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Short communication

Isolation and functional characterization of the *FLOWERING LOCUS T* homolog, the *LsFT* gene, in lettuce

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ABSTRACT

High temperature-induced bolting of lettuce is undesirable agriculturally, making it important to find the mechanism governing the transition from vegetative to reproductive growth. *FLOWERING LOCUS T* (*FT*) genes play important roles in the induction of flowering in several plant species. To clarify floral induction in lettuce, we isolated the *FT* gene (*LsFT*) from lettuce. Sequence analysis and phylogenetic relationships of *LsFT* revealed considerable homology to *FT* genes of *Arabidopsis*, tomato, and other species. *LsFT* induced early flowering in transgenic *Arabidopsis*, but was not completely effective compared to *AtFT*. *LsFT* mRNA was abundant in the largest leaves under flowering-inducible conditions (higher temperatures). Gene expression was correlated with flower differentiation of the shoot apical meristem. Our results suggest that *LsFT* is a putative *FT* homolog in lettuce that regulates flower transition, similar to its homolog in *Arabidopsis*. This is the first information on the lettuce floral gene for elucidating regulation of the flowering transition in lettuce.

Introduction

Lettuce is a popular leafy vegetable that is harvested and consumed during its vegetative growth. The transition from vegetative to reproductive growth is induced by high temperatures (Thompson and Knott, 1933; Fukuda et al., 2009), which is followed by rapid stem elongation and flowering. In lettuce, stem elongation is undesirable agriculturally, signaling the end of its marketability as a vegetable. Hence, preventing stem elongation in lettuce cultivation is important, which may be achievable by determining the mechanism that induces flowering in lettuce. Besides high temperature, exogenous gibberellins (GAs) induce flowering in lettuce. We reported that bioactive GA_1 increased with the upregulation of *LsGA3ox1* expression, which metabolizes GA_{20} to GA_1 in the lettuce stem at high temperature (Fukuda et al., 2009). Waycott and Taiz (1991) and Mazier et al. (2007) demonstrated the importance of GA in regulating lettuce stem growth with GA mutants of lettuce, but they showed a severe dwarf phenotype. Although some options to control *LsGA3ox1* expression or LsGA3ox1 activity and others to reduce GA_1 level only in stems are known, an additional factor related to flowering must be considered to control stem elongation of lettuce.

Flowering is one of the most critical events in higher plants. The genetic networks of flowering have been elucidated in *Arabidopsis* and some other plants (Amasino, 2010). The initiation of flowering in *Arabidopsis* is controlled via four major pathways: photoperiod, vernalization, GA, and autonomous pathways. Floral induction signals from these flowering pathways, except the GA pathway, are transmitted to two central flowering regulators, *CONSTANS* (*CO*) and *FLOWERING LOCUS C* (*FLC*). The *CO* gene mediates the photoperiodic pathway, whereas the *FLC* gene mediates the autonomous and vernalization pathways. These signals converge to regulate genes that are often referred to as 'floral integrators,' *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) and *FLOWERING LOCUS T* (*FT*), which act upstream from the genes involved in floral morphogenesis, such as *APETALA1* (*AP1*) and *LEAFT* (*LFY*) (Jack, 2004). Of these genes, *FT* plays an important role because several flowering pathways are integrated into the regulation of *FT* expression (Corbesier and Coupland, 2006). *FT* genes of many plants have been isolated, and overexpression lines induce early-flowering phenotypes (Hsu et al., 2006; Lifschitz et al., 2006; Hayama et al., 2007; Lin et al., 2007; Kotoda et al., 2010). *FT* is generally expressed in the phloem of leaves (Takada and Goto, 2003) and the FT proteins of some plants are present in the phloem sap, and may act as a mobile flower-inducing signal, such as the proposed 'florigen' signal (Corbesier et al., 2007; Lin et al., 2007; Tamaki et al., 2007).

In lettuce, no reports have described a gene related to the flowering pathway. As described above, *FT* is a potent factor that seems to integrate the flowering signals. In this study, we isolated and

Abbreviations: *FT*, *FLOWERING LOCUS T*; GA, gibberellin; LB, left border; LD, long-day; NJ, neighbor-joining; npt II, neomycin phosphotransferase II gene; OE, overexpression; Pnos, nos promoter; RACE, rapid amplified cDNA ends; RB, right border; SAM, shoot apical meristem; Tnos, 3′ region of nos; ZT, zeitgeber time.

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Table 1
Primers used in this study.

Gene		Sequences
LsFT fragment	Forward	5′-TGGTTGGTBACYGATATHCCWGC-3′
	Reverse	5′-GTRTTGAAVTTCTGRCGCCAYNCC-3′
LsFT fragment (nested)	Reverse	5′-DGSATAMACHGTYTGCCKNCC-3′
RACE	Sense	5′-CCACGGGAGCACGTTTTGGCCAAGA-3′
	Antisense	5′-GGAATAACACAAAAACCATGCGATGA-3′
LsFT full-length cDNA	Forward	5′-CCACGGGAGCACGTTTTGGCCAAGA-3′
	Reverse	5′-GGAATAACACAAAAACCATGCGATGA-3′
LsFT genomic DNA	Sense	5′-AATATAGTGATAGACAATGGATTGCTCC-3′
	Antisense	5′-CTTCGGTTTAACCCCACTATTAAAGG-3′
LsFT transformation	Forward	5′-ATTCTAGAATGATGCCTAGGGAGAGGA-3′
	Reverse	5′-GAGCTCTTATCTTCTTCGCCCACCAAAC-3′
Tubulin qRT-PCR	Forward	5′-GGCAAAATGAGCACGAAAGAG-3′
	Reverse	5′-GATCCATTCCACAAAGTAAGACGAG-3′
LsFT qRT-PCR	Forward	5′-CGATATACCAGCGACCACGGGAGCA-3′
	Reverse	5′-TGACGCCATCCAGGGGCATACACA-3′

Underlined sequences correspond to the *Xba*I/*Sac*I restriction sites, respectively.

characterized the *FT* homolog of lettuce and analyzed transcripts of lettuce *FT* gene to clarify floral induction in lettuce.

Materials and methods

Plant material

Lactuca sativa L. (cv. Leaf Lettuce Green, leaf lettuce, early bolting) seeds were germinated in cell trays filled with soil in growth chambers at 25/15 °C under a 14-h light/10-h dark photoperiod provided by metal halide lamps (MLBOC400C-U, Mitsubishi/Osram) (about 220 $\mu mol\ m^{-2}\ s^{-1}$). Three weeks after sowing, the seedlings were transplanted into 7-cm pots, some of which were transferred to growth chambers at 35/25 °C (light/dark). Leaves were harvested from five plants.

Isolation of an FT-like gene from lettuce

Total RNA was isolated from lettuce leaves using the RNA Plant Mini Kit (QIAGEN) and treated with RNase-free DNase (QIAGEN). First-strand cDNA was prepared using the Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics) with 1 μg of DNase-treated RNA. Nested PCR was performed with degenerate primers. DNA was sequenced using a BigDye terminator cycle sequencing kit (Applied Biosystems) and a 3730xl DNA Analyzer (Applied Biosystems). The sequences were analyzed using MEGA4 software. Rapid amplified cDNA ends (RACE) reactions were done using a Marathon cDNA amplification kit and Advantage cDNA PCR kit (Clontech). End-to-end PCR was carried out to obtain the full-length cDNA of lettuce using 5′- and 3′-end primers. The amplified PCR fragment was cloned into a pCR-Blunt-TOPO vector (Invitrogen) and sequenced. Genomic DNA was isolated from leaves using ISOPLANT (Nippon Gene). PCR was performed to determine the genomic sequence with the above primers using for full-length cDNA and gene-specific sense and antisense primers (Table 1).

Sequence analysis

The *FT* sequence was identified through blast searches in GenBank (http://www.ncbi.nlm.nih.gov/BLAST/) and aligned with *FT* homologs using ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html). The phylogeny based on the amino acid sequences was analyzed using the neighbor-joining method in MEGA4 (http://www.megasoftware.net/) (Tamura et al., 2007). Sequence data from this article have been deposited in the DNA Data Bank of Japan under accession numbers AB602322 (mRNA) and AB602323 (genome).

DNA hybridization

Genomic DNA was isolated from lettuce leaves using the CTAB method, as described by Roger and Bendich (1988). Approximately 10 μg of genomic DNA was digested with *Eco*RV or *Bgl*II, and the resulting fragments were electrophoresed in 1.5% agarose gels and transferred to a Hybond N+ membrane (GE Healthcare) by capillary blotting (Sambrook and Russell, 2001). The full coding sequence of the *LsFT* gene was labeled with alkaline phosphatase using the AlkPhos Direct Labeling and Detection System (GE Healthcare).

Construction of the transformation vector and Arabidopsis transformation

For *Arabidopsis* transformation, wild-type plants (Columbia) were used. The protein-coding region of *LsFT* cDNA was PCR-amplified using primers containing *Xba*I and *Sac*I restriction sites (Table 1). It was cloned directionally into the *Xba*I/*Sac*I sites of the pBI121 binary vector, which is downstream from the CaMV 35S promoter. *Arabidopsis* plants were transformed using the floral dipping method (Clough and Bent, 1998). Kanamycin-resistant transformants were grown at 22 °C under long-day (LD) conditions (16-h light/8-h dark). The T_2 generation was analyzed morphologically. Analysis of variance was performed with SAS (SAS Institute). Means were separated using Tukey's test in SAS.

Analysis of the LsFT transcript in lettuce

Lettuce cDNAs were prepared as described above. Quantitative RT-PCR (qRT-PCR) was performed with a LightCycler 480 (Roche Diagnostics) and the LightCycler 480 SYBR Green 1 Master Kit (Roche Diagnostics) with gene-specific primers (Table 1). The absence of unwanted byproducts was confirmed by automated melting curve analysis. The transcripts were normalized to the expression of the *beta-tubulin* (*BTUB1*) gene.

Results

Identification of the FT-like gene from lettuce

A lettuce *FT*-like sequence was identified by PCR-based cloning using primers designed from the *Arabidopsis*, apple, citrus, poplar, and tomato *FT* sequences in GenBank. One partial cDNA sequence was identified in lettuce and named *LsFT*. RACE was performed using gene-specific primers based on the nucleotide sequence. The predicted coding regions of *LsFT* encoded polypeptides of 175 amino acids, and had a high identity with *FT*-like members, such as *Chrysanthemum* × *morifolium FT*, *SP3D*, *MdFT1*, *CiFT*, and *AtFT* (97, 86, 82, 80, and 76%, respectively) (Fig. 1A and B) (for the accession numbers, see the legend of Fig. 1). The clone was characterized by DNA blot analysis using the full-length cDNA as a probe (Fig. 1C). A single band was detected in the *Bgl*II digest and two bands were detected in the *Eco*RV digest because of a restriction site in the third intron (data not shown). These results suggest that only one copy of the *FT* gene exists in lettuce. The genomic sequence of *LsFT* consisted of 201, 62, 41, and 224 bp exons, which resembles the genomic structure of other *FT* genes (Fig. 1D).

Functional complementation of LsFT in Arabidopsis

To assess the biological function of *LsFT*, the transcript was expressed ectopically in *Arabidopsis*. We generated transgenic *Arabidopsis* plants with *LsFT* or *AtFT* under control of the CaMV 35S promoter and a gene for kanamycin resistance to transgenic selection (Fig. 2A). We obtained more than 10 independent transgenic lines for Pro35S:*AtFT* and Pro35S:*LsFT*, which were selected for