Promoting flowering, lateral shoot outgrowth, leaf development, and flower abscission in tobacco plants overexpressing cotton FLOWERING LOCUS T (FT)-like gene GhFT1

Chao Li¹, Yannan Zhang¹, Kun Zhang¹, Danli Guo¹, Baiming Cui¹, Xiyin Wang²* and Xianzhong Huang¹, ²*

¹ Plant Genomics Laboratory, College of Life Sciences, Shihezi University, Shihezi, China, ² Plant Genome Mapping Laboratory, University of Georgia, Athens, GA, USA

FLOWERING LOCUS T (FT) encodes a mobile signal protein, recognized as major component of florigen, which has a central position in regulating flowering, and also plays important roles in various physiological aspects. A mode is recently emerging for the balance of indeterminate and determinate growth, which is controlled by the ratio of FT-like and TERMINAL FLOWER 1 (TFL1)-like gene activities, and has a strong influence on the floral transition and plant architecture. Orthologs of GhFT1 was previously isolated and characterized from Gossypium hirsutum. We demonstrated that ectopic overexpression of GhFT1 in tobacco, other than promoting flowering, promoted lateral shoot outgrowth at the base, induced more axillary bud at the axillae of rosette leaves, altered leaf morphology, increased chlorophyll content, had higher rate of photosynthesis and caused flowers abscission. Analysis of gene expression suggested that flower identity genes were significantly upregulated in transgenic plants. Further analysis of tobacco FT paralogs indicated that NtFT4, acting as flower inducer, was upregulated, whereas NtFT2 and NtFT3 as flower inhibitors were upregulated in transgenic plants under long-day conditions, but downregulated under short-day conditions. Our data suggests that sufficient level of transgenic cotton FT might disturb the balance of the endogenous tobacco FT paralogs of inducers and repressors and resulted in altered phenotype in transgenic tobacco, emphasizing the expanding roles of FT in regulating shoot architecture by advancing determine growth. Manipulating the ratio for indeterminate and determinate growth factors throughout FT-like and TFL1-like gene activity holds promise to improve plant architecture and enhance crop yield.

Keywords: florigen, FLOWERING LOCUS T (FT), floral transition, lateral shoot, leaf morphology, abscission, tobacco

Introduction

Plants sense multiple environmental cues and endogenous signals to determine the appropriate timing of flowering, which is an orchestrated process through the integration of multiple environmental cues and endogenous signals. Genetic and molecular analyses of flowering-time mutants in Arabidopsis have established the current model,
in which five major pathways mainly control the transition from the vegetative to reproductive phase. The photoperiodic and vernalization pathways are responsive to the appropriate environmental conditions, whereas the autonomous, gibberellin, and age pathways reflect the internal status of plants (Srikanth and Schmid, 2011; Yamaguchi and Abe, 2012), which all converge on the ‘hubs’ known as the integrator genes. Among them, FLOWERING LOCUS T (FT) and its paralog TWIN SISTER OF FT (TSF), encodes a ~20 kDa globular proteins of the phosphatidylethanolamine-binding protein (PEBP) family, which has a central position in mediating the onset of flowering (Kardailsky et al., 1999; Kobayashi et al., 1999; Yamaguchi and Abe, 2012; Hiraoka et al., 2013). FT as well as TSF proteins including tomato SINGLE FLOWER TRUSS (SFT) and rice HEADING DATE 3a (Hd3a; Li et al., 2006; Corbesier et al., 2007; Mathieu et al., 2007; Tamaki et al., 2007; Notaguchi et al., 2008), nicknamed florigen, were produced in the phloem companion cells. They are subsequently transported to the shoot apical meristem (SAM), where they form a complex involving a bZIP transcription factor FLOWERING LOCUS D (FD) to activate the expression of floral meristem identity genes, including SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1), APETALA1 (API), and LEAFY (LFY); Abe et al., 2005; Wigge et al., 2005; Yoo et al., 2005; Kaufmann et al., 2010), which are important regulatory hubs in the control of flowering time.

Phylogenetic studies of PEBP-like genes in angiosperms revealed that they fall into three subfamilies: the FT-like, the TERMINAL FLOWER (TFL1)-like and the MOTHER OF FT AND TFL1 (MFT)-like (Chardon and Damerval, 2005; Hedman et al., 2009). FT-like and TFL1-like genes modulate flowering transition and inflorescence architecture (Kobayashi et al., 1999; Hanzawa et al., 2005; Ahn et al., 2006), but their functions in flowering control are opposite. FT promotes the transition to reproductive development and flowering, whereas TFL1 represses this transition.

Numerous studies have concluded, FT orthologs possessing floral inductive function in woody perennials (Hisada et al., 1997; Endo et al., 2005; Böhlenius et al., 2006; Hsu et al., 2006; Carmona et al., 2007; Kotoda et al., 2010; Song et al., 2013); grasses (Yan et al., 2006; Tamaki et al., 2007; Kikuchi et al., 2009; Meng et al., 2011; Wu et al., 2013; Coelho et al., 2014); legumes (Kong et al., 2010; Ono et al., 2010; Hecht et al., 2011; Laurie et al., 2011); ornamental (Hayama et al., 2007; Hou and Yang, 2009; Imamura et al., 2011), CsFTL3 from chrysanthemum (Chrysanthemum cinsicus; Oda et al., 2012; Xiang et al., 2012; Li et al., 2013); and others such as BvFT2 from sugar beet (Beta vulgaris; Pin et al., 2010), NtFT4 from tobacco (Nicotiana tabacum; Harig et al., 2012), SISPD3 form potato (Solanum tuberosum; Navarro et al., 2011), AcFT2 from onion (Allium cepa; Lee et al., 2013), PaFT from avocado (Persea americana; Ziv et al., 2014), LsFT from lettuce (Lactuca sativa; Fukuda et al., 2011; Above information was listed in Supplementary Table S1). Previously study suggests a conserved ancestral function of FT-like proteins in transmitting inductive signals in plants. However, recent studies showed that FT-like genes in numerous species play important roles in various physiological aspects other than flowering (Pin and Nilsson, 2012). In Arabidopsis, FT and TSF regulates stomatal guard cells opening by activating H+ -ATPase (Kinoshita et al., 2011), meristem maintenance in cooperation with SHOOT MERISTEMLESS (STM) and FD during inflorescence development (Smith et al., 2011), and prevention of indeterminate growth, floral reversion and aerial rosette (Melzer et al., 2008). FT and TSF modulate lateral shoot outgrowth in Arabidopsis, and link the floral transition and lateral shoot development to maximize the reproductive success of a plant (Hiraoka et al., 2013). FT has also been demonstrated to be involved in multiple steps of axillary bud development, likely to coordinate axillary shoot development with flowering (Niwa et al., 2013). Ectopic overexpression of FT in cotton through virus-induced flowering uncouples flowering from photoperiodic regulation and promotes determinate growth habit in all aerial organs (McGarry and Ayre, 2012). In tomato, SINGLE FLOWER TRUSS (SFT) regulates reiterative growth and termination of shoots, influences leaf maturation, compound leaf architecture, stem growth, and abscission zone formation (Shalit et al., 2009). Florigen is thus established as a plant protein functioning as a general growth hormone. Also, allelic variation at the SFT locus is implicated in heterosis of yield (Krieger et al., 2010), suggesting a single overdominant gene may improve productivity in other agricultural organisms, which supports the overdominance model for heterosis. PtFT1 controls short-day (SD) induced growth cessation and bud set in autumn (Böhlenius et al., 2006). Some members of FT-like gene family modulate growth of underground storage organs. StSP6A functions as a mobile ‘tuberigen’ that induces the photoperiod-sensitive process of tuberization in potato (Navarro et al., 2011), and AcFT1 and AcFT4 play role in bulb formation in onion (Lee et al., 2013).

The Gossypium (Cotton) is one of the most important cash crops worldwide, having a large impact on our economy and everyday life. Gossypium species are naturally a photoperiodic that does not flower until the shorter days of late summer or fall. Domestication of the two allotetraploid that comprise the majority of world-wide cultivations, Gossypium hirsutum and G. barbadens gradually lose their photoperiod sensitivity (McGarry and Ayre, 2012). Cotton originated from a tropical region, and its growth is very sensitive to low temperature and soil conditions in temperate cultivation regions. Flowering earliness is an important objective in most cotton breeding programs. However, the molecular mechanisms regulating the transition from vegetative to reproductive growth in cotton are less well characterized than in other plant species, mostly due to the complexity of cotton genome and scarcity of cotton flowering time mutants. In previous study, we isolated and characterized an FT-like gene GhFT1 from G. hirsutum, and we investigated its temporal and spatial expression profile during cotton multiple develop stages (Guo et al., 2015). Overexpression of GhFT1 in Arabidopsis obviously generated early flowering phenotypes in both LD and SD conditions, showing that GhFT1 is a putative FT ortholog in G. hirsutum that regulates floral transition, similar to Arabidopsis (Guo et al., 2015). In this study, we further dissected its roles by ectopic expression of GhFT1 in wild-type (WT) tobacco. As expected, GhFT1 obviously promotes the floral transition in transgenic tobacco plants by producing
terminal flower. However, boosting flowering is just one of the pleiotropic functions of GhFT1. In addition to precocious flowering, we observed that tobacco plants carrying 35S::GhFT1 had more lateral shoots outgrowth at the base, axillary buds at rosette axil, altering leaves morphology and causing flower abscission. Our data suggests that sufficient level of transgenic cotton FT homolog might disturb the balance of endogenous FT-like proteins and disorder the ratio of inducer and repressors, resulting in inflorescence and plant architecture change.

Materials and Methods

Plant Materials and Growth Conditions

The seeds of N. tabacum cv. NC89 and N. benthamiana preserved in our lab were surface-sterilized for 20 min with 2.8% sodium hypochlorite solution containing 0.1% surfactant (Triton X-100, Sigma-Aldrich, Munich, Germany), and rinsed several times with sterile water. Then seeds were stratified for 3 days at 4°C in darkness and then plated on the Petri dishes with half-strength Murashige and Skoog (MS) medium containing MS salt (pH 5.7; Duchefa, Haarlem, the Netherlands) mixture, 1% (w/v) sucrose and 0.8% (w/v) agar. Petri dishes were then placed in light growth incubator at 28°C for 15 days under SD conditions (8 h light/16 h dark). The aseptic seedlings of N. tabacum for transformation were then transferred into a sterile flask containing half-strength MS medium at 28°C for another 30 days. The N. benthamiana seedlings for transient expression assay were transplanted into soil after germination and grown in phytotron under long-day (LD) conditions (16 h light/8 h dark), and the light intensity for tobacco growth is 200 μmol m⁻² s⁻¹.

Constructions of Overexpression Vectors

35S::GhFT1, 35S::GFP, and 35S::GhFT1-GFP constructs were the same vectors that were used in Guo's study (Guo et al., 2015). We first replaced the GUS fragment in the binary vector pCAMBIA1301 (CAMBIA, Canberra, ACT, Australia) by 525 bp of GhFT1 encoding sequence (digested with Nco I and Bst EII restriction, respectively) to construct pCAMBIA1301-GhFT1. The 5.7 kb upstream sequence of Arabidopsis thaliana FT was amplified by polymerase chain reaction (PCR) using pDONR207-8.1kbAtFTpro plasmid as template and next cloned as an internal control. At least three replicate assays were performed with independently isolated RNA for all experiments. Each RT reaction was loaded in triplicate for qRT-PCR analysis. qRT-PCR data were analyzed using the PCR analysis program 7500 software v2.0.6 (Life Technologies, Foster City, CA, USA).

Tobacco Transformation

Tobacco plants (N. tabacum cv. NC89) were transformed with 35S::GhFT1, 35S::GhFT1-GFP, and 5.7kbAtFTpro::GhFT1 vectors to construct the 5.7kbAtFTpro::GhFT1 plasmid. 35S::GhFT1, 35S::GhFT1-GFP and 5.7kbAtFTpro::GhFT1 were all transfected into Agrobacterium tumefaciens GV3101(pMP90RK) by electroporation.

Subcellular Location Analysis

For analysis of the subcellular localization of GhFT1 protein using GFP reporter gene, the 35S::GhFT1-GFP construction (Guo et al., 2015) was transformed into N. tabacum cv. NC89 plants stably by A. tumefaciens strain GV3101(pMP90RK). Hypocotyl of selected 35S::GhFT1-GFP homozygous transformants were used to detect GFP fluorescent by confocal laser scanning microscopy (CLSM; Zeiss, LSM510, Jena, Germany). The transient expression assays in tobacco were performed according to the method described by Voinnet et al. (2003). The A. tumefaciens strain GV3101 (pMP90RK) carrying 35S::GhFT1-GFP was grown at 28°C in LB medium with kanamycin and rifampicin to OD₆₀₀ = 0.5–0.6. The agrobacteria cells were centrifuged and re-suspended in 10 mmol L⁻¹ MgCl₂, 10 mmol L⁻¹ MES-KOH (pH 5.7) and 150 μmol L⁻¹ acetosyringone to OD₆₀₀ = 0.5. The agrobacteria cells were left to standing for 3 h at room temperature and then infiltrated into the abaxial side of leaves of 4-weeks-old N. benthamiana plants. After 3–5 days the infiltrated leaves were selected to detect GFP fluorescent by CLSM.

Gene Expression Analysis

Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The cdNA synthesis reactions were performed using the Superscript® First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions with 1 μg of total RNA per reaction used as template. qRT-PCR was carried out using Applied Biosystems 7500 Fast Real-Time PCR System and Fast SYBR® Green Master Mix (Life Technologies, Foster City, CA, USA) to detect the expression of GhFT1 and endogenous genes in transgenic tobacco lines and WT plants. Primers information used in this research are listed in Supplementary Table S5. NtActin-F and NtActin-R were used to amplify the NtActin gene (GenBank accession no. U60495), which was used as an internal control. At least three replicate assays were performed with independently isolated RNA for all experiments. Each RT reaction was loaded in triplicate for qRT-PCR analysis. qRT-PCR data were analyzed using the PCR analysis program 7500 software v2.0.6 (Life Technologies, Foster City, CA, USA).

Semi-quantitative RT-PCR was performed as described by Xu et al. (2013). Gene-specific primers GhFT1-F2 and GhFT1-R2 were used to analyze the expression of GhFT1 in 35S::GhFT1-GFP transgenic tobaccos, and NtActin was used as an internal control. Amplification was performed for 28 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. PCR products were subsequently separated on a 1.2% (w/v) agarose gel, then stained with ethidium bromide and photographed under UV light.

Chlorophyll Content Determination

Determination of chlorophyll content in transgenic plants grown under LD and SD conditions were estimated according to the method described by Xu et al. (2013). 0.1 g plant tissue was...
Overexpression of GhFT1 in tobacco

homogenized in 80% acetone and incubated in dark for 6 h. The homogenate was centrifuged at 10,000 rpm for 10 min. Supernatant obtained was read at 649, 665 nm in Spectra Max plus-384 (Molecular device, USA).

**Leaf Mass Per Area (LMA) Measurements**

Leaf area was measured using paper-cutting method described by Hattersley (1984) with some modification. We digitally photographed leaves of different transgenic tobacco lines and WT plants. Photos of different lines were printed randomly with A4 paper (No. 3954, Deli, Ningbo, China). Then the printed photos were cut off carefully. Weight of leaves (W1) and their corresponding cut-off papers (W2) were weighed. Then LMA was calculated using the following formula:

\[
\text{LMA} = \frac{7 \times W_1}{1000 \times W_2} \text{ cm}^{-2}
\]

At least three replicate assays were performed independently in this experiment.

**Photosynthetic Rate Curve**

The photosynthetic rate of tobacco plants under LD and SD conditions were measured by LI-6400 (LI-COR Inc., Lincoln, NE, USA) with auto-measure program. The CO₂ concentrations in sample phytotron were controlled at 400 µmol CO₂ mol⁻¹. And different red-blue light intensity 2000, 1800, 1500, 1200, 1000, 800, 500, 300, 200, 100, 50, and 0 µmol m⁻² s⁻¹ were applied to measure the net CO₂ uptake rate. Light curve data were analyzed using the built-in program in LI-6400 system.

**Results**

**Cytoplasm and Nucleus Location of GhFT1 Protein**

We previously confirmed that GhFT1 located in the cytoplasm and nucleus by detecting the fused green fluorescence protein (GFP) in the *Arabidopsis* root cells carrying 35S::GhFT1-GFP (Guo et al., 2015). In this study, we initially generated up to 12 independent 35S::GhFT1-GFP transgenic tobacco plants by transformation with *A. tumefaciens*. We next observed the green fluorescence using CLSM. As expected, root tip cells expressing GhFT1-GFP fusion protein revealed strong fluorescence in the nucleus, and GFP signal was also obvious in the cell membrane (Supplementary Figure S2), which was similar to that of the 35S::GhFT1-GFP transgenic *Arabidopsis* seedling (Guo et al., 2015). To exclude the possibility of cell wall association of GhFT1-GFP, we performed the plasmolysis assay by sucrose treatment. However, we were not able to observe an ideal picture of plasmolysis due to the very thick cell wall of tobacco root tips. Furthermore, the resulting construct of 35S::GhFT1-GFP (Supplementary Figure S1A) was transformed transiently into *N. benthamiana*. We next monitored the subcellular location of the GhFT1-GFP fusion protein by CLSM in the leaf epidermal cells of *N. benthamiana*. Green fluorescence was detected in the peripheral cytoplasm (surrounding the vacuole) as well as in the nucleus, which was similar to the cells expressing GFP alone (Figure 1). As our previous report (Guo et al., 2015), we further confirmed that GhFT1 localized in both the cytoplasm and nucleus in plant cells.

We next transferred all the 35S::GhFT1-GFP transgenic tobacco plants into pots containing soil. Under SD conditions, these transgenic plants flowered at 63 ± 6.6 days after sowing with 14.5 ± 0.6 leaves (Supplementary Figure S3A), compared with 94.5 ± 3.3 days in the WT tobacco plants with 15.7 ± 0.5 leaves (Supplementary Table S2). To investigate whether GhFT1 was highly expressed in the 35S::GhFT1-GFP transgenic lines, semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) was performed. As shown in Supplementary Figure S3B, GhFT1 was expressed in all the selected transgenic lines, and...
Li et al. Overexpression of GhFT1 in tobacco

the flowering time was positively correlated with expression level of GhFT1 in transgenic lines.

Ectopic Expression of GhFT1 Promoted Flowering in N. tabacum

Our previous research showed that overexpression of cotton GhFT1 in Arabidopsis caused early flowering both LD and SD conditions (Guo et al., 2015). To explore the potential of GhFT1 in the regulation of flowering in tobacco, this gene was overexpressed in N. tabacum under the control of the strong and constitutive cauliflower mosaic virus (CaMV) 35S promoter by transformation with 35S::GhFT1 construct (Supplementary Figure S1B). We obtained numerous transgenic lines from two times of independent transform assays, and all of them were confirmed by PCR (data was not provided). The majority of 35S::GhFT1 primary transformants flowered much earlier than the WT, both in terms of time and the number of leaves before flowering (Supplementary Table S2). In the homozygous T3 plants, 14 showed significantly early flowering phenotype under LD (line 1 and line 2 are shown as an example in Figure 2A), and 12 also showed precocious flowering compared with the WT plants under SD conditions (line 15 and line 16 are shown as an example in Figure 2B). In LD conditions, the flowering time in the 35S::GhFT1 transgenic lines was 45.8 ± 4.8 days after sowing by producing 8 ± 0.7 leaves, compared with 106.3 ± 4.8 days by producing 16 ± 0.8 leaves in the WT (Supplementary Table S2). Likewise, under SD conditions, the flowering time of the 35S::GhFT1 transgenic lines was about 57.2 ± 4.9 days by producing 7 ± 0.9 leaves, compared with 94.5 ± 3.3 days by producing 15.7 ± 0.5 leaves in the WT siblings (Supplementary Table S2). In addition, transgenic lines had rapidly elongated internodes and reduced internodal length, and thereby developed dwarf stature when flowering than the controls under both conditions.

Previously, it has been shown that a transgene consisting of 5.7-kb sequence upstream of the Arabidopsis FT translation start site fused to the FT cDNA was sufficient to rescue the late flowering phenotype of ft-10 plants grown under inductive conditions. 5.7kbAtFTpro::GhFT1 transgenic tobacco lines grew in phytotron under SD conditions. 5.7kbAtFTpro::GhFT1 transgenic tobacco lines grown in phytotron under SD conditions. Scale bars: 3.0 cm. Values are mean ± SE of results from three independent replicates (n = 3).
extended SD conditions (Adrian et al., 2010). To further analyze the function of GhFT1 in promoting flowers, we designed the 5.7kb AtFTpro::GhFT1 construction by using 5.7-kb Arabidopsis FT gene promoter fused to the GhFT1 cDNA (Supplementary Figure S1C). We next generated up to 14 independent transgenic lines expressing GhFT1 cDNA by transformation with A. tumefaciens. All of them flowered earlier than the WT plants under non-inductive SD conditions (Figure 2E). The average flowering time for these 5.7kbpro::GhFT1 transgenic lines was approximately 50.5 ± 2.1 days average by producing 13.5 ± 1.0 leaves, whereas the flowering time in WT sibling was 94.5 ± 3.3 days by producing 15.7 ± 0.5 leaves (Supplementary Table S2). These data combined with previous report by Guo et al. (2015) further supported that the gene product of GhFT1 function as a floral activator to promote flowering in cotton.

To explore whether the early flowering phenotype correlated with GhFT1 expression in the transgenic tobacco lines, we used quantitative Real-time PCR (qRT-PCR) methods to analyze gene expression level. As shown in Figures 2B,D,F, higher GhFT1 expression was observed in early flowering transgenic lines more than in those with a less phenotype, whereas no GhFT1 expression was seen in WT.

**Ectopic Expression of GhFT1 Caused Lateral Shoot Outgrowth in Tobacco**

*Nicotiana tabacum* is an annually grown herbaceous plant with little branches. Many flowered inflorescences arise at the terminal after floral transition of the plant (Amaya et al., 1999). Axillary buds are formed at the axillae of foliage leaves, and will further develop into an inflorescence shoot (Figure 3A). Surprisingly, we also observed that the 35S::GhFT1 transgenic plants produced more axillary buds after floral transition under LD conditions, but these axillary buds could not further develop into lateral shoots (Figures 3B,D), whereas the WT tobacco plants could. Conversely, more lateral shoots were generated at the base of transgenic plants, which do not usually appear in the WT plants of laboratory accession, *N. tabacum* cv. NC89 (Figures 3CE; Supplementary Figures S4A,B). Under SD conditions, the formation of axillary buds in all the 35S::GhFT1 transgenic plants were not as obvious as in the LD conditions. They also could not develop into lateral shoots finally, and more lateral shoots were generated from stem base at early buds stage (Figures 3F,G; Supplementary Figure S4C). These observations suggested that ectopic expression of GhFT1 in tobacco could modulate lateral shoot outgrowth and axillary bud set in additional to floral transition.

**Overexpression of GhFT1 Influenced Leaf Morphology in Tobacco**

Surprisingly, we also noted that change of leaf morphology appeared in all the 35S::GhFT1 transgenic plants. To decipher the function of GhFT1 further, the 35S::GhFT1 transgenic line 5 and line 6 were used to observe their leaves phenotype under LD and SD conditions. We compared leaves morphology from apical to basal position among the transgenic lines and the WT tobacco siblings. The leaf area in the transgenic lines was significantly smaller than in the WT plants. Under LD conditions, the leaves in line 5 appeared to be much longer and narrower than that in the WT plants, but leaves in line 6 appeared to be much shorter and wider (Figure 4A). We next measured the leaf length to width (L/W) ratio in the transgenic lines and WT tobacco plants, respectively. Accordingly, line 5 had the largest L/W ratio value, followed by the WT plant and Line 6, respectively (Figure 4B). Strikingly, the leaves of all the 35S::GhFT1 transgenic lines looked much more green and fleshy than the WT plants. We then measured chlorophyll content, suggesting both line 5 and line 6 had higher total chlorophyll content than the WT plants (Figure 4C). Similar phenotype was also observed in the 35S::GhFT1 transgenic lines in SD conditions (Supplementary Figure S5A). Leaf mass per area (LMA) is a key trait in plant growth and an important indicator of plant strategies, which is most closely correlated with a relative growth rate, and has been used wildly in plant ecology, agronomy, and forestry (Poorter et al., 2009). The transgenic lines showed higher LMA values than the WT plants (Figure 4D), contributing to more fleshy...
FIGURE 4 | Overexpression of GhFT1 in tobacco has a powerful influence on leaf development. (A) Comparison of apical (left), medial (middle two), and basal (bottom) leaves among WT plant and the 35S::GhFT1 transgenic line 5 and line 6 (at 7 weeks) under LD conditions. Scale bar: 1 cm. The ratio of leaf length to width (L/W; B), chlorophyll content (C) and Leaf mass per area (LMA; D) were determined among WT plant and the 35S::GhFT1 transgenic tobacco line 5 and line 6 (at 7 weeks) under LD conditions, respectively. Data represent the mean ± SE of three independent experiments (n = 4). The asterisks indicate significant differences compared with the WT plants (P < 0.05, Student’s t-test).

leaves compared with the WT plants in LD conditions. Similar to LD conditions, all the 35S::GhFT1 transgenic plants (line 15 and line 16 were shown as an example) had higher L/W ratio, chlorophyll content and LMA values than the WT under SD conditions (Supplementary Figure S5).

We next set out to explore whether the change of leaf morphology and increasing of chlorophyll content in transgenic plants could enhance photosynthesis. The efficiency of photosynthesis was determined by LI-6400 portable photosynthesis system in different light intensity. As shown in Figure 5, the transgenic tobacco lines showed higher photosynthetic efficiency than the WT plant in LD as well as SD conditions. This suggests, GhFT1 might play important roles in the modulation of leaf development in cotton by increasing photosynthesis and chlorophyll content other than floral transition.

Ectopic Overexpression of GhFT1 Caused Flowers Abscission

Nicotiana tabacum cv. NC89 is a typical cymose inflorescence in which the first-formed flower develops from the growing region at the top of the flower stalk, and the development of the flower at the apex is followed by two new flower axes developing from buds opposite on another (Amaya et al., 1999; Figure 6A). Both LD and SD conditions, strikingly, overexpression of GhFT1 in tobacco caused extremely early flowering (Figure 2). In addition, 86% tobacco plants overexpressing GhFT1 showed obvious premature flowering abscission in early flower developmental stages. For example, in the 35S::GhFT1 line 18, after the first flower opened, it abscised form the stalk (Figure 6B) under SD conditions, so it produced very few flowers and few mature seeds. In transgenic line 17, it could not present the regular flowers, due to their abscission before opening (Figure 6C). One of the reasons may be their flowers failed to enter meiosis, and eventually the plants did not produce any seed capsules. No cymose inflorescences similar to the WT plants (Figure 6A) were formed in all these transgenic lines. Similar to SD conditions, we also observed that these transgenic lines showed buds abscission at the initiation of early bud set under LD conditions (Figures 6D,E). However, the extent of floral bud abscission was alleviated, and flowers could normally open and seed set at later developmental stage (Figures 2A,C).

Viewed form outside, the transgenic plants showed normal flower development, produced fertile flowers and normal seeds (Supplementary Figures S6A,E). To investigate whether overexpression of GhFT1 affected flower organs development in tobacco, we dissected the flowers of −1 days of anthesis (DOA) and 0 DOA in the WT and 35S::GhFT1 transgenic
Overexpression of GhFT1 in tobacco

Li et al.

Overexpression of cotton GhFT1 increased photosynthetic efficiency in tobacco. Photosynthetic efficiency among the WT and the 35S::GhFT1 transgenic tobacco lines was determined using LI-6400 portable photosynthesis system at different light intensity under LD conditions (A) and SD conditions (B), respectively. The WT plant and transgenic plants were 7 weeks-old after transfer to the phytotron. Data represent the mean ± SE of three independent experiments (n = 4). Asterisk denotes significant difference compared with WT plants at P < 0.05, according to the Student's t-test, respectively.

Influence of GhFT1 Overexpression on the Expression Level of Other Genes in Tobacco

In the present model, FT protein, is now widely established as a major component of florigen, a systemic signal that induces flowering in response to daylength, which is translocated through the phloem to the SAM (Corbesier et al., 2007; Mathieu et al., 2007; Notaguchi et al., 2008), where they form a complex involving a bZIP transcription factor FD to promote the transition to flowering by activating the expression of multiple flower meristem identity genes, such as SOC1 and AP1 (Abe et al., 2005; Fornara et al., 2010). The MADS-domain transcription factor AP1 is a key regulator of Arabidopsis flower development, controlling the onset of flower development (Wigge et al., 2005; Kaufmann et al., 2010). SOC1 integrates multiple flowering signals including photoperiod, temperature, hormone, and age-related signals, involving in the process of floral organ formation, meristem determinacy, and prevention of secondary growth and shoot longevity (Lee and Lee, 2010; Hiraoka et al., 2013). LFY, which encodes a plant specific transcription factor, plays dual roles in determining floral meristem identity and floral organ patterning via AP1 and other floral homeotic genes (Moyroud et al., 2010). We next detected the expression profiles of the tobacco flower meristem identity genes among the transgenic and the WT siblings by qRT-PCR. The results indicated that NtAP1, NFL (the likely Nicotiana FLO/LFY homolog; Amaya et al., 1999) and NtSOC1 were obviously upregulated in the transgenic lines under LD conditions (Figures 7A–C). Under SD conditions, the three genes were also obviously upregulated in 35S::GhFT1 transgenic tobacco plants (Figures 7D–F).

Four FT-like genes have been identified in N. tabacum genome, NtFT1, NtFT2, NtFT3 and NtFT4, which acts antagonistically to regulate floral initiation (Harig et al., 2012). The NtFT1, NtFT2, and NtFT3 proteins are floral inhibitors, whereas NtFT4 is a floral inducer (Harig et al., 2012). To explore whether the early flowering phenotype was correlated with the endogenous NtFTs expression in 35S::GhFT1 transgenic lines, we next further detected the expression profiles of the four FT paralogs. As is shown in Figure 8A, higher NtFT4 expression was observed in line 5 and line 6 than the WT plants under LD conditions. NtFT4 was also observed highly expressed in transgenic tobacco plants under SD conditions (Figure 8B).
FIGURE 7 | Expression patterns of tobacco endogenous flowering-related gene in WT and 35S::GhFT1 transgenic lines. Total RNA isolated from the 35S::GhFT1 transgenic tobacco line 5 and line 6 and one WT tobacco plant under LD and SD conditions was used as template, respectively. The expression level of NtAP1 (A,D), NtSOC1 (B,E) and NFL1 (C,F) was determined by qRT-PCR, respectively. Data represent the mean ± SE from three biological replicates (n = 3), and NtActin was used as internal control. NtAP1, JQ686939.1; NFL1, JQ686928.1; NtSOC1, JQ686938.1; NtActin, U60495. The asterisks indicate significant differences compared with the WT plants (P < 0.05, Student's t-test).

Surprisingly, the expression of NtFT2 and NtFT3 were also upregulated in the 35S::GhFT1 transgenic line 5 and line 6 under LD condition (Figures 8C,E). However, both NtFT2 and NtFT3 were downregulated expressed in line 5 and line 6 in SD conditions (Figures 8D,F). It was previously reported that overexpression of NtFT2 and NtFT3 showed a delayed flower phenotype, but the exact biological functions of NtFT2 and NtFT3 remain unclear. We were unable to detect NtFT1 expression under both conditions, while Harig et al. (2012) were unable to detect the expression of these genes under LD condition, which may be associated with the different tobacco varieties.

Discussion

Conserved Functions of FT-Like Proteins as Floral Promoters

A wide spectrum of research of FT orthologs from angiosperms has been demonstrated their conserved function in the regulation of flowering time (Pin and Nilsson, 2012). However, the developmental mechanisms targeted by FT orthologs to transform vegetative meristems into reproductive organs remain unclear. We previously identified a FT-like gene GhFT1 from cotton (G. hirsutum), which was highly expressed in all the tissues except in root, and the strong sequence identity and critical amino acids residues Tyr88 (Y) and Gln144 (Q) to FT-orthologous genes of other species indicates that the GhFT1 might be also involved in the control of flowering. Ectopic expression of GhFT1 promoted precocious flowering under both LD and SD conditions in Arabidopsis (Guo et al., 2015), suggesting that GhFT1 is a potential FT ortholog that regulates floral transition in cotton. To investigate the developmental mechanism targeted by GhFT1 protein, we further unveiled its overall growth effects by overexpressing GhFT1 in tobacco.

The transgenic tobacco plants carrying the 35S::GhFT1 construct flowered earlier and had fewer leaves at flowering than the WT plants in both LD and SD condition. Furthermore, the 5.7kbAtFTpro::GhFT1 construction by using 5.7-kb Arabidopsis FT gene promoter fused to the GhFT1 cDNA could also accelerate flowering in transgenic tobacco (Figure 2E). The precocious flowering phenotype regardless of photoperiod indicated its conserved roles in floral induction.
Although FT orthologs have been identified and characterized from numerous plant species, the subcellular distributions of many of them have not been clearly studied. Here, we examined the distribution of functional GhFT1-GFP fusion protein expressed in leaf epidermal cells of N. benthamiana or in hypocotyl of tobacco by transform with 35S::GhFT1-GFP. In both cases, GhFT1-GFP was observed in the nucleus and cytoplasm (Figure 1; Supplementary Figure S2), which was consistent with Guo et al. (2015) results. Similar results using a GFP or YFP-tagged FT were also reported in Arabidopsis (Abe et al., 2005), rice (Taoka et al., 2011), tomato (Lifschitz et al., 2006), and tobacco (Harig et al., 2012).

FT-GFP fusion protein in transgenic plant has been detected to move through the phloem from the leaves as the place of light perception to the shoot apex as the position of flower formation only in limited plant species (Supplementary Table S3). FT-GFP fusion proteins induced early flowering were previously reported in Arabidopsis (Corbesier et al., 2007), rice (Tamaki et al., 2007), and tomato (Shalit et al., 2009). However, the size of the fusion protein restricted the long-range function of FT. The larger FT-GFP protein may move less effectively to the SAM from the minor veins than from the larger veins (Supplementary Table S3). The 35S::GhFT1 with C-terminal translational fusion of GFP induced precocious flowering in tobacco, indicating GhFT1-GFP protein has similar activity like the WT FT protein. Previously publication have shown that overexpression of FT orthologs in tobacco could accelerate flowering in different plant species (Supplementary Table S4), including tomato (Shalit et al., 2009), fig (Ikegami et al., 2013), spring orchid (Xiang et al., 2012), London plane (Zhang et al., 2011), and tobacco itself (Harig et al., 2012; Guo et al., 2015; Wickland and Hanzawa, 2015).

Gene expression using qRT-PCR analysis revealed that the expression of NAP1, NtSOC1, and NFL1 were significantly more upregulated in transgenic lines than in the WT plants under LD or SD conditions (Figure 7). These results were consistent with the finding that NAPI and NFL1 were highly expressed in the flower buds of 35S::CgFT tobacco plants (Xiang et al., 2012). These data indicates that the expression of NAPI, NtSOC1, and NFL1 may be regulated by FT. Therefore, GhFT1 might have upregulated them to regulate flowering in transgenic tobacco plants.
Beyond Flowering Promotion: Pleiotropic Functions of GhFT1

Tobacco is a determinate species in which main shoot terminates by converting into a flower, with subsequent growth occurring only from lower meristems (Figure 3A). A number of axillary meristems generated below the apex also develop into terminal flowers in a cymose pattern (Amaya et al., 1999). Although florigen was originally proposed as a flowering hormone, it is now apparent that FT is a universal growth factor affecting several aspects of plant architecture. In addition to promoting flowering, we observed that the transgenic tobacco plants showed pleiotropic phenotype different from the WT control, suggesting that GhFT1 played multifaceted roles during plant development.

That Arabidopsis FT is involved in the promotion of lateral shoot outgrowth and axillary bud initiation were previously proposed (Hiraoka et al., 2013; Niwa et al., 2013), but the single overexpression of Arabidopsis FT gene is insufficient to promote initiation or early development of axillary buds, and must combined with LFY (Niwa et al., 2013). However, ectopic overexpression of GhFT1 in tobacco resulted in more axillary buds transition (Figures 3B,D) and more lateral shoots generation at the base of main shoot (Figures 3C,E–G; Supplementary Figure S4).

It has been previously reported that elevation of FT concentration promotes more determinate habit, and influences leaf development (Shalit et al., 2009; McGarry and Ayre, 2012). Here, we also observed that leaf morphology of the 35S::GhFT1 transgenic tobacco lines was very different from that of WT. Firstly, the leaves of transgenic lines appeared much more dark green and fleshy compared with WT plants. Accordingly, the chlorophyll content in transgenic lines was higher than that in the control plants (Figure 4C; Supplementary Figure SSC). Secondly, compared with other WT plants, leaves were shorter and wider in some transgenic lines, whereas leaves were longer and narrower in other transgenic lines. However, both had bigger value of L/W ratio and LMA under LD and SD conditions than the WT plants (Figures 4B,D; Supplementary Figures SSB,D). Likewise, transgenic lines showed higher photosynthetic efficiency than the WT plants (Figure 5). The results suggested that high GhFT1 level could function to modulate leaf development by increasing L/W ratio, LMA and photosynthesis, and developing into smaller leaf. We surmised that GhFT1 could link the transition to floral with leaf development.

The leaf morphological change in the 35S::GhFT1 transgenic tobacco lines is reminiscent of resent reports on overexpression FT orthologs in different plant species. It was previously reported that the florigen-dependent SFT/SP regulatory hierarchy could determine leaf architecture in tomato and overexpression of SFT induced simple lanceolate-like leaves (Shalit et al., 2009). When Arabidopsis FT is ectopically overexpressed in ancestral cotton accession TX701 through virus-induced flowering, it also generated the lanceolate leaf shape (McGarry and Ayre, 2012). Endo et al. (2005) reported that constitutive expression of CiFT in trifoliate orange altered the leaf shape and color; the leaf in the transgenic plants containing 35S::CiFT was small, lacked color on the margin, and had a leaflet at the center of trifoliate leaf that was smaller than other leaflets. Xiang et al. (2012) reported that transgenic tobacco lines expressing CgFT showed the early release of axillary buds, and the rapid elongation of internodes enabled the formation of thinner stems and reduced leaf sizes. Teper-Bamnolker and Samach (2005) reported that overexpression of Arabidopsis FT induced the high level expression of FUL and SEPTAL (SEP3) in Arabidopsis and led to small-sized leaves. Overexpression of tobacco NFL1 in tobacco resulted in dwarfer stature, reduced internode length, and thickened leather-like leaves (Ahearn et al., 2001). Furthermore, Flachowsky et al. (2010) reported that ectopic expression of Arabidopsis LFY in apple showed an altered phenotype, which is similar to the columnar phenotype, and leads to shortened internodes and a significantly reduced length of the regrowing shoot. The high expression of NFL1 (Figure 7) in tobacco driven by the overexpression of GhFT1 might contribute to the leaf shape and plant architecture. Ectopically overexpressed transgenic plants containing FT orthologous genes exhibited similar phenotype in leaf shape and plant architecture, suggesting that the function of FT-like gene family is highly conserved during evolution.

Surprisingly, we also observed obvious premature flowering abscission in early developmental stages in the extremely early flowering transgenic lines carrying 35S::GhFT1, resulting in fewer mature seeds (Figure 6). In tomato, SFT was also reported to accelerate mature and promote abscission zone formation (Shalit et al., 2009). Overexpression of Arabidopsis FT in the ancestral cotton accession TX701 delivered by virus-induced flowering caused many of flowers abscission before producing mature bolls (McGarry and Ayre, 2012). The abscission trait is considered as an innovation in angiosperms, and is regulated by multifactor, including auxin, ethylene, and jasmonic acid even day length (Shalit et al., 2009). Further study would clarify the possible mechanism for the precocious floral organ abscission in tobacco plants overexpressing cotton GhFT1.

Overexpression of GhFT1 Might Disturb the Balance between Inducer and Repressor of FT Paralogs in Tobacco

It is now apparent that the relative ratios of FT to other members of the PEBP gene family have influenced the balance of indeterminate and determinate growth in many plant species, and play important role in the floral transition and architecture formation. For example, the tomato SFT/SP ratio regulates the reiterative growth and termination cycles typical of perennial plants, accelerates leaf maturation, influences the complexity of compound leaves, the growth of stems and the formation of abscission (Shalit et al., 2009). The recent report showed N. tabacum possesses four FT-like proteins (Harig et al., 2012), suggesting that the balance of FT-clade in tobacco plays important roles in the floral transition and plant architecture. However, the exact mechanism in the control of floral transition remains very unclear.

As expected, NtFT4 showed significantly upregulated expression in all the transgenic tobacco plants under both light conditions (Figures 8A,B). Surprisingly, the expression of NtFT2 and NtFT3 were upregulated in transgenic plants under LD conditions.
light conditions, but their expression levels were downregulated in SD conditions (Figures 8C–F). The contrast expression profiles of NtFT2 and NtFT3 under LD and SD conditions leads us to have a profound consideration of the florigen paradigm for influencing plant architecture. The balance model predicts that FT and TFL1 concentration fluctuate, and balance are re-fined in local tissues to give rise to different architecture (McGarry and Ayre, 2012). The expression levels of GhFT1 in LD condition tobaccos were much higher than that in SD condition (Figure 2), suggesting that in LD and SD conditions, tobacco might have different concentration of florigen for mediating floral transition. High expression of cotton GhFT1 completely influenced the expression level of endogenous FT genes in tobacco. As a floral inducer, NtFT4 expression was highly upregulated under both LD and SD conditions, whereas NtFT2 and NtFT3 expression, acting as a floral repressor, were upregulated in LD condition and downregulated in SD, resulting in disorders of the balance between inducers and repressors in transgenic tobacco plants, therefore influences the FT/TFL1 genes expression, and further changes FT/TFL1 proteins concentration in the transgenic tobacco. The problem balance will further influence the expression levels of flowering meristem identity genes, such as NAPI, NFL1, and NISOCI (Figure 7), resulting in developing multifaceted phenotypes: early flowering, axillary buds set, lateral shoot outgrowth, leaf development change and flower abscission. However, further extensive research is needed to clarify these scenarios.

Took together, overexpression of cotton GhFT1 in tobacco promotes precocious flowering uncouple from photoperiod, showing that FT paralog evokes a conserved function of floral promoter in fiber plants. Introducing of transgenic cotton FT disturbs the balance of endogenous FT paralogs including inducers and repressors, and further disturbs other PEBP family members balance through antagonistic functions. We here present evidences that sufficient levels of FT activity might modulate auxillary and lateral shoot outgrowth, influence leaf development and promote flower abscission, supporting the view that florigen functions as general growth hormone mediating growth and termination. These finding further extends the knowledge for plant florigen. Judicious manipulation of the ratio for indeterminate and determinate growth factors, mediated by a balance of FT-like and TFL1-like gene activities by transgenic technology, holds promise for improved plant architecture optimized for region-specific environment and enhanced crop yield in order to meet the agricultural demand of the rapidly expanding global population.

Author Contributions

XH and XW designed the experiments and organized the manuscript. CL, YZ, KZ, and DG performed the experiments. XW and BC edited the manuscript. All the authors discussed the results and contributed to the manuscripts.

Acknowledgments

The authors sincerely thank Dr. Zhixi Tian (The State Key of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology – Chinese Academy of Sciences) for kindly providing us with the pDONR207-8.1kbAtFTpro vector, and Dr. Andrew H. Paterson (Plant Genome Mapping Laboratory, University of Georgia) for critical reading of the manuscript. This work was supported by the National Natural Science Foundation of China (grant no. 31363066); the Program for New Century Excellent Talents in University (grant no. NCET-12-1072); the Doctor Science Foundation of Xinjiang Production and Construction Corps (grant no. 2012BB007).

Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpls.2015.00454

References

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y., et al. (2005). FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science 309, 1052–1056. doi: 10.1126/science.115983

Adrian, J., Farrona, S., Reimer, J. J., Albani, M. C., Coupland, G., and Turck, F. (2010). cis-Regulatory elements and chromatin state coordinately control temporal and spatial expression of FLOWERING LOCUS T in Arabidopsis. Plant Cell 22, 1425–1440. doi: 10.1105/tpc.110.074682

Ahearn, K. P., Johnson, H. A., Weigel, D., and Wagner, D. R. (2001). NFL1, a Nicotiana tabacum LEAFY-like gene, controls meristem initiation and floral structure. Plant Cell Physiol. 42, 1130–1139. doi: 10.1093/pcp/pec143

Ahn, J. H., Miller, D., Winter, V. J., Banfield, M. J., Lee, J. H., Yoo, S. Y., et al. (2006). A divergent external loop confers antagonistic activity on floral regulators FT and TFL1. EMBO J. 25, 605–614. doi: 10.1038/sj.emboj.7600950

Amaya, I., Ratcliffe, O. J., and Bradley, D. J. (1999). Expression of CENTRORADIALIS (CEN) and CEN-like genes in tobacco reveals a conserved mechanism controlling phase change in diverse species. Plant Cell 11, 1405–1418. doi: 10.1105/tpc.11.8.1405

Böhlenius, H., Huang, T., Charbonnel-Campaa, L., Brunner, A. M., Janssos, S., Strauss, S. H., et al. (2006). CO/FT regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312, 1040–1043. doi: 10.1126/science.1126038

Carmona, M. J., Calonje, M., and Martinez-Zapater, J. M. (2007). The FT/TFL1 gene family in grapevine. Plant Mol. Biol. 63, 637–650. doi: 10.1007/s11103-006-9113-z

Chardon, F., and Damerval, C. (2005). Phylogenomic analysis of the PEBP gene family in cereals. J. Mol. Evol. 61, 579–590. doi: 10.1007/s00239-004-0179-4

Coelho, C. P., Minow, M. A., Chalfun-Júnior, A., and Colasanti, J. (2014). Putative sugarcane FT/TFL1 genes delay flowering time and alter reproductive architecture in Arabidopsis. Front. Plant Sci. 5:221. doi: 10.3389/fpls.2014.00221

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., et al. (2007). FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science 316, 1030–1033. doi: 10.1126/science.114752

Endo, T., Shimada, T., Fujii, H., Kobayashi, Y., Araki, T., and Omura, M. (2005). Strigolactone-mediated flowering in citrus is controlled by the STRIGOLACTONE INSENSITIVE (STRIGA) gene. Planta 221, 1075–1083. doi: 10.1007/s00425-005-1676-2

Ectopic expression of an FT homolog from Citrus confers an early flowering phenotype on trifoliate orange (Poncirus trifoliata L. Raf.). Transgenic Res. 14, 703–712. doi: 10.1007/s11248-005-6632-3

Endo, T., Shimada, T., Fujii, H., Kobayashi, Y., Araki, T., and Omura, M. (2005). Transgenic Res. 14, 703–712. doi: 10.1007/s11248-005-6632-3

Frontiers in Plant Science | www.frontiersin.org

12 June 2015 | Volume 6 | Article 454
Imamura, T., Nakatsuka, T., Higuchi, A., Nishihara, M., and Takahashi, H. (2010). Isolation and functional characterization of the FLOWERING LOCUS T homolog, the LsFT gene, in lettuce. J. Plant Physiol. 168, 1602–1607. doi:10.1016/j.jplph.2011.02.004

Guo, D., Li, C., Dong, R., Li, X., Xiao, X., and Huang, X. (2015). Molecular cloning and functional analysis of the FLOWERING LOCUS T (FT) homolog GhFT1 from Gossypium hirsutum. J. Integr. Plant Biol. 57, 522–533. doi:10.1111/jipb.12316

Hanzawa, Y., Money, T., and Bradley, D. (2005). A single amino acid converts a repressor to an activator of flowering. Proc. Natl. Acad. Sci. U.S.A. 102, 7748–7753. doi:10.1073/pnas.0500932102

Harig, L., Beinecke, F. A., Oltmanns, J., Muth, J., Müller, O., Rüping, B., et al. (2010). Florigen is involved in axillary bud control of photoperiodic flowering in soybean. Plant Physiol. 154, 1232–1238. doi:10.1104/pp.110.160796

Kotoda, N., Hayashi, H., Suzuki, M., Igarashi, M., Hatsuuya, Y., Kidou, S., et al. (2010). Molecular characterization of FLOWERING LOCUS T-like genes of apple (Malus domestica Borkh.). J. Plant Physiol. 51, 561–575. doi:10.1093/jpc/pcp021

Krieger, U., Lippman, Z. B., and Zamir, D. (2010). The flowering gene SINGLE FLOWERING TRUSS drives heterosis for yield in tomato. Nat. Genet. 42, 459–463. doi:10.1038/ng.550

Laurie, E. R., Diwadkar, P., Joubel, M., Zhang, L., Hecht, V., Wen, J., et al. (2011). The Medicago FLOWERING LOCUS T homolog, MtFT1a, is a key regulator of flowering time. Plant Physiol. 156, 2207–2224. doi:10.1104/pp.111.180182

Lee, J., and Lee, I. (2010). Regulation and function of SOC1, a flowering pathway integrator. J. Exp. Bot. 61, 2247–2254. doi:10.1093/jxb/erq098

Lee, R., Baldwin, S., Kenel, F., McCullum, J., and Macknight, R. (2013). FLOWERING LOCUS T genes control onion bulb formation and flowering. Nat. Commun. 4, 2884. doi:10.1038/ncomms3884

Li, X. F., Jia, L. Y., Xu, J., Deng, X. J., Wang, Y., Zhang, W., et al. (2013). FT-like NFT1 gene may play a role in flower transition induced by heat accumulation in Narcissus tazetta var. chinensis. Plant Cell Physiol. 54, 270–281. doi:10.1093/pcp/pcs181

Lisichitz, E., Eviatar, T., Rozman, A., Shaltit, A., Goldshmidt, A., Amsellem, Z., et al. (2006). The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proc. Natl. Acad. Sci. U.S.A. 103, 6398–6403. doi:10.1073/pnas.0601078103

Mathieu, J., Warthmann, N., Küttner, F., and Schmid, M. (2007). Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Curr. Biol. 17, 1055–1060. doi:10.1016/j.cub.2007.05.009

McGarry, R. C., and Ayre, B. G. (2012). Geminivirus-mediated delivery of florigen promotes determinate growth in aerial organs and uncouples flowering from photoperiod in cotton. PLoS ONE 7:e36746. doi: 10.1371/journal.pone.0036746

Melzer, S., Jens, F., Gennens, J., Vanneste, S., Rohde, A., and Beckman, T. (2008). Flowering-time genes modulate meristem determinacy and growth form in Arabidopsis thaliana. Nat. Genet. 40, 1489–1492. doi:10.1038/ng.253

Meng, X., Muszynski, M. G., and Danilevskaya, O. N. (2011). FT-like ZCN8 gene functions as a floral activator and is involved in photoperiod sensitivity in maize. Plant Cell 23, 942–960. doi:10.1105/tpc.109.1081406

Moyroud, E., Kusters, E., Monniaux, M., Koes, R., and Parcy, F. (2010). LEAFY interacts antagonistically with FT and TFL1 to regulate floral initiation in tobacco. Proc. Natl. Acad. Sci. U.S.A. 107, 5460–5465. doi:10.1073/pnas.1006695107

Ono, N., Ishida, K., Yamashino, T., Nakashima, H., Sato, S., Tabata, S., et al. (2010). Genome-wide characterization of the light-responsive and clock-controlled genes in Arabidopsis. J. Exp. Bot. 61, 2247–2254. doi:10.1093/jxb/erq098

Oda, A., Narumi, T., Kando, T., Higuchi, Y., Sumitomo, K., et al. (2010). Coordination of flowering time by the FLOWERING LOCUS T ortholog F2L15 is essential for flowering in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 107, 13635–13640. doi:10.1073/pnas.1007004107
control of flowering time in sugar beet. Science 330, 1397–1400. doi: 10.1126/science.1197004
Pin, P. A., and Nilsson, O. (2012). The multifaceted roles of FLOWERING LOCUS T in plant development. Plant Cell Environ. 35, 1742–1755. doi: 10.1111/j.1365-3040.2012.02558.x
Poirier, H., Niinemets, U., Poirier, L., Wright, I. J., and Villar, R. (2009). Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. New Phytol. 182, 565–588. doi: 10.1111/j.1469-8137.2009.02830.x
Shalit, A., Rozman, A., Goldshmidt, A., Alvarez, J. P., Bowman, J. L., Eshed, Y., et al. (2009). The flowering hormone florigen functions as a general systemic regulator of growth and termination. Proc. Nat. Acad. Sci. U.S.A. 106, 8392–8397. doi: 10.1073/pnas.0810810106
Smith, H. M., Ung, N., Lal, S., and Courtier, J. (2011). Specification of reproductive meristems requires the combined function of SHOOT MERISTEMLESS and floral integrators FLOWERING LOCUS T and FD during Arabidopsis inflorescence development. J. Exp. Bot. 62, 583–593. doi: 10.1093/jxb/erq296
Song, G. Q., Walworth, A., Zhao, D., Jiang, N., and Hancock, J. F. (2013). Overexpression of QhFT1 in tobacco. Plant J. 73, 2661–2675. doi: 10.1105/tpc.105.035766
Voinnet, O., Rivas, S., Mestre, P., and Baulcombe, D. (2003). The enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. Plant J. 33, 949–956. doi: 10.1046/j.1365-313X.2003.01676.x
Wickland, D. P., and Hanzawa, Y. (2015). The flower synchronizer (FT) ortholog from Arabidopsis thaliana. Plant Physiol. 170, 1327–1338. doi: 10.1104/pp.15.00712