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Original Article

Overexpression of *PvSVP1*, an *SVP*-like gene of bamboo, causes early flowering and abnormal floral organs in *Arabidopsis* and rice

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Abstract

Bamboo is a nontimber woody plant featuring a long vegetative stage and uncertain flowering time. Therefore, the genes belonging to flowering repressors might be essential in regulating the transition from the vegetative to reproductive stage in bamboo. The Short Vegetative Phase (*SVP*) gene plays a pivotal role in floral transition and development. However, little is known about the bamboo *SVP* homologues. In this study, *Phyllostachys violascens* *PvSVP1* is isolated by analysis of the *P. edulis* transcriptome database. Phylogenetic analysis shows that *PvSVP1* is closely related to *OsMADS55* (rice *SVP* homolog). *PvSVP1* is ubiquitously expressed in various tissues, predominantly in vegetative tissues. To investigate the function of *PvSVP1*, *PvSVP1* is overexpressed in *Arabidopsis* and rice under the influence of the
Overexpression of *PvSVP1* in *Arabidopsis* causes early flowering and produces abnormal petals and sepals. Quantitative real-time PCR reveals that overexpression in *Arabidopsis* produces an early flowering phenotype by downregulating *FLC* and upregulating *FT* and produces abnormal floral organs by upregulating *AP1*, *AP3* and *PI* expressions. Simultaneously, overexpression of *PvSVP1* in rice alters the expressions of flowering-related genes such as *Hd3a*, *RFT1*, *OsMADS56* and *Ghd7* and promotes flowering under field conditions. In addition, *PvSVP1* may be a nuclear protein which interacts with *PvVRN1* and *PvMADS56* on the yeast two-hybrid and BiFC systems. Our study suggests that *PvSVP1* may play a vital role in flowering time and development by interacting with *PvVRN1* and *PvMADS56* in the nucleus. Furthermore, this study paves the way toward understanding the complex flowering process of bamboo.

**Keywords**: *Phyllostachys violascens*, *PvSVP1*, ectopic expression, flowering time, floral organs, yeast-two hybrid and BiFC

**Introduction**

The world is facing the dual pressures of an energy shortage and environmental protection. Thus, bioenergy resource development is an inevitable choice to achieve sustainable economic and social development. Bamboo is considered a new renewable lignocellulosic material that has excellent potential to produce energy [1–4]. Bamboo is a perennial flowering plant with a distinctive life cycle. For many bamboo species, flowering is often followed by the death of bamboo forests [5,6]. In many tropical and subtropical regions, gregarious flowering results in substantial economic loss and ecological crisis. However, the mechanism of bamboo flowering is still unclear, although this phenomenon has been recorded and studied for a long time [7–14]. Therefore, the study of bamboo flowering will play an essential role in the development of bamboo industry.

In *Arabidopsis thaliana*, the Short Vegetative Phase (*SVP*) and Agamous-like 24 (*AGL24*) genes are flowering pathway integrators that encode a MADS-box transcription factor. They play a decisive role in the flowering transition [15,16]. Although the two genes belong to the StMADS11 subfamily, they perform opposite functions in flowering time [15,17]. *AGL24* can promote the transition from the vegetative phase to the reproductive phase by directly activating the transcription of
Suppressor of Overexpression of Constans 1 (SOC1) [18]. In contrast, SVP delays the floral transition, and the elimination of SVP gene function causes early flowering under long and noninductive short days [15].

To prevent plants from going into the reproductive phase, SVP represses the expression of *Flowering Locus T* (*FT*) in the phloem and SOC1 in the shoot apical meristem (SAM) by directly binding to the GArG boxes in their promoters [17,19]. Moreover, SVP can interact with *Flowering Locus C* (*FLC*), another central repressor of flowering time [20]. The SVP-FLC dimer directly represses the expression of SOC1 in SAM and FT expression in the leaves [17,21]. Moreover, transgenic plants overexpressing SVP or AGL24 have floral abnormalities, such as aberrant flowers and shoot-like structures [22,23]. Previous studies indicated that *Apetala 1* (*AP1*) and *Sepallata 3* (*SEP3*) are components of the SVP and AGL24 interactome [24], and these interactions contribute to flower meristem identity [22,25,26]. Therefore, SVP/AGL24 regulates flowering time by controlling FT, SOC1 or FLC and affects the structure of floral organs by regulating AP1 and SEP3.

In contrast, SVP/AGL24 homologues from monocots mainly regulate floral meristem identity. In barley (*Hordeum vulgare*), ectopic expressions of BM1, BM10 and HvVRT2 inhibit floral development and cause floral reversion [27]. *Oryza sativa* has three SVP-like genes (*OsMADS22*, *OsMADS47* and *OsMADS55*) involved in shoot development and brassinosteroid signaling [28]. Transgenic rice overexpressing *OsMADS22* shows abrupt floral morphogenesis, such as a disorganized palea, elongated glume and two-floret spikelets [29]. Heterologous expressions of *OsMADS22* and *OsMADS47* in *Arabidopsis* cause only altered flower development but do not complement the flowering phenotypes of *svp* and *agl24* mutants [30]. Unlike *OsMADS22* and *OsMADS47*, overexpression of *OsMADS55* delays the flowering time of wild-type plants and rescues the early flowering phenotype of the *svp* mutant in *Arabidopsis* [31]. Recently, Xie et al. [32] reported that wheat *TaVRT2* is a floral activator in the vernalization regulatory pathway. These studies show that the functions of *OsMADS55* and *TaVRT2* genes in flowering time are more similar to the canonical roles of SVP and AGL24, respectively. Until now, the homologues SVP/AGL24 from monocots have been found to have higher similarity to SVP than AGL24 [30,33], so they have been identified as SVP-like genes. From the above studies, SVP homologues are conserved in regulating the structure of floral organ but divergent in controlling flowering time. Additionally, SVP homologues are involved
in other growth phases, such as regulating inflorescence branching, the formation of pedicel abscission zones, prophyll development and bud dormancy [34‒38].

Therefore, in the present study, to analyze the function of SVP in *Phyllostachys violascens*, we isolated the *PvSVP*-like gene using the *P. edulis* transcriptome database [39] and named it *PvSVP1*. Systematic phylogenetic analyses revealed that the *PvSVP1* protein is more closely related to rice OsMADS55. Furthermore, *PvSVP1* was overexpressed in wild-type *Arabidopsis* and rice. The flowering time, floral organ identity and expression levels of related genes from wild-type and transgenic lines were analyzed. Our results indicated that *PvSVP1* might mediate flowering time and floral organ identity. Moreover, this study also revealed that *PvSVP1* could interact with *PvVRN1* and *PvMADS56*.

Materials and Methods

Plant materials and growth conditions

In this study, bamboo samples used for gene cloning and expression analysis were collected from the Bamboo Garden of Zhejiang Agriculture and Forestry University. To analyze the temporal and spatial expression of *PvSVP1*, samples including different tissues harvested at different time points [March 15 (T1), March 29 (T2), and April 12 (T3) in 2016] were obtained as described previously [11]. For all studies, *A. thaliana* (ecotype Columbia), including the wild-type and transgenic plants, was grown on soil under 16-h light/8-h dark conditions at 22°C. All rice of transgenic and wild-type plants used in this study were from *Oryza sativa* L. ssp. japonica (cv. Nipponbare) grown in the paddy field of Nanbin farm (Sanya, China).

Cloning and sequence analysis of genes

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, USA). According to the manufacturer’s instructions, first-strand cDNA was synthesized by using Reverse Transcriptase M-MLV (TaKaRa, Dalian, China). The open reading frame (ORF) sequence of *OsMADS55* (an SVP homolog from rice) was used to identify the *SVP* gene from the transcriptome database of *P. edulis* (species affinis of *P. violascens*) [39] by using BioEdit. A sequence (ID: PH01000077G1380) with the highest similarity to *OsMADS55* was identified in the database. Then, the corresponding genomic sequence (PH01000077) was filtered from the genome database of *P. edulis* and used to design primers to isolate the *PvSVP1* gene from *P. violascens*. The ORF
for *PvSVP1* was amplified by PCR using primers shown in Supplementary Table S1. The PCR products were purified and cloned into the pMD20-T vector, and then confirmed by sequencing performed in Huada (Shanghai, China).

**Quantitative real-time PCR (RT-qPCR)**

Total RNA was isolated from different tissues (young leaf, mature leaf, culm, rhizome, bamboo shoot and flower) and at different dates T1, T2 and T3 (young leaf and flower) of flowering and nonflowering plants. RT-qPCR primers were designed according to the *PvSVP1* full-length ORFs, and *PheUBC18* was used for normalizing cDNA [40] (Supplementary Table S1). SYBR Premix Ex Taq II mix (TaKaRa) was used for RT-qPCR in the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, USA). Amplification involved the following cycling parameters: 95°C for 3 min, followed by 40 cycles of amplification (95°C for 10 s, 60°C for 20 s). Reactions were performed in 20 µL mixtures containing 10 µL 2×SYBR Premix Ex Taq II mix, 0.5 µL each forward or reverse primer, 1 µL cDNA template and 8 µL ddH₂O. RT-qPCR experiments were performed with three biological replicates (three technical replicates for one biological replicate). The data were analyzed by the 2^−ΔΔCT method [41]. Relative mRNA expression levels were normalized to those of *PheUBC18*.

**Ectopic expression of *PvSVP1* in *Arabidopsis* and rice**

The full-length coding sequence (CDS) of *PvSVP1* was cloned into the pCAMBIA1301 vector under control of the cauliflower mosaic virus (CaMV) 35S promoter. After confirmation of the sequence, the constructed plasmid was transformed into *Agrobacterium tumefaciens* strain GV3101 and then introduced into wild-type *Arabidopsis* according to the floral dip method as described previously [42]. Rice was transformed by transforming the *A. tumefaciens* strain EHA105 with the above recombinant construct according to the *Agrobacterium*-mediated cocultivation method [43]. Positive transgenic plants that survived in medium containing 50 µg/mL hygromycin were transferred to soil and confirmed by genomic PCR. Three homozygous T₃ transgenic lines were selected for further analysis, and wild-type plants were used as the control.

Twenty-five seedlings for each line, including transgenic and wild-type plants, were used for the analysis of flowering time. The flowering time of *Arabidopsis* was
calculated as the rosette leaf number and days counted when the height of the primary bolts was approximately 1 cm. The days to rice heading were measured as the flowering time when the inflorescence was almost 1 cm long. The expressions of the \( FLC, FT, SOC1, AP1, AP3 \) and \( PI \) genes in \textit{Arabidopsis} and \( Hd1, Hd3a, RFT1, OsMADS50 \) and \( Ghd7 \) in rice, including transgenic and wild-type lines, were analyzed by RT-qPCR using gene-specific primers (Supplementary Table S1). \textit{Actin1} and \textit{OsUbq} were used as internal controls in \textit{Arabidopsis} and rice, respectively [44,45]. Total RNA was extracted from leaves of 25-day-old \textit{Arabidopsis} and 70-day-old rice in the T3 generation. The mixed leaves of three seedlings of every transgenic line as one biological repeat (three biological replicates) were used for RNA extraction. The data analysis was performed as previously described [41].

Subcellular localization analysis

The full-length CDS of \textit{PvSVP1} without a terminator codon (TAA) was cloned into the CaM35S-gfp vector to generate a \textit{PvSVP1}-GFP fusion protein for the investigation of subcellular localization in onion epidermal cells. Transient expression assays were performed using the particle bombardment method [46]. To further determine \textit{PvSVP1} subcellular localization, the \textit{PvSVP1}-GFP construct was transformed into \textit{Arabidopsis} protoplasts in the bimolecular fluorescence complementation (BIFC) assay described below. A confocal laser scanning microscope (LSM510; Zeiss, Oberkochen, Germany) was used to observe onion epidermal cells or protoplasts after 20 h of transfection.

Yeast two-hybrid and BIFC assays

To generate the constructs for yeast two-hybrid assays, the ORFs for \textit{PvSVP1}, \textit{PvMADS56} and \textit{PvVRN1} were amplified using specific primers (Supplementary Table S1). PCR products were ligated into the pGADT7 (AD) or pGBK7 (BD) plasmid. All clones were further sequenced for verification. The resulting constructs were denoted AD-\textit{PvSVP1} and BD-\textit{PvSVP1}, AD-\textit{PvVRN1} and BD-\textit{PvVRN1}, and AD-\textit{PvMADS56} and BD-\textit{PvMADS56}. The prey pGADT7 vector and constructs AD-\textit{PvSVP1}, AD-\textit{PvVRN1}, and AD-\textit{PvMADS56} were transformed into yeast strain Y2H Gold, and the bait pGBK7 vector and constructs BD-\textit{PvSVP1}, BD-\textit{PvVRN1}, BD-\textit{PvMADS56} were transformed into yeast strain Y187 using the Yeast Transformation System 2 (Clontech, Mountain View, USA) and the lithium acetate
method. The cells did not become blue (no autoactivation) when incubated at 30°C for 4-6 days on the selection medium SD/-Leu/X-α-gal and SD/-Trp/X-α-gal. pGBK7-53 + pGADT7-T and pGBK7-Lam + pGADT7-T were positive and negative controls, respectively. For the selection or interaction tests, all yeast transformants were grown on SD/-Trp/-Leu/-His/-Ade/X-α-gal medium.

The CDSs of *PvSVP1, PvVRN1* and *PvMADS56* were amplified using primers listed in Supplementary Table S1 and then cloned into pSAT1-nEYFP-C1 (nYFP) or pSAT4-cEYFP-C1(B) (cYFP) using the ClonExpress II One Step Cloning kit (xx, xx, xx) to generate the constructs *PvSVP1-cYFP, PvVRN1-nYFP*, and *PvMADS56-nYFP*. Protoplast cells were isolated from the leaves of 3-week-old *Arabidopsis* plants and *PvSVP1-cYFP/PvVRN1-nYFP* and *PvSVP1-cYFP/PvMADS56-nYFP* plasmids were cotransformed into *Arabidopsis* protoplasts using the polyethylene glycol (PEG)-mediated transformation method as described previously [47]. As controls, the plasmids *PvSVP1-cYFP/pSAT1-nYFP, pSAT4-cYFP/PvVRN1-nYFP*, and *pSAT4-cYFP/PvMADS56-nYFP* were also cotransformed into protoplast cells. After incubation for 20 h, protoplast cells were observed with a confocal laser scanning microscope (LSM510; Zeiss) at 488 nm excitation and 594 nm emission.

**Bioinformatics analysis**

The *PvSVP1* protein sequence was BLAST searched in the NCBI database ([http://www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) and bamboo protein database (*P. edulis*, *Bonia amplexicaulis*, *Guadua angustifolia*, and *Olyra latifolia*) [48‒50]. The high-similarity sequences obtained from BLAST were used to construct the phylogenetic tree. The SVP-like gene amino acid sequences were aligned by using DNAMAN 7.0 software. A neighbor-joining (NJ) phylogenetic tree was constructed using MEGA 5.0 with 1000 bootstrap replications [51]. Primers were designed with Vector NTI and Premier Primer 5. ProtParam software from ExPASy ([http://exPasy.org](http://exPasy.org)) was used to analyze the physical and chemical properties of the protein. Subcellular localization of the protein was predicted by using WoLF PSORT ([http://www.genscript.com/psort/wolf.psort.html](http://www.genscript.com/psort/wolf.psort.html)).

**Statistical analysis**

Statistical analysis was performed using SPSS 21.0. Differences were analysed by one-way ANOVA followed by Tukey’s test. *P*<0.05 was considered statistically
significant.

Results

Identification and phylogenetic analysis of *PvSVP1*

According to sequence alignment analysis with the *P. edulis* transcriptome database, an *SVP*-like gene was isolated from *P. violascens*, which is named *PvSVP1*. *PvSVP1* contains a 681-bp ORF that encodes 226 amino acid residues. It possesses a MADS-box and a K-box, which are highly conserved in the SVP subfamily of the MADS-box family (Figure 1A). Furthermore, a phylogenetic tree was constructed using a neighbor-joining method. *PvSVP1* is grouped with monocot Poaceae SVP-like proteins and has high similarities to *OsMADS55* (*Oryza sativa*), *TaVRT2* (*Triticum aestivum*) and SVP-like proteins from other bamboo species, including *P. edulis*, *Bonia amplexicaulis*, *Guadua angustifolia*, and *Olyra latifolia* (Figure 1B). This protein is more closely related to *OsMADS55*, with 81.9% identity, and *TaVRT2*, with 76.6% identity (Figure 1B,C).

Spatiotemporal expression patterns of *PvSVP1*

To gain insight into the possible roles of *PvSVP1* in tissue development, we analysed its temporal and spatial expression patterns. *PvSVP1* was expressed in all tested tissues, including young leaves, mature leaves, culms, rhizomes, bamboo shoots and flowers of nonflowering and flowering *P. violascens* (Figure 2A). The mRNA level of *PvSVP1* was the highest in culm and rhizome and lowest in young leaf and shoot in flowering plants. However, the expression of *PvSVP1* was the highest in young leaves and lowest in culm and rhizome in nonflowering plants (Figure 2A). Generally, *PvSVP1* had a higher expression level in the flowering stage than in the nonflowering stage, indicating its putative functional roles in regulating the bamboo flowering transition.

We analysed *PvSVP1* expression in different flowering stages to determine whether *PvSVP1* is related to flowering in *P. violascens*. *PvSVP1* expression changed with the flowering stage from 15 March to 12 April (Figure 2B). *PvSVP1* expression gradually increased from T1 to T2 and then decreased from T2 to T3 in flowering plant leaves (FL) and nonflowering plant leaves (VL) (Figure 2B). However, *PvSVP1* expression in flower (FF) tissue showed the opposite trend: the expression gradually decreased from T1 to T2 and then increased from T2 and T3 (Figure 2B). Moreover, *PvSVP1*
expression was higher in FF than in VL and FL at T1 and T3.

Ectopic expression of \textit{PvSVP1} caused early flowering and abnormal floral morphologies in transgenic \textit{Arabidopsis}

To investigate the role of \textit{PvSVP1} in plant development, the \textit{PvSVP1} gene was ectopically expressed in \textit{Arabidopsis} plants. Transgenic plants were generated with 35S::\textit{PvSVP1} constructs. A total of 30 transgenic \textit{Arabidopsis} \textit{T1} plants were obtained. Among these, 11 independent lines in the homologous \textit{T3} generation for each genotype were screened under controlled conditions. Then, three lines for each set were randomly selected as representatives for phenotype observation. \textit{PvSVP1} overexpression lines displayed early flowering phenotypes (Figure 3A). Compared with the wild-type plants, \textit{Arabidopsis} plants containing 35S::\textit{PvSVP1} (lines 25, 27 and 43) flowered significantly earlier, at 7.1 days on average (Figure 3B; \textit{P}<0.01). Meanwhile, the number of rosette leaves at the time of bolting was lower in the three \textit{Arabidopsis} plant lines carrying 35S::\textit{PvSVP1} than in the control plants, with 3.7 leaves on average (Figure 3C; \textit{P}<0.01). RT-qPCR analysis showed that the promotion of flowering time was associated with \textit{PvSVP1} expression (Figure 3D). For example, \textit{Arabidopsis} line 25 from 35S::\textit{PvSVP1} was the earliest flowering among the three lines and exhibited the highest mRNA level of \textit{PvSVP1} (Figure 3B,D). Moreover, 35S::\textit{PvSVP1} transgenic lines produced abnormal floral organs (Figure 4). The sepals decreased and had leaf-like shapes and did not enclose inner organs (Figure 4A–E) and were present until the capsule matured (Figure 4F,G). The petals showed an open phenotype (Figure 4H,I).

\textit{PvSVP1} overexpression altered the expressions of some flowering-related genes in transgenic \textit{Arabidopsis} plants

In \textit{Arabidopsis}, \textit{FT}, \textit{SOC1}, \textit{FLC}, \textit{AP1}, \textit{AP3} and \textit{PI} play a central role in modulating floral transition and development [52]. \textit{PvSVP1} overexpression was assumed to affect the expressions of these genes, thereby changing flowering time and floral organs in transgenic \textit{Arabidopsis} lines. Compared with the wild-type plants, transgenic lines with \textit{PvSVP1} overexpression showed significantly decreased \textit{FLC} (a repressor) expression, with 70.2% reduction on average (Figure 5). The expression of \textit{FT} (an activator) was significantly increased (317.7%), but that of \textit{SOC1} (another flowering
promoter) was not significantly changed with PvSVP1 overexpression (Figure 5). Thus, early flowering caused by PvSVP1 overexpression might be mainly due to upregulation of FT expression or downregulation of FLC expression to promote flowering in Arabidopsis.

According to previous studies, we chose AP1 (class A gene) and AP3/PI (class B gene), which are essential for flower organ development [53], for further expression analysis. AP1, AP3 and PI expressions were significantly upregulated in 35S::PvSVP1 transgenic plants compared with those in the wild-type plants (Figure 5). RT-qPCR results indicated that AP1 and PI expressions were positively associated with PvSVP1 expression in transgenic lines (Figure 5). These results suggest that overexpression of PvSVP1 in Arabidopsis affects the development of floral organs by modulating AP1, AP3 and PI expressions.

Overexpression of PvSVP1 caused early flowering by regulating flowering-related genes in transgenic rice plants

The relationship between bamboo and Arabidopsis is distant. Therefore, to further confirm the function of PvSVP1, PvSVP1 was overexpressed in rice, which belongs to the Poaceae family, the same as bamboo. We confirmed 52 independent 35S::PvSVP1 transgenic rice T1 lines by genomic PCR; 10 independent lines in the homologous T3 generation were screened, and three lines (line 1, line 5 and line 6) were further chosen to analyze the phenotypic changes. Compared with the wild-type rice, rice plants containing the 35S::PvSVP1 constructs (line 1, line 5 and line 6) flowered significantly earlier, by 6.1 days on average (Figure 6A,B; P<0.01). The promotion of flowering time was also associated with PvSVP1 expression. Among the three lines analysed, line 6 with higher PvSVP1 expression flowered earlier than lines 1 and 5 (Figure 6B,C).

Genes such as Hd3a/RFT1 (FT homolog), OsMADS50/OsMADS56 (SOC1 homolog), and Ghd7 (unique gene) contribute to the modulation of flowering time in rice [54]. To determine whether PvSVP1 overexpression affects the expressions of these genes, we analyzed their expressions by RT-qPCR. Compared with the wild-type rice, rice with PvSVP1 overexpression showed decreased the expressions of OsMADS56 and Ghd7 (as repressors), with reductions of 56.2% and 44.2% on average, respectively (Figure 7). Meanwhile, the expressions of Hd3a and RFT1 (as activators) were greatly increased by 348.1% and 507.5%, respectively, but
OsMADS50 (another flowering promoter) was not significantly affected by PvSVP1 overexpression (Figure 7). These results suggested that PvSVP1 overexpression in rice caused early flowering by inducing Hd3a/RFT1 expression or inhibiting OsMADS56 and Ghd7 expressions.

**Effect of PvSVP1 on transgenic rice stem and panicle elongation**

For rice, plant height is a decisive factor in plant architecture and an important agronomic trait that is directly linked to yield potential [55,56]. The stems and panicles of 35S::PvSVP1 transgenic rice lines showed dwarf phenotypes and were 20.4 cm and 4.6 cm less than those of wild-type rice, respectively (Figure 8A–C and Figure 6A; P<0.01). Qi et al. [56] reported that the OsEATB gene could reduce rice height and panicle length at maturity, so we examined its expression by RT-qPCR. The expression of OsEATB was significantly higher in transgenic plants than in wild-type plants and showed a negative relationship with stem and panicle length (Figure 8D).

**PvSVP1 is located in the nucleus and interacts with PvVRN1 and PvMADS56**

PvSVP1 is located in the nucleus according to WoLF PSORT prediction. To confirm this, the subcellular localization of the PvSVP1 protein was determined by the particle bombardment method using a fusion construct of the PvSVP1 ORF with a green fluorescent protein (GFP). The PvSVP1-GFP fusion protein was found to be located in the nucleus of onion epidermal cells, whereas the control GFP was uniformly distributed in the whole onion cell (Figure 9). To further confirm this observation, the PvSVP1-GFP fusion protein was transiently expressed in Arabidopsis protoplasts by PEG-mediated transformation. Consistent with the results of the former method, PvSVP1-GFP is located in the nucleus of Arabidopsis protoplasts (Figure 10). These results indicated that PvSVP1-GFP is a nuclear protein.

In Arabidopsis, SVP interacts with AP1 and SOC1 [24,57]. Therefore, we also tested the interaction of PvSVP1 with an AP1 homolog (PvVRN1) [58] and SOC1 homolog (PvMADS56) in P. violascens [11]. The PvSVP1, PvVRN1 and PvMADS56 genes in pGBK7T7 or pGADT7 showed white colonies in SD/-Leu/X-gal or SD/-Trp/X-gal media, respectively (Supplementary Figure S1). These results eliminated the possibility of self-activation in the yeast two-hybrid assay. The positive control showed blue colonies on SD/-Trp/-Leu/-His/-Ade/X-alpha-gal, but the negative
control failed to grow. Meanwhile, yeast zygotes containing pGBK7-PvSVP1 with pGADT7-PvVRN1 and pGADT7-PvMADS56 constructs developed blue colonies (Figure 11). These results indicate a direct positive interaction of PvSVP1 with PvVRN1 and PvMADS56 in yeast.

In addition, their interactions were further verified by BIFC in transient assays. The CDSs of \(\text{PvSVP1}, \text{PvVRN1}\) and \(\text{PvMADS56}\) were cloned into pSAT1-nYFP and pSAT4-cYFP to generate BIFC constructs, which were cotransformed into \(\text{Arabidopsis}\) protoplasts by PEG-mediated transformation. A strong YFP signal was detected only in the nucleus of protoplasts transformed with plasmids \(\text{PvSVP1-cYFP/PvMADS56-nYFP}\) and \(\text{PvSVP1-cYFP/PvVRN1-nYFP}\) (Figure 12). BIFC analysis confirmed the direct interaction of PvSVP1 with PvVRN1 and PvMADS56 in plant cells.

Discussion
To date, a few \(\text{SVP/AGL24}\) homologues from plants have been cloned, and their functions have been investigated, but \(\text{SVP/AGL24}\)-like genes in bamboo are still unknown. In the current study, we identified and characterized an \(\text{SVP/AGL24}\)-like MADS-box gene from \(\text{P. violascens}\). Sequence comparison and phylogenetic analysis showed that this protein clusters with \(\text{SVP}\) homologous proteins from monocots and is closely related to \(\text{OsMADS55}\) from \(\text{O. sativa}\). Moreover, it has higher similarity to SVP (52.7%) than to AGL24 (46.7%) (Supplementary Figure S2), so the bamboo \(\text{SVP/AGL24}\)-like gene was named \(\text{PvSVP1}\). To explore its function, overexpression experiments were conducted in \(\text{Arabidopsis}\) and rice. \(35S::\text{PvSVP1}\) transgenic \(\text{Arabidopsis}\) and rice plants showed abnormal floral organs. These results are consistent with \(\text{SVP/AGL24}\) MADS-box genes from \(\text{Arabidopsis}\), rice, and barley, which induce flower abnormalities and floral reversion [17,27,59,60].

In \(\text{Arabidopsis}\), \(\text{AP1}\) (A-function genes) and \(\text{AP3/PI}\) (B-function genes) are required for producing floral organs, including sepals and petals [61–63]. In the current study, the \(35S::\text{PvSVP1}\) transgenic \(\text{Arabidopsis}\) plants displayed abnormal sepals and petals, and the transcript levels of \(\text{AP1}, \text{AP3}\) and \(\text{PI}\) were significantly increased. Among these three genes, the expressions of \(\text{AP1}\) and \(\text{PI}\) were positively associated with that of \(\text{PvSVP1}\) (Figure 6). These results suggest that \(\text{PvSVP1}\) might be involved in the development of floral organs by directly upregulating \(\text{AP1}\) and \(\text{PI}\).
or indirectly upregulating AP3 in transgenic Arabidopsis. Therefore, we proposed that SVP genes in regulating floral meristem identity may be well conserved between bamboo and other species. SVP is a negative regulator of flowering time in Arabidopsis. Its homologues from other dicots also delay flowering time when overexpressed in Arabidopsis [64]. Overexpression of OsMADS55 in wild-type Arabidopsis plants results in delayed flowering, but that in rice does not change the flowering time [28,30]. In contrast, ectopic expression of PvSVP1 in wild-type Arabidopsis and rice caused early flowering, suggesting that PvSVP1 has an opposite function for regulating flowering time. Obviously, SVP-like genes are subject to functional differentiation in bamboo and rice. This divergence might be due to the unique flowering characteristics of bamboo, which are different from those of other species. Low expression of floral pathway integrator genes, including the homologues of SVP, SOC1 and FT, in floral tissues of P. edulis suggested that bamboo flowering does not depend on these known pathways [39]. Furthermore, studies have shown that bamboo PvMADS56 (a floral activator) and PvPin1 (a floral repressor) have opposite functions in flowering time compared with their homologues, such as rice OsMADS56 (a floral repressor) and Arabidopsis Pin1At (a floral activator), respectively [11,13]. In addition, consistent with PvSVP1, wheat TaVRT2 (SVP homolog) promotes flowering in the vernalization regulatory pathway [32], even though both have higher similarity to SVP than to AGL24. Therefore, SVP homologous genes might play different roles in different species in regulating flowering time. These results suggest that bamboo has its own particular flowering mechanism.

Previous studies have shown that SVP can delay flowering by directly regulating FLC, SOC1 and FT expressions in Arabidopsis [17,19]. Overexpression of MiSVP1/MiSVP2 from Mangifera indica in Arabidopsis delays or accelerates flowering time by regulating AtFLC, AtSOC1 and AtFT expressions [65]. To explore the reason behind the earlier flowering of 35S::PvSVP1 Arabidopsis plants, we examined and compared the gene expressions of these genes in transgenic and wild-type Arabidopsis. The results showed that the transcript levels of FLC and FT were associated with PvSVP1 expression. Furthermore, FLC and FT gene expression was downregulated or upregulated, respectively, in Arabidopsis plants containing the 35S::PvSVP1 construct. These results suggest that PvSVP1 affects flowering time by directly regulating FLC and FT expressions in transgenic Arabidopsis plants.

The rice homologues of the FT, Hd3a and RFT1 genes are also essential for
flowering [66]. 

RFT1 expression is induced via a unique pathway in which OsMADS50 (SOC1 homolog) plays a pivotal role, enabling rice plants to flower [66-67]. OsMADS56 (another SOC1 homolog) and Ghd7 (unique gene) are rice-specific and important floral repressor genes [68]. In this study, we found that 35S::PvSVP1 transgenic rice plants with higher expression of Hd3a/RFT1 and lower expressions of OsMADS56 and Ghd7 showed the phenotype of early flowering. Moreover, similar to SOC1, OsMADS50 expression did not significantly differ between transgenic and wild-type lines. Only OsMADS56 expression was positively associated with the flowering time and PvSVP1 expression of transgenic lines. Taken together, these results suggest that PvSVP1 overexpression in Arabidopsis and rice causes early flowering by regulating some important promoters and repressors directly or indirectly. Interactions between proteins are essential for their functioning and the biological processes they control. During the formation and growth of the floral meristem of Arabidopsis, protein heterodimers such as AP1-SVP, AP1-AGL24 and SEP3-SVP are essential to establish floral meristem identity and simultaneously repress genes that control the formation of floral organs [25, 57, 69]. Similar interactions between SVP-like and AP1-like proteins exist in Prunus avium, Poncirus trifoliate, Antirrhinum majus, Lolium perenne, Triticum aestivum, Mangifera indica and Cymbidium goeringi (Supplementary Table S2). In yeast two-hybrid assays, INCO interacts with SQUA [31], LpMADS10 interacts with LpMADS1 (LpVRN1) [70] and TaVRT2 interacts with TaVRN1 [32,71]. Like SVP homologues, PvSVP1 could interact with PvVRN1 (a VRN1 homolog that belongs to the FUL1 clade of Poaceae AP1/SQUA-like genes) in yeast two-hybrid assays and BIFC experiments. Therefore, the regulatory mechanism involving interactions of SVP-like with AP1-like proteins in controlling flower development may be conserved in bamboo and other plants.

SVP represses SOC1 transcription in the shoot apex and leaf by directly binding to SOC1 CArG motifs that further regulate flowering time [17]. However, unlike SVP in Arabidopsis, SVP homologues from Brassica juncea (BjuSVP) [72] and Cymbidium goeringii (CgSVP) [73] can directly interact with SOC1 homologues (BjuSOC1 or CgSOC1) in vitro and in vivo (Supplementary Table S2). Similarly, PvSVP1 interacts with PvMADS56 (SOC1 homolog), as demonstrated by the yeast two-hybrid assay and BIFC analysis. These data indicated that SVP-like genes are diverse in their regulatory mechanisms.
Ma et al. [58] reported that 35S::PvVRN1 transgenic plants only displayed abnormal floral organs. In the same year, Liu et al. [11] reported that when overexpressed, PvMADS56 in Arabidopsis exhibits an early flowering phenotype and abnormal floral organs. Therefore, it was speculated that PvSVP1 might affect flowering time and flower development by interacting with PvVRN1 and PvMADS56 directly in bamboo.

Several SVP-like genes play a role in stem development. BM1 expression is vital for barley in the nodes and internodes, and its overexpression in the stem enhances stem elongation [27]. In rice, OsMADS55 and OsMADS22 are highly expressed in stems, and a double RNAi experiment demonstrated that OsMADS22 assists the functioning of OsMADS55 in controlling stem elongation [28,31]. Further experiments showed that OsMADS55 overexpression induces stem elongation, but OsMADS22-overexpressing plants produce shortened stems [28]. In this study, we found that PvSVP1 was also strongly expressed in culm of flowering plants, so the role of PvSVP1 in culm development could be predicted from its expression pattern. 35S::PvSVP1 transgenic rice plants exhibited a significant phenotype of dwarfism, suggesting that PvSVP1 is involved in stem elongation in transgenic rice, similar to OsMADS22 instead of OsMADS55. Additionally, a shorter panicle was observed in transgenic rice plants. Qi et al. [56] reported that OsEATB could reduce rice height and panicle length at maturity. Here, RT-qPCR results suggested that PvSVP1 affects the stem and panicle growth of transgenic rice by positively downregulating OsEATB expression.

Supplementary Data
Supplementary data is available at Acta Biochimica et Biophysica Sinica online.

Funding
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Conflict of Interest
The authors declare that they have no conflict of interest.
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Chromosome-level reference genome and alternative splicing atlas of moso
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Figure legends

**Figure 1. Sequence and phylogenetic analyses of *PvSVP1*** (A) Conserved functional analysis of the *PvSVP1* domain. (B) Phylogenetic tree inferred from amino acid sequences of *PvSVP1* and its orthologues from other plant species. Amino acid sequences were aligned by using Clustal W, and the tree was constructed by the neighbor-joining method with Mega 7.0. Bootstrap values were calculated from 1000 replicates. Sequence data were from *Hordeum vulgare* (BM1 and BM10), *Lolium perenne* (LpMADS10), *Zea mays* (ZMM19, ZMM20, ZMM21 and ZMM26), *Triticum aestivum* (TaVRT2), *Oryza sativa* (OsMADS22, OsMADS47 and OsMADS55), *Solanum tuberosum* (StMADS11 and StMADS16), *Eucalyptus occidentalis* (EgSVP), *Arabidopsis thaliana* (SVP and AGL24), *Brassica rapa* (BcSVP), *Antirrhinum majus* (INCO), *Ipomoea batatas* (IbMADS3), *Citrus trifoliata* (PtSVP), *Phyllostachys edulis* (PeSVP1), *Bonia amplexicaulis* (BamSVP1.1, BamSVP1.2 and BamSVP1.3), *Guadua angustifolia* (GanSVP1.1, GanSVP1.2 and GanSVP1.3), *Olyra latifolia* (OlaSVP1.1 and OlaSVP1.2) and *Raddia guianensis* (RguSVP1). (C) Multiple alignment of the deduced amino acid sequences of *PvSV*

**Figure 2. Spatial and temporal expression of *PvSVP1*** in flowering and nonflowering *P. violascens* (A) Relative expression of *PvSVP1* in different tissues of flowering and nonflowering plants. (B) Relative expression of *PvSVP1* in leaves of
nonflowering plants (VL), flowering plants (FL) and flowers (FF) of flowering plants during flowering development. T1, T2 and T3 represent the sampling times. T1 is the time when the floral bud formed and switched from the vegetative phase to the reproductive stage (15 March); T2 is the time when the inner organs of the flower began to form, which was examined by anatomy under a stereomicroscope (29 March); and T3 is the bloom stage when the anther was outcropped from palea (12 April). Data are shown as the mean ± SEM from three biological replicates.

Figure 3. Phenotypic analysis of wild-type (WT) and transgenic Arabidopsis plants
Overexpression of PvSVP1 promoted flowering in WT Arabidopsis. Three independent lines (25, 27, and 43) in the T3 generation were analyzed. (A) Early flowering phenotype of 35S::PvSVP1 transgenic Arabidopsis plants. Days to flowering (B) and rosette leaf number (C) for 35S::PvSVP1 transgenic plants. (D) RT-qPCR analysis of PvSVP1 expression in transgenic plants overexpressing PvSVP1. Data are shown as the mean ± SEM from three biological replicates. Scale bar: 5 cm. **P<0.01.

Figure 4. Flower phenotypes of wild-type (WT) and 35S::PvSVP1 Arabidopsis plants
Inflorescence and close-up view of a flower of wild-type (A, B, D and H) and 35S::SVP1 (C, E and I) plants. Siliques of WT (F) and 35S::PvSVP1 pants (G). Scale bar: 1 mm.

Figure 5. RT-qPCR analysis of FT, SOC1, FLC, AP1, AP3 or PI expressions in 35S::PvSVP1 transgenic plants and WT Arabidopsis
Data are shown as the mean ± SEM from three biological replicates. **P<0.01.

Figure 6. Overexpression of PvSVP1 promoted flowering in wild-type (WT) rice
(A) 35S::PvSVP1 transgenic plants showed the phenotypes of early flowering and dwarfism. (B) Days to heading T3 homozygous 35S::PvSVP1 transgenic lines (lines 1, 5 and 6). (C) RT-qPCR analysis of PvSVP1 expression in transgenic plants. Scale bar: 5 cm. Data are shown as the mean ± SEM from three biological replicates. **P<0.01 by Student’s t tests.

Figure 7. RT-qPCR analysis of OsMADS50, OsMADS56, Hd3a, RFT1 and Ghd7
expressions in 35S::PvSVP1 transgenic and wild-type (WT) rice plants. Data are shown as the mean ± SE from three biological replicates. **P < 0.01 by Student’s t tests.

Figure 8. Phenotypes of transgenic rice plants overexpressing PvSVP1. (A) Plant height of wild-type (WT) and 35S::PvSVP1 plants (lines 1, 5 and 6) (n=20). (B) Panicle size of WT and 35S::PvSVP1 plants (lines 1, 5 and 6) (n=20). (C) Panicle morphology for wild-type (WT) and 35S::PvSVP1 plants. (D) OsEATB expression in wild-type (WT) and 35S::PvSVP1 plants (Lines 1, 5 and 6). Data are shown as the mean±SEM from three biological replicates. **P < 0.01 by Student’s t tests.

Figure 9. Subcellular location of GFP and PvSVP1-GFP in onion cells.

Figure 10. Subcellular location of GFP and PvSVP1-GFPs in Arabidopsis protoplasts. Scale bar: 15 μm.

Figure 11. PvSVP1 interacts with PvVRN1 and PvMADS56. Examination of the interactions between these proteins by yeast two-hybrid assay. Yeast containing dual vectors of pGBK7T-53 and pGAD7-T was used as the positive control, and yeast containing pGBK7-Lam and pGAD7-T was used as the negative control. SD/-Leu/-Trp/-His/-Ade medium was used for clone selection. Positive interaction is indicated by X-α-gal activity.

Figure 12. BIFC assay of protein/protein interactions of PvSVP1 and PvVRN1, PvSVP1 and PvMADS56 in Arabidopsis cells. Images were captured 20 h after transient expression under an Olympus confocal microscope. Scale bar: 15 μm.
99x72mm (300 x 300 DPI)
99x42mm (300 x 300 DPI)
149x110mm (300 x 300 DPI)
150x57mm (300 x 300 DPI)
149x87mm (300 x 300 DPI)
Bright light  GFP  Chlorophyll  Merged

GFP

PvSVP1-GFP

78x33mm (300 x 300 DPI)
BD-PvSVP1 + AD-PvVRN1

AD-PvSVP1 + BD-PvVRN1

Positive

BD-PvSVP1 + AD-PvMADS56

AD-PvSVP1 + BD-PvMADS56

Negative

313x198mm (300 x 300 DPI)
93x102mm (300 x 300 DPI)
**Arabidopsis thaliana**

**PvSVP1**

**FLC**
**FT**
**AP1**
**AP3**
**PI**

**PvMADS56**

**Hd3a**
**RFT1**

**OsMADS56**

**Oryza sativa**

**Bamboo**
Highlight

The *Short Vegetative Phase* (*SVP*) gene plays a pivotal role in floral transition and development. Here, *PvSVP1*, an *SVP*-like gene from *Phyllostachys violascens*, is isolated and characterized. In addition, to investigate its function, *PvSVP1* is overexpressed in *Arabidopsis* and rice.

- *PvSVP1* is expressed in tested organs but preferentially in vegetative tissues.
- Ectopic expression of *PvSVP1* in *Arabidopsis* causes early flowering and abnormal floral organs by regulating the expressions of *FLC, FT, AP1, AP3* and *PI* genes.
- Overexpression of *PvSVP1* in rice promotes flowering by altering the expressions of flowering-related genes such as *Hd3a, RFT1, OsMADS56* and *Ghd7*.
- *PvSVP1* may be a nuclear protein and interacts with *PvVRN1* and *PvMADS56* in yeast-two hybrid and BiFC assays.
**Supplementary Table S1. Oligonucleotide sequences used for expression analysis and cloning in this study**

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<th>Primer application</th>
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<td></td>
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<td><strong>PvMADS56</strong></td>
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Supplementary Table S2. Interactions of SVP-like proteins with AP1-like or SOC1-like proteins in previously reported species and *Phyllostachys violascens*

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<td>SVP/SOC1</td>
<td><em>Mangifera indica and Brassica juncea</em></td>
<td><em>Cymbidium goeringii and Phyllostachys violascens</em></td>
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Supplementary Figure S1. Self-activation of these three proteins were examined by yeast two-hybrid assay. The positive control (PCL) turned blue, while the PvSVP1, PvVRN1 or PvMADS56 remained white whether they were served as prey or as bait.
Supplementary Figure S2. Multiple alignment of the deduced amino acid sequences of PvSVP1, SVP and AGL24