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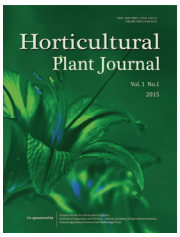

The K Domain Mediates Homologous and Heterologous Interactions Between FLC and SVP Proteins of *Brassica juncea*

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Abstract

The transcription factors FLOWERING LOCUS C (FLC) and SHORT VEGETATIVE PHASE (SVP) can interact to form homologous and heterologous protein complexes that regulate flowering time in *Brassica juncea* Coss. (Mustard). Previous studies showed that protein interactions were mediated by the K domain, which contains the subdomains K1, K2 and K3. However, it remains unknown how the subdomains mediate the interactions between FLC and SVP. In the present study, we constructed several mutants of subdomains K1–K3 and investigated the mechanisms involved in the heterologous interaction of BjFLC/BjSVP and in the homologous interaction of BjFLC/BjFLC or BjSVP/BjSVP. Yeast two-hybrid and *β*-galactosidase activity assays showed that the 19 amino acids of the K1 subdomain in BjSVP and the 17 amino acids of the K1 subdomain in BjFLC were functional subdomains that interact with each other to mediate hetero-dimerization. The heterologous interaction was enhanced by the K2 subdomain of BjSVP protein, but weakened by its interhelical domain L2. The heterologous interaction was also enhanced by the K2 subdomain of BjFLC protein, but weakened by its K3 subdomain. The homologous interaction of BjSVP was mediated by the full K-domain. However, the homologous interaction of BjFLC was regulated only by its K1 and weakened by its K2 and K3 subdomains. The results provided new insights into the interactions between FLC and SVP, which will be valuable for further studies on the molecular regulation mechanisms of the regulation of flowering time in *B. juncea* and other Brassicaceae.

Keywords: *FLC*; *SVP*; protein interaction; flowering time; *Brassica juncea* Coss.

1. Introduction

Brassica juncea Coss. (mustard) is economically important as a vegetable crop in China, an oil crop in India and a condiment crop in Europe. It was selected for canola quality recently in Canada and Australia (Yang et al., 2014). The species possesses unique traits, including very wide morphological variation in leaves, roots, stems, seed stalks and oil types (Qi et al., 2007). The production and quality of *B. juncea* are affected greatly by flowering time, which is regulated

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by complex genetic networks that monitor various environmental and endogenous signals (Boss et al., 2004; Yang et al., 2014). Genetic analyses of flowering control have identified four major floral promotion pathways: The photoperiod and vernalization pathways mediate the response to environmenta signals; whereas the autonomous and gibberellin (GA) pathways appear to act independently of these signals. These pathways eventually converge with the flowering integrators to regulate flowering time (Boss et al., 2004; He and Amassino, 2005; Moon et al., 2005).

The FLOWERING LOCUS C (FLC) and SHORT VEGETATIVE PHASE (SVP) are two important core transcription factors that determine the fate of the flowering integrators (Sheldon et al., 2002; Lee et al., 2007). They form protein complexes that selectively bind to the CArG motif of the integrator promoter region [e.g. to regulate transcription and expression of *SOC1* and *FLOWERING LOCUS T* (*FT*) genes], and delay flowering time (Mathieu et al., 2007; Li et al., 2008; Yant et al., 2009). FLC and SVP are mainly expressed in active regions, such as the stem apex and the root apical meristem (Wigge et al., 2005). The *FLC* gene plays a key role in flowering-time control by responding to signals from *FRIGIDA* (*FRI*) in the autonomous and vernalization pathways (Michaels and Amasino, 2001; Sheldon et al., 2006), while the *SVP* gene is regulated by autonomous, vernalization and GA pathways (Hartmann et al., 2000; Li et al., 2008). The loss of *SVP* function significantly inhibits the late-flowering phenotype of *FRI* in plants with high levels of *FLC* expression. However, the loss of *FLC* function represses the late-flowering phenotype of *35S::SVP*. Therefore, the functions of FLC and SVP are interdependent (Michaels and Amasino, 2001). *Arabidopsis thaliana* FLC can interact with SVP *in vivo* and *in vitro* (Fujiwara et al., 2008; Li et al., 2008; Jung and Müller, 2009; Gregis et al., 2013) to form the heterologous protein complex FLC/SVP, which co-mediates environmental signals (Li et al., 2008). The *Arabidopsis* FLC protein can also form the homodimer FLC/FLC in plants (Helliwell et al., 2006). He et al.(2014) showed recently that AGAMOUS-LIKE16 is a potential partner of flowering repressor complexes targeting *FT*. AGAMOUS-LIKE16 can interact directly with SVP and indirectly with FLC, two proteins that form a complex to repress the expression of *FT*.

In *B. juncea*, Yang et al. (2014) identified four *FLOWERING LOCI C* (*FLC1–FLC3* and *FLC5*) genes. Similarly, in a previous study, we cloned five *FLC* genes (the three respective homologs for *B. napus FLC1*, *FLC2* and *FLC5*, and the other two homologs of *B. napus FLC3*) and one *SVP* gene in *B. juncea*. Amino acid sequence analysis showed that the five *B. juncea FLC*s encoded a total of four FLC proteins: FLC1–FLC3 and FLC5. However, transgenic assays showed that *B. juncea* FLC2 and *B. juncea* SVP (NCBI accession numbers KJ489426 and KJ489427) have predominant functions in regulating flowering in *B. juncea*. Similar to *B. juncea* FLC2, a genetic–genomics approach revealed that BrFLC2 is a major regulator of flowering time in *B. rapa* (Xiao et al., 2013; Li et al., 2015). Hence, *B. juncea* FLC2 and *B. juncea* SVP (respectively named BjFLC and BjSVP in the present study) were used for further experiments on protein interactions.

BjFLC and BjSVP also interact to form a stable heterologous protein complex BjFLC/BjSVP (Tang et al., 2011), as well as homologous complexes BjFLC/BjFLC and BjSVP/BjSVP (Tang et al., 2012, 2013). BjFLC and BjSVP belong to MIKC-type proteins, which have MADS-box (M), intervening (I), keratin-like (K) and C-terminal (C) domains. The M and K domains in MIKC-type proteins are highly conserved compared with the I and C domains (Hartmann et al., 2000; Martinez-Castilla and Alvarez-Buylla, 2003; Kaufmann et al., 2005; Lee et al., 2007; Alexandre and Hennig, 2008).

Tang et al. (2012, 2013) indicated that the K domain is the key domain mediating the protein interactions of BjFLC/BjSVP, BjFLC/BjFLC and BjSVP/BjSVP. Based on the K-domain sequence alignments and α-helix predictions of MIKC-type proteins from *Arabidopsis* and other plant species, the K domains of both FLC and SVP proteins were found to contain three subdomains, K1–K3, each forming a single α-helix (Fan et al., 1997; Yang et al., 2003). Yang et al. (2003) also divided the K domain of APETALA3 and PISTILLATA into K1–K3 subdomains and found that each subdomain made different contributions to the interaction of APETALA3/PISTILLATA in *Arabidopsis*. However, it is unclear how the subdomains mediate the homologous and heterologous protein interactions of FLC and SVP. It also remains unknown whether the interactions of K1–K3 in FLC/SVP are similar to those of APETALA3/PISTILLATA.

Hence, we constructed several mutants of the K subdomains in BjFLC and BjSVP, screened subdomains that mediated the homologous and heterologous interactions, and analyzed the molecular mechanisms involved in target sequence-specific recognition. The findings have significant implications for the regulation of FLC–SVP polymerization as well as flowering signal integration.

2. Materials and methods

2.1. Material

Mustard and yeast recombinant plasmids pGADT7BjFLC, pGADT7BjSVP, pGBKT7BjFLC, and pGBKT7BjSVP were provided by the Key Laboratory of Horticulture Science for Southern Mountainous Regions, Chongqing, China. The Matchmaker™ Gold Yeast two-hybrid system and amino