effect of hormones on *FaFT1* expression, GA₃ solution was sprayed onto the surface of strawberry leaves. The treated plants were at flowering and were grown in the same solar greenhouse. The treatment started on April 25, 2019, which is an LD condition for strawberry. A stock solution of 100 mM GA₃ (BBI) was made in ethanol and diluted with water before application. Based on the previous reports (Thompson and Guttridge 1959; Kang et al. 2013), the final treatment concentration of GA₃ in this study was 500 μM. The GA₃ solution was sprayed every 2 days. Water was sprayed onto the mock control. Considering that frequent leaf removal of the strawberry plant may affect its vegetative/reproductive development balance, which then should affect the expression of *FaFT1* in the samples, we sprayed GA₃ or water at different times before sampling (−144 h, −96 h and −48 h). All the samples were collected at the same time, including the mock control. For the mock control, water was sprayed at each spraying time point but was only sampled on the last day. Treated leaf samples were collected at 9:00 AM, which is about 4.5 h after dawn.

Quantitative PCR (qRT-PCR) was conducted using SYBR® Premix Ex Taq™ II (TaKaRa) with the gene-specific primer sets *FaFTF* and *FaFTR* on an ABI 7500 Real-Time PCR system (Applied Biosystems). Three biological and three technical replicates were performed to calculate the relative expression level using the comparative Ct(2^{−ΔΔCt}) method after normalization to the 26S rRNA internal transcript control.

Table 1 Primers used in this study

Name	Primers sequences (5′–3′)	PCR conditions	Expected amplification sizes
<i>FaFTfwd</i>	ATGCCTAGGGACAGGGACCCC	32 cycles of 94 °C for 40 s, 56 °C for 1 min, 72 °C for 0.5 min (or 2 min)	531 bp for cDNA segment
<i>FaFTrev</i>	TTACGATGATCTTCTCCTTCC		2.1 kb for DNA segment
PFF	CGATATTTTCATTAGCGAATCGATTCCA	35 cycles of 94 °C for 30 s, 51 °C for 30 s, 72 °C for 1.5 min	~1.5 kb
PFR	CAGAACATCACCTATGACTCTTCCAC		
<i>FaFTF</i>	TCTCAGGGTGACTTACACTTCT	40 cycles of 95 °C for 15 s, 60 °C for 1 min, 72 °C for 20 s	261 bp
<i>FaFTR</i>	GTTGGCCGTGGACTTTCATA		
<i>Fa26SF</i>	TAACCGCATCAGGTCTCCAA	40 cycles of 95 °C for 15 s, 60 °C for 1 min, 72 °C for 20 s	164 bp
<i>Fa26SF</i>	CTCGAGCAGTTCTCCGACAG		

Ectopic Expression of *FaFT1* in *Arabidopsis*

The *FaFT1* overexpression construct was made by inserting a full-length cDNA clone of *FaFT1* in sense orientation into the *Nco* I and *Bgl* II sites of the plasmid pCAMBIA1304 under the control of the CaMV 35S promoter (Fig. S2). The target plasmid was then introduced into the disarmed strain of *Agrobacterium tumefaciens* GV3101.

Arabidopsis plants (*Col*) were used for transformation with floral-dip method (Clough and Bent 1998). After hygromycin selection and PCR confirmation, the transformants were transplanted into moistened potting soil consisting of vermiculite and perlite [1:1 (V:V)] after they developed 2–5 adult leaves. Both the transformants and the WT were all grown in a chamber with a short-day (SD) treatment (8 h light/16 h dark). The chamber temperature was set at 23 °C. The phenotypes of 12 plantlets in each typical independent T3 generation line were analyzed. The day of sowing was counted as day 0.

Flowers and siliques from different plants of the lines were collected for qRT-PCR detection of *FaFT1*. Leaves were collected for PCR confirmation of the *FaFT1* insertion. Primers for both qRT-PCR and PCR were *FaFTF* and *FaFTR*. The amplified *FaFT1* segment was 261 bp in length.

Results

Isolation and Characterization of *FaFT1*

Based on the sequence information of *FvFT1* in diploid strawberry, the gene-specific primers *FaFT*fw and *FaFT*rev

were used to isolate the *FT* homolog in cultivated strawberry. One cDNA fragment which is about 500 bp was amplified and then cloned into the T-vector. Sequencing results showed that it is 531 bp in length and encodes a putative protein with 176 amino acid residues. This *FT* homolog was named as *FaFT1* (GenBank: JQ364958). Sequence alignment indicated that there are 3 nucleotide changes in *FaFT1* and *FvFT1*, resulting in one amino acid difference between their coding proteins.

Alignment of *FaFT1* with other FT-like proteins showed that they share the typical D-P-D-x-P and G-x-H-R motifs (Fig. 1), which are identical to PEBP family proteins (Banfield and Brady 2000). Multiple sequence alignment also revealed that *FaFT1* contains the conserved key amino acid residue Tyr84 (Y) in the position corresponding to Tyr85 in *AtFT*, which has been reported to be the crucial position for differentiating FT from TFL1 (TERMINAL FLOWER 1) (Hanzawa et al. 2005).

Phylogenetic analysis indicated that *FaFT1* falls into a small clade with other *FaFT1*s (KP184716, AB840273 and LC017713) and the *F. vesca* *FvFT1*, distinctly separated from TFL1-like proteins (Fig. 2A). *FaFT* (FR729042), together with the other two FT homologs of *F. vesca*, *FvFT2* and *FvFT3*, falls into another small clade.

The genomic DNA sequence of *FaFT1*, *gFaFT1*, was also isolated, and sequencing results showed that it is 2104 bp in length. Alignment of *gFaFT1* with its cDNA sequence revealed that there are three introns with nucleotide lengths of 238 bp, 1233 bp and 102 bp, respectively (Fig. 2B). The number of introns is similar to that of other FT-like genes, such as *MdFT2* of apple and *PtFT* of poplar. However, the position and length of their introns differ.

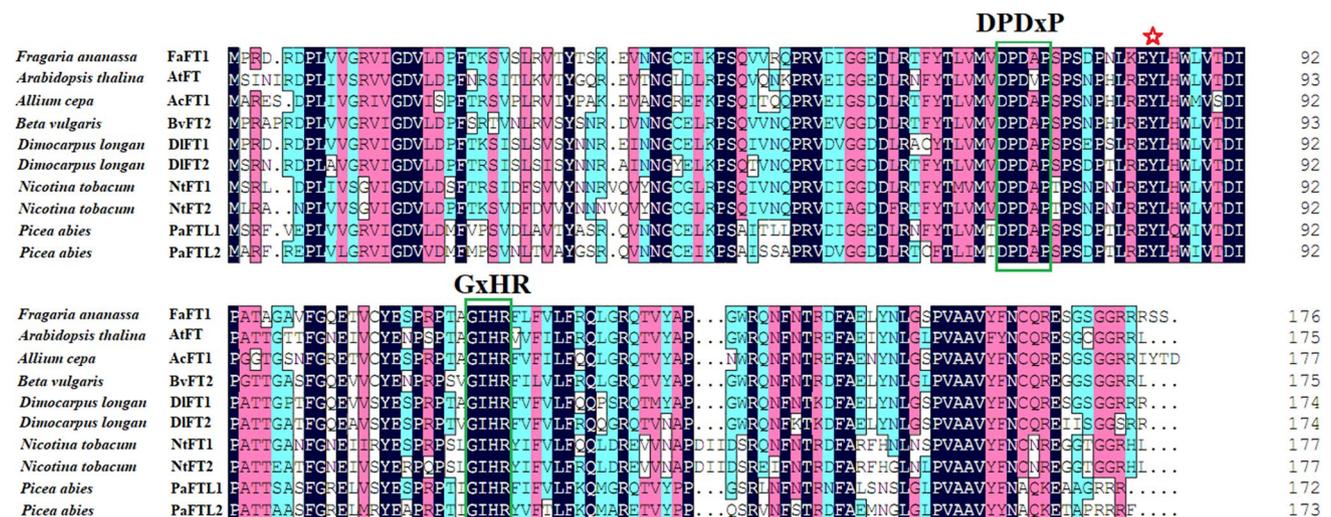


Fig. 1 Sequence alignment of FT-like proteins from different plants. The DPDxP motif and GxHR motif are shown with green boxes. The red star indicates the conserved key amino acid residue Tyr84 (Y) (Color figure online)

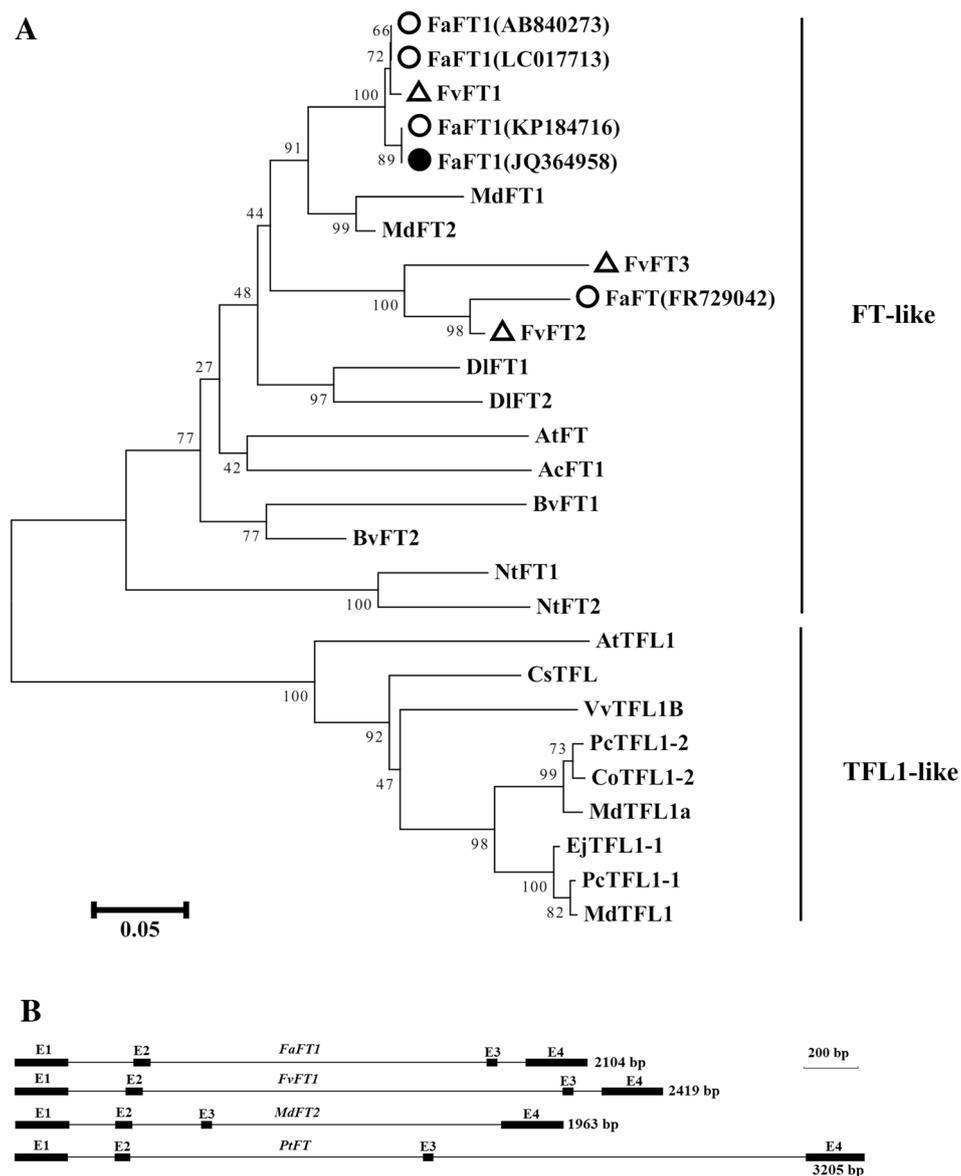


Fig. 2 **A** Phylogenetic analysis of FaFT1 with other FT/TFL1 homologous proteins. FaFT1(JQ364958) is indicated with a black circle, other FaFTs and FaFT are indicated with a white circle. Three FT homologs in *Fragaria vesca* are indicated with triangles. **B** The intron (line) distributions of several FT-like genes. Sequence length is shown at the end of each gene. Proteins used in phylogenetic tree construction are: *Fragaria ananassa* FaFT1(AB840273), BAP18900, FaFT1(LC017713), BAR63708, FaFT1(KP184716), AKO90119, FaFT1(JQ364958), AFA42331, FaFT(FR729042), CBY25183; *Fragaria vesca* FvFT1, AEP23098, FvFT2, XP_004297273, FvFT3, XP_004295610; *Arabidopsis thaliana* AtFT, NP_176726; *Malus domestica* MdFT1, NP_001280791, MdFT2,

NP_001280810; *Allium cepa* AcFT1 AGO81838; *Beta vulgaris* BvFT1 XP_010690385, BvFT2, XP_010673871; *Dimocarpus longan* DIFT1, AEZ63949, DIFT2, AEZ63950; *Nicotiana tomentosiformis* NtFT1, XP_009592530, NtFT2, XP_016459944; *Fragaria ananassa* FaTFL1, BAO03909; *Fragaria ananassa* FaTFL1-like, AFA42328, *Pyrus bretschneideri* PcTFL1-2, NP_001289244; *Eriobotrya japonica* EjTFL1-1, BAD10966; *Pyrus bretschneideri* PcTFL1-1, XP_009374732; *Malus domestica* MdTFL1a, NP_001280794, MdTFL1, NP_001280887; *Vitis vinifera* VvTFL1B, NP_001267931; *Citrus sinensis* CsTFL, NP_001275848; *Cydonia oblonga* CoTFL1-2, BAD10970; *Arabidopsis thaliana* AtTFL1, NP_196004

gFaFT1 and *gFvFT1* shared not only the same exon number but also the same length for each exon. However, the introns of these genes also differ from each other, especially for their second introns.

Cis-Acting Regulatory Element Analysis of the *FaFT1* Promoter

Based on the primer pair PFF and PFR, a DNA fragment of

1,588 bp was isolated. Compared with the complete cDNA sequence of another reported octoploid strawberry *Ft* homolog *Flowering Locus T-like 1* (LC017713), the 5'UTR of *FaFTI* is 53 bp in length and the promoter we isolated is 1,481 bp in length.

Sequence analysis showed that the *FaFTI* promoter contained several typical TATA-boxes, the core promoter elements, and CAAT-boxes, the common cis-acting elements. In addition to these fundamental elements, many plant hormone-responsive elements were detected, including two ABA-responsive elements, three GA-responsive elements and one MeJA-responsive element (Fig. 3, Table 2). We also identified two *CREs* that are associated with low-temperature in the promoter region of *FaFTI*.

In the photoperiod flowering pathway, transcription of *Ft* is normally activated by CONSTANS (CO) by binding

at the CO response element (CORE). However, in the isolated 1481-bp *FaFTI* promoter, no typical CORE motif (TGTG(N2-3)ATG) was found. While three NF-Y complex binding sites (CCAAT-box), which are also possible CO binding sites, were detected.

Expression Profile of *FaFTI* in Strawberry Tissues and Organs

Expression detection of *FaFTI* in vegetative tissue/organs showed that a higher expression level was found in leaves than in stolon stems and stolon buds (Fig. 4A). This result is consistent with the expression profile of other *Ft* homologs, such as the *AtFT* of *Arabidopsis*, whose expression is also activated in leaves.

Fig. 3 Promoter analysis of *FaFTI*. Motifs in green are *CREs* responsible for plant hormones, motifs in purple are *CREs* responsible for low-temperature, motifs in red are the potential CONSTANS binding sites (CCAAT-box) (Color figure online)

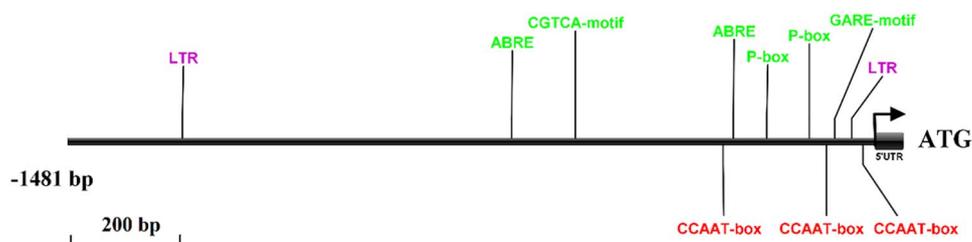


Table 2 Cis-acting regulatory elements (*CREs*) detected in the 1481 bp promoter of *FaFTI* identified by the PlantCARE database

Responsive factors	<i>CREs</i> name	Factors	Sequence pattern	Position	Strand
Hormone	ABRE	ABA	ACGTG	-666	+
			GACACGTACGT	-259	+
	CGTCA-motif	MeJA	CGTCA	-549	-
	GARE-motif	GA	TCTGTTG	-73	-
	P-box	GA	CCTTTTG	-198	-
Abiotic stress	LTR	Low-temperature		-120	-
			CCGAAA	-1270	+
				-42	+

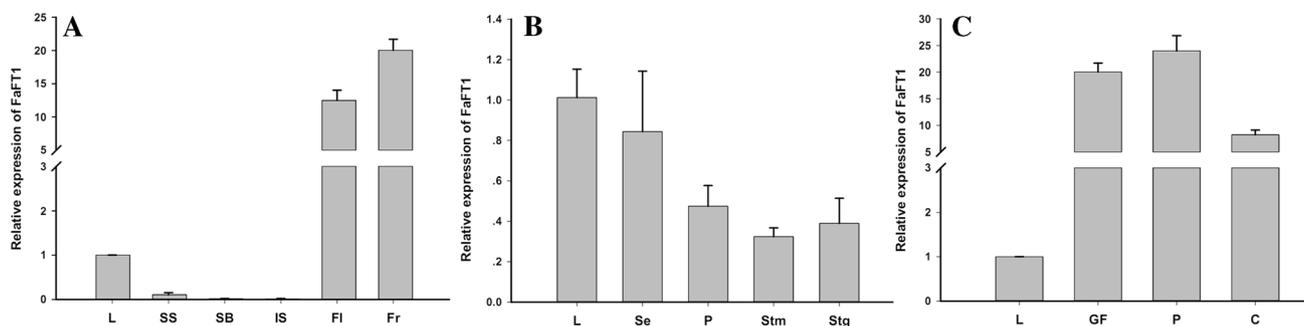


Fig. 4 Expression detection of *FaFTI*. **A** Expression in different tissues/organs. L: leaf, SS: stolon stem, SB: stolon bud, IS: inflorescence stem, FI: flower, Fr: fruit. **B** Expression in floral organs. L: leaf, Se: sepal, P: pistil, Stm: stamen, Stg: stigma. **C** Expression in fruit.

GF: green fruit, P: pith, C: cortex. The average expression level of three biological replicates is shown for each sample, all normalized to the expression level of *Fragaria × ananassa* 26S rRNA. Error bars indicate \pm SD

Interestingly, unexpectedly high expression levels were also detected in most of the detected reproductive tissues/organs. *FaFT1* showed notably high expression levels in the flowers and the fruits of strawberry, with approximately 12-fold and 20-fold higher expression levels compared with that in leaves, respectively. For this unexpected expression result, we further detected its expression in flower and fruit in detail.

For the four whorls of floral organs, *FaFT1* showed a similar transcription pattern in sepals as in leaves, while a considerably lower expression level was detected in the other three whorls of floral organs (Fig. 4B).

Meanwhile, we detected the expression of this gene in green fruit and separated fruit tissues, including the pith and cortex. The results showed that green fruit indeed showed considerably stronger *FaFT1* transcription with an approximately 20-fold higher expression level than that in the leaf. Stronger expression was also detected in pith and cortex tissues of the fruit individually. In addition, our results also indicated that much higher *FaFT1* expression level was found in the pith than in the cortex (Fig. 4C).

GA₃ Regulates the Expression of *FaFT1*

CRE analysis of the *FaFT1* promoter suggested that the expression of this gene might be regulated by plant hormones. To test this hypothesis, we also detected changes in *FaFT1* expression after treatment with GA₃.

The expression of *FaFT1* in leaves was significantly upregulated at 48 h after GA₃ spraying with approximately 1.7-fold expression change compared to the mock (Fig. 5). Furthermore, *FaFT1* expression was detected to be continuously upregulated following the repetitive spraying of GA₃. After 6 days, at which point GA₃ had been sprayed three times, the expression level of *FaFT1* in leaves was ninefold higher than that in the mock.

Phenotypic Analysis of *FaFT1* Transgenic *Arabidopsis*

The *FT*-like gene generally promotes the flowering process of transformants overexpressing it for its well-known function as ‘Florigen’. To evaluate the function of *FaFT1*, we generated transgenic *Arabidopsis* plants carrying *FaFT1* driven by the CaMV35S promoter. The T3 generation transgenic lines were used for further analysis in this study.

When grown in the noninductive SD conditions, most of the *Arabidopsis* transformants showed an early flowering phenotype compared with the wild type. Three typical independent T3 lines (T3-1, T3-2 and T3-3) were selected for further analysis. Insertion of *FaFT1* in the genomic DNA was confirmed in the selected lines by PCR using primers FaFTF and FaFTR (Table 1, Fig. S3). Although the flowering times of these lines were not consistent with each other,

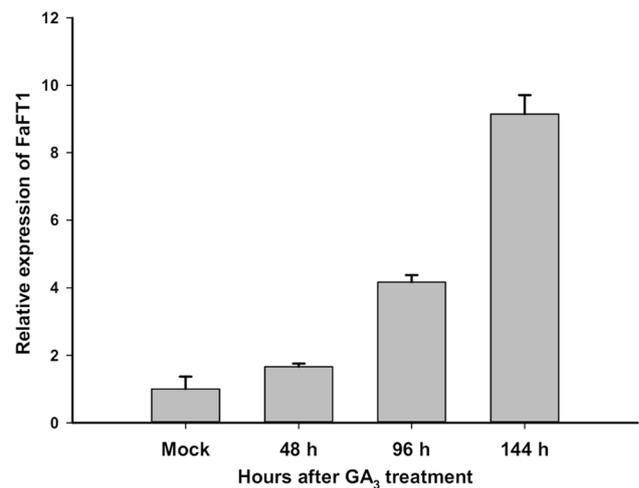


Fig. 5 Expression of *FaFT1* in *Fragaria* × *ananassa* leaves in response to GA₃ treatment. Changes in *FaFT1* expression after every 2 days of spraying with 500 μM GA₃ are shown. The average expression level of three biological replicates is shown for each time point, all normalized to the expression level of *Fragaria* × *ananassa* 26S rRNA. Error bars indicate ±SD

all of them showed early flowering compared with the WT line (Figs. 6A, 7A).

Plantlets of line T3-1 showed the most severe early flowering phenotype compared with the WT, exhibiting flowering approximately 11 days earlier than the control. At the same time, the decreased rosette leaf numbers and the reduced leaf size at flowering also indicated shortened vegetative development (Figs. 6A, 7B). The other two lines also showed early flowering and shortened vegetative development, although their phenotypes were not as severe as those in line T3-1. Even in line T3-3, the latest flowering line among the three lines, plantlets flowered 2 days earlier on average than the control.

High *FaFT1* expression levels were detected in the reproductive tissues of strawberry, such as fruit and flower. However, phenotypes of the siliques and flowers of the transformants were similar to those in WT (Fig. 6B, C), while notable *FaFT1* expression was detected in flowers (Fig. 8A) and siliques (Fig. 8B) of three lines by qRT-PCR.

Discussion

FaFT1 is a Typical *FT*-Like Homolog of Strawberry

The physiology underlying flowering in cultivated strawberry has been extensively studied for nearly a century. However, due to the complex genetic background of this berry, studies aimed at elucidating the genetic basis of the flowering character via genetic mapping have led to varying

Fig. 6 Phenotypes of *FaFT1* transgenic plants. **A** Early flowering phenotypes of three lines (T3-1, T3-2 and T3-3) in the T3 generation are shown, while WT were still in vegetative growth development. **B** Siliques of the WT (left) and the transformants (T3-1, right). **C** Flowers of the WT (left) and the transformants (T3-1, right) (Color figure online)

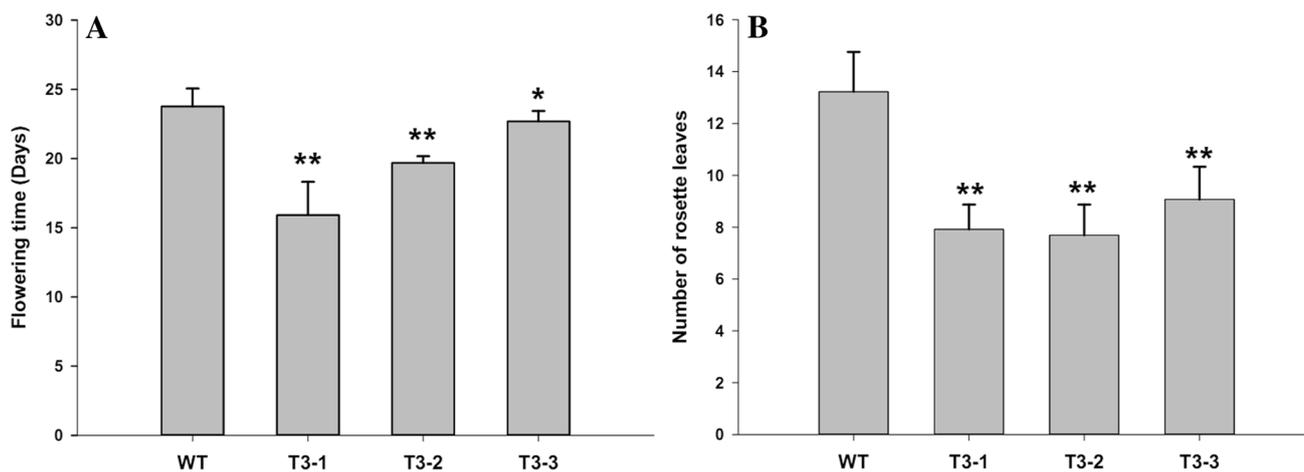


Fig. 7 Promoted flowering process and reduced vegetative growth of the transformants. **A** Average days needed for WT and transformants to be flowering. **B** Number of rosette leaves when flowering in WT

plants and transgenic lines. Error bars indicate \pm SD (n=13). Asterisks indicate significant differences between WT and transgenic lines (Student's *t*-test, * $P < 0.05$, ** $P < 0.01$)

results (Ahmadi et al. 1990; Weebadde et al. 2008). The closely related diploid woodland strawberry *F. vesca* has been widely used in functional genetic studies to elucidate the molecular pathways controlling flowering in *Fragaria*. Considerable progress has been made on the strawberry *TERMINAL FLOWER1* gene (*TFL1*) (Koskela et al. 2016,

2017; Iwata et al. 2012; Rantanen et al. 2015), the competitor of FT in binding with FD (Hanano and Goto 2011).

However, the role of the *FT* homologue in flowering regulation of strawberry was not well elucidated as the *TFL1* homolog. However, in *F. vesca*, *FvFT1* was found to be expressed specifically under LDs and negatively correlated

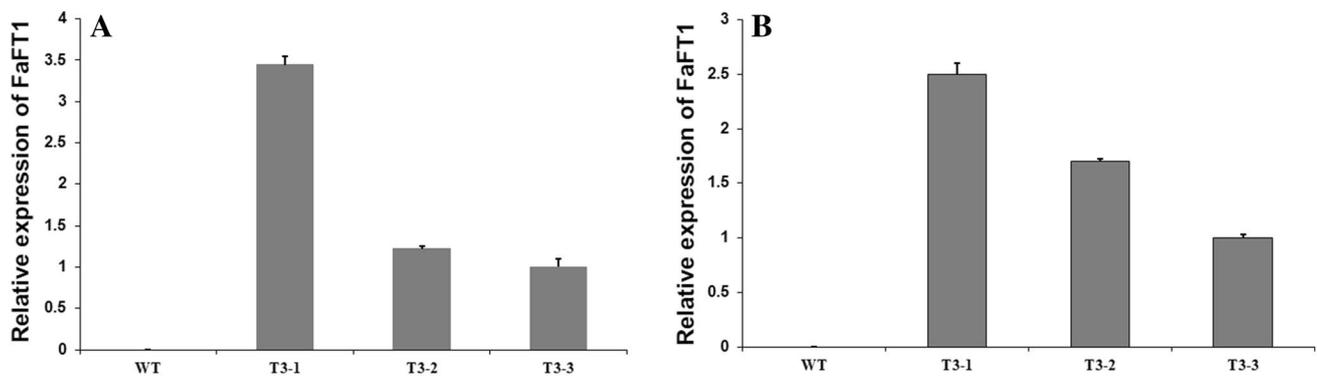


Fig. 8 Expression detection of *FaFT1* in *Arabidopsis* flowers **A** and siliques **B**. The average expression level of three biological replicates is shown for each sample, all normalized to the expression level of *Fragaria × ananassa* 26S rRNA. Error bars indicate \pm SD

with the flower induction in SD genotypes of *F. vesca* (Koskela et al. 2012). A similar result was also found in the octoploid Junebearing strawberry (Nakano et al. 2015). Moreover, Koskela et al. (2012) reported that the *FvFT1* RNAi lines, which were produced in an everbearing H4 background, grown in LD conditions showed a clearly late flowering phenotype. These results indicated that the role of *FT* homologs in *Fragaria* is more complex than that of the relevant genes in *Arabidopsis* or other plants. Characterization of *FT* homologs in cultivated strawberry is strongly warranted.

In this study, we isolated and characterized an *FT*-like gene, *FaFT1*, from cultivated strawberry. Sequence analysis showed that *FaFT1* is a typical *FT*-like PEBP family member. In addition to the conserved key amino acid residue Tyr84 (Y), several other important amino acid residues that have been reported to be responsible for the *FT*-like function are also detected, such as Tyr133(Y) and Trp137(W) (Klintenäs et al. 2012; Wickland and Hanzawa 2015). The genomic sequence of *FaFT1* consisted of four exons, which resembled the genomic structure of other *FT* genes. Such information about the *FaFT1* sequence suggested its potential role in flowering regulation.

Several plant hormone-responsive and low-temperature responsive elements were detected in the promoter region of *FaFT1*, suggesting that *FaFT1* expression may be affected by phytohormones and temperature. Further study (Fig. 5) confirmed that GA_3 treatment significantly upregulated the expression of *FaFT1*. A previous study reported that treatment with bioactive GAs inhibited the flowering process of strawberry, while runner production was enhanced (Thompson and Guttridge 1959; Tafazoli and Vince-Prue 1978; Choma and Himelrick 1984). Further work is needed to clarify the possible role of *FaFT1* in the GA regulation of flowering or runner formation in strawberry.

The CO-*FT* module plays central, crucial roles in the photoperiodic flowering pathway (Turck et al. 2008). Although

the typical CORE motif was not found in the identified *FaFT1* promoter, several CCAAT boxes (NF-Y complex binding sites) were detected, indicating that the expression of *FaFT1* may also be regulated by the *CONSTANS* homolog of strawberry. In *F. vesca*, it was reported that *FvCO* up-regulates *FvFT1* in light (Kurokura et al. 2017).

Expression Profile of *FaFT1* Implies Its Function in Reproductive Development

Normally, florigen is synthesized in leaves (Turck et al. 2008). For the vegetative organs, *FaFT1* showed a higher expression level in leaves than in others, such as stolon stems or stolon buds. Interestingly, compared with the expression in vegetative organs, its expression in the reproductive organs was found to be considerably higher (Fig. 4A). There are also some other similar reports indicating that *FT*-like genes are abundantly expressed in reproductive organs, such as flowers and pods in soybean (Sun et al. 2011), flowers and berries in grape (Sreekantan and Thomas 2006), and fruit skin and fruit pulp in banana (Chaurasia et al. 2017). Such findings suggest that *FaFT1* may also play roles in reproductive development processes other than flowering.

Although the expression of *FaFT1* is considerably higher in an integrated flower, its expression in each of the four whorls of flower organs individually is not higher than in the leaf (Fig. 4B). As a kind of accessory fruit, in addition to the four whorls of flower organs, the receptacle is also an integral part of the flower. The existence of the receptacle may be the reason that *FaFT1* expression in an integrated flower is considerably higher than that in the leaf.

We further detected the expression of *FaFT1* in the fruit, which developed from the receptacle. Gene expression results (Fig. 4C) confirmed our hypothesis, and its expression is considerably higher in the green fruit than in the leaf. Notably higher expression levels were also detected in the pith and cortex. For the receptacle tissue, the expression of

FaFT1 is considerably higher in the pith than in the cortex. Considering the vital role of the receptacle in the development of strawberry fruit, this expression profile of *FaFT1* implies its possible function in the regulation of strawberry fruit development.

FaFT1* Activates the Flowering Process of *Arabidopsis

There are some different reports about the function of the *Fragaria FT* homolog in the regulation of the flowering process. In *F. vesca*, downregulation of *FvFT1* in Hawaii-4 by RNAi resulted in a clearly late flowering phenotype, which indicated that *FvFT1* functions as a flowering activator in *F. vesca* LD accessions (Koskela et al. 2012). In this study, when ectopically expressed in *Arabidopsis*, *FaFT1* could promote the *Arabidopsis* flowering process under noninductive SD conditions, indicating that it could promote the flowering process in *Arabidopsis*.

While when *FaFT* (FR729042), another reported *FT* homolog of cultivated strawberry, ectopically expressed in tobacco, the transformants showed a moderately late flowering phenotype (Wang et al. 2017). Koembuoy et al. (2017) also reported that ectopic expression of *FaFT1* (AB840273) in *Arabidopsis* only caused the aerial rosette phenotype under SD, and they deduced that *FaFT1* (AB840273) acts at the vegetative propagation stage.

For those confusing results, we further checked the deduced protein sequence similarity coded by five reported *FaFTs* (JQ364958, AB840273, LC017713 and FR729042). The alignment results (Fig S4) showed that protein sequence of the *FaFT1* (JQ364958) we isolated is same as the *FaFT1* (KP184716) reported by Lei et al (2015), while *FaFT1* (AB840273) and *FaFT1* (LC017713) are the same one. Early flowering phenotype of the transgenic *Arabidopsis* in both our finding and Lei et al. (2015) indicate that *FaFT1* (JQ364958/KP184716) could function as a flowering activator when ectopically expressed in *Arabidopsis*.

FaFT1 (JQ364958/KP184716) and *FaFT1* (AB840273/LC017713) showed a high similarity with only two amino acid variations. In the study reported by Koembuoy et al. (2017), the role of *FaFT1* (AB840273) in flowering induction was evaluated by counting *Arabidopsis* leaf numbers at the flowering stage, not only the rosette leaf number but also the cauline leaf number. The flowering time data of transgenic *Arabidopsis* was not shown. Different evaluation methods may result in the different conclusions deduced by our study and that study.

While sequence of *FaFT* (FR729042) owns much more variation compared with the other two *FaFT1* sequences. Considering the late flowering phenotype in tobacco caused by ectopic expression of this gene, *FaFT* (FR729042) may function as a flowering inhibitor, just as the different roles of *BvFT1* and *BvFT2* in sugar beets (Pin et al. 2010).

However, further work should be performed to clarify the detailed function of *FaFTs* in strawberry. The expression of *FaFT1* (AB840273) was induced in strawberry grown under noninductive LD and high-temperature conditions (Nakajima et al. 2014). Thompson and Guttridge (1959) reported that GA treatment on strawberry could suppress flower initiation and activate runner initiation, which normally occurs in strawberry plants grown under LD conditions. In this study, we found that GA₃ treatment could activate the expression of *FaFT1* (Fig. 5). All these findings imply the function of *FaFT1* not as a flowering inducer in strawberry, at least in SD genotypes of strawberry. However, when ectopically expressed in *Arabidopsis*, *FaFT1* promoted the flowering process of *Arabidopsis*, even in the non-inductive photoperiod. Such different results regarding the function of *FaFT1* obtained from its expression character and its ectopic expression in *Arabidopsis* may derive from the varied flowering regulation mechanisms in strawberry and *Arabidopsis*. In the meantime, the extremely high expression level in strawberry fruit tissue (Fig. 4) implies that it should be involved in strawberry fruit development. However, *Arabidopsis* owns the silique fruit, which is different from the berry fruit of strawberry that developed from the expanded receptacle. The possible role of *FaFT1* in strawberry fruit development cannot be verified by this work for the different fruit development mechanisms between *Arabidopsis* and strawberry. Further analysis of the possible function of *FaFT1* in flowering and fruit development by overexpression or knockout in strawberry is required.

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Author Contributions YL designed the research. WC, HL, DZ, YY, CL and AY performed the experiments. YL, WC, HL and ZZ wrote the manuscript.

Declarations

Conflict of interest The authors declare no conflict of interest.

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