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Research article

Photoperiodic control of *FT*-like gene *ClFT* initiates flowering in *Chrysanthemum lavandulifolium*

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ABSTRACT

The *FLOWERING LOCUS T* (*FT*) gene plays crucial roles in regulating the transition from the vegetative phase to the reproductive phase. In this study, we isolated an *FT* homologous gene (denoted as *ClFT*) from *Chrysanthemum lavandulifolium*. The sequencing analysis indicated that the promoter of the *ClFT* gene contains many elements, such as light response, abscisic acid, drought-inducibility response and CIRCADIAN clock elements. The expression patterns of *ClFT* in different tissues/organs at different developmental stages and its responses to different photoperiods were observed. *ClFT* is expressed in all tested organs/tissues, with the highest expression level being observed in the leaves of plants with visible floral buds under the short day (SD) condition. Next, we studied the rhythmic expression of *ClFT* during different photoperiod treatments and found that the level of *ClFT* increases with additional hours of continuous dark. *ClFT* accumulates when the continuous dark period is 12 h, regardless of the duration of light period. The ectopic expression of the *ClFT gene* in wild type *Arabidopsis* (Col-0) results in early flowering, with high expression levels of endogenous *LFY* and *SOC1* being observed in transgenic *Arabidopsis*. All results indicated that the *ClFT* gene plays an evolutionarily conserved role in promoting flowering in inductive short days in *C. lavandulifolium* and that this gene could serve as a vital target for the genetic manipulation of flowering time in chrysanthemums.

1. Introduction

Plants initiate flowering after a period of vegetative development. The initiation of flowering in plants is controlled by endogenous and environmental signals. The timing of floral transition is controlled by sophisticated regulatory networks that monitor changes in the environment, which ensures that the successful reproduction and the persistence of populations and species are guaranteed. In the model plant species *Arabidopsis thaliana*, approximately 180 genes have been implicated in the control of flowering time, and many of these genes occur in a network of six major pathways: the photoperiod pathway, vernalization pathway, ambient temperature pathway, age pathway, autonomous pathway and gibberellin pathway (Fornara et al., 2010). The six pathways converge to regulate a small number of 'floral integrator genes', which include *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *LEAFY* (*LFY*). Of these genes, *FT*, which is a well-known floral integrator gene, plays an important role in controlling flowering time (Kardailsky et al., 1999; Kobayashi et al., 1999), and recent studies have demonstrated that the FT protein is the long-sought florigen (Taoka et al., 2013).

The FT protein belongs to the phosphatidylethanolamine-binding protein (PEBP) family, which consists of six members: FT, TFL1 (TERMINAL FLOWER 1), MFT (MOTHER OF FT AND TFL1), ATC (ARABIDOPSIS THALIANA CENTRORADIALIS HOMOLOG), TSF (TWIN SISTER OF FT) and BFT (BROTHER OF FT AND TFL1) (Kobayashi et al., 1999). Phylogenetic analysis has resolved three major clades within this family, namely FT-like, TFL1-like and MFT-like (Chardon and Damerval, 2005). *FT*-like and *TFL1*-like genes primarily function in controlling flowering time, and *FT*-like genes promote flowering. In contrast, *TFL1*-like genes delay flowering and prevent the conversion of the shoot apical meristem (SAM) into a floral meristem (Hanano and Goto, 2011; Mathieu et al., 2007).

Among these *FT*-like genes, most of these genes have been proven to promote flowering (Laurie et al., 2011; Fukuda et al., 2011; Hayama et al., 2007; Kojima et al., 2002; Hou and Yang, 2009); however, *FT*-like flowering repressors were also recently

Abbreviations: ATC, ARABIDOPSIS THALIANA CENTRORADIALIS HOMOLOG; BFT, BROTHER OF FT AND TFL1; CO, CONSTANS; *FT, FLOWERING LOCUS T*; *LFY, LEAFY*; MFT, MOTHER OF *FT* AND *TFL1*; MTP, metal tolerance protein; qRT-PCR, quantitative real-time RT-PCR; RACE, rapid amplification of cDNA ends; SAND, SAND family protein; SD, short day; *SOC1, SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*; TFL1, TERMINAL FLOWER 1; TSF, TWIN SISTER OF *FT*; *TUB2*, β-tubulin; WT, wild type.

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described (Harig et al., 2012; Winterhagen et al., 2013; Pin et al., 2010). Furthermore, *FT*-like genes also have other functions in plants. For example, *PaFT4* in Norway spruce is a key integrator of photoperiodic and thermal signals in the control of growth rhythms, which can control active growth and dormancy (Gyllenstrand et al., 2007). *StSP6A* in potato is involved in the tuberization transition (Navarro et al., 2011).

Chrysanthemums (*Chrysanthemum morifolium* Ramat.) are one of the most important ornamental plants, which enjoy a major share of the cut flower and potted mum market (Anderson, 2006). Chrysanthemums are typical short day (SD) plants and the variations in the flowering time (from early summer to winter) under natural conditions are mainly dependent on their critical photoperiod. Therefore, this photoperiod is the primary target in the industrial development of chrysanthemums to breed new cultivars that can be produced all year and that are not sensitive to photoperiods. Researching photoperiod-sensitive genes could lay the foundations for modifying the flowering time at the molecular level. With obvious traces of inter-specific hybridization and artificial selection in its origins, the chrysanthemum is classified as a hybrid cultigen complex (Dai et al., 2002). Therefore, the genetic intractability of the chrysanthemum is primarily because chrysanthemum cultivars are generally polyploid and self-incompatible. In 2012, Oda (Oda et al., 2012) used a wild diploid chrysanthemum, *Chrysanthemum seticuspe*, to illuminate the photoperiodic flowering in chrysanthemums. Three *FT*-like genes were isolated, and only the *CsFTL3* gene coincided with the induction of flowering. However, there were also some other questions to be clarified further, which included the expression patterns of *FT*-like genes under different photoperiods and the effect of leaf age on the expression of *FT*-like genes. The characteristics of *cis*-acting regulatory elements in the promoter of the *FT*-like gene have not been studied; therefore, at present, it is difficult to reveal how the trans-acting factor interacts with *cis*-acting regulatory elements. *Chrysanthemum lavandulifolium*, which is a wild species with a close relation to cultivated chrysanthemums, was used as an ideal model plant for molecular biological research on chrysanthemum because of its simple genetic background (Zhang and Dai, 2009). We have previously reported that *C. lavandulifolium* is an SD plant that is widely distributed in the northeast regions of China (Ding et al., 2012) and have studied the molecular mechanism of flower transitioning and flower development in this species under natural conditions (Ding et al., 2012; Ma et al., 2008). Understanding the expression patterns of key genes that are involved in the photoperiod flowering of *C. lavandulifolium* will help us to understand the flowering mechanism and promote molecular breeding in chrysanthemums to ease the difficulty of year-round production.

In this study, we isolated an *FT*-like gene (denoted *ClFT*) using the traditional RT-PCR and rapid amplification of cDNA ends (RACE) method. Next, we analyzed the expression patterns of *ClFT* in different tissues/organs at different developmental stages and the diurnal rhythm expression patterns in different photoperiods. The promoter of this gene was isolated, and its *cis*-acting regulatory elements were analyzed. Furthermore, we also provided evidence that the alteration in flowering time by the overexpression of *ClFT* in transgenic *Arabidopsis* plants is due to the induction of flowering time-related genes, such as *LFY* and *SOC1*.

2. Results

2.1. Isolation and characterization of ClFT from C. lavandulifolium

Degenerate primers were designed according to the conserved regions of the closely related species of *FT*-like sequences. Using the RT-PCR and RACE methods, one *FT*-like gene (hereafter called *ClFT*) was isolated from the mixed sample of young, fully expanded leaves and shoot apices. The full-length sequence of *ClFT* (GenBank accession number: GU120195) cDNA contains 837 nucleotides. This sequence encodes a 174 amino acid polypeptide, which is flanked by a 15 bp 5′-untranslated region and a 297 bp 3′-untranslated region. The alignment of the predicted amino acid sequence of the ClFT protein shows 99%, 90% and 90% sequence identity to CsFT3, CsFT1 and CsFT2 proteins, respectively (Fig. 1A). The conserved and functionally crucial amino acids of the *Arabidopsis* FT (Tyr85/Gln140) are presented at positions 84 and 139 in ClFT protein sequences, respectively (Fig. 1A). Furthermore, the ClFT sequence contains the conserved 14 amino acid segment (LGRQTVYAPGWRQN) and the LYN triad (Fig. 1A), which have been described in the conserved domains of FT homologous proteins in various plant species (Ahn et al., 2006).

To further evaluate the relationship of the ClFT protein with FT/TFL1 proteins in other plants, we performed a phylogenetic analysis. There are six family members in the FT/TFL1 family in *Arabidopsis*, which include the FT-like clade (including FT and TSF proteins), TFL1-like clade (including TFL1, BFT and ATC proteins) and MFT-like clade (including MFT protein) (Chardon and Damerval, 2005; Karlgren et al., 2011). According to the phylogenetic analysis, the ClFT protein belongs to the FT-like clade, with a close relationship to CsFT3 (Fig. 1B).

We also analyzed the genomic structure of the *ClFT* gene (GenBank accession number: KF752602) and compared this structure with the structures of the *FT* genes of *Arabidopsis* and apple. The position and number of introns are identical, but their lengths are different (Fig. 1C).

To estimate the copy number of *FT* like genes in *C. lavandulifolium*, we performed Southern hybridization with a probe that was derived from the coding region of the *ClFT* gene. There are one or two bands on the autoradiogram (Fig. 1D). According to our analysis of the restriction sites of the cDNA or gDNA of *ClFT*, *Hind*III, *Xba*I or *Sca*I restriction sites do not exist in the cDNA of *ClFT*, but exist in the gDNA of *ClFT*. Therefore, there is only one copy of the *ClFT* gene in the genome of *C. lavandulifolium*.

2.2. Sequence analysis of the ClFT promoter

Using the Genome Walker method, we isolated a 911 bp promoter fragment that was upstream of the ATG start codon of the *ClFT* gene (GenBank accession number: KF752603, Fig. 2). The prediction of *cis*-acting regulatory elements in this promoter sequence was analyzed using the PLACE and Plant CARE databases. The TATA-box and CAAT-box, which are conserved in the promoters of eukaryotes, are also found in the promoter of the *ClFT* gene. Several elements are related to light response, including AE-box, Box 4, Box I and G-Box (Fig. 2 and Table 1). The abscisic acid response element ABRE and drought-inducibility element MBS are also found in the promoter. The putative circadian control element CIRCADIAN is also observed (Table 1 and Fig. 2).

2.3. Expression patterns of ClFT in C. lavandulifolium

To investigate the temporal and spatial expression patterns of *ClFT*, quantitative real-time RT-PCR (qRT-PCR) was performed in different tissues/organs (leaves, shoot apices, petioles, stems, roots, floral buds and flowers) at different developmental stages of *C. lavandulifolium*. *ClFT* mRNA is expressed in all tested organs/tissues with different expression levels (Fig. 3). The *ClFT* mRNA level in leaves increases from young seedlings to floral transition plants and then decreases after the formation of floral buds. The highest