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ORIGINAL ARTICLE

# Ectopic expression of *PvSOC1*, a homolog of *SOC1* from *Phyllostachys violascens*, promotes flowering in *Arabidopsis* and rice

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**Abstract** MADS-box protein SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1) plays an important role in the regulation of flowering by integrating multiple flowering signals in *Arabidopsis thaliana*. *SOC1*-like genes have been isolated from many plants and their functions received wide attention. To investigate *SOC1* homologs in bamboo, a *SOC1* homolog, referred to as *PvSOC1*, was isolated from *Phyllostachys violascens*, and characterized. PvSOC1 encoded a protein of 226 amino acids containing a highly conserved SOC1 motif. *PvSOC1* transcript was expressed in all examined tissues but preferentially in leaves. Ectopic expression of *PvSOC1* in both *Arabidopsis* and rice caused early flowering, abnormal floral organs and leaves. A P-box, the gibberellin response element existed in the promoter region of *PvSOC1* but not in that of *SOC1* and *OsSOC1*. Upon gibberellic acid treatment, *PvSOC1* transcript was upregulated in the leaves of *Ph. violascens* seedlings, suggesting a positive response to gibberellin acid. Thus, our experiments suggest *PvSOC1* to be a multifunctional gene, which not only regulates flowering, but also affects leaf development.

**Keywords** *Phyllostachys violascens* · *PvSOC1* · Flowering · Activator

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S. Liu and T. Ma contributed equally to this work.

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## Introduction

To produce progeny and perpetuate the species, flowering plants must flower in time, where a successful initiation of flowering is critical and known controlled by multiple endogenous signal pathways under the influences of environmental factors. The well-studied pathways include photoperiod, autonomous, vernalization and gibberellin-dependent signaling (Johanson et al. 2000; Kin et al. 2009). Although they interact with each other and form complex regulatory networks, they converge onto a handful of floral integrator genes including *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*), *FLOWERING LOCUS* (*FT*), *LEAFY* (*LFY*) and *FLOWERING LOCUS C* (*FLC*) (Boss et al. 2004; Amasino and Michaels 2010; Fornara et al. 2010).

*SOC1* is a crucial integrator that aggregates signals from different flowering pathways and a flowering activator in *Arabidopsis* (Lee et al. 2000; Samach et al. 2000; Moon et al. 2003). In the photoperiod pathway, *SOC1* is regulated by *CO* through *FT*, causing an early flowering (Yoo et al. 2005). Under short days, GA can activate the expression of *SOC1* (Moon et al. 2003). As a strong repressor of flowering, *FLC* is the merging point of the autonomous and vernalization pathways (Moon et al. 2003), and can inhibit *SOC1* expression directly and indirectly by binding to the *SOC1* regulatory region (Hepworth et al. 2002). In addition

to control flowering time, *SOC1* can regulate floral meristem determinacy and floral patterning by activating *LFY* and *AP1* (Liu et al. 2009; Melzer et al. 2008; Lee and Lee 2010). To date, *SOC1*-like genes have been cloned and studied from many plants including *Oryza sativa* (*OsSOC1*), *Triticum aestivum* (*WSOC1*), *Zea mays* (*ZmMADS1/3*), *Vigma unguiculata* (*VuSOC1*), and *Vitis vinifera* (*VvSOC1*). The results demonstrate that they are widely expressed in various tissues such as roots, leaves, shoot meristems and floral organ primordia (Nakamura et al. 2005; Papaefthimiou et al. 2012). Additionally, *SOC1*-like genes promote flowering when overexpressed, similar to *SOC1*, but some involve in the floral development as well. Although *SOC1* genes are conserved and have similar expression patterns among plant species, their functions can be divergent in angiosperms (Lee and Lee 2010; Zhong et al. 2012). Ectopic expression of *UNSHAVEN* (*UNS*), a *SOC1*-like gene of Petunia hybrid, leads to ectopic trichome formation on floral organs and conversion of petals into organs with leaf-like features accompany by early flowering phenotype (Ferrario et al. 2004; Zhong et al. 2012). A homolog of *SOC1* in *Prunus armeniaca*, *ParSOC1*, may be involved in the regulation of dormancy break of the vegetative shoots (Trainin et al. 2013). Moreover, *SOC1* also involves in stomatal regulation (Kimura et al. 2015). Thereby, a detail study may be required to uncover its functional conservation and divergence for a specific plant.

Bamboo belongs to grass family but differs from many other members in the kingdom; it has elusive characteristics in flowering. The fact that it has an unpredictable and long vegetative period before flowering, and undergoes death after flowering brings challenges to investigate its flowering molecular mechanisms. Additionally, numerous factors, such as biological clock, nutrition, temperature and soil conditions, and endogenous hormones (CTK, GA and ABA), all are reported to affect bamboo flowering (Janzen 1976; Keeley and Bond 1999). However, the flowering mechanism of bamboo still remains to be elucidated.

*SOC1*-like genes have been identified and found to play a conserved role as a key flowering gene in eudicot and monocot plants, but *SOC1* homologs in bamboo have not been reported. In our study, a *SOC1*-like gene was isolated, referred to as *PvSOC1*, from *Phyllostachys violascens* (Poaceae: Bambusoideae), and characterized its biological properties. The gene was analyzed through its sequences structure, expression pattern in bamboo, and transgenic expression in *Arabidopsis* and rice. The results showed that the gene is conservative in the promotion of bamboo flowering and regulation of floral organs development, but different from most known *SOC1*-like genes, it might additionally regulate leaf development.

## Materials and methods

### Plant materials and growth conditions

All *Ph. violascens* samples for isolation and quantitative real-time PCR were collected in the campus of Zhejiang A & F University. Wild-type and *soc1* mutant (SALK_138131C) *Arabidopsis thaliana* in Columbia-0 background as well as *35S::PvSOC1* transgenic *Arabidopsis* plants of the same background were cultivated under the condition of 16 h light/8 h dark at 22 °C. All rice of transgenic lines and wild-type plants used here were from *O. sativa* cv. Zhonghua11, which were cultivated in the paddy field of Lin'an (Hangzhou, north latitude 30°14′ and east longitude 119°42′).

### Isolation of *PvSOC1* cDNA and its promoter from *Ph. violascens*

Isolation of total RNA from *Ph. violascens* was performed using a Trizol Method. To isolate a *SOC1*-like gene from *Ph. violascens*, we compared the amino acid sequences of *SOC1* homologs from grass family plants including *O. sativa*, *T. aestivum* and *Zea mays* (Vandenbussche et al. 2003; Nakamura et al. 2005), and designed the primers (S1 and S2 in Table 1) from the conserved regions. Specific *SOC1*-like cDNA fragment (approximately 250 bp) was amplified using the pair of primers (S1 and S2). The cDNA fragment was then ligated into the pMD18-T vector, and confirmed as partial sequence of a *SOC1*-like gene base on sequencing. To acquire the full-length sequence of this cDNA, 3′-RACE (Rapid Amplification of cDNA ends) was performed with the gene-specific primers (3′GSP1 and 3′GSP2, Table 1) base on the manufacturer's recommendation (Invitrogen Company). The 5′ sequence of the gene was obtained by sequence alignment against the known *PvSOC1* partial sequence and the RNA-seq database of *Ph. violascens*. Eventually, the full-length cDNA sequence was obtained by the primers (FGSP1 and FGSP2) based on the known 5′ and 3′ sequences. Genomic DNA was isolated from the collected leaves using modified CTAB method. Two primers (WGSP1 and WGSP2, Table 1) were designed according to the known 5′UTR sequence. The 800 bp fragment was isolated from the DNA using BD Genome-Walker kit following the manual instruction. A sequence of 1496 bp was then obtained by the primers S3 and S4 that were designed by using the acquired sequence and *Phyllostachys edulis* transcriptome database (Peng et al. 2013).

### Bioinformatics analysis

The proteins sequences of homologs were obtained through BLAST searches in the NCBI database. The phylogenetic

**Table 1** Primers used in this study

| Primer | Sequences (5′–3′) | Purpose |
|---|---|---|
| S1 | GGGAAGACGCAGATGAAGCGGAT | Clone cDNA fragment |
| S2 | ACTTGCTCTATATCTTGCTGTAC | |
| 3′GSP1 | GCACCAAGACAGTAGAGCAAGAT | 3′-RACE |
| 3′GSP2 | GATGCTGAGGGCTTGGCAA | |
| FGSP1 | GTGGTCTGGATTTGGGGGCT | Clone full-length cDNA fragment |
| FGSP2 | CGGTTGATTTTAGGGTGTCATAGC | |
| RT-F | ATGGCTTGGCAAAGACACT | RT-qPCR |
| RT-R | GCTGAGTCTTCCTTCCCCTGA | |
| Actin-F | CTCCTCGTCTCCTTCCCGAA | RT-qPCR |
| Actin-R | GTCCGTTGCTGTAAATGTGTGG | |
| WGSP1 | GGCATCAAACAGACATACAACACAATG | Genome walking |
| WGSP2 | CAACACAGCACAAGCATAACATAAC | Genome walking |
| S1 | ATATGGAGCCACATGTCAACAAGAT | Clone promoter |
| S2 | CATCTTGGACCAACCGACGGATGGA | Clone promoter |

tree was built using neighbor-joining method of MEGA version 5.0 software with bootstrap values obtained form 1000 replications (Tamura et al. 2011). The molecular weights (MW) and theoretical isoelectric point (pI) of the protein was analyzed using the software ProtParam from ExPASy (http://exPasy.org).

### Gene expression assay

Total RNAs from collected samples of the non-flowering and flowering *Ph. violascens* was extracted. The gene-specific primers were designed in accordance with the *PvSOC1* ORF while *PheUBC18* was used as a reference (Qi et al. 2013) (Table 1). RT-qPCR reactions were run on the instrument of CFX96TM Real-Time PCR Detection System (Bio-Rad) following the 2 × SYBR Premix Ex Taq II mix manuals. The amplification was 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 consisting of 10 μL 2 × SYBR Premix Ex Taq II mix, 0.5 μL each of forward or reverse primer (Table 1), and 1 μL cDNA template (50 ng/μL). The sample was made up with water to a final volume of 20 μL. The data was analyzed by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

### Plasmid construction and transformation

The full-length ORF of *PvSOC1* gene was ligated into the binary vector *pCAMBIA*1301 to generate the plasmid *35S::PvSOC1*. The plasmid was transferred into wild-type *Arabidopsis* plants (Col-0) using the floral dipping method mediated by *Agrobacterium tumefaciens* strain GV3101 (Clough and Bent 1998). All transgenic *Arabidopsis* seeds were picked out on 1/2 MS solid medium containing 50 mg/L hygromycin. The same construct was also transformed into *A. tumefaciens* strain EH105 and then into wild-type rice plants (Zhonghua 11) according to Agrobacterium-mediated co-cultivation methods (Lee et al. 1999). Positive transgenic lines were confirmed by genomic PCR.

### GA treatment

The experiment of GA treatment was performed as described by Liu et al. (2016). $GA_3$ treatment was performed by ejecting the leaves of *Ph. violascens* seedlings with the $GA_3$ solution (40 mg $L^{-1}$ $GA_3$, 0.02 % Silwett-77) or water as mock solution once a day for 10 days. $GA_3$ treatment was carried out with two biological replicates. Then total RNA of the leaves was extracted for further detecting the *PvSOC1* transcript level.

## Results

### Isolation of *PvSOC1* gene

To isolate *SOC1*-like gene in bamboo, a cDNA fragment (approximately 250 bp) was obtained by amplification using primers designed based on the conserved regions of the gene in the known grass family plants. The 5′ sequence was obtained by blasting the 250 bp sequence and *Ph. violascens* RNA-seq database (data not shown), while the 3′ sequence was amplified by 3′-RACE. The full-length cDNA of 1025 bp was cloned by aligning the 5′ and 3′ sequences, referred to as *PvSOC1*, which contains a 681 bp ORF that encoding a protein of 226 amino acids. Analysis of PvSOC1 amino acid sequence showed the theoretical values pI/Mw of 8.99/25653.3. Like other MADS-box

proteins, PvSOC1 also contains the more conserved M and K domain as well as the less conserved I domain and the divergent C-terminal region (Fig. 1). Phylogenetic comparison of the PvSOC1 protein with its orthologs in other plants showed that PvSOC1 was closely related to these from the monocots rather than eudicots, especially the monocot *Ph. edulis* and *O. sativa* (Fig. 2).

## Expression pattern of *PvSOC1*

To characterize the expression pattern of *PvSOC1*, tissue-specific expression analysis was carried out by RT-qPCR using aerial tissues. Although *PvSOC1* transcripts were detectable almost in all tested organs (Fig. 3), including culm, leaf bud, young leaf, mature leaf, as well as floral bud, they were mainly expressed in leaves (leaf bud, young and mature leaf). Comparison of *PvSOC1* expression in non-flowering and flowering plants showed that the expression levels of *PvSOC1* exhibited no remarkable change in culms but significant change in leaf buds, young or mature leaves. In the non-flowering plants, the expression level of *PvSOC1* decreased with differentiation and development of leaf from leaf bud, young leaf to mature leaf but increased with leaf development in the flowering *Ph. violascens* plants. In addition, *PvSOC1* expression level in the floral bud was medium among other tissues.

## Comparison of the promoter sequences for *PvSOC1, SOC1* and *OsSOC1*

To analyze the correlation between the expression patterns of *PvSOC1*, *AtSOC1* and *OsSOC1* genes and the regulation of their promoters, their promoter sequences were assessed. A 1496 bp 5′ flanking sequence of *PvSOC1* gene was

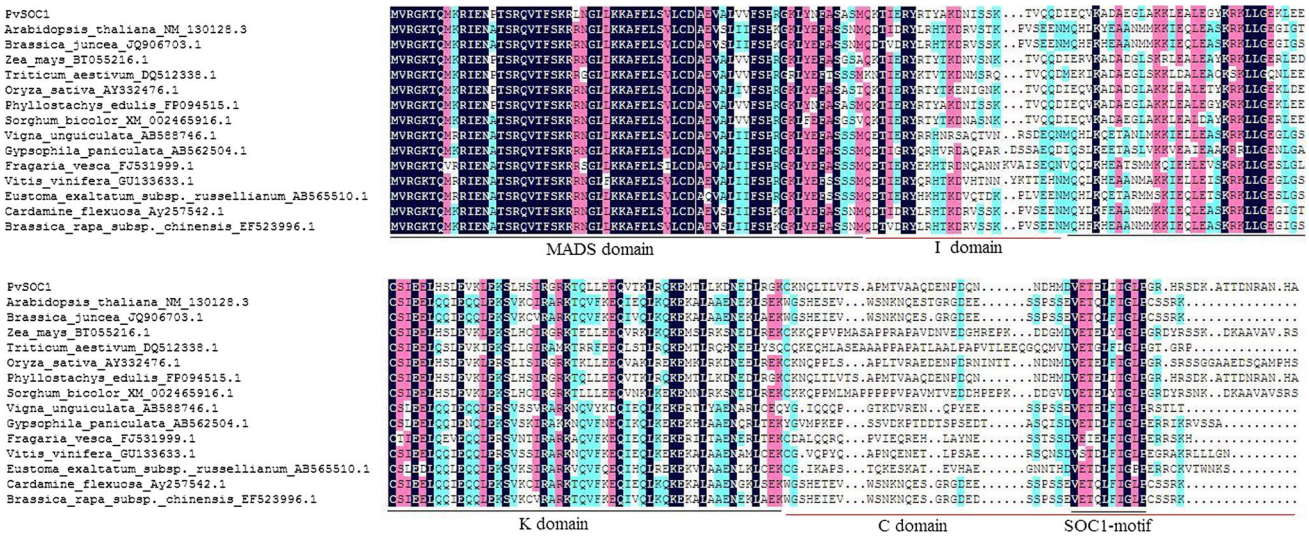


**Fig. 1** Alignment of the amino acid sequences of PvSOC1 and its orthologs from other plant species. The regions of MADS domain, K domain and the specific SOC1 motif are *underlined*

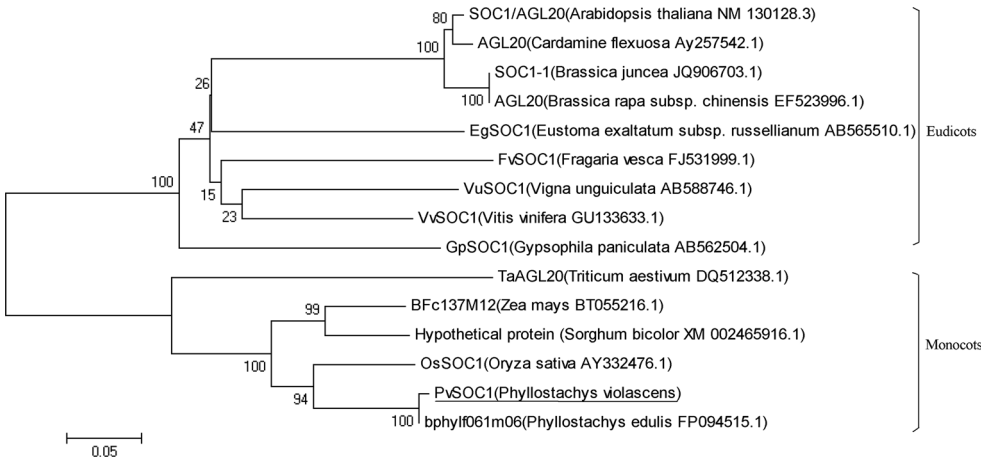


**Fig. 2** Phylogenetic analysis of PvSOC1 and its orthologs from various plant species. The phylogenetic tree was generated with the neighbor-joining algorithm. Bootstrap values (>50 %) in 1000 replicates are indicated next to the nodes. The PvSOC1 is *underlined*

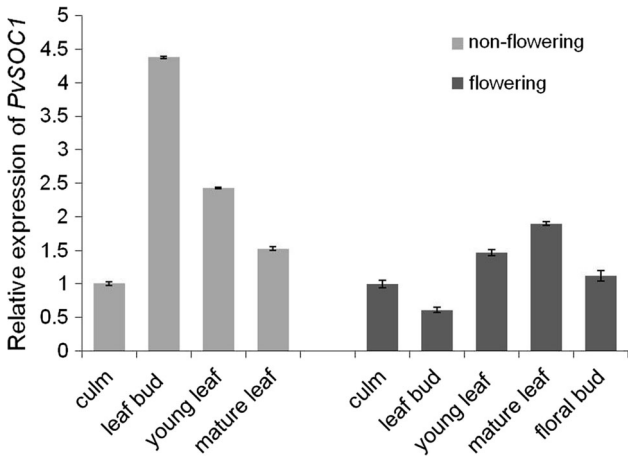


**Fig. 3** Relative expression level of *PvSOC1* in different aerial tissues of flowering and non-flowering *Ph. violascens*

cloned, and the promoter sequences of *AtSOC1* and *OsSOC1* in the same length were extracted from NCBI database. *PvSOC1* shared 36.26 and 45.51 % similarity with *SOC1* and *OsSOC1* by sequence alignment, respectively. Using PlantCARE, we predicted the existence of potential *cis*-acting regulatory elements. The typical CAAT-box, TATA-box core elements, and other elements associated with light responsive and hormone such as G-box and Sp1 were commonly found in these three promoters (Fig. 4). However, the promoter sequences of *PvSOC1* and *OsSOC1* shared more of the same *cis*-acting elements, such as A-box, CCGTCC-box, MBS and TCA element, while G-Box, LTR, P-box and dOCT were only found in the promoter of *PvSOC1* (Table S1).

## Ectopic expression of *PvSOC1* promotes to flower in *Arabidopsis*

To explore the functional role of *PvSOC1*, its open reading frame was fused to the pC1301 vector with CaMV *35S* promoter and introduced into wild-type *Arabidopsis*. Two independent lines in the homozygous $T_3$ generation were chosen for further analysis. The *35S::PvSOC1* transgenic

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                                                          CAAT-box      TATA-box TATA-box   CAAT-box
-1496 TATGGAGCCA CATGTCAACA AGATATCGAT GAGTAAATAC TCAAATGTAC CTATTTATAG TAATAAATCC TCAATAATTC
        TATA-box                                                        CAAT-box
-1426 ATTTAAAAAC ATATGGCCCA AACCAAACTT CAGCAACAGA TGTATTCACC TTACAAATGG ATCATCTGCA TTGAATGCAA
                       G-Box                    MBS     CAAT-box       CAAT-box       CAAT-box
-1346 AGGATCTACA GTGGCACGTA TTAGAAACCG ATATCAACTG TCCAAATCAC CCGATACAAT GTTTGCTACA ATAATATCAA
                                                          TATA-box                       dOCT
-1266 CACAGTAAAT ATCCACGGTA GAGCTACAGT ACCTGCTACA GTATAACTAT AGAGTACTAC TCACTCGCTC ACGGATCTTG
-1186 TTCACACAGT TCGGATCCAT TCATCCCGTG CGGCAGCAAC AAGAAGCTGG AGCAGTGCTA GTGCCTGCGG AAACCAACTT
-1106 GCAGCTGCGA CACACACGTC GTGGTGAGGC AGCAGCAGTC CAAGCCGTCC TGCCTCATCT GCTGTTGCTA GACTTGTTTA
                                                                                      LTR
-1026 GATGCCGAAC CGGGCCGGGC TTGAAAAGCC CGGTCTGAAA CAGGCAGGCC CGAGCTCGGC CCCGTCCGAA AGTTGAGTTT
         TATA-box          TATA-box              TATA-box
 -946 TTCATTTTAA AATATTATTG TAATAATTTT CCAGAAAAAG TAATATTTAA TTTATTTTCA GAATTAAAAA ATAATTTTTC
        TATA-box
 -866 ATATTTTTAA TTGAATTGTT TGGACCTTGC TTTCGGACTG GAAATACAGT CTCAGACTTA GCCTGACTGC CTCGGACTCG
 -776 GGCCGGACCG CCGGCCAGGC CGCCATGAAC ACCTCTAGCT GCTGCCCGTG GTCGTGGACT CGGCGGGCGG ATGCGCGCGC
                                        CAAT-box
 -696 GAGTGAAAGC CTCTGGTAGC ATAGTACCGC AGCGCAAATA GCGCGTCTTC CCGGGTGGTT AACCGCAAAG GCGCCTTGTC
                         LTR                          CAAT-box
 -616 CGCTTAATTT CCCTTCCCGA AAAGAGACAG TAGCACCCGC CCATCCCCAA TGGGGAGATA ATCTCTTTTC CTCGGATGGG
 -536 AAATTCTGGT ATCGCTTTCC TTCTTTATTT GGCTTGGCCC GGGAGGGGAT GCGCCGCCTG CTAGTGCTAG GGCTGCCACC
                                                                    P-box
 -456 TTTTTCTTGC CCATCGCCCC GCACCCCCAT AAAACGGCAG GATACGCCGC CCCGCCGACC AAACCCGTTT CGCTTCCGCC
                                                                                        LTR
 -376 CTGCGTTGCG TTGCGTTGCG TCCCTCTTCC CCCACCCCAC TCATAAAACA TTGATTGAGA CGAGACCCCT GCAGGTACCG
 -296 AAAAGGAAAG CCGAGGAGGC GGACTGCAGG AGGAGGAGGA GGAGGGGGAG GAGAGAGAGA TAGGTGGTGG GCAAGAAGCG
 -216 GCTACGGCCA AACCCTACAC CACCACCGCC CTCCTTGCCA TTTTTGGTGA GTCCCCCTTT GCCTTCCCCA GATCCCCTTG
                                        CAAT-box                  TATA-box
 -136 CTTGTGTTTC CACTTTTTGC CTCGCCTGCC GCCCCAATTG GGATAAGCTC TTGTTTCTTT TATCCTCCCT GTTCCGACGC
  -56 GCAGGCTGCC GATCGGGGAG GATTTTGGTT TGGATCCATC CGTCGGTTGG TCCAAGATG
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**Fig. 4** The sequence of promoter from *PvSOC1*. Some specific elements were underlined

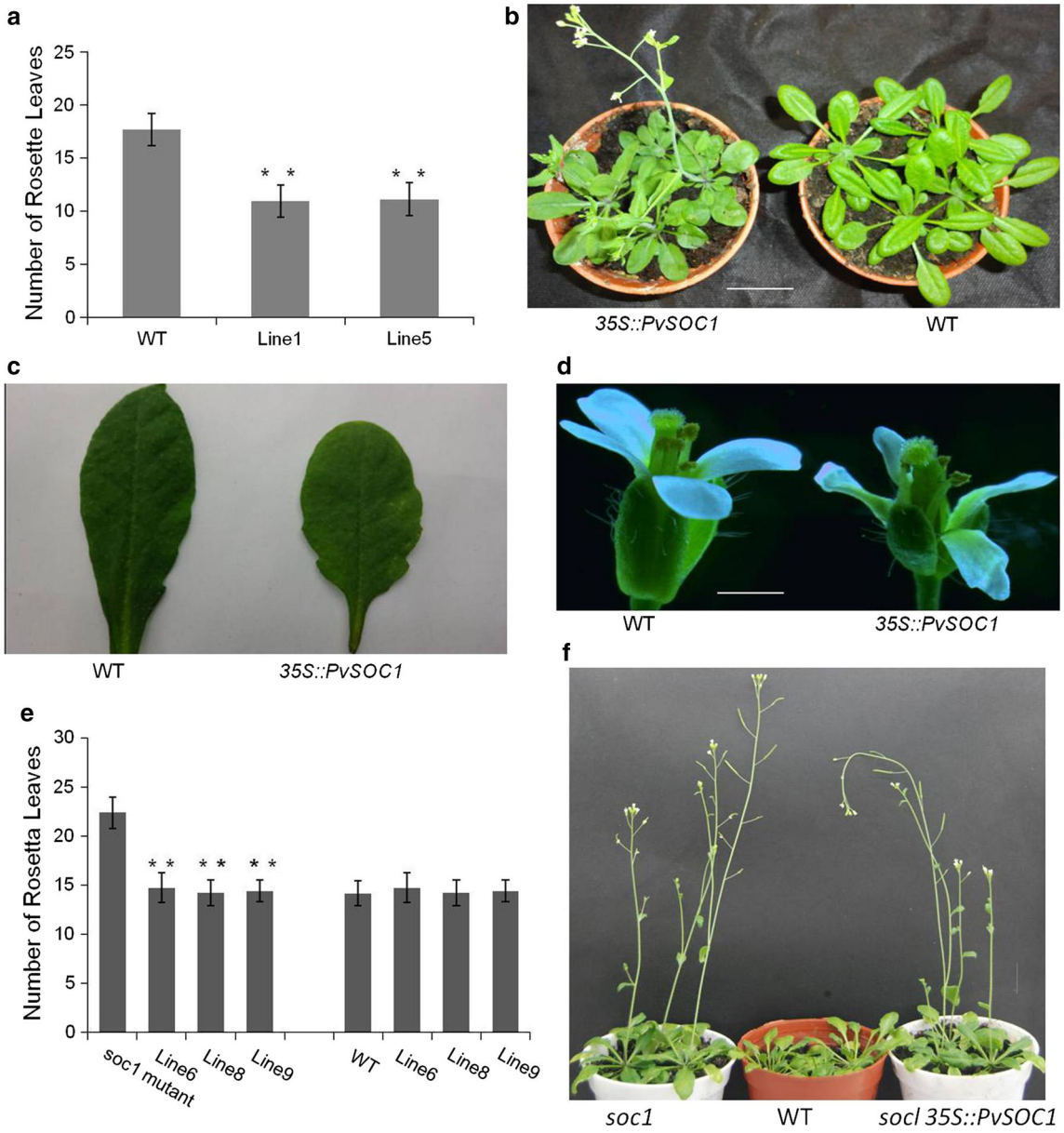


**Fig. 5** Overexpression of *PvSOC1* in *Arabidopsis* caused early flowering. **a** Flowering time of *35S::PvSOC1* (*Line1* and *Line5*) ($n = 20$) in $T_3$ homozygous transgenic plants under LD conditions by counting the number of rosette leaves, **b** *35S::PvSOC1* in wild-type *Arabidopsis* accelerated flowering, *bar* 3 cm, **c** Leaves phenotype *35S::PvSOC1* plants, **d** flower organs phenotypes of *35S::PvSOC1* plants, *bar* 1 cm, **e** flowering time (*Line6*, *Line8* and *Line9*) ($n > 20$) in $T_3$ homozygous transgenic plants carrying *35S::PvSOC1* in *soc1* background, **f** *bar* 2 cm. **The difference was extremely significant as compared to wide type ($p < 0.01$)

*Arabidopsis* plants displayed the phenotype of earlier flowering (Fig. 5a, b) with abnormal flower organs and leaves. Although both petals and sepals were smaller in size, petals exhibited wrinkles and valgus (Fig. 5d). In addition, the leaves of the transgenic plants became wider and shorter with more oval margin (Fig. 5c). Besides, we further overexpressed *PvSOC1* in *soc1* mutants *Arabidopsis* to testify if *PvSOC1* could compensate for the phenotype of delayed flowering because of the loss of *SOC1*. As shown in Fig. 5e, f, *PvSOC1* in *socl* mutant completely rescued the late flowering phenotype.

## *PvSOC1* overexpression accelerates flowering and produce shortened panicles and stems in rice

To further recapitulate its function, *PvSOC1* was transformed into *O. sativa* (rice) that is another member in the same grass family as bamboo. Four independent lines in the homozygous $T_3$ generation were selected to analyze the flowering time. An early flowering phenotype was observed in *35S::PvSOC1* transgenic rice plants (Fig. 6a, d). Moreover, not only overexpression of *PvSOC1* in rice resulted in shortened panicles (Fig. 6c, e) and stems

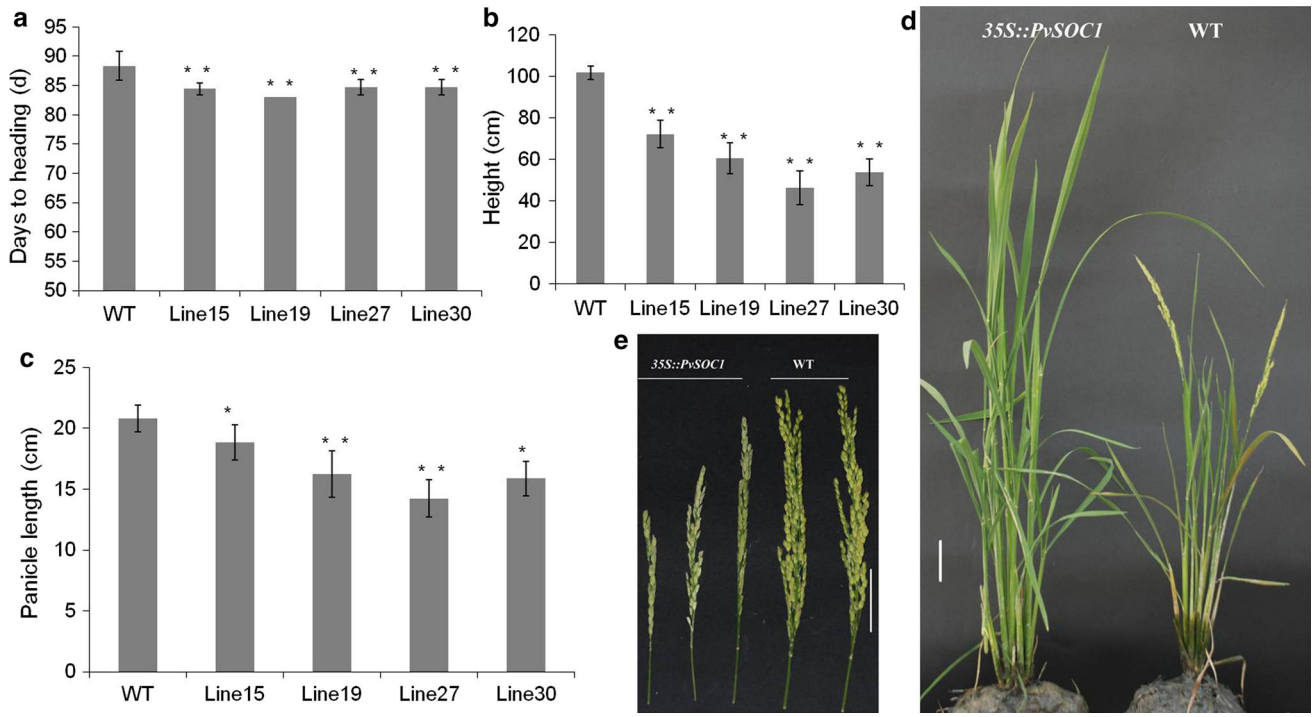


**Fig. 6** Overexpression of *PvSOC1* in rice caused early flowering. **a–c** Days to heading, height and panicle length of *35S::PvSOC1* (Line13, 19, 27 and 30) in $T_3$ homozygous transgenic plants under LD conditions ($n = 10$), respectively; *the difference was significant as compares to wild type ($p < 0.05$); **The difference was extremely significant as compared to wide type ($p < 0.01$), **d** *35S::PvSOC1* promoted flowering and made the heighted reduced, **e** *35S::PvSOC1* displayed shortened panicle. *Bar* 5 cm

(Fig. 6b, d), but also smaller leaves and lower fertility (Fig. 6e).

### GA increases *PvSOC1* expression level in the leaf of *Ph. violascens* seedlings

Comparison of the promoter sequences among *PvSOC1*, *SOC1* and *OsSOC1* indicated that a P-box element regulated by gibberellic acid is present only in *PvSOC1* promoter. To determine if GA affects *PvSOC1* expression, $GA_3$ treatment was carried out in *Ph. violascens* plants. The result showed that the level of *PvSOC1* mRNA in the leaf was significantly higher in the $GA_3$-treated seedlings than in the mock (Fig. 7).

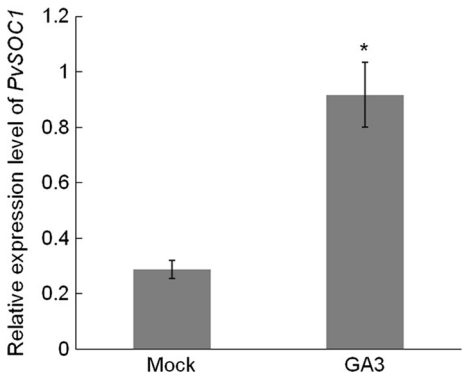


**Fig. 7** Effects of $GA_3$ on the expression level of *PvSOC1* in leaf of *Ph. violascens* seedlings. *Asterisks* indicate significant differences ($*p < 0.05$); *error* indicates the standard deviation

## Discussion

We isolated and characterized a *SOC1* homolog, *PvSOC1*, from *Ph. violascens*. The gene encodes a protein containing a conserved SOC1-motif that exists only in *SOC1* homologs from angiosperms and gymnosperms (Vandenbussche et al. 2003; Nakamura et al. 2005). Base on phylogenetic analysis, *PvSOC1* can be categorized into monocot group. Its protein sequence was more similar to SOC1-like proteins from *Ph. edulis* (with 99.56 % identity) and *O. sativa* (with 80.17 % identity) compared with homologs from other plants, suggesting that *PvSOC1* is a *SOC1* homolog in *Ph. violascens*. In *Arabidopsis*, *SOC1* gene acts as an activator and acts a critical part in mediating flowering time (Parcy 2005). When it is overexpressed, *OsSOC1* promotes flowering in *Arabidopsis* and rice (Tadege et al. 2003; Lee et al. 2004). Studies also show that other *SOC1*-like genes are conserved in promoting flowering (Shitsukawa et al. 2007; Zhong et al. 2012). In this paper, an early flowering phenotype was too observed in both

*35S::PvSOC1 Arabidopsis* and rice plants. Meanwhile, overexpression of *PvSOC1* completely rescued the delayed flowering phenotype in *soc1* mutant. These results suggest *PvSOC1* is conservative in the promotion of flowering time.

Besides its function in regulating flowering, *SOC1*-like genes are found to affect flower development in some plants. Downregulation of *SOC1* transcript in floral meristems is liable for the precocious development of floral organs, while its upregulation partially results in the reversion of inflorescence meristems into floral meristems (Gregis et al. 2006; Liu et al. 2007, 2009). Overexpression of *GhSOC1* in gerbera messes up the development of floral organ (Ruokolainen et al. 2011). In petunia this leads to unshaven floral organs with ectopic trichome formation and leaf-like petals (Ferrario et al. 2004). In orchids, this suppresses the development of perianth organs (Ding et al. 2013). Likewise, we proved that overexpressed *PvSOC1* in *Arabidopsis* led to smaller floral organs, wrinkled and valgus petals. Moreover, shortened panicles and low fertility were observed in *35S::PvSOC1* transgenic rice plants. These data suggest that *PvSOC1* might involve in floral organs development.

*SOC1*-like genes from *Arabidopsis*, *O. sativa*, *Hordeum vulgare* and *T. aestivum* are highly expressed in leaves but less in flowers (Shitsukawa et al. 2007; Komiya et al. 2009; Papaefthimiou et al. 2012). For instance, rice *SOC1* ortholog, *OsSOC1*, is scarcely expressed in the inflorescence and floral meristems (Komiya et al. 2009). Its expression pattern shows that the gene mediates flowering by manipulating other flowering regulators in leaves (Ding et al. 2013). Unlike *OsSOC1*, *PvSOC1* was expressed in tested organs but preferentially in leaves in both flowering and non-flowering *Ph. violascens*. Besides, the abnormal leaves were observed in *35S::PvSOC1* transgenic *Arabidopsis* and rice plants. Therefore, we speculate that unlike other *SOC1*-like genes, *PvSOC1* might involve in leaf development in addition to promoting flowering. This notion is supported by the observations that *PvSOC1* expression level decreased with differentiation and development of leaves in the non-flowering *Ph. violascens* while it increased with leaf development in the flowering plants. Previous data suggest that compared with the leaves of non-flowering bamboo, the leaves of flowering bamboo were quite abnormal. They became smaller in size and weaker photosynthesis, and were susceptible to senescence during leaf development (Qiu et al. 2002). These data suggest that an underlying connection may exist between physiological characteristics and the different expression pattern of *PvSOC1* in the flowering and non-flowering bamboo.

To clarify if the different expression patterns of *PvSOC1*, *OsSOC1* and *SOC1* were correlated with the regulation of their promoters, we compared their promoter sequences. It turned out that some regulatory elements are unique and not shared among the three promoters. Notably, a P-box, the *cis*-acting element involving in the GA response, exists only in *PvSOC1* promoter. Our experiments show that GA can upregulate the expression level of *PvSOC1* upon $GA_3$ treatment. The flower bud of *Ph. violascens* is easier to differentiate under the low level of GA (Lu et al. 2012). These data suggest that GA might regulate *PvSOC1* expression, which in turn mediates bamboo flowering and leaf development.

In conclusion, we found that the sequence and expression pattern of *PvSOC1* are evolutionarily conserved compared to *SOC1* orthologs from other plants, in particular, monocots. *PvSOC1* may not only regulate flowering time but also affect flower organs as well as leaf development by responding to GA. Putting together, our data lay a solid foundation for further disclosing the flowering mechanism in bamboos.

**Author contribution statement** Shinan Liu involved cultivation of *Arabidopsis* and rice, data analysis, and wrote the manuscript. Tengfei Ma involved in gene and its promoter cloning, and cultivation of *Arabidopsis*. Xinchun Lin and Luyi Ma designed the experiments and revised the manuscript. All authors have declared no conflicting interests.

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