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ORIGINAL ARTICLE

Ectopic expression of *LoSVP*, a MADS-domain transcription factor from lily, leads to delayed flowering in transgenic *Arabidopsis*

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Abstract

Key message **A MADS-domain transcription factor *LoSVP*, which could delay flowering through vernalization pathway, was isolated from lily.**

Abstract MADS-domain transcription factors play important roles in plant growth and development, especially in the transition from vegetative phase to reproductive phase. However, their functions in bulbous flowering plants are largely unknown. In this work, a SHORT VEGETATIVE PHASE (SVP) encoding genes *LoSVP* from oriental lily was isolated. Bioinformatic analyses demonstrated that *LoSVP* encodes a type II MADS-box protein containing a conserved MADS-box, as well as a conserved K-box domain. Semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) revealed ubiquitous expression of *LoSVP* in various tissues, including petals, stamens, pistils, leaves and scales. Real-time polymerase chain reaction (PCR) analyses demonstrated that *LoSVP* was predominantly expressed in the early stage of developing flowers. Constitutive expression of *LoSVP* in *Arabidopsis* led to significantly delayed flowering of transgenic plants. These results suggest that *LoSVP* is involved in plant flowering and could be used as a potential candidate gene for the genetic regulation of flowering time in higher plants.

Keywords MADS-box · Lily · *LoSVP* · Transgenic plant

Introduction

In plants, after the formation of embryo, different organs develop from a population of undifferentiated cells called meristem. In the meristem tissue, stem cells located in the central region remain active, while cells in the peripheral part differentiate into organs. In flowering plants, such as *Arabidopsis*, primordia from stem apex meristem (SAM) develop into leaves during the vegetative phase (Langensgerrits et al. 2003; Lee et al. 2011). Changes in subsequent generative stages are called floral transition, which is regulated by multiple genes controlled by both environment and endogenous cues. During the floral transition, SAM undergoes fate changes and becomes an inflorescence meristem (IM) (Melzer et al. 2008). During the developmental process, floral organ identity is specified mainly by a set of flower organ identity genes (Scortecci et al. 2003; Li et al. 2008; Becker and Theissen 2003; Gregis et al. 2009). To date, many genes regulating flowering time have been cloned and identified. For examples, *AGAMOUS-LIKE 20* (*AGL20*), *AGAMOUS-LIKE 24* (*AGL24*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) and *FLOWERING LOCUS T* (*FT*) promoted flower opening in plants (Hussain et al. 2019), whereas *FLOWERING LOCUS C* (*FLC*), *FLOWERING LOCUS M* (*FLM*) and *FRIGIDA* (*FRI*) inhibited plant flowering (Borner et al. 2000; Liu et al. 2008; Son et al. 2014).

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SHORT VEGETATIVE PHASE (*SVP*) is a MADS-box transcription factor which has an inhibitory effect on flowering (Hartmann et al. 2000). It could integrate flowering signal from both autonomous and vernalization pathways to regulate the flowering time of plants (Nilsson 2013). Gibberellin pathway could negatively regulate the expression of *SVP*. The expression level of *SVP* increased in *Arabidopsis gal-3* mutant, but decreased in wild-type plant after treatment with gibberellin (Wang et al. 2018). Consistently, *SVP*-like genes in *Arabidopsis* and barley acted as negative regulators of flowering (Liu et al. 2007; Trevaskis et al. 2006). In wheat, *TaVRT2*, encoded by an *SVP*-like gene, interacted with *VRN1* and *VRN2* to regulate the flowering time, by binding to the CArG-box in the promoter of *VRN1* (Kane et al. 2007). In *Arabidopsis*, two flowering signal integrators SVP and FLC could form protein complexes in vivo and in vitro (Jung and Mller 2009). Further studies revealed that SVP–FLC protein complex could inhibit the expression of both FT and SOC1 (the target gene of FT), and SVP negatively regulated flowering integron FT by directly binding to the CArG motif in FT sequence, thereby regulating plant flowering time (Lee et al. 2007a, b).

Lily (*Lilium longiflorum*), commonly known as cut flower plant, is a leading bulbous crop worldwide (Langensgerrits et al. 2003). Like many other ornamental bulbs, lily has a crucial transition feature from growth to dormancy and to flower during its annual growth cycle. Previously, we reported that low-temperature (4 °C) treatment significantly promoted the flowering of oriental lily hybrid 'Sorbone' (Liu et al. 2014). Here, we report the isolation and characterization of *LoSVP*, a MADS-domain transcription factor gene from oriental lily hybrid 'Sorbone'. We demonstrate that *LoSVP* was down-regulated by low temperature and its heterologous expression resulted in delayed flowering in transgenic *Arabidopsis* plants.

Materials and methods

Plant materials and growth conditions

Oriental lily hybrid 'Sorbone' bulbs were sanitized at 4 °C for 12 weeks. Then they were planted in a greenhouse under natural light conditions at the National Floriculture Engineering Research Center, Beijing, China (116.3°E, 40.0°N) from March 1 to July 15, 2017. The temperature was kept at 22–25 °C in daytime and 17–20 °C at night. Samples were collected, frozen in liquid nitrogen, and stored at − 80 °C for related experimental analyses. For low-temperature treatment, lily bulbs were kept at 4 °C for 1, 3, 6, 9, and 12 weeks and stem tips were collected for gene expression analyses.

For the growth of *Arabidopsis*, seeds in Columbia (Col) background were grown in greenhouse under short-day condition (8 h light/16 h dark) for 2 weeks, then under long-day condition (16 h light/8 h dark) for another 1 or 2 weeks. The temperature was kept at 25 °C in daytime and 20 °C at night with 60% humidity.

RNA isolation and cDNA synthesis

Total RNA was extracted from various tissues of 9-week-old oriental lily hybrid 'Sorbonne' or 4-week-old *Arabidopsis* plants with RNAiso Plus (TaKaRa, Total RNA extraction reagent) according to the operation manual. RNA quality and quantity were examined with Agilent 2100 BioAnalyzer (Agilent Technologies, CA, USA). Qualified total RNA samples were used for cDNA synthesis with TransScript One-Step gDNA Removal and cDNA Synthesis SuperMix (Transgen, China). Residual gDNA was removed with gDNA remover. Primers used for cDNA synthesis were random primer and anchored oligo$(dT)_{18}$. The reaction was carried out with 30 min incubation at 42 °C and 5 min heating at 85 °C. Finally, the quality and quantity of synthesized cDNA were determined with Agilent 2100 BioAnalyzer.

Isolation of LoSVP

Synthesized cDNA was used as template and the full-length gene was amplified by RACE technique according to the User Manual (SMART™ RACE cDNA Amplification Kit). SVP CDS sequence of lily was blast-searched with the homologous fragments in *Arabidopsis thaliana* (AK226537.1) from NCBI. The gene found in our previous EST database shares the highest identity with *AtSVP* (AK226537.1) and was named as *LoSVP*. Gene-specific primers for the amplification of 5′ and 3′ ends in this experiment were 5f: ′-ACTCATCGGACGACTT CACCTGACG-3′ and 3r: 5′-GTCTCCTTGTAACCGCATCTAATGCG-3′. Primers were designed according to the sequence of the cDNA fragments in our previous transcriptomic sequencing data. Gene fragment obtained was ligated into the pEASY®-T5 Zero Cloning Vector (Transgen, China) after purification. Finally, full-length *LoSVP* cDNA was isolated.

Bioinformatic analysis of LoSVP

The sequencing results were compared with the SVP sequences obtained from the transcriptomic database of lily. Conserved domain search (CD Search) was used to predict the conserved domain of the gene, and the homologous alignment was performed by BlastX. DNAMAN software was used to infer the gene. Encoded amino acid sequence was subjected to multiple sequence alignment with the program ClustalW (Thompson et al. 1994). Amino acid physicochemical properties were predicted with ProtParam. Protein transmembrane domain was

predicted with TMHMM. Protein signal peptide was predicted with SignaIP. ProtScale was used to predict hydrophobicity. WOLF PSORT was used to predict subcellular localization. GOR4 was used to predict protein secondary structure. SWISS-MODEL was used to model protein tertiary structure, and MEGA5.0 was used to construct the phylogenetic tree (Tamura et al. 2007). As a rule, nodes with bootstrap values greater than 70 are significantly supported with 95% probability (Hillis and Bull 1993). Genbank accession numbers for the amino acid sequences of the SVP genes in other plants used are: *Arabidopsis thaliana* (BAE98676.1, NP_001324584.1, AFU85632.1), *Lilium longiflorum* (AXE75656.1), *Nelumbo nucifera* (XP_010254525.1, XP_010277489.1), *Elaeis guineensis* (AAW66885.1, XP_010926365.1), *Populus trichocarpa* (XP_002310310.1), *Vitis vinifera* (XP_019073897.1), *Coffea arabica* (AHW58026.1), *Sesamum indicum* (XP_020554864.1) and *Malus domestica* (XP_028953815.1).

RT-PCR and quantitative real-time PCR analyses

For gene expression analyses, RT-PCR and quantitative real-time PCR were performed using SYBR Premix Ex Taq (TaKaRa, Dalian, China) and the Rotor-Gene 3000 Series real-time DNA amplification system. Amplification conditions were: 95 °C for 30 s; 40 cycles of 95 °C for 5 s, 60 °C for 15 s, 72 °C 30 s. Gene-specific primers LoSVP forward (5′-CAGTCCTATA GACCGCTGGA-3′) and LoSVP-R reverse (5′-TCTACTGTCCTGACGACC TG-3′) for *LoSVP*, *LoACT* forward (5′-CGAAGCCAGAAA CGGAGAAGAAT-3′) and *LoACT* reverse (5′-GGGTAG GGTGGATTGGGAAGA-3′) for *LoACTIN*, and *AtACT* forward (5′-TGGCTT CACAGTCTATCCCTC-3′) and *AtACT* reverse (5′-GGGACAAGATTGGTCTGGAAC-3′) for *AtACTIN* were used. Expressions of *AtACTIN* (GenBank accession number: AT2G37070) and *LoACTIN* (GenBank accession number: JX826390) served as internal control. For *LoSVP* expression analysis in flowers, total RNA in flower buds at five flowering stages (S1: the preliminary stage of flower bud differentiation; S2: the second stage of flower bud differentiation; S3: the third stage of flower bud differentiation; S4: the fourth stage of flower bud differentiation; S5: the final stage of flower bud differentiation) were reverse transcribed and cDNA was used as template. The relative expression level was calculated by $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001), and the regulation of *LoSVP* gene on different developmental stages of flowers was analyzed. At the same time, the total RNA of leaves, petals, stamens, pistils and bulbs was extracted, and the tissue-specific expression of *LoSVP* gene was analyzed by RT-PCR.

Vector construction and *Arabidopsis* transformation

LoSVP was excised from PGEM-T easy vector using restriction enzymes *Xba* I and *Sma* I, and cloned into the binary plant expression vector PBI121 under the control of cauliflower mosaic virus (CaMV) 35S promoter. The resultant construct pBI121-35S:*LoSVP* was transformed into *Agrobacterium tumefaciens* strain GV1301. *Arabidopsis thaliana* ecotype Columbia (Col) plants were transformed as described previously (Clough and Bent 1998). Homozygous T_3 plants were grown in a greenhouse for flowering time analysis.

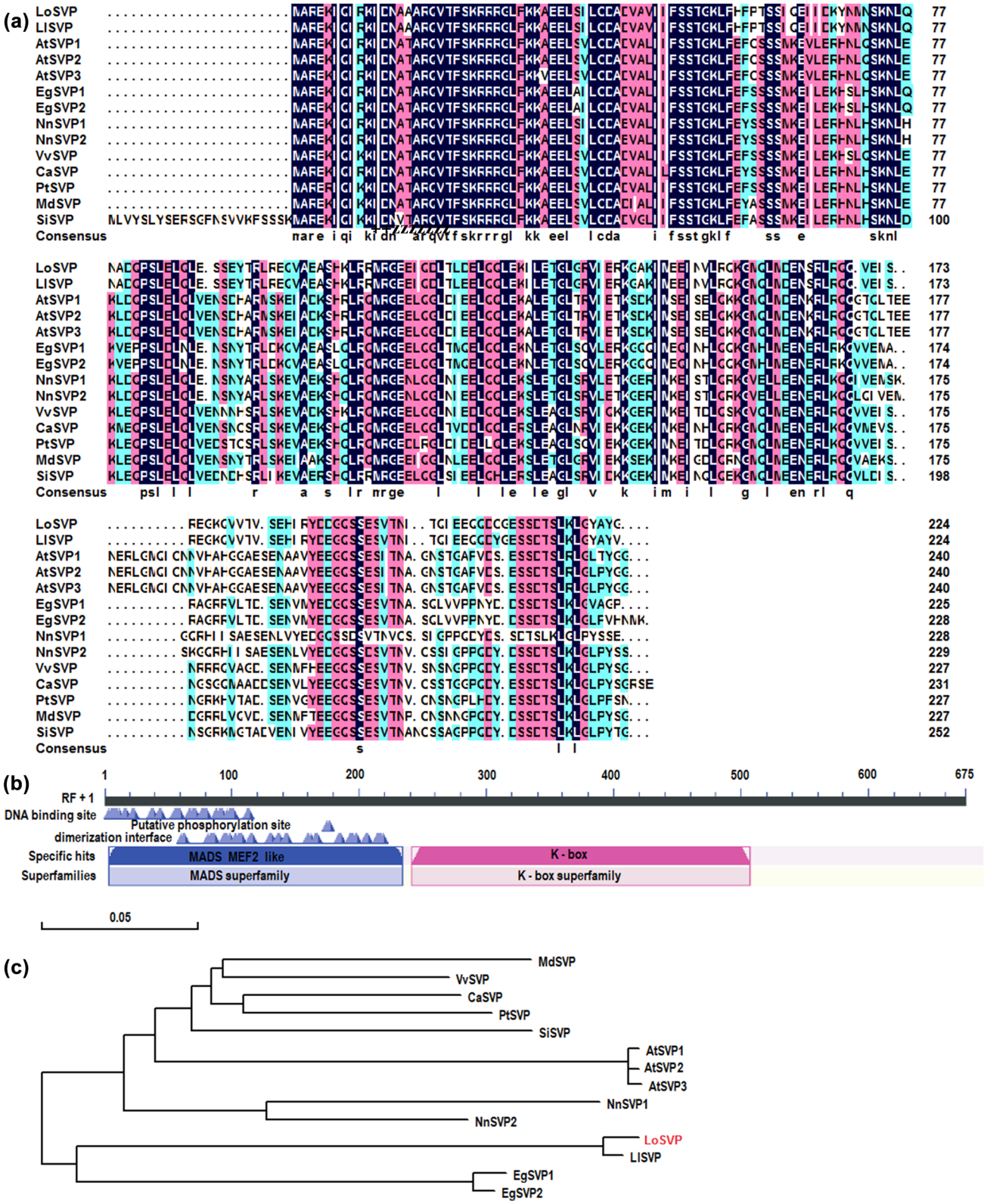
Statistical analyses

All data were mean values ± SD of three experiments. For qPCR analysis, the ΔΔCq method was used. ANOVA was applied to analyze the significant differences and *P* value was kept below 0.05. Sigma Plot 12.0 was used for plotting.

Results

Isolation and sequence analysis of LoSVP

Based on our previous transcriptomic database, we identified a high expressing transcript homologous to *AtSVP*, a floral repressor which could repress the expression of *FT* gene via directly binding to the vCArG III motif in its promoter. To understand its possible function in flowering transition in lily, we cloned the full-length sequence of *LoSVP* containing the transcript homolog (GenBank accession No. MF693882). The 675-bp *LoSVP* encodes a 224-amino acid protein (*LoSVP*) with a calculated molecular mass of ~ 37 kD and a pI value of 4.85. The total number of positive and negative charge residues is 33 and 37, respectively, indicating that *LoSVP* is negatively charged in the neutral environment, with the highest glutamic acid content (12.1%), followed by leucine (9.4%) and serine (8.9%), as predicted by ProtParam (http://www.expasy.org/tools/) (Fig. 1a). *LoSVP* shares a 99% amino acid sequence identity with the Madonna lily *LlSVP*. It also shares high amino acid sequence homology with SVPs from other higher plants, such as the *Arabidopsis AtSVP1* (69% identity), *AtSVP2* (69% identity) and *AtSVP3* (69% identity), the sesame S*iSVP* (67% identity), the oil palm *EgSVP* (67% identity), the indian lotus *NnSV*P (67% identity), the coffee tree C*aSVP* (66% identity), the apple *MdSVP* (67% identity) and the grape *VvSVP* (67% identity). Like other SVPs, *LoSVP* possesses a MADS-box and a K-box, which are highly conserved



in the SVP subfamily of MADS-box family (Fig. 1b). We also constructed a phylogenetic tree including the SVPs in *Arabidopsis* and other plants. Again, *LoSVP* was highly homologous to *LlSVP* in Madonna lily, one of the most closely related organisms to the oriental lily hybrid (Fig. 1c).

◂**Fig. 1** **a** The protein sequence multiple alignment of the deduced amino acid sequences of *LoSVP* with other plant SVPs. **b** The conserved functional domain analysis of *LoSVP* protein. **c** Phylogeny of *LoSVP* and other plant SVP genes. A consensus phylogenetic tree created using the protein parsimony method of the PHYLIP package (http://evolution.genetics.washington.edu/phylip.html). Bootstrap support values from 1000 bootstrap replicates are shown for each node as percentages. The GenBank accession numbers of SVP are as follows: *Arabidopsis thaliana* (BAE98676.1, NP_001324584.1, AFU85632.1), *Lilium longiflorum* (AXE75656.1), *Nelumbo nucifera* (XP_010254525.1, XP_010277489.1), *Elaeis guineensis* (AAW66885.1, XP_010926365.1), *Populus trichocarpa* (XP_002310310.1), *Vitis vinifera* (XP_019073897.1), *Coffea arabica* (AHW58026.1), *Sesamum indicum* (XP_020554864.1), and *Malus domestica* (XP_028953815.1)

Bioinformatic analysis of LoSVP

Signal peptide is a kind of short peptide usually composed of 15–30 amino acids at the N-terminal of the secreted protein. SignalP prediction analysis revealed that *LoSVP* protein contained no signal peptide at its N-terminal and was a non-secreted protein (http://www.cbs.dtu.dk/services/SignalP-2.0/) (Fig. 2a). We then performed ProtScale prediction (http://www.psort.org). The result indicated that the most hydrophilic regions in *LoSVP* were located at the 165th and 208th amino acid residues, with the lowest value of − 2.611, whereas the most hydrophobic region was located at the 47th amino acid residue, with the highest value of 2.189. Most amino acid residues in *LoSVP* were located in the hydrophilic regions, indicating that *LoSVP* was a hydrophilic protein (Fig. 2b). The transmembrane domain of *LoSVP* was also predicted by TMHMM (www.cbs.dtu.dk/services/TMHMM-2.0/). *LoSVP* peptide chain was located outside of the membrane, suggesting that it may not have a transmembrane domain and belong to a non-transmembrane protein (Fig. 2c). Subcellular localization prediction showed an 87.0% probability of *LoSVP* in the nucleus and a 13.0% probability in the mitochondria (http://wolfpsort.seq.cbrc.jp). So *LoSVP* protein may be located in the nucleus, although subsequent experiments have to be performed. To complete the construction of the active functional domain conformation, protein polypeptide chains are usually coiled and folded into a relatively stable secondary structure. Two-dimensional structure modeling analysis demonstrated that the secondary structure of *LoSVP* contained 136 α-helices (60.71%), 65 random curls (29.02%) and 23 extended strands (10.27%). Tertiary structure prediction showed that *LoSVP* may exist as a homotetramer. To further determine the evolutionary relationship of *LoSVP*, we constructed a phylogenetic tree with the MADS-box gene family in *Arabidopsis* and found that *LoSVP* was clustered into the SVP subfamily (Fig. 2e).

Expression profile of LoSVP

As a first step to know the expression profile of *LoSVP* gene in lily, we examined its relative expression levels in different tissues of 9-week-old oriental lily hybrid 'Sorbonne' plants under normal growth condition by RT-PCR (Fig. 3a). *LoSVP* was ubiquitously expressed in various tissues including petals, stamens, pistils, leaves and scales, with a relatively higher expression in the leaves and scales (Fig. 3b). To analyze the expression of *LoSVP* in different flower developing stages, flower buds of at different developing stages (S1: the preliminary stage of flower bud differentiation; S2: the second stage of flower bud differentiation; S3: the third stage of flower bud differentiation; S4: the fourth stage of flower bud differentiation; S5: the final stage of flower bud differentiation) were collected from plants grown under normal growth condition, and quantitative real-time PCR was carried out (Fig. 3c). The most abundant expression of *LoSVP* was observed in flowers at stage 2 (Fig. 3d). To further understand the possible function of the vernalization of lily, we treated the bulbs with low temperature (4 °C) for different time periods and examined the expression of *LoSVP* in the stem tips. We found that low temperature significantly down-regulated the expression of *LoSVP* (Fig. 4).

Ectopic expression of LoSVP leads to delayed flowering in transgenic *Arabidopsis*

Since low temperature (4 °C) promoted the flowering of oriental lily hybrid 'Sorbone' and decreased the expression of *LoSVP*, we speculated that expression of *LoSVP* may postpone the flowering in other plants. To testify this possibility, a construct (pBI121-35S:*LoSVP*) containing the open reading frame of *LoSVP* was transformed into *Arabidopsis* by *Agrobacterium tumefaciens*-mediated transformation (Fig. 5a). At least 20 independently derived transgenic lines were obtained, and 10 transgenic lines (L1–10) were selected by PCR analyses to confirm the integration of transgene into the *Arabidopsis* genome (Fig. 5b). Real-time PCR analyses further confirmed the expression of *LoSVP* in all the selected transgenic lines, but not in the wild-type and transgenic plants transformed with pBI121 (Fig. 5c). Wild-type and transgenic plants were subsequently transplanted in pots and grown in greenhouse for flowering experiments. As expected, constitutive expression of *LoSVP* delayed the flowering of transgenic plants (Fig. 6a, b). It took transgenic plants 24 and 28 days, whereas the wild-type and pBI121 transgenic plants 18 and 22 days, to bolt and flower, respectively (Fig. 6a, b).

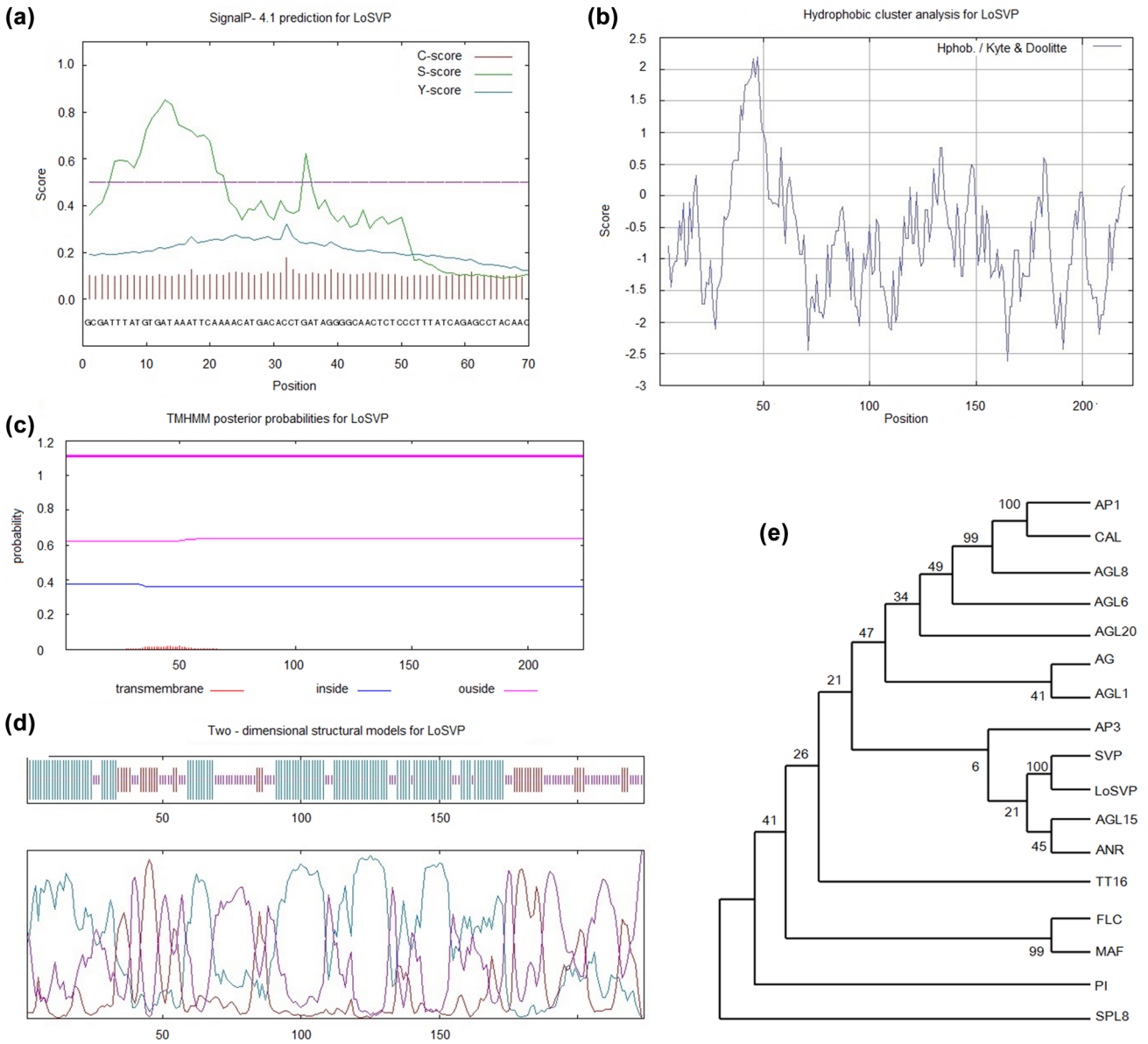


Fig. 2 The signal peptide analysis, hydrophobic cluster analysis, transmembrane structure, two-dimensional structural model and phylogenetic analysis of *LoSVP* protein. **a** The signal peptide analysis of *LoSVP* by Signal P. **b** The hydrophobic cluster analysis of *LoSVP*. **c** The transmembrane domain analysis of *LoSVP* protein with TMHHM. **d** The two-dimensional structural model of *LoSVP* protein. **e** Phylogeny of *LoSVP* and *Arabidopsis* MADS-box genes. A consensus phylogenetic tree created using the protein parsimony method of the PHYLIP package (http://evolution.genetics.washington.edu/phylip.html). Bootstrap support values from 1000 bootstrap replicates are shown for each node as percentages

Discussion

In the MADS-box transcription factor family, both SVP and AGL24 belong to the STMADS11 family, but have completely opposite functions in the regulation of flower development. AGL24 promotes, but SVP inhibits flowering. SVP plays a crucial role in the growth and impacts flower transition from vegetative to reproductive growth in both monocot and dicot plants (Jiang et al.2007; Khan et al. 2013; Trevaskis et al. 2003; Yu et al.2011). SVP determines the specificity of flower meristem at the early flower development stage and directly inhibits the expression of B and C flower homologous genes along with AP1 and AGL24, thereby inhibiting the flower transformation process. Based

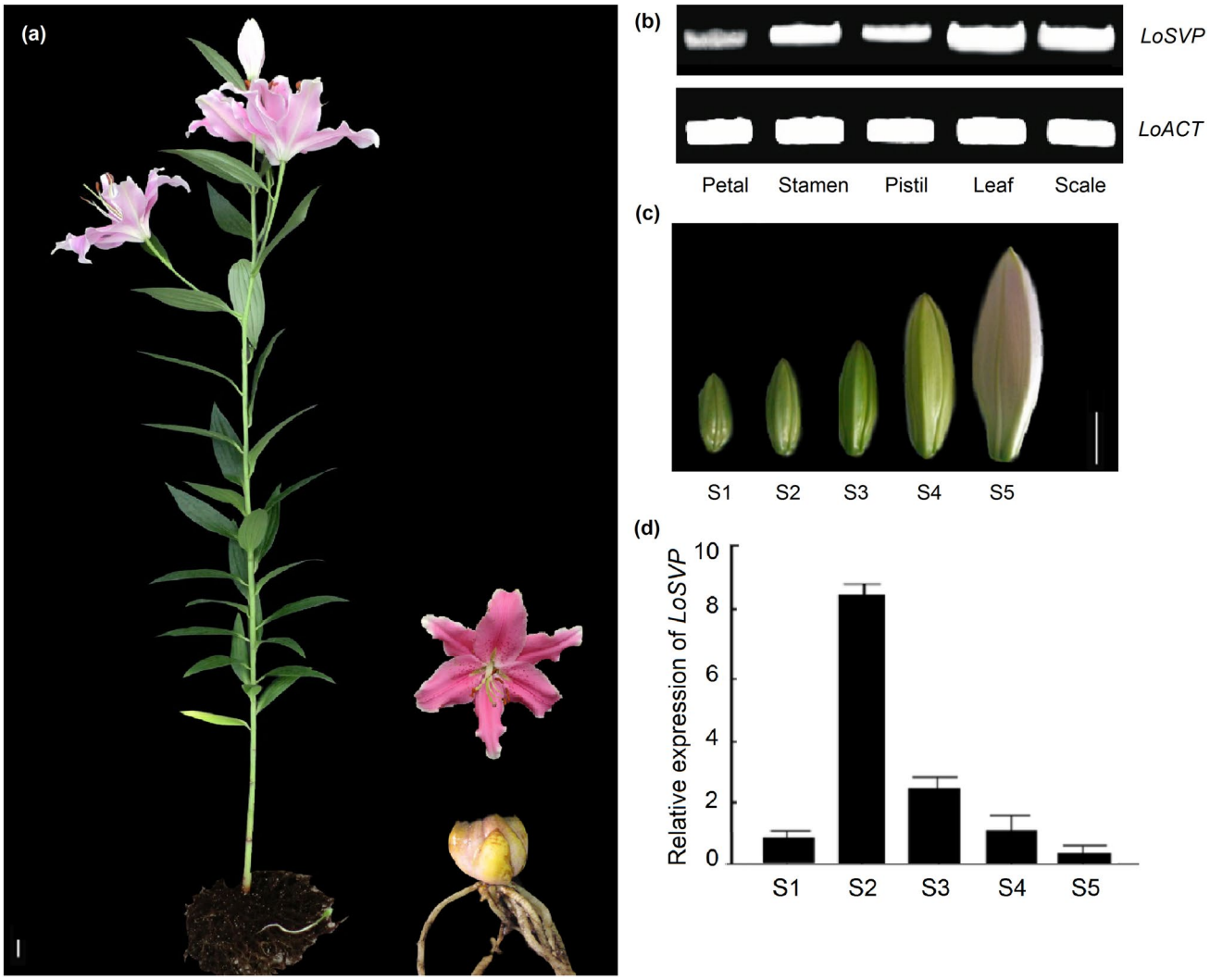


Fig. 3 Expression pattern of *LoSVP* in Oriental lily hybrid 'Sorbonne'. **a** Growth phenotypes of lily plant for 9 weeks. **b** Tissue-specific analysis of *LoSVP* by RT-PCR. **c** Stages of lily flower during flower development for RT-PCR (S1: the preliminary stage of flower bud differentiation; S2: the second stage of flower bud differentiation; S3: the third stage of flower bud differentiation; S4: the fourth stage of flower bud differentiation; S5: the final stage of flower bud differentiation). **d** Relative expression of *LoSVP* in lily during different stages of flower development

on the observation in our previous study that low temperature decreased the transcription of *LoSVP*, we speculated that it may play a biological function in the flowering regulation in lily (Liu et al. 2014). To investigate the molecular mechanism and the relationship between floral induction and *LoSVP* expression, we isolated the *LoSVP* gene from lily and investigated its biological function in *Arabidopsis*. *LoSVP* shares very high amino acid sequence identity with the SVPs from *Arabidopsis* and other plants (Fig. 1a). Like other SVPs, *LoSVP* contains a conserved MADS-box and belongs to the SVP subfamily (Figs. 1b, 2e).

In different plant species, MADS-domain transcription factor showed different degrees of sub-functionalization and new functionalization (Becker and Theissen 2003). In *Arabidopsis*, *SVP* gene was equivalently expressed in vegetative tissues, but was specifically expressed in flower primordia, not in flowers and reproductive tissues (Hartmann et al. 2000). In grapes, *SVP* gene was expressed in both vegetative and reproductive organs, with a relatively lower expression in flowers (Melzer et al. 2008). In kiwi fruit, *SVP* gene was only expressed in vegetative organs and not in flowers (Wu et al. 2012). In our study, we found

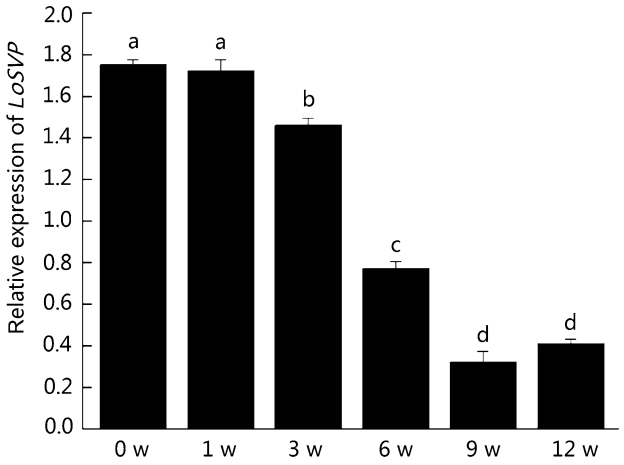


Fig. 4 Relative expression of *LoSVP* in lily stem tips during different stages under 4 °C low-temperature storage. For *LoSVP* expression analysis during low-temperature treatment, total RNA isolated from lily stem tips stored at 4 °C for 1, 3, 6, 9 and 12 weeks were reverse transcribed and cDNA was used as template for gene expression analyses, respectively

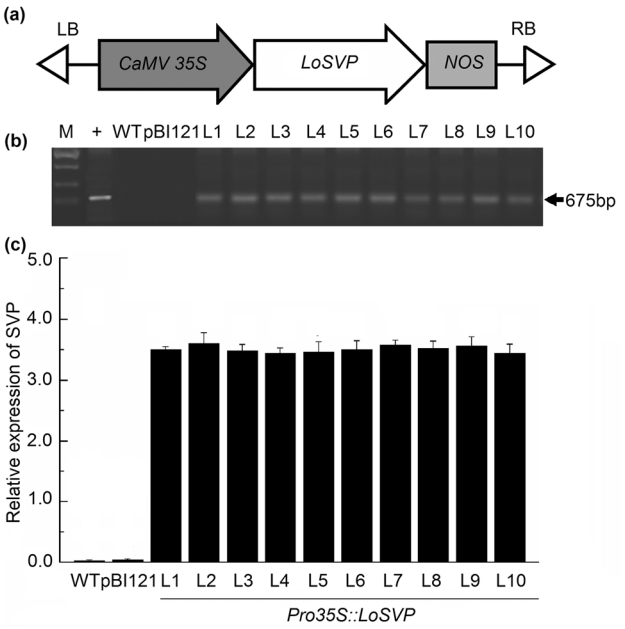


Fig. 5 **a** The plant expression vector constructed of *LoSVP*. **b** The detection of *LoSVP* expression of wild-type, empty vector and Pro35S::*LoSVP* transgenic plants transgenic *Arabidopsis* plants. Total RNAs isolated from three 14-day-old 35S:: *LoSVP* transgenic *Arabidopsis* plants and from one untransformed wild-type plant and empty vector plant were used as template. L1–L10 showed later flowering, whereas wild-type and empty vector showed early flowering. The expression of *LoSVP* was detectable in 35S:: *LoSVP* transgenic *Arabidopsis* plants, whereas *LoSVP* expression was undetectable in untransformed wild-type and empty vector plants. A fragment of the ACTIN (*AtACT*) gene was amplified as an internal control. **c** Relative expression levels of *LoSVP* gene at the shoot apex of WT, empty vector and Pro35S::*LoSVP* transgenic plants determined by RT-qPCR. Three biological replicates and three technical replicates were performed using 14-day-old long-day-grown seedlings. The *AtACT* gene was used as a reference

that *LoSVP* was ubiquitously expressed in all tested tissues, with a relatively higher expression in leaves and scales (Fig. 3b). It is well known that the expression level of *SVP* genes was closely related with the flowering transition. We observed that *LoSVP* was mainly expressed at the early stage in the developing flowers of lily (Fig. 3c, d) and was significantly down-regulated by low-temperature treatment (Fig. 4). This is consistent with our previous observation that low temperature promoted flowering of lily (Liu et al. 2014).

To further clarify the biological function of *LoSVP*, we expressed it in *Arabidopsis*, driven by the 35S promoter (Fig. 5a–c). Compared to control plants (wild-type and transgenic plants transformed with pBI121 vector), constitutive expression of *LoSVP* delayed the flowering of transgenic plants by 6 days (Fig. 6a–c). Consistent results were also observed with the *Arabidopsis svp-32* and *svp-41* mutants. Under normal growth condition, *svp-32* mutants showered early flowering (Lee et al. 2007a, b). Expression of the Chinese cabbage *BcSVP* gene under the control of *AtSVP* promoter successfully restored the flowering time of the mutant plants. Similarly, the flowering time of *svp-41* mutants was significantly earlier than that of the wild-type plants, and the early flowering phenotype could be rescued by the introduction of *PtSVP* gene in the mutant (Li et al. 2010). All these results imply that *LoSVP* is functionally conserved and has an important regulatory effect on the flowering of lily. Further studies on the regulation of its down-stream genes in wild-type and transgenic lily plants will add more information to the molecular mechanisms underlying the transition from vegetative to reproductive growth of plants, and possibly lead to new techniques for the engineering of cut flower plants with well-controlled flowering time.

Fig. 6 Plants of *LoSVP* ectopic expression in transgenic *Arabidopsis* that flowered later than plants with all the other lines. **a** Growth phenotypes of 20-day-old *Arabidopsis* plants. WT and vector were bolting, but *LoSVP* transgenic *Arabidopsis* did not. **b** Growth phenotypes of 30-day-old *Arabidopsis* plants. WT and vector flowered, but *LoSVP* transgenic *Arabidopsis* did not. **c** Days plants with different lines needed to flower. Data are shown as mean ± SD. Values marked with different letters are significantly different (scale bar = 2 cm)

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Author contribution statement XT, ML, JH, and JC performed the experiments and analyzed the data. HZ and XL conceived the study. HZ and XL wrote the manuscript. All authors read and agreed at the last version of the manuscript.

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Compliance with ethical standards

Conflict of interest We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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