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CsTFL1b may regulate the flowering time and inflorescence architecture in cucumber (*Cucumis sativus* L.)

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ABSTRACT

Cucumber is an important vegetable with indeterminate growth habit which is beneficial to its yield. In this study, we cloned the *TFL1* homolog *CsTFL1b* in cucumber. CsTFL1b shares highly sequence similarity to TFL1 from *Arabidopsis* and has conservative histidine amino acid residue which is necessary for TFL1 function. However, phylogenetic analysis suggested that cucurbits TFL1s (CsTFL1b of cucumber and CmTFL1 of melon) formed a subclade which is far from the AtTFL1 in *Arabidopsis* or CEN in *Antirrhinum*. *CsTFL1b* was highest expressed in male flower but barely expressed in SAM which was different from *TFL1* in *Arabidopsis* with highly transcription accumulation in SAM and *CsTFL1b* was located in nucleus and cytoplasm. Upon ectopic expression of *CsTFL1b* in *Arabidopsis*, the flowering time of transgenic plants was significantly delayed in both wild type and *tfl1-11* mutant background but the terminal flower phenotype of *tfl1-11* mutant was partially rescued. These results may underlie the discrepant function of *CsTFL1b* in cucumber from that in *Arabidopsis*.

1. Introduction

Flower development is a highly complex process in which shoot apical meristem (SAM) accepts various environment signals and endogenous signals and flower transition promoters such as *LEAFY* (*LFY*) and *APETALA1* (*AP1*) were stimulated to produce the flower meristems (FM) [1–3]. However, it is not a single gene but the interaction involved numerous genes specify the meristem fate [4,5]. Among these, the floral integrator *FLOWERING LOCUS T (FT)* which belongs to phosphatidyl ethanolamine-binding proteins (PEBPs) family plays a key role in controlling flower transition and FT protein has been shown to act as a mobile signal transported from leaves to the shoot apex to promote flowering [6,7]. On the contrary, *TERMINAL FLOWER 1* (*TFL1*) have antagonistic role to *FT* and acts as a suppressor of flower regulation although TFL1 also belongs to PEBP family [8,9]. There have been reported that a single amino acid of H (Histidine) 88 and the relevant Y (Tyrosine) 85 is necessary for TFL1 and FT to act as a suppressor and promoter [10]. In addition, both TFL1 and FT need the existence of the bZIP protein FD to perform their function of suppressing and promoting flower transition, respectively [11,12].

In *Arabidopsis*, *TFL1* is expressed in the central region of SAM whereas TFL1 protein is a mobile signal and controls flowering in the whole SAM [13–15]. Overexpression of *TFL1* results in the late flowering and both the vegetative growth and reproductive growth stage were obviously extended [8]. On the contrary, the flowering time of *tfl1* mutant was earlier than the wild type and transformed the indeterminate growth habit to determinate growth habit by formed the terminal flower structure in *Arabidopsis* [16,17]. Similarly, the corresponding *centroradialis* (*cen*) mutant of *Antirrhinum* also converted the indeterminate inflorescence to determinate inflorescence and produced the terminal flower structure [18]. In tomato, the *TFL1* homolog *SELF-PRUNING* (*SP*) plays an important role in regulating the switching of vegetative to reproductive growth stage [19]. The *TFL1* homologs of rice and maize were also involved in regulating flowering time and inflorescence architecture [20,21].

Cucumber (*Cucumis stativus* L.) is an important vegetable and cultivated all over the world. However, the vegetative growth and reproductive growth stage of cucumber can't be clearly divided as that of *Arabidopsis*. In cucumber, leaves were sequentially produced from SAM with flower buds initiated in axil after a short juvenile stage [22]. In production, growth habit is very important to the yield of cucumber. Cucumber with indeterminate growth habit will

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extend the harvest time and produced more fruits than that with determinate growth habit. At present, *TFL1* genes have been reported to play an important role in regulating inflorescence structure and flowering time in many species [8,9]. However, the role of *TFL1* gene in cucumber is still unknown.

In this study, phylogenetic analysis, expression analysis and genetic transformation methods were performed to explore the function of the *TFL1* homolog *CsTFL1b* in cucumber. Phylogenetic tree of TFL1 proteins suggested that CsTFL1b was far from the AtTFL1 in *Arabidopsis* or CEN in *Antirrhinum* although all of them have the conserved histidine amino acid residue. RT-PCR results indicated that *CsTFL1b* was expressed highest in male flowers and lowest in SAM which was different from *TFL1* in *Arabidopsis* [13]. Ectopic expression of *CsTFL1* in *Arabidopsis* leads to the delayed flowering time in both WT and *tfl1-11* mutant background but the terminal flower structure of *tfl1-11* mutant can't be fully rescued. These results may underlie the discrepant function of *CsTFL1b* in flowering regulation of cucumber.

2. Materials and methods

2.1. Plant materials and growth conditions

The northern China type cucumber inbred line R1461, similar to the Chinese long 9930 which was sequenced in 2009 [23], was used for expression analysis and grown in the greenhouse of China Agricultural University under standard water management and pest control. The Columbia (Col) *Arabidopsis* and the *tfl1-11* mutant [24] were obtained from the *Arabidopsis* Biological Resource Center (ABRC) and grown in a growth chamber: 16 h/8 h of light/dark at 22 °C.

2.2. Gene cloning and structure analysis

Quick RNA isolation Kit (Waryoung, Beijing, China) was used to extract Total RNA from the male flowers of cucumber line R1461 and TianScript II RT Kit (Tiangen Biotech, Beijing, China) was used for synthesizing cDNA. The full-length coding region of *CsTFL1b* (GeneBank accession number: NP_001267654.1) was amplified by gene-specific primers (Supplemental Table 1). The online software GSDS 2.0 (http://gsds.cbi.pku.edu.cn/) was used to analyze the gene structure of *AtTFL1* and *CsTFL1b*.

2.3. Protein alignment and phylogenetic analysis

To obtain the TFL1 homologs in cucumber and other species, the full-length amino acid sequence of AtTFL1 was used in *Cucurbitaceous* species (http://www.icugi.org/) [23] and online BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST/). ClustalW in the MEGA5 software and the BoxShade web site (http://www.ch.embnet.org/software/BOX_form.html) were used to perform protein alignment. The neighbor-joining (NJ) method with 1000 bootstrap replicates in MEGA5 software was used to perform the phylogenetic analysis [25]. Gene information used in phylogenetic analysis was listed in Supplemental Table 2.

2.4. Quantitative real time RT-PCR

Quick RNA isolation Kit (Waryoung, Beijing, China) was used to extract total RNA of different cucumber organs and *Arabidopsis* young inflorescences. cDNA was synthesized by the TianScript II RT Kit (Tiangen Biotech, Beijing, China). Quantitative real time RT-PCR experiments were performed by ABI PRISM 7500 Real-Time PCR System (Applied Biosystems, USA). Three biological and three technical replicates were performed in each gene. The cucumber *Ubiquitin extension protein* (Csa000874) and *Arabidopsis ACTIN2* (AT3G18780) genes were used as internal references. The primers information for RT-PCR was listed in Supplemental Table 1.

2.5. Subcellular localization

The *CsTFL1b* coding sequence without the termination codon was associated to the GFP coding sequence, and then the fused fragment was inserted into the plasmid pUC-SPYNE through SpeI and SmaI cleavage sites. Onion (*Allium cepa*) epidermal cells were used to perform the subcellular localization as previously described [26]. The confocal laser-scanning microscope (Carl Zeiss LSM 510, Germany) was used to take images excited at a 488 nm wavelength. The primer information for subcellular localization was listed in Supplemental Table 1.

2.6. Ectopic expression of CsTFL1b in Arabidopsis

To make the *CsTFL1b* overexpression construct, the full-length of *CsTFL1b* coding sequence was amplified and inserted into the pBI121 binary vector through XbaI and XmaI cleavage sites. The recombinant construct was transferred into Agrobacterium by electroporation and then transformed into Col (WT) and *tfl1-11* mutant *Arabidopsis* by the floral dip method [27]. Murashige and Skoog (MS) medium with 40 mg/L kanamycin was used to screen the transgenic plants. The primers information for vector construction was listed in Supplemental Table 1.

3. Results

3.1. Cloning of CsTFL1b in cucumber

Previously, six PEBP family members had been reported in cucumber [28]. However, we performed BLAST search in the Cucurbit Genomics Database [23] using the full amino acid sequence of PEBP family members in *Arabidopsis* and found that there are seven PEBP family members were really existed (Supplemental Table 3). Amino acid sequences comparison of PEBP family members between cucumber and *Arabidopsis* suggested that Csa3G776350 shared the highest similarity (71.91%) to AtTFL1 (Supplemental Table 3). Therefore, we chose Csa3G776350 for the further study and named Csa3G776350 as CsTFL1b consistently [28].

Next, Cucumber male flowers were taken as template to clone the full coding region sequence and genome sequence of *CsTFL1b*. Gene structure analysis suggested that CsTFL1b encodes 178 amino acids and contains four exons and three introns which is similar to that of AtTFL1 in *Arabidopsis* (Fig. 1A). According to the protein alignment performed by ClustalW in the MEGA5 software, CsTFL1b shared 71.91%, 71.58% and 70.79% sequence similarity with TFL1 homologs from *Arabidopsis* (AtTFL1), *Antirrhinum* (CEN) and tomato (SP), respectively (Fig. 1B). Previous research has shown that histidine 88 of TFL1 and tyrosine 86 of FT is very important for each function in *Arabidopsis* [10]. Conformably, CsTFL1b and TFL1 homologs (AtTFL1, CEN and SP) have conservative histidine in the corresponding position (thin red rectangle) (Fig. 1B). On the contrary, AtFT and FT homolog from rice (Hd3a) have conservative tyrosine in the relevant position (Fig. 1B).

3.2. Phylogenetic analysis of TFL1 homologs

In order to investigate the genetic relationship between CsTFL1b and TFL1 homologs from other species, the phylogenetic tree was constructed using 40 species including cucumber, melon (CmTFL1), *Arabidopsis* (AtTFL1) and *Antirrhinum* (CEN). As shown in Fig. 1C, cucurbits TFL1s (CsTFL1b of cucumber and CmTFL1 of melon)

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