Conservation and divergence of four kiwifruit SVP-like MADS-box genes suggest distinct roles in kiwifruit bud dormancy and flowering

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Abstract

MADS-box genes similar to Arabidopsis SHORT VEGETATIVE PHASE (SVP) have been implicated in the regulation of flowering in annual species and bud dormancy in perennial species. Kiwifruit (Actinidia spp.) are woody perennial vines where bud dormancy and out-growth affect flower development. To determine the role of SVP-like genes in dormancy and flowering of kiwifruit, four MADS-box genes with homology to Arabidopsis SVP, designated SVP1, SVP2, SVP3, and SVP4, have been identified and analysed in kiwifruit and functionally characterized in Arabidopsis. Phylogenetic analysis indicate that these genes fall into different sub-clades within the SVP-like gene group, suggesting distinct functions. Expression was generally confined to vegetative tissues, and increased transcript accumulation in shoot buds over the winter period suggests a role for these genes in bud dormancy. Down-regulation before flower differentiation indicate possible roles as floral repressors. Over-expression and complementation studies in Arabidopsis resulted in a range of floral reversion phenotypes arising from interactions with Arabidopsis MADS-box proteins, but only SVP1 and SVP3 were able to complement the svp mutant. These results suggest that the kiwifruit SVP-like genes may have distinct roles during bud dormancy and flowering.

Key words: Actinidia species, AGL24, Arabidopsis, budbreak, bud dormancy, flowering, hydrogen cyanamide, kiwifruit, SVP.

Introduction

Flowering in trees is often associated with bud endodormancy, a physiological state typical for most perennials, where growth is repressed by plant growth regulators within the bud (Bohlenius et al., 2006; Hsu et al., 2011). Plants that are endodormant will not resume growth even when placed in favourable environments, with adequate moisture, long photoperiods, and warm temperatures. In the perennial kiwifruit (Actinidia spp.) vines, flowering is spread over two growing seasons (Walton et al., 2001). During the first season, meristems with a potential to differentiate flowers, are initiated in the lateral buds of developing shoots (Walton et al., 1997; Snelgar et al., 2007). By autumn, these lateral buds have ceased growth and become dormant. A period of low temperatures or treatment with dormancy-breaking chemicals is needed to release dormancy. Flower differentiation commences as kiwifruit buds resume growth during the spring of the second growing season (Brundell, 1975; Polito and Grant, 1984; Walton et al., 1997, 2001).

In order to understand the molecular mechanisms of dormancy and flowering in kiwifruit, a study of MADS box genes with potential roles in dormancy and flowering was undertaken. Two closely related MADS-box genes, SHORT VEGETATIVE PHASE (SVP) and AGAMOUS-LIKE 24 (AGL24), have been implicated in floral transition and
development in Arabidopsis. SVP functions in the maintenance of vegetative shoot identity, while AGL24 promotes inflorescence identity. The svp mutant flowers early, while constitutive expression of SVP in Arabidopsis delays flowering and affects flower development by converting flowers into shoot-like structures and giving rise to leaf-like sepals (Hartmann et al., 2000; Gregis et al., 2006). By contrast, agl24 mutants are late flowering, while ectopic expression of AGL24 in Arabidopsis accelerates flowering, but also results in the development of leaf-like sepals and secondary flowers, mimicking the mutant Arabidopsis ap1-I phenotype (Yu et al., 2002; Masiero et al., 2004; Liu et al., 2008). Genetic interaction models have shown that a complex of SVP and AGL24 genes, together with SUPPRESSOR OF OVER-EXPRESSION OF CONSTANS1 (SOC1), are necessary to prevent premature differentiation of floral meristems and to determine the appropriate timing of floral organ development (Gregis et al., 2009; Liu et al., 2009; Lee and Lee, 2010). The mechanism of SVP and AGL24 action was established through interactions with other proteins in complexes that play important roles during various phases of plant development. In the vegetative phase, a floral repressor complex, comprised of SVP and FLOWERING LOCUS C (FLC), down-regulates the key flowering genes SOC1 and FLOWERING LOCUS T (FT) to suppress flowering (Lee et al., 2007b; Li et al., 2008), while AGL24 promotes SOC1 expression during the floral transition (Michaels et al., 2003; Liu et al., 2008). In the early stages of flower development, SVP and AGL24 act redundantly and interact with APE-TAL1 (API) to maintain floral meristem identity through direct repression of B, C, and E class floral homeotic genes (Gregis et al., 2006, 2008, 2009; Liu et al., 2009).

Considerable effort has been made to identify and characterize SVP- and AGL24-like genes in many monocot and dicot, and annual and perennial species. Genes with homology to SVP and AGL24 have been studied in potato (Carmona et al., 1998; Garcia-Maroto et al., 2000), Chinese cabbage (Lee et al., 2007a), ryegrass (Petersen et al., 2006), barley (Trevisakis et al., 2007), rice (Fornara et al., 2008), and the woody perennials, sapphire dragon tree (Prakash and Kumar, 2002) and eucalyptus (Brill and Watson, 2004). Furthermore, SVP-like genes have been associated with the onset and/or release of endodormancy in raspberry (Mazzitelli et al., 2007), Japanese apricot (Yamane et al., 2008; Sasaki et al., 2011; Yamane et al., 2011), leafy spurge (Horvath et al., 2010), grapevine (Diaz-Riquelme et al., 2009), and citrus (Li et al., 2010). Most notably, the deletion of six SVP-like genes (PpDAM1–6) resulted in a complete lack of dormancy in the terminal shoot meristems in the peach evergrowing (evg) mutant (Bielenberg et al., 2004, 2008). These genes had a different pattern of expression over the growing season in the wild-type peach, with PpDAM5 and PpDAM6 showing elevated expression during endodormancy and rapid down-regulation upon application of the dormancy breaking chemical, suggesting that these genes play a role in the establishment and maintenance of bud endodormancy (Li et al., 2009; Yamane et al., 2011).

To study the role of SVP-like genes in dormancy and flowering of kiwifruit, four MADS-box genes with homology to Arabidopsis SVP were identified and characterized by expression analysis, ectopic transgenic analysis in Arabidopsis and analysis of protein interactions. Seasonal fluctuation that correlated with bud dormancy and growth cycles were observed with some of these genes. The effect of dormancy-breaking chemical application on budbreak, flower differentiation, and SVP-like gene expression was also investigated. Finally, functional conservation in Arabidopsis was investigated and protein interactions were examined to determine the mechanism of SVP-like gene action and to address distinct flowering time and floral morphology phenotypes. Based on these results, the potential roles of kiwifruit SVP-like genes are postulated.

Materials and methods

Plant material

Kiwifruit plant material used in this study was collected from a female cultivar ‘Hayward’ (Actinidia delicosa (A. Chev.) C.F. Liang et A.R. Ferguson). The kiwifruit vines were grown in the field under natural conditions, trained on a T-bar training system and underwent standard orchard management practice. Root, stem, leaf, flower, and fruit tissue collection was carried out on vines growing at the Plant and Food Research orchard near Kerikeri, New Zealand, during the spring and summer season of 2005–2006. Actively-growing apical and axillary buds were collected in early autumn 2006 from vines growing at the Plant and Food Research orchard near Te Puke, New Zealand.

For the annual cycle of bud and flower development, the 1995–1996 sample series of Walton et al. (2001) collected at the Blands Research orchard near Hamilton was utilized and additional samples were collected during 2008–2009 at the Plant and Food Research orchard near Kerikeri. The minimum and maximum daily temperatures were recorded during sampling time. Mean winter temperatures were 10.8 °C and 14.5 °C at Hamilton and Kerikeri, respectively.

To monitor the effect of the dormancy-breaking chemical hydrogen cyanamide (HC), a 5% solution of Hi-Cane® (NuFarm, New Zealand, active ingredient hydrogen cyanamide 520 g l⁻¹) was applied at 700 l ha⁻¹ on plants grown at the Blands Research orchard near Kerikeri on 23 August 2004. Bud samples were collected weekly from HC-treated and non-treated vines for seven weeks. The buds were dissected and their length was measured before RNA extraction. Bud length above 15 mm was recorded as budbreak.

Identification and phylogenetic study of kiwifruit SVP-like genes

Kiwifruit SVP-like genes were identified from the Plant and Food Research Actinidia EST database (Crowhurst et al., 2008) by BLAST alignment (Altschul et al., 1990) to Arabidopsis SVP and further amplified from A. delicosa bud cDNA using gene-specific oligonucleotide primers (see Supplementary Table S1 at JXB online). Sequence alignment was performed using Vector NTI version 9.0. (Invitrogen, Carlsbad, CA) Clustal W (opening 15, extension penalty 0.3). Phylogenetic analyses were conducted using MEGA version 4.0 (Kumar et al., 2004), using a minimum evolution phylogeny test with 1000 bootstrap replicates and above 50% cut-off value for the condensed tree. The predicted amino acid sequences used in phylogenetic analyses were: Arabidopsis SVP (AT2G22450), AGL24 (AT4G24640), potato SMADS11 (AF008652), SMADS16 (AY643736), rice OsMADS22 (AB107957), OsMADS47 (AY345221), OsMADS55 (AY345223), barley HvBMI1 (AJ249142), HvBIM10 (EF043040), Chinese cabbage BcSVP (DQ922945),
eucalyptus EgrSVP (AY273873), Japanese apricot PmDAM6 (AB437345), leafy spurge EeCDAM2 (EU339320), trifoliate orange CsSVP (FJ373211), peach PpDAM1, PpDAM2, PpDAM3, PpDAM4, PpDAM5, and PpDAM6 (Bielenberg et al., 2006), sapphire dragon tree PKMADS1 (AF060880), grape VvSVP1, VvSVP4, VvSVP5, VvSVP6, VvSVP7, VvSVP8, VvSVP9, VvSVP10 (Diaz-Riquelme et al., 2009), apple MdMADS16 (EBI14714), MdMADS20 (EBI37980), MdMADS25 (CO723380), MdMADS201 (CO899324), and poplar PtMADS7, PtMADS21, PtMADS26, PtMADS27, PtMADS28, PtMADS29, PtMADS47, PtMADS48 (Leseberg et al., 2006). Arabidopsis FLC (AT5G10140), API (AT1G69120), and SOC1 (AT2G45660) were used as an outgroup.

**RNA extractions and quantitative RT-PCR (qRT-PCR) analysis**

Total RNA was extracted from kiwifruit tissue as described by Chang et al. (1993). Total RNA was isolated from Arabidopsis seedlings using the Trizol reagent (Invitrogen, Carlsbad, CA). Five μg of total RNA were treated with DNaseI (Ambion, Austin, TX) and reverse-transcribed at 50 °C using Superscript III (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. RNA and cDNA were stored at −80 °C for long-term use.

Gene-specific primers for qRT-PCR (see Supplementary Table S2 at JXB online) were designed using Primer 3 software (Rosen and Skaltsky, 2000) to amplify products between 150–300 bp in size. Kiwifruit Actin (FG403300; Walton et al., 2009), was used as a standard for qRT-PCR analyses performed on kiwifruit cDNA, based on low variability of expression and stability index values similar to the stable reference genes proposed in poplar (Brunner et al., 2004). Similar expression profiles were detected when using kiwifruit ubiquitin and alpha-tubulin (see Supplementary Fig. S1 at JXB online). Arabidopsis Actin (At3g18780) was used as a standard for transgenic plant analyses. Amplification and quantification were carried out using the FastStart DNA Master SYBR Green I reaction mix and the Lightcycler 1.5 instrument (Roche). PCR reaction mix and parameters were as described previously by Walton et al. (2009). Each PCR reaction was performed in triplicate and a non-template control was included in each run. PCR reaction efficiency for each primer pair was determined by a standard curve and a non-template control was included in each run. PCR reaction efficiency for each primer pair was determined by a standard curve and a non-template control was included in each run. PCR reaction efficiency for each primer pair was determined by a standard curve and a non-template control was included in each run. PCR reaction efficiency for each primer pair was determined by a standard curve and a non-template control was included in each run.

**Results**

**Identification and phylogenetic analysis of kiwifruit SVP-like genes**

Four Actinidia chinensis MIKC-type II MADS-box genes with homology to SVP genes were identified from the Actinidia EST database (Crowhurst et al., 2008). A near-identical sequence was also identified from a closely related A. delicosa and additional identical or near-identical sequences were amplified from A. delicosa cDNA (Table 1). Each candidate gene has a single long open reading frame encoding a predicted protein of between 216 and 233 amino acids, comprising the MADS-box, I-box, K-box, and C-terminal domains. The similarity with Arabidopsis SVP and AGL24 is most prominent in the MIK region, but similarity is also detected in the less conserved C-terminal end of predicted proteins (Fig. 1A). The sequence analysis using deduced amino acid sequences of kiwifruit SVP-like genes revealed that kiwifruit SVP1 was most similar to Arabidopsis SVP, with 71% identity. SVP2 and SVP3 are closely related, sharing 88% identity, and are also similar to both Arabidopsis SVP and AGL24, with approximately 60% identity. SVP4 shares 58% identity with Arabidopsis SVP and is most similar to the potato StMADS11, with 61% identity. Predicted proteins of all four kiwifruit SVP-like genes also shared high amino acid identity with AGL24 (see Supplementary Table S2 at JXB online). Further evidence of possible evolutionary association was seen when predicted SVP-like proteins from other plant species were considered (Fig. 1B). The phylogenetic study indicated that four kiwifruit SVP-like genes fell into three
distinct sub-clades. SVP1 is closely related to Arabidopsis SVP, along with SVP-like members from some woody perennial species (grape, eucalyptus, sapphire dragon tree, citrus, and poplar). SVP2, SVP3, and two poplar MADS box genes form a distinct sub-clade, and SVP4 is closely related to the members of the StMADS11 clade, which includes five poplar MADS box genes, three grape SVP-like, and a leafy spurge dormancy-associated MADS-box (DAM2). Predicted proteins of six peach PpDAM genes and the Japanese apricot PmDAM6 genes form a separate sub-clade within the eudicot SVP-like group, closely related to three apple MADS box genes. The SVP-like genes from rice and

Table 1. Kiwifruit (Actinidia) SVP-like genes

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<th>Gene name</th>
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<th>Kiwifruit species</th>
<th>Total EST numbers</th>
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<td>3</td>
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<td>A. delicosa</td>
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<td>JF838213</td>
<td>A. delicosa</td>
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<td>JF838215</td>
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</table>

Fig. 1. Comparison of predicted SVP-like protein sequences. (A) Alignment of kiwifruit SVP-like amino acid sequences with Arabidopsis SVP, AGL24, and potato StMADS11. The amino acid residues in the alignment are coloured according to the following scheme: white on black, identical or conservative residue; white on grey, similar residues; black on white, non-similar or weakly similar residues. (B) Phylogenetic tree based on the amino acid alignment of kiwifruit SVP-like predicted proteins and SVP-like proteins from other plant species. The tree was generated with MEGA4.1 software, using the Neighbor–Joining method and 1000 bootstrap replicates. Bootstrap values above 50% are indicated. At, Arabidopsis thaliana; Bc, Brassica campestris; Ee, Euphorbia esula; Egr, Eucalyptus grandis; Hv, Hordeum vulgare; Os, Oryza sativa; Pk, Paulownia kawakamii; Pm, Prunus mume; Pp, Prunus persica; Ct, Poncirus trifoliata, syn. Citrus trifoliate; St, Solanum tuberosum; Vv, Vitis vinifera; Md, Malus × domestica Borkh.; Pt, Populus trichocarpa. Kiwifruit SVP-like genes are indicated by arrows.
barley form another independent sub-clade, the monocot SVP-like gene group.

Expression profiles of kiwifruit SVP-like genes

Transcripts of all four kiwifruit SVP-like genes accumulated in vegetative tissues, but most prominently in buds, especially SVP1 and SVP2 (Fig. 2A). The transcripts were mostly absent from the flower and fruit tissues. SVP3 accumulated to similar amounts in the bud, stem, and leaf tissue and SVP4 was most abundant in leaf and bud tissue. In general, accumulation was relatively low compared with kiwifruit actin.

Further, expression analysis was performed using axillary buds, which represent sites of kiwifruit inflorescence and floral initiation and development. Distinct profiles were revealed in axillary buds collected in regular intervals over the period of one year and repeated in two different years at different locations (Fig. 2B). All four kiwifruit SVP genes were expressed at low levels during the summer and early autumn (December to March). The level of SVP1 transcript increased during the winter in both seasons and locations, peaking in the early spring (before budbreak), and then declined. Transcription accumulation started earlier in the samples collected from the colder region. The levels of SVP2 and SVP4 transcripts increased in the autumn, remained at elevated levels during winter, and declined in spring. This decline occurred earlier than that detected for SVP1. The accumulation of SVP4 transcript was more prominent in the colder growing region. No significant changes in the accumulation of the SVP3 transcript were detected.

Kiwifruit SVP-like genes are down-regulated before flower differentiation

The application of hydrogen cyanamide (HC) can advance kiwifruit budbreak and flowering by two to three weeks (Walton et al., 2009) and provided an opportunity to see if reduced accumulation of SVP-like genes correlated with advances in budbreak and flower differentiation.

To monitor bud development with and without HC application, buds were dissected and measured. The recorded budbreak was approximately 35 d from application on HC-treated vines and 49 d on non-HC-treated vines (Fig. 3A). The kiwifruit cell cycle gene, CDKB1 (Walton et al., 2009), was used to monitor cell division. An increased transcript level of the CDKB1 gene was detectable at 14 d

![Fig. 2. Expression profiling of kiwifruit SVP-like genes. (A) Relative expression of kiwifruit SVP-like genes in root, stem, leaf, flower, fruit, shoot tip, and shoot axillary bud. The level of expression was normalized to kiwifruit Actin. Error bars represent SE for three replicate reactions. (B) Relative expression of kiwifruit SVP-like genes in axillary buds during the growth and dormancy cycle. The solid line represents samples collected from Kerikeri, New Zealand, in 2008–2009, and the dotted line represents samples collected from Hamilton, New Zealand, in 1995–1996. The expression levels were normalized to kiwifruit Actin. Data points represent the mean ± SE of three replicate reactions and are expressed as a ratio to the first November sample point, which was set arbitrarily to 1. The vertical dashed and solid lines indicate budbreak time in Hamilton and Kerikeri, respectively.](image-url)
on HC-treated vines and after 21 d on non-HC-treated vines (Fig. 3B). Ongoing floral differentiation was monitored through increased accumulation of kiwifruit floral organ identity genes (Varkonyi-Gasic et al., 2011). Accumulation of the kiwifruit B class gene PISTILATA (PI) was detectable after 21 d on treated vines and after 35 d on non-treated vines. A small increase in the C class AGAMOUS (AG) transcript accumulation was detected as early as 14 d after application on treated vines and was followed by a significant increase after 35 d (Fig. 3B).

An initial reduction in transcript levels for all kiwifruit SVP-like genes was detected 7 d after HC application and further reduction was detectable up to 21 d post application (Fig. 3C).

Expression of kiwifruit SVP-like genes in Arabidopsis affects flowering time and flower development

SVP in Arabidopsis directly represses the B and C floral homeotic genes to maintain floral meristem identity (Gregis et al., 2009). To investigate the potential role for kiwifruit SVP-like genes in floral meristem identity function, they were expressed under the control of the cauliflower mosaic virus (CaMV) 35S promoter and transformed into wild type, and svp41 and agl24-2 mutant Arabidopsis.

Constitutive expression of kiwifruit SVP-like genes in Col-0 (Fig. 4A) resulted in different degrees of flowering time phenotype compared with wild-type Col-0, 35S:SVP and 35S:AGL24 plants used as controls. Expression of kiwifruit SVP1 and SVP3 resulted in delayed flowering, while 35S:SVP2 and 35S:SVP4 plants showed no significant flowering time changes (Fig. 4B). Over-expression of four kiwifruit SVP-like genes in Arabidopsis resulted in plants with morphological abnormalities of inflorescence and floral structures, which resembled floral defects obtained upon ectopic expressions of Arabidopsis SVP and AGL24 (Fig. 4C–P). Plants expressing high levels of kiwifruit SVP transgenes developed aberrant flowers with enhanced vegetative traits, most prominently leaf-like sepalas that failed to enclose before anthesis and were enriched with trichomes. The severity of phenotypes varied between the transgenes. Transgenic SVP1 plants showed mild defects of floral phenotype, with normal petals surrounded by four big leaf-like and trichome-enriched sepals (Fig. 4J). Constitutive over-expression of SVP2 and SVP3 resulted in plants with severely enhanced vegetative traits that often developed secondary flowers in the axils of primary flowers (Fig. 4L) and were largely sterile. Transgenic plants expressing SVP4 exhibited milder phenotypes.

Only kiwifruit SVP1 and SVP3 showed the ability to complement the early flowering phenotype of the svp41 mutant when ectopically expressed (Fig. 5A, B). None of the kiwifruit SVP-like genes could substitute for the lack of functional AGL24 in the agl24 mutant even when expressed at high levels (Fig. 5C, D). Transgenic svp41 plants exhibited various degrees of abnormal inflorescence and floral development (Fig. 5E–N), in particular, those
expressing SVP2 (Fig. 5J) and SVP3 (Fig. 5L), which resulted in development of secondary flowers. Similar abnormal floral phenotypes were observed in transgenic agl24 plants (data not shown).

Kiwifruit SVP-like protein interactions resemble AGL24 and SVP protein interactions

The observed over-expression phenotypes might have been caused by interactions with Arabidopsis MADS-box proteins required for normal flower development. In addition, the unexpected differential ability of two highly homologous genes, SVP2 and SVP3 to delay flowering and rescue the svp41 phenotype, could have been caused by their differential ability to bind the floral repressor FLC in a complex crucial for the control of floral induction (Li et al., 2008). To evaluate the presence and intensity of protein interactions, a yeast two-hybrid analysis was performed using proteins known to interact with SVP and AGL24. The coding sequences of SVP and AGL24 fused to the BD and AD domains were used for comparison. Kiwifruit SVP1 interacted with all Arabidopsis SVP partners with similar interaction intensities, except for SEP3, where the interaction was weaker. In addition, SVP1 was capable of forming heterodimers with SVP2 and SVP3, but failed to interact with Arabidopsis SVP and kiwifruit SVP4. SVP2 and SVP3 had very similar interaction capacities. They interacted with most of the AGL24 and SVP partners at strong interaction intensity. SVP2 and SVP3 both had a capacity to interact with FLC with similar interaction intensity, which was higher than that detected for SVP1 and Arabidopsis SVP. They were also capable of forming homodimers, in particular SVP2, as well as heterodimers with each other, with SVP1, and weakly with SVP4. Of all the Arabidopsis proteins tested, kiwifruit SVP4 interacted only with SOC1 (Table 2; see Supplementary Fig. S2 at JXB online).

Discussion

Evolutionary and functional conservation and divergence within the kiwifruit SVP gene family

Interogation of the Actinidia EST database showed the presence of four kiwifruit SVP-like genes. They were derived from vegetative tissues, developing buds, and young leaves and represented with a single A. chinensis sequence each, with the exception of SVP3 where several sequences were present. This reflected a low representation of vegetative transcripts in the EST collection, which mainly contains
fruit transcripts (Crowhurst et al., 2008) and the relatively low expression levels of these genes, in particular SVP1, SVP2, and SVP4, as confirmed in subsequent expression analysis. Only the SVP3 sequence was found in both A. chinensis and A. deliciosa ESTs, reflecting the relatively high abundance of this transcript. Additional amplification of predicted coding regions confirmed the presence of all four genes in both A. deliciosa and A. chinensis. Phylogenetic analysis suggested that SVP1 and SVP4 might be orthologues of Arabidopsis SVP and potato StMADS11, respectively. None of the kiwifruit SVP genes were highly similar to the peach DAM gene group with a demonstrated role in regulation of dormancy (Bielenberg et al., 2004, 2008; Yamane et al., 2008, 2011).

Members of the SVP-like gene family have been identified in a wide range of species and have been shown to perform diverse functions. In Arabidopsis, the family is represented by two paralogous genes, SVP and AGL24, which perform opposite functions during floral initiation (Hartmann et al., 2000; Yu et al., 2002), but act redundantly during the early stages of flower development (Gregis et al., 2008). Thus, conservation of biological function of kiwifruit SVP-like genes was assessed in overexpression and complementation tests performed in Arabidopsis. As expected, based on similarity to SVP, kiwifruit SVP1 was able to complement for the loss of SVP but not AGL24 function; constitutive overexpression caused a significant delay in flowering and SVP1 protein interactions resembled closely those detected with Arabidopsis SVP. These data indicate that kiwifruit SVP1 is a functional equivalent of SVP and suggest conservation of this gene across eudicot genera. The most divergent of the kiwifruit genes (SVP4) was similar to the StMADS11-like genes and did not complement either of the Arabidopsis mutants; overexpression had no effect on flowering time and most transgenic plants exhibited relatively mild floral phenotypes. Accordingly, yeast two-hybrid assays failed to detect interactions with Arabidopsis floral organ identity genes.

SVP2 and SVP3 are the most intriguing members in the kiwifruit SVP-like gene family. High sequence similarity, similar amino acid identity to both SVP and AGL24, and similar protein interaction patterns would have suggested similar over-expression and complementation results. Constitutive over-expression of SVP2 and SVP3 phenocopied the

Fig. 5. The effect of constitutive expression of kiwifruit SVP-like genes in Arabidopsis svp41 and agl24 mutant plants. (A) Relative expression of kiwifruit SVP-like genes in 10-d-old transgenic Arabidopsis svp41 seedlings. (B) Flowering time of transgenic Arabidopsis svp41 plants. (C) Relative expression of kiwifruit SVP-like genes in 10-d-old transgenic Arabidopsis agl24 seedlings. (D) Flowering time of transgenic Arabidopsis agl24 plants. Two independent lines were tested for each construct. The expression of each gene was normalized against Arabidopsis Actin and is represented as mean ± SE of three replicate reactions. Flowering time was recorded as the rosette leaf number when the primary inflorescence stems were 1.5 cm long and is represented as mean ± SE of 12 individuals for each genotype. (E–N) Phenotypes of transgenic Arabidopsis svp41 plants. (E, F) Early flowering svp41 mutant plant and flower. Plant and flower resulting from constitutive expression of kiwifruit SVP1 (G, H), SVP2 (I, J), SVP3 (K, L), and SVP4 (M, N). Scale bar represents 2 mm.
over-expression of SVP and AGL24 (Yu et al., 2004; Liu et al., 2007), resulting in floral reversion in Arabidopsis, but only SVP3 delayed flowering and successfully rescued the svp mutant phenotype. This may be the result of SVP3-mediated repression of SOCI or FT transcription, as demonstrated for Arabidopsis SVP (Lee et al., 2007b; Li et al., 2008), which is predicted to occur upon SVP interaction with FLC (Li et al., 2008). Intriguingly, similar FLC interaction potentials of both SVP2 and SVP3 proteins in the yeast two-hybrid assay suggest additional mechanisms.

Potential roles of kiwifruit SVP-like genes

Distinct expression patterns in kiwifruit organs and timing of accumulation in dormant shoot buds suggests that kiwifruit SVP-like genes may perform similar, but distinct functions. This hypothesis is supported by different flowering time phenotypes observed in wild-type and mutant Arabidopsis constitutive expression of kiwifruit SVP3-like genes and distinct protein interaction networks.

Table 2. Kiwifruit SVP protein interactions with Arabidopsis MADS box proteins detected by yeast two hybrid assays. ++; very strong interaction, +; strong interaction, +/-; weak interaction, -; no interaction.

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Down-regulation of SVP-like genes also preceded floral differentiation, suggesting a role for at least some of these genes as floral repressors. Rapid accumulation of B and C class gene transcripts occurred upon reduction in SVP-like transcript levels, consistent with the role in the maintenance of floral meristem identity during dormancy. This role has been demonstrated in Arabidopsis, where Arabidopsis SVP and AGL24 together with LEAFY, AP1, and SOC1 specify floral meristem identity and both SVP and AGL24 genes are repressed before floral differentiation to prevent the development of abnormal flowers observed upon ectopic expression of SVP and AGL24 genes (Gregis et al., 2009; Liu et al., 2009).

Expression in kiwifruit dormant buds, where potentially floral merisms have been iniated (Walton et al., 2001), combined with functional conservation of two kiwifruit SVP genes, suggests that these genes may function as internal suppressors during bud dormancy, to prevent premature development and growth of flowers during unfavourable winter periods.

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References


