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A conserved role of *SHORT VEGETATIVE PHASE* (*SVP*) in controlling flowering time of *Brassica* plants

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Abstract

The control of flowering time in *Brassica* plants is an important approach for improving productivity, as vegetative tissues are not produced after the floral transition in *Brassica* plants. In order to determine the feasibility of modulating flowering time in Chinese cabbage plants, genes homologous to *Arabidopsis SHORT VEGETATIVE PHASE* (*AtSVP*) were isolated from spring-type and fall-type cultivars of Chinese cabbage plants, and their functions were determined. Their deduced amino acid sequences were 91–93% identical with that of AtSVP. The expression of *BcSVP* was ubiquitously detected, and was unaffected by vernalization. Constitutive *BcSVP* expression induced late flowering with additional floral defects. This delayed flowering was attributed to the repression of *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*). *BcSVP* expression under the control of the *AtSVP* promoter also resulted in the complementation of the *svp* mutation in *Arabidopsis*. These results indicate that *BcSVP* is a functional equivalent of *AtSVP* and also suggest that *BcSVP* may prove useful for the genetic manipulation of flowering time in *Brassica* plants.

Keywords: Chinese cabbage; *BcSVP*; Vernalization; Floral repressor; Flowering time; Genetic manipulation

1. Introduction

The genus *Brassica,* which includes the cabbage, Chinese cabbage, cauliflower, mustard, and rape, contains more important agricultural and horticultural plants, and is of particular economic importance in Asia. *Brassica* plants are frequently served as freshly cooked vegetables in stir-fry dishes as well as in processed forms, such as pickled, and over 40 million tons of *Brassica* plants are consumed annually in Asia [1]. In addition, *Brassica* plants are utilized in a variety of practical applications: the anti-carcinogenic compounds harbored by *Brassica* plants are employed in medical treatments, and rapeseed oil has been identified as a potential fuel substitute [2,3].

The production of vegetative tissues in *Brassica* plants has been closely associated with the changes in flowering time, as the majority of *Brassica* plants are annual plants and vegetative tissues are not produced after flower development. This characteristic has led to many attempts to control flowering times of *Brassica* plants. One approach has been to extend vegetative growth by delaying flowering with the aim of increasing the production of plant biomass [4]. Another approach has been to accelerate flowering and thus shorten the life cycle in order to facilitate introgression of the useful agronomic traits of wild relatives of *Brassica* plants that may prove useful for molecular breeding, such as male sterility, disease resistance, and tolerance against harsh environmental conditions [5]. The modulation of flowering time is, therefore, considered to be a practical means to improve the agronomic value of *Brassica* plants.

In *Arabidopsis*, a close relative of the Chinese cabbage, a variety of genes that control flowering time have been identified, revealing an intricate network of signaling pathways operant in flower development [6]. Given the close phylogenetic relationship and the physiological similarities between *Arabidopsis* and *Brassica* plants, it appears likely that the functions of the genes involved in the regulation of flowering time tend to be conserved. For example, two major quantitative

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trait loci (QTL) that exert significant effects on flowering time in *Brassica* plants are collinear with the *FRIGIDA* (*FRI*) and *FLOWERING LOCUS C* (*FLC*) loci, which have important functions in distinguishing winter-annual habits and summer-annual habits in *Arabidopsis* [7,8]. Several *FLC*-related genes isolated from *Brassica* species appear to affect flowering time in *Arabidopsis* and *B. rapa* [9,10]. These findings support the view that the structures and functions of flowering time genes are largely conserved between *Arabidopsis* and *Brassica* species.

We report here the characterization of *BcSVP*, a gene cloned from Chinese cabbage plants, and subsequently determined to be homologous to *SHORT VEGETATIVE PHASE* (*SVP*) in *Arabidopsis*. We assessed the temporal and spatial expression patterns of *BcSVP* in Chinese cabbage plants. We determined the function of *BcSVP* by introducing *BcSVP* into *svp* loss-of-function mutants and by assessing the overexpression of *BcSVP* in transgenic *Arabidopsis* plants. Our results suggest that *BcSVP* is a functional equivalent of *AtSVP* and that the function of *SVP* genes in controlling flowering time is conserved in Chinese cabbage and *Arabidopsis* plants.

2. Materials and methods

2.1. Plant material and growth conditions

Two spring-type cultivars (Norangbom and Yeorumsinkwan) and one fall-type cultivar (Jangmi) of Chinese cabbage plants (*Brassica campestris* L. ssp. *Pekinensis*) were used in this study. For the vernalization treatment, Chinese cabbage plants were grown in a greenhouse for 8 weeks at 23 °C, then at 4 °C for 2–6 weeks, as described previously [11].

Wild-type *Arabidopsis* plants (ecotype Columbia) and transgenic *Arabidopsis* plants were grown in soil or Murashige and Skoog (MS) medium at 23 °C under long-day conditions [16/8-h (light/dark)] with light supplied at an intensity of 120 μmol m^{-2} s^{-1}. *svp-32* [12] was employed for the functional analysis of *BcSVP*.

2.2. Cloning of BcSVP

A full-length open reading frame (ORF) of *BcSVP* was cloned via reverse transcription (RT)-PCR using primers JH4173 (5′-CCATTTCAGTCGTCTTGTCACTCT-3′) and JH4174 (5′- AAAACATCTTCCTCGGACAGAAAC-3′).

2.3. Expression analysis

Gene expression levels were determined via semi-quantitative RT-PCR, as described previously [13]. Total RNA was extracted from a variety of tissues using Trizol reagent (Invitrogen, Carlsbad, Calif.), and first-strand complementary DNA (cDNA) was synthesized from 1 μg of total RNA, in accordance with the manufacturer's instructions (Promega, Madison, Wis.). The following oligonucleotides were employed as gene-specific primers in the RT-PCR analyses: for *BcSVP*, JH2595 (5′-CAGCTGGAAAAGGCCCTTGA-3′) and JH2598 (5′-CGGAGCTCTCGGAGTCAACA-3′); for *BcFLC*, MB73 (5′-GTAGCCGACAAGTCACCTTCTCCA-3′) and JH2926 (5′-GAGATTTGTCCAGGTGACATCT-3′); for 25S rRNA, JH2924 (5′-ATGCCCGCGTCGCAATAG-3′) and JH2925 (5′-TGGATCGTGGCAGCAAGG-3′); for *UBQ10*, JH1011 (5′-GATCTTTGCCGGAAAACAATTGGAGGATGGT-3′) and JH1012 (5′-CGACTTGTCATTAGAAAGAAAGAGATAACAGG-3′). To detect *BcSVP* transcript specifically, JH2595 and JH2598 primers were designed to bind the K and C regions, which are less conserved among MADS box proteins, of *BcSVP*.

To analyze the expression patterns of flowering time genes in transgenic plants, T_1 plants that were selected for Kanamycin resistance and their rosette leaves were harvested for RNA extraction. In the case of T_2 plants, the leaves of plants that showed 3:1 segregation ratio of Kanamycin resistance were harvested for RNA extraction.

2.4. Generation of transgenic plants

We amplified a full-length ORF of *BcSVP* from cDNAs prepared from total RNA and subcloned this amplified product into the pCHF3 binary vector, which harbors the 35S promoter. The resulting recombinant plasmid, pSHP003, which was referred to as *35S::BcSVP* in this study, was then introduced into wild-type *Arabidopsis* plants to assess the constitutive expression of *BcSVP*. For the complementation test, we generated a chimeric plasmid harboring *BcSVP* ORF fused with the *AtSVP* promoter. The 1.7-kb promoter region of *AtSVP* was amplified from a bacterial artificial chromosome (BAC) clone (F14M13) with the primers JH3118 (5′-gaattcGTGGTCGACACTTTTTATTTTA) and JH2600 (5′-tctagaCACAACGAACAAAAAAACCC-3′) (the synthetic restriction enzyme sites are indicated in lowercase lettering). This amplified fragment was then fused with a full-length ORF of *BcSVP*, resulting in the pSHP011 clone, which is referred to as *AtSVP::BcSVP* in this study.

Transgenic plants were generated using the floral dip method, with minor modifications [14]. Flowering time was measured by scoring the total number of primary leaves of at least 10 plants.

3. Results and discussion

3.1. Isolation of BcSVP from Chinese cabbage

We amplified and cloned genes homologous to *AtSVP* from three Chinese cabbage cultivars (*B. campestris*) using oligonucleotides predicted from the conserved sequences in the *SVP* homologues (AY356367 and AY356366) deposited in the GenBank database. We analyzed the sequences of 7–8 independent clones from each cultivar and found that all clones isolated from a single cultivar were identical. When we compared the nucleotide sequences between cultivars, we found that the nucleotide sequences of the ORF regions amplified from the two spring-type cultivars (Norangbom and Yeorumsinkwan) were identical, whereas the nucleotide sequence of ORF region from the fall-type cultivar (Jangmi) differed slightly from that of the spring-type cultivars. The deduced amino acid sequences of the ORF regions isolated from the spring-type and fall-type cultivars showed 93 and 91% identity with AtSVP [15], respectively (Fig. 1A). The amino acid sequences of these clones were 56% identical with that of AGAMOUS-LIKE24 (AGL24) [16], the closest AtSVP homolog in the *Arabidopsis* genome. Phylogenetic analysis showed that these clones were clustered in a group that was distinct from other MADS box proteins, such as BrFLC and BrSOC1 (Fig. 1B). We designated the clones isolated from the spring-type cultivars as *BcSVP*. The nucleotide sequences of these clones from cultivars Norangbom, Yeorumsinkwan, and Jangmi were deposited into the GenBank database as DQ922945, DQ922944, and DQ641675, respectively.

It appeared that the gene structures of *AtSVP* and *BcSVP* are conserved. A comparison of the genomic sequences of *AtSVP* and *BcSVP* [17] revealed that their exon/intron structures and boundaries were conserved (Fig. 1C), with the exception that intron 2 of *BcSVP* was slightly shorter than that of *AtSVP*. A similar genomic structural organization was reported in the *FLC* homologs isolated from *B. rapa* [9], despite the fact that several *FLC* homologs have been identified in *Brassica* species [10,11]. The presence of multiple homologs within the *Brassica* genome