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Molecular Cloning and Expression Analysis of a FT Homologous Gene from *Solanum tuberosum*

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Abstract

A homologue of flowering locus T gene, designated *StFT*, was isolated from *Solanum tuberosum* by reverse transcriptase-polymerase chain reaction (accession no. GU223211). The DNA sequence of *StFT* was 1 626 bp long and contained four exons and three introns. The open reading frame of the gene was 522 bp long and encoded a putative protein of 173 amino acids with a molecular weight of 19.75 kD and a theoretical *p*I of 7.76. StFT protein had a conserved PBP domain and a higher degree of identity with FT homologous members from other species. Analysis on the mRNA levels of *StFT* showed that it was highly expressed in leaves, apical buds, flowers, and swelling stolons. Further analysis indicated that its expression was regulated by *CONSTANS* gene in *StCOL*-antisense transgenic potato plants.

Key words: cloning, expression, *StFT* gene, *Solanum tuberosum*

INTRODUCTION

Fluctuations in day length determine the time to flower in many plants. During the growth of plants, day-length duration is sensed by light receptor phytochrome B (Schepens *et al*. 2004), which activates the abundance of CONSTANS (CO) protein with cryptochromes, and transforms the signal into a systemic signal and induces flower development (Valverde *et al*. 2004).

Flowering locus T (FT), a 20-kD protein, is a member of the small CETS protein family (Kardailsky *et al*. 1999; Kobayashi *et al*. 1999). FT acts as a potent promoter of flowering in long-day plants (LDP) such as *Arabidopsis thaliana*, short-day plants (SDP) such as rice and *Pharbitis nil*, and day-neutral plants (DNP) such as tomato (Turck *et al*. 2008). In *Arabidopsis,* the expression of FT in cotyledons and leaves is induced by the B-box zinc-finger protein CO (Suárez-López *et al*. 2001; Valverde *et al*. 2004). Then FT protein interacts with a bZIP transcription factor FD and activates transcription of meristem identity genes such as *APETALA1* (*AP1*), causing flower bud formation (Abe *et al*. 2005; Notaguchi *et al*. 2008). Up to date, *FT* gene has been cloned in *Arabidopsis* (Kobayashi *et al*. 1999), rice (Kojima *et al*. 2002), wheat (Yan *et al*. 2006), tomato (Lifschitz *et al*. 2006), Japanese morning glory (Hayama *et al*. 2007), grapevine (Carmona *et al*. 2007), cucurbit (Lin *et al*. 2007), barley (Hemming *et al*. 2008), *Chenopodium rubrum* (Cháb *et al*. 2008), *Sinapis alba* (Aloia *et al*. 2009), orchid (Hou and Yang 2009), apple (Kotoda *et al*. 2010), sunflower (Blackman *et al*. 2010), etc. However, the cloning of *FT* gene in potato has not been reported.

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In potato, flowering and tuberizing signals might be similar (Rodríguez-Falcón *et al*. 2006; Jackson 2009). Martínez-García (2002) reported that constitutive overexpression of *AtCO* in transgenic potato plants resulted in an impairment of tuberization under short days. Sarkar (2008) suggested that *AtCO* acts together with *PHYB* and produces the systemic signals consisting of inductive as well as inhibitory signals. A relative balance between these two opposite signals determines flowering or tuberization. As one of the main targets of CO in flowering regulatory pathway, whether FT involves in tuberization regulation in potato? Cloning *FT* gene from potato might be put forward for understanding CO mediated signal pathway of tuberization regulation. In this paper, we reported the results on the cloning of *StFT* cDNA from potato leaf using reverse transcriptase-polymerase chain reaction (RT-PCR) technique and its expression pattern in different tissues of donor plants and *StCOL*-antisense transgenic potato plants.

MATERIALS AND METHODS

Plant materials

In vitro plantlets of the potato cultivar Désirée (*Solanum tuberosum* L.) and *StCOL*-antisense transgenic Désirée (constructed by our laboratory) were transplanted to pots at the beginning of March 2009 and grown in a greenhouse at 23-25°C under a 10-12 h photoperiod, and watered every two days. Two months later, leaves of Désirée plants were collected for cloning of *StFT* cDNA. About three months later, different organs of the two genotypes above were harvested for expression analysis of *StFT*.

Isolation of full-length cDNA and genomic DNA of StFT

Total RNA was extracted from 0.1 g of fresh leaves with total RNA isolation reagent (Tiangen, China) following the manufacturer's instructions. First-strand cDNA was synthesized with M-MLV reverse transcriptase from Promega (USA) according to the manufacturer's instructions.

To clone the conserved region of *StFT* cDNA, a pair of primers, F1 (5′-TTCTACACTCTGGTCATGGTG-3′) and F2 (5′-CGCCACCCTGGAGCATACAT-3′) were designed according to the conserved regions of *FT* genes from other plants using the DNAssist 2.0 software. The PCR product was firstly incubated at 94°C for 5 min, and then incubated by a stepped program (94°C for 30 s, 51°C for 30 s, 72°C for 30 s) for 30 cycles, and by an extension at 72°C for 10 min.

To obtain 5′ end sequence, a primer F3 (5′-ATGCCTAGAGTTGATCCATTGATAG-3′) was designed according to 5′ end sequence of *FT* from *Ipomoea nil* (accession no. EU178860). The PCR was carried out using F3 and F2, according to the following program: 94°C for 4 min, followed by 30 cycles (94°C for 30 s, 55°C for 40 s, 72°C for 40 s) and by extension at 72°C for 10 min.

To obtain 3′ end sequence, a primer F4 (5′-TCATCGTCTCCGGCCTCC-3′) was designed according to 3′ end sequence of *FT* from *Ipomoea nil* (accession no. EU178860). Primary amplification was carried out using F1 and F4, under the following PCR condition: 94°C for 4 min, followed by 30 cycles (94°C for 30 s, 52°C for 30 s, 72°C for 30 s) and by extension at 72°C for 10 min. An aliquot of 1 μL (1:20 diluted) primary amplification products was used for 3′ end nested amplification under the same PCR condition using gene specific primers F5 (5′-CCGGTCAC AGATATCCCAGC-3′) designed according to the conserved region of *StFT* cDNA and F4.

The full length of the gene cDNA was amplified with gene-specific primers F3 and F4 after assembling the full-length sequence of *StFT*. PCR conditions were: 94°C for 4 min, followed by 30 cycles (94°C for 30 s, 53°C for 40 s, 72°C for 40 s) and with a final extension step at 72°C for 10 min.

The genomic DNA sequence of *StFT* was obtained from direct PCR of genomic DNA using the specific primers F3 and F4. PCR was carried out under the following conditions: 95°C for 5 min, then incubated by a stepped program (94°C, 40 s; 53°C, 40 s; 72°C, 2 min) for 30 cycles, and an extension at 72°C for 10 min.

All PCR products were separated on 1% agarose gels and target DNA bands were recovered by gel extraction and cloned into pMD18-T vector (TaKaRa Biotech, Dalian, China), and finally transformed into competent

cells of *E. coli* strain DH5α. White clones were checked by PCR, and the positive clones were sequenced (Invitrogen Biotech, Shanghai, China).

Sequence analysis

Sequencing data accumulation, processing and sequence alignment analysis were performed using DNAMAN. BLASTp was performed on http://www.ncbi.nlm.nih.gov/blast. The open reading frame (ORF) of the sequence was predicted using GenBank ORFfinder (http://www.ncbi.nih.gov/gorf/gorf.html). The protein primary structure (theoretical isoelectric point and mass values) and domains analysis were predicted respectively using ProtParam (http://www.expasy.org/tools/protparam.html) and Smart (http://smart.embl-heidelberg.de/smart/) programs. The tertiary structure of the protein was predicted using online Swiss Model server (http://swissmodel.expasy.org/). The phylogenetic tree was constructed with DNAMAN-TreeView.

Expression analysis of *StFT* in different tissues

For the organ expression studies, white fibrous roots, stems, mature leaves, apical buds, flowers, initial stolons, stolons, swollen stolons (that is stolons with enlarged tips), little tubers, and mature tubers were harvested from both Désirée and *StCOL*-antisense transgenic Désirée plants cultivated in a greenhouse. The materials were frozen immediately in liquid nitrogen and stored at -70°C. A fragment of the potato GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was amplified as a positive control using the primer GAPDHS (5′-TCAACGAGAATGAATACAAGCCA-3′) and GAPDHA (5′-TCGACAACAGAAACATCAGCAGT-3′). The initial denaturation step of 4 min at 94°C was followed by 40 s at 94°C, 40 s at 54°C and 40 s at 72°C for 26 cycles, and the final extension was carried out for 10 min at 72°C. The corresponding amount of cDNA was used as template among samples with *StFT* specific primers F2 and F3 using the same reaction conditions described above, but with only 26 cycles.

RESULTS

Cloning of *StFT* gene

The cloning of *StFT* was stated by amplifying an approximate 230 bp fragment of the conserved region, followed by generation of a 270 bp 3′ fragment and a 400 bp 5′ fragment. The full length of the *StFT* cDNA was obtained using a pair of specific primers designed according to the assembled sequence of the gene (accession no. GU223211). The cDNA contained an open reading frame of 522 bp coding a protein of 173 amino acids, corresponding to a 19.75 kD polypeptide with an isoelectric point of 7.76. Its corresponding genomic sequence was 1 626 bp long and consisted of four exons and three introns (first exon 1-180; first intron 181-491; second exon 492-551; second intron 552-1 145; third exon 1 146-1 186; third intron 1 187-1 481; four exon 1 482-1 626) (Fig.1). This structure was similar to those of other plants, even though there was a big difference in the length of the introns among these species (Table 1).

Fig. 1 Schematic representation of *StFT* gene. A, schematic diagram of the *StFT* cDNA ORF (black boxes) with 3′ and 5′ UTR (thin lines). B, exon-intron organization of the *StFT* gene with location of introns (thin lines) and exons (black boxes). The number 1 indicates the location of the translational start codon as determined. Numbers delineate addresses in nucleic acid sequences.

Table 1 Organization of introns and exons in *FT* genomic sequence from different plants

Name of organisms	Accession no.	No. of introns	Length (bp)	No. of exons	Length (bp)	No. of amino acid residues
Arabidopsis thaliana	NC003070	3	814, 712, 123	4	201, 61, 40, 223	175
Solanum tuberosum	GU223211	3	310, 593, 294	4	195, 59, 40, 231	173
Solanum lycopersicum	AY186737	3	771, 518, 236	4	195, 61, 40, 124	140
Malus × *domestica*	DQ535887	3	105, 190, 801	4	198, 61, 40, 223	174
Brassica napus	FJ848914	3	809, 342, 75	4	201, 61, 40, 223	175
Vitis labrusca × *Vitis vinifera*	EF203919	3	137, 907, 679	4	198, 61, 40, 223	174
Phyllostachys meyeri	AB498761	3	164, 125, 90	4	201, 61, 40, 232	178

Sequence analysis of the StFT protein

An alignment of the predicted amino acid sequences of *S. tuberosum* StFT, *A. thaliana* AtFT (accession no. NP176726), *Ci. unshiu* CiFT2 (accession no. BAF96644), *C. sativus* CsFT (accession no. BAH28253), *M. domestica* MdFT (accession no. ACL98164), and *I. nil* InFT (accession no. ABW73563) was conducted using the DNAMAN program. The result showed that StFT protein had a higher identity with MdFT (80%), CsFT (79%), InFT (77%), CiFT2 (75%), and AtFT (74%) at the overall amino acid level, and especially in their N-and C-terminal regions (Fig.2).

Online Smart Server analysis found that in N-terminal at position 18-163, there was a conserved domain named PBP, a specific phosphatidylethanolamine-binding protein domain (Kardailsky *et al*. 1999), which was similar to the *Arabidopsis* FT protein structure. The tertiary structure of the StFT protein was very similar to *Arabidopsis* FT, too (Fig.3).

A phylogenetic tree was constructed using the sequences of FT/FT-like proteins downloaded from different species from GenBank (Fig.4). The phylogenetic tree analysis showed that all members could be

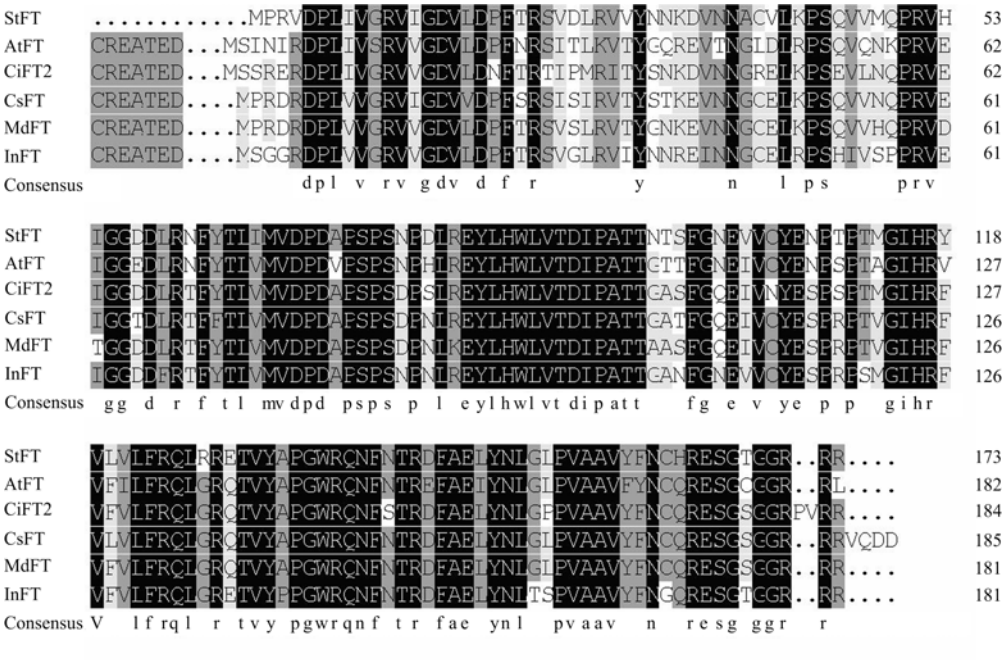

Fig. 2 Alignment of the amino acid sequence of StFT (accession no. ADA77529) with that of other homologous AtFT (accession no. NP176726), CiFT2 (accession no. BAF96644), CsFT (accession no. BAH28253), MdFT (accession no. ACL98164), InFT (accession no. ABW73563). The identical amino acids are shaded in black and the conserved amino acids are in gray.

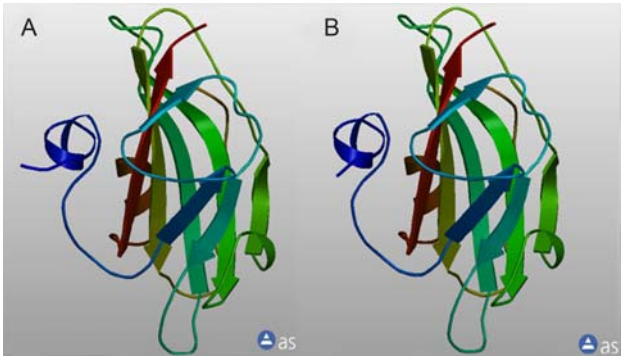

Fig. 3 Tertiary structure model of AtFT1 (A) and StFT (B).

divided into four divergent groups and that the StFT protein was clustered to InFT and had a high homology with CiFT2 from *Ci. unshiu* and AtFT from *A. thaliana*.

Expression of *StFT* gene in different tissues

The results on expression analysis of *StFT* gene in different tissues were presented in Fig.5. *StFT* was expressed in all organs and the level of transcript varied among the different tissues. High level of *StFT* expres-

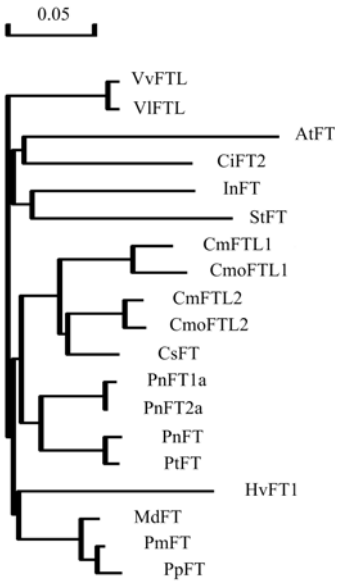

Fig. 4 Phylogenetic analysis of StFT and the FT or FT-like proteins from different plant species. The tree is displayed as a phylogram in which branch lengths are proportional to distance. The proteins are as follows: AtFT (NP176726), CiFT2 (BAF96644), CmFTL1 (ABI94605), CmFTL2 (ABI94606), CmoFTL1 (ABR20498), CmoFTL2 (ABR20499), CsFT (BAH28253), HvFT1 (AAZ38709), InFT (ABW73563), MdFT (ACL98164), PmFT (CAQ16124), PnFT1a (BAD01612), PnFT2a (BAD01561), PnFT (BAD02371), PpFT (ACH73165), PtFT (ABD52003), VlFTL (ABN46891), VvFTL (ABN46890), and StFT (ADA77529).

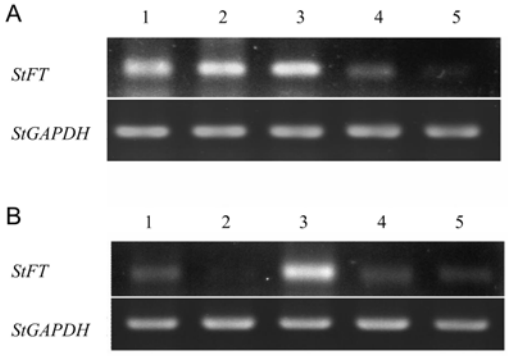

Fig. 5 Expression analysis of *StFT* in different tissues. A: 1, flowers; 2, leaves; 3, apical buds; 4, stems; 5, roots. B: 1, initial stolons; 2, stolons; 3, swelling stolons; 4, small tubers; 5, mature tubers.

sion was observed in leaves, apical buds and flowers. For determining the function of *StFT* in tuberization, the expression of *StFT* in mRNA in tubering organs was detected. mRNA accumulation of *StFT* was obviously increased at the stage of tuber initiation, compared with other stages. This result shows that *StFT* might be involved in the stolon-to-tuber transition of potato.

Expression of *StFT* gene in *StCOL*-antisense transgenic potato plants

To verify the relationship of *StFT* and *StCOL* in tuberization, we analyzed the expression of *StFT* in the tubering organs of *StCOL*-antisense transgenic potato plants. The level of *StFT* transcript in the transgenic plants was so low that it was not detected in all tissues tested, contrasted to in the wild control plants (Fig.6). This result indicates that *StFT* expression is regulated by *StCOL*.

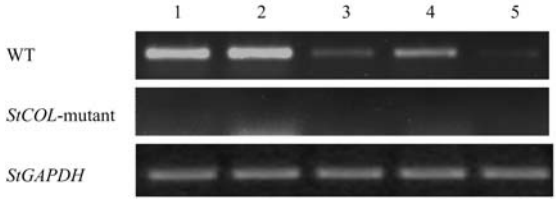

Fig. 6 Expression analysis of *StFT* in mutant of potato. 1, leaves; 2, apical buds; 3, stems; 4, stolons; 5, mature tubers. WT, wild-type; *StCOL*-mutant, mutant of antisense-*StCOL* transgenic potato.

DISCUSSION

It has been known that *AtFT* gene cDNA contained an open reading frame of 528 bp coding a protein of 175 amino acids which possessed a conserved PBP domain and its DNA sequence contained four exons and three introns (Kardailsky *et al*. 1999; Kobayashi *et al*. 1999). Our analysis showed that *StFT* and its coding protein were very similar to *AtFT* and AtFT from *Arabidopsis* in gene and protein structure. Phylogenetic analysis placed the StFT and the AtFT into the same cluster. This result suggests that StFT is a FT homologue.

FT function has been studied in several plants, especially in cereals (Kojima *et al*. 2002; Yan *et al*. 2006), tomato (Lifschitz *et al*. 2006), cucurbits (Lin *et al*. 2007), *Arabidopsis* (Notaguchi *et al*. 2008), and orchid (Hou and Yang 2009), which mainly concerns flower development. In our study, *StFT* transcript was detected in potato leaves, apical buds, flowers, stolons, and tubers. This result reveals that FT might also be involved in other biological processes of plant growth and development, in addition to regulating plant flowering.

Photoperiod is an important environmental factor influencing tuberization in potato. CO is a transcriptional factor situated in the downstream of PHYB and plays a negatively regulating role in potato tuber formation (Rodríguez-Falcón *et al*. 2006). To verify the role of endogene CO in tuberization, we cloned *StCOL* gene (Guo *et al*. 2007) and constructed *StCOL*-antisense transgenic potato plants in previous experiment. The *StCOL* inhibitory expression in the *StCOL*-antisense plants caused normal tuberization under long-day conditions and an increase in tuber number under short-day conditions (data not shown). As one of the targets of CO, FT functions as an integrator of the different flowering regulatory pathways. In order to know whether it also plays a role on tuberization induction, in this study *StFT* expression in the *StCOL*-antisense plants was analysed. *StFT* mRNA was not detected in the stolons and tubers of these plants, while in its wild plants there was expression with variable levels. This result reveals that StFT is in the downstream of StCOL and might be involved in the *StCOL*-mediated regulation of potato tuberization.

CONCLUSION

In this paper, we reported a *StFT* gene cloned from potato cultivar Désirée. The gene was expressed in leaves, apical buds, flowers, and swelling stolons of the donor plants, but its transcript was not detected in all organs examined of *StCOL*-antisense transgenic potato plants, suggesting that *StFT* could be involved in the regulation of potato tuberization as downstream of *StCOL* gene, besides flowering induction.

Acknowledgements

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