Isolation and Functional Characterization of *SOC1*-like Genes in *Prunus mume*

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ABSTRACT. The MADS-box gene *SOC1/TM3* (suppressor of overexpression of constans 1/tomato MADS-box gene 3) integrates multiple flowering signals to regulate the transition from vegetative to reproductive development in arabidopsis (*Arabidopsis thaliana*). Although *SOC1*-like genes have been isolated from a wide range of plant species, their orthologs are not well characterized in mei (*Prunus mume*), an important ornamental and fruit plant in east Asia. To better understand the molecular regulation of flower development in mei, we isolated and characterized three putative orthologs of arabidopsis *SOC1*, including *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*. The phylogenetic tree revealed that these genes fall into different subgroups within the *SOC1*-like gene group, suggesting distinct functions. *PmSOC1-1* and *PmSOC1-3* were mainly expressed in vegetative organs and at low expression levels in floral parts of the plants, whereas *PmSOC1-2* was expressed only in vegetative organs. Furthermore, the expression level decreased significantly during flower bud differentiation development, suggesting a role for these genes in the transition from the vegetative to the reproductive phase. Overexpression of *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3* in arabidopsis caused early flowering. Early flowering also increased expression levels of four other flowering promoters, agamous-like 24 (*AGL24*), leafy (*LFY*), apetala 1 (*API*), and fruitfull (*FUL*). Moreover, the overexpression of *PmSOC1-1* and *PmSOC1-2* resulted in a range of floral phenotype changes such as sepals into leaf-like structures, petal color into green, and petal into filament-like structures. These results suggested that the genes *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3* play an evolutionarily conserved role in promoting flowering in mei, and may have distinct roles during flower development. Our findings will help elucidate the molecular mechanisms involved in the transition from vegetative to reproductive development in mei.

The floral transition is one of the most dramatic developmental switches in the life cycle of flowering plants. The transition from vegetative to reproductive phase is regulated by a complex genetic network that monitors the developmental cues and environmental signals. Six genetic pathways, namely, photoperiod, vernalization, autonomous, gibberellin (GA), ambient temperature, and age control flowering in the model plant arabidopsis (Fornara et al., 2010; Simpson and Dean, 2002; Srikanth and Schmid, 2011). These six pathways converge to regulate a small number of “floral integrator genes,” including *SOC1* and flowering locus T (*FT*), which mediate other regulators such as the floral meristem identity gene, *LFY*, to determine the formation of floral meristems (Borner et al., 2000; Lee et al., 2000; Liu et al., 2008; Moon et al., 2003; Samach et al., 2000; Wang et al., 2009).

In the photoperiod pathway, constans (*CO*), which plays a central role and mediates the circadian clock, activates *SOC1* mainly through *FT* (Valverde et al., 2004; Wigge et al., 2005; Yanovsky and Kay, 2002; Yoo et al., 2005). Conversely, in the

vernalization and autonomous pathways, flowering locus C (*FLC*) acts as a floral transition repressor, and suppresses the expression of *SOC1* by directly binding to the promoters of *SOC1* (Michaels and Amasino, 1999; Searle et al., 2006). In the GA pathway, GA regulates *SOC1* at the shoot apex to influence phase transition. Moreover, the *soc1* null mutants demonstrate a reduced sensitivity to GA, and overexpression of *SOC1* rescues the nonflowering phenotype of *gal-3* in short-day (SD) conditions (Lee and Lee, 2010). *SOC1* is also regulated by an age-dependent mechanism involving *SPL9* and *microRNA156*. *SPL9*, as a *miRNA156*-targeted squamosa promoter binding protein-like (*SPL*) transcription factor, shows low expression at the early seedling stage. However, *SPL9* subsequently increases its expression from the vegetative phase until and through the transition of flowering, independent of the photoperiod, and binds to the first intron of *SOC1*. Together, these data indicate that *SPL9* is a positive age-related regulator of *SOC1* independent of FT/flowering locus D (FD) (Wang et al., 2009). Recent studies have revealed other interesting functions of *SOC1*. For example, *SOC1* directly represses C repeat/dehydration-responsive element (CRT/DRE)-binding factors (*CBFs*) to negatively regulate the cold response pathway, whereas overexpression of *CBFs* increases the *FLC* transcript level and causes delayed flowering (Seo et al., 2009).

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SOC1 belongs to the *TM3* clade of MADS-box genes, which possess a highly conserved DNA-binding domain and is conserved among angiosperms including both monocotyledons and dicotyledons (Cseke et al., 2003; Ferrario et al., 2004; Lee et al., 2000, 2004, 2008; Nakamura et al., 2005). Expression analyses showed that *SOC1* is mainly expressed in developing leaves and shoot apical meristems and its expression level increases with the developmental age in arabidopsis (Borner et al., 2000; Lee et al., 2000; Samach et al., 2000; Wang et al., 2009). Arabidopsis contains six *AtSOC1*-like genes, but only a few have been studied in detail (Becker and Theissen, 2003). Some of these genes, such as *AtAGL14*, are expressed only in roots, whereas *AtAGL19* is also expressed in rosette leaves and seedlings except roots (Schönrock et al., 2006). *AtAGL71* is expressed in arabidopsis seedlings (Ma et al., 2005), whereas *AtAGL72* has not been explored further. Both *AtAGL71* and *AtAGL72* were investigated in the comprehensive arabidopsis MADS domain protein–protein interaction mapping assay (de Folter et al., 2005). *AtAGL42* is mainly expressed in arabidopsis roots, where its expression can be used as a marker for the quiescent center (Nawy et al., 2005).

So far, *SOC1*-like genes in a wide range of plant species have been isolated, such as UNSHAVEN (*UNS*) [petunia (*Petunia hybrida*)], *GhSOC1* [gerbera (*Gerbera hybrida*)], *OsSOC1* [rice (*Oryza sativa*)], *DOSOC1* [dendrobium orchid (*Dendrobium*)], *GmSOC1* [soybean (*Glycine max*)], *EgrSOC1* [lisianthus (*Eustoma grandiflorum*)], *ZmMADS1* [maize (*Zea mays*)], *TrcMADS1* [trillium (*Trillium camtschaticense*)], *ClSOC1* [chrysanthemum (*Chrysanthemum lavandulifolium*)], *HvSOC1* [barley (*Hordeum vulgare*)], *PTM5* [poplar (*Populus tremuloides*)], *ETL* [tasmanian bluegum (*Eucalyptus globulus*)], and *VvMADS8* [grape (*Vitis vinifera*)] (Cseke et al., 2003; Decroocq et al., 1999; Ding et al., 2013; Ferrario et al., 2004; Fu et al., 2013; Heuer et al., 2001; Lee et al., 2004; Na et al., 2013; Nakamura et al., 2005; Nakano et al., 2011; Papaefthimiou et al., 2012; Ruokolainen et al., 2011; Sreekantan and Thomas, 2006). Nevertheless, very few of these genes are functionally characterized in woody perennials. Flowering in woody perennials differs significantly from herbaceous species such as arabidopsis in having distinct juvenile and adult periods during development. First, most woody perennials have a longer juvenile phase, which lasts for several years, during which time no flowering occurs. This is a great disadvantage for fruit breeding. Second, when attaining the adult stage, flowering in most woody trees (especially fruit trees) is spread over two growing seasons. During the first season, meristems with a potential to differentiate flowers are initiated in the lateral buds of developing shoots. By autumn, the second season, these buds have ceased growth and become dormant (Lang, 1987). A certain amount of chilling accumulation or treatment with dormancy-breaking chemicals is needed to release dormancy (Faust et al., 1997; Horvath et al., 2003). Recent studies suggest that the application of *SOC1* orthologs has a potential to reduce the juvenile phase and promote floral transition in woody plants. In grape, the overexpression of *SOC1* ortholog (*VvMADS8*) in arabidopsis promotes flowering, indicating that this gene functions as a promoter of flowering (Sreekantan and Thomas, 2006). Ectopic expression of *CsSL1*, a *SOC1*-like gene in sweet orange (*Citrus sinensis*), causes early flowering in arabidopsis wild-type ecotypes Columbia and C24. The expression of *CsSL1* functionally complements the late flowering phenotype of the *soc1* mutant; it exhibits an expression pattern

similar to arabidopsis *SOC1* (Tan and Swain, 2007). In the woody perennial, rose gum (*Eucalyptus grandis*), the overexpression of *SOC1*-like genes (*EgrMADS3* and *EgrMADS4*) in the arabidopsis ecotype Columbia, accelerate flowering time under SD conditions, implicating their role as floral activators (Watson and Brill, 2004). Therefore, further functional characterization of *SOC1*-like genes in woody plants is required to facilitate the breeding of woody plants.

Mei, a rosaceous woody perennial tree, has been cultivated in China and other east Asian countries for over 3000 years for its ornamental and economic value (Chen, 1996). As an early-blooming garden ornamental, mei possesses many conspicuous ornamental characteristics such as colorful corollas, pleasant fragrance, and various types of flowers (Chen, 1996; Sun et al., 2013). Moreover, mei is also an important commercial fruit tree due to its flavorful fruit. These unique properties endow mei with a high cultural and commercial value in China and elsewhere. There is an urgent need to cultivate new mei cultivars with enhanced ornamental and nutritional value, suitable for consumer needs. However, mei takes a long time to reach its reproductive age, which reduces its breeding efficiency and, thus, its yield. Therefore, it is essential to study the expression patterns of floral integrator genes in mei, understand its flowering mechanisms, and promote its molecular breeding. Our ultimate objective is to use this knowledge to develop practical applications in mei such as artificial induction of flowering and reduction of the juvenile period.

The completed sequenced genome of mei (Zhang et al., 2012) enabled us to isolate and characterize three *SOC1*-like genes (denoted *PmSOC1-1*–*PmSOC1-3*) from mei. We analyzed the phylogenetic relationships and expression patterns of these genes, and their effects on the phenotypes of transgenic arabidopsis ectopically expressing them.

Materials and Methods

PLANT MATERIALS AND GROWTH CONDITIONS. Mei cultivar Changrui Lve was used in this study [the wild species ‘Zang mei’ in Tongmai town (Tibet, China) was the genotype used for mei genome sequencing]. 5-year-old adult trees were grown in Beijing Jiufeng International Plum Blossom Garden (Beijing, China), and 1-month-old seedlings were grown in a greenhouse at 16 to 25 °C with 60% relative humidity under a 12-h light/dark cycle. The wild-type arabidopsis ecotype Columbia-0 (Col-0), 35S::*PmSOC1-1*, 35S::*PmSOC1-2*, and 35S::*PmSOC1-3* transgenic plants of the same ecotype were grown in soil at 22 ± 1 °C under long-day (LD) (16 h light/8 h dark) or SD (8 h light/16 h dark) conditions. In all cases, samples were collected and immediately frozen in liquid nitrogen and stored at –80 °C until RNA extraction. Samples were collected from three plants to provide three biological replicates.

TOTAL RNA EXTRACTION AND COMPLEMENTARY DNA SYNTHESIS. Total RNA was extracted from leaf buds using TRIzol reagent (Invitrogen, Carlsbad, CA) following the manufacturer’s instructions. Potentially contaminating genomic DNA was eliminated by treatment with RNase-free DNase I according to the manufacturer’s instructions (Promega, Madison, WI). First strand complementary DNA (cDNA) synthesis was carried out from 2 µg of total RNA using the TIANScript First Strand cDNA Synthesis Kit (Tiangen, Beijing, China) according to the manufacturer’s protocol.

GENE CLONING. Mei genome sequences were downloaded from the mei genome project (Zhang et al., 2012). To identify

SOC1-like genes in mei, the arabidopsis gene *AtSOC1* (accession no. AT2G45660.1) protein sequences was downloaded from The Arabidopsis Information Resource (Rhee et al., 2003) and used as initial query to perform BLASTP searches against the mei genome database. Three *SOC1*-like genes, named *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*, were identified in mei. For obtaining the complete cDNA sequences of *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*, combinations of primers were designed based on the nucleotide sequence from mei genome sequences (Supplemental Table 1). Polymerase chain reaction (PCR) assays were performed in a total volume of 25 μ L containing 1 μ L cDNA, 0.8 U Taq DNA polymerase (Promega), 0.5 μ M of each primer, 1 $\times$ PCR buffer (Promega), 1.5 mM MgCl₂, and 0.2 mM dNTP mix (Promega). The PCR conditions were as follows: 94 °C for 5 min, 30 cycles of 94 °C denaturation for 30 s, 57 °C annealing for 30 s, and 72 °C extension for 1 min, with a final extension at 72 °C for 7 min. The amplified products were separated in 1.0% agarose gels, cloned into the pMD18-T vector (Takara Biotechnology, Dalian, China) following manufacturer's instructions and subsequently sequenced in both directions. The full-length cDNA sequence of *PmSOC1-2* and *PmSOC1-3* were deposited at GenBank (accession no.s KP938964 and KP938965), whereas *PmSOC1-1* was found to have 100% identity with the sequence JF806632.1 in GenBank.

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSIS. The protein sequences of the *SOC1*-like genes aligned in this study were retrieved from the National Center for Biotechnology Information database and plant genome database [Phytozome (Goodstein et al., 2012)]. Multiple sequence alignments were performed using DNAMAN version 5.2.2 and Clustal W (Thompson et al., 1994). Proteomics Server of the Expert Protein Analysis System of the Swiss Institute of Bioinformatics (Gasteiger et al., 2003) was used to predict the conserved protein domain sequences of *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*. A phylogenetic tree was generated using Molecular Evolutionary Genetics Analysis (MEGA) version 5.1 (Tamura et al., 2011) and the neighbor-joining method with the following parameters: Poisson correction, complete deletion, and bootstrap (1000 replicates).

PARAFFIN SECTIONING AND IDENTIFICATION OF FLORAL BUD DIFFERENTIATION STAGE. To analyze the expression patterns of *PmSOC1*-like genes during mei floral bud differentiation, we used paraffin sections to identify floral bud differentiation stage. For paraffin sectioning, floral bud development was categorized into eight stages (predifferentiation stage, flower primordium differentiation stage, sepal differentiation stage, petal differentiation stage, stamen differentiation stage, pistil differentiation stage, pistil elongation stage, and ovule and anther formation stage). Floral buds from pre-differentiation stage to ovule and anther formation stage were collected and fixed with formalin–acetic acid–alcohol fixative (70% ethanol, 5% glacial acetic acid, 5% formaldehyde) by vacuum infiltration. The samples were then dehydrated in an ethanol series before being embedded in paraffin. Vertical sections were cut using a microtome (RM2235; Leica, Wetzlar, Germany). The sections were stained in safranin and fast green staining solution successively and photographed using a microscope (BX53; Olympus, Tokyo, Japan).

REAL-TIME QUANTITATIVE REVERSE TRANSCRIPTION-PCR. The tissue-specific and developmental expression patterns of *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*, were investigated

by real-time quantitative reverse transcription PCR (qRT-PCR). For the analysis of the spatial localization patterns of *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3* in various organs, young unfolded leaves, mature expanded leaves, stems, leaf buds, floral buds, fully opened floral organs (sepals, petals, stamens, and pistils), seeds, young fruit ($\approx$ 45 d after full bloom, $\approx$ 10 mm diameter), and mature fruit ($\approx$ 95 d after full bloom, $\approx$ 30 mm diameter) of adult trees, and roots, stems, and leaves of 1-month-old seedlings were sampled. For the analysis of *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3* in the floral bud at different developmental stages, two samples of floral buds that were similar were collected weekly from June to Dec. 2013. One sample was used to identify floral bud differentiation stage by paraffin sections, and the other was frozen in liquid nitrogen and stored at -80 °C until RNA extraction.

qRT-PCR was performed using the PikoReal real-time PCR system (Thermo Fisher Scientific, Wilmington, DE). Each 20- μ L quantitative RT-PCR mixture contained 1 μ L of diluted cDNA, 200 nM of each primer, and 10 μ L of SYBR Premix Ex Taq II (Takara Biotechnology). The following conditions were used: 30 s at 95 °C, 40 cycles of 5 s at 95 °C, and 30 s at 60 °C. All qRT-PCR reactions were carried out in biological and technical triplicates, and a nontemplate control was also included in each run. PCR reaction efficiency for each primer pair was determined by a standard curve generated with a cDNA serial dilution. The relative expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method with the protein phosphatase 2A (*PP2A*) gene of mei as the internal control gene (Wang et al., 2014). Relevant primers were designed using Primer Express 3 software (version 0.4.0; PE Applied Biosystems, Foster, CA) to amplify products between 150 and 300 base pairs (bp) in size and their sequences are shown in Supplemental Table 1. The reverse primer used for analyzing gene expression was carefully designed on the 3' exon–exon junction of the open reading frame (ORF).

VECTOR CONSTRUCTION AND PLANT TRANSFORMATION. The *Bgl* II–*Bst* E II fragment of *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3* were generated by PCR with specific primers (Supplemental Table 1). The fragments were separately cloned into the pMD18-T (Takara Biotechnology) vector and confirmed by sequencing. After digestion with restriction enzymes *Bgl* II and *Bst* E II, the fragments were cloned into the same sites of the pCAMBIA1304 vector under the control of CaMV 35S promoter. 35S::*PmSOC1-1*, 35S::*PmSOC1-2*, and 35S::*PmSOC1-3* constructs were transformed separately into wild-type arabidopsis plants ecotype Col-0 using the floral-dip method according to Clough and Bent (Clough and Bent, 1998) with *Agrobacterium tumefaciens* strain EHA105. The seeds of transgenic arabidopsis plants were selected on Murashige and Skoog medium containing 50 mg·L⁻¹ kanamycin at 4 °C for 2 d, and then were transferred to the greenhouse under LD or SD conditions at 22 ± 1 °C for 10 d. Subsequently, the seedlings were transplanted to soil. As a control reference, untransformed control seeds were planted at the same time on the above medium minus kanamycin. The homozygous T3 plants were used for gene function analyses.

GENE EXPRESSION ANALYSIS AND PHENOTYPIC OBSERVATION OF TRANSGENIC PLANTS. Rosette leaves from transgenic and wild-type arabidopsis plants were collected for RT-PCR and qRT-PCR verification. The CaMV 35S promoter-specific forward primer and *SOC1*-like (*PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*) gene-specific reverse primers were designed (Supplemental

Table 1). For analysis of the expression levels of *LFY*, *API*, *AGL24*, and *FUL* in the transgenic arabidopsis lines, 6-, 12-, and 16-d-old transgenic seedlings were sampled for qRT-PCR assay. The *TUB2* gene of arabidopsis was used as an internal control; and the gene-specific primers of *TUB2*, *LFY*, *API*, *AGL24*, and *FUL* are shown in Supplemental Table 1. Flowering time was scored by counting the days from sowing to the opening of the first flower. Furthermore, the number of rosette leaves was counted when the first flower opened. According to a completely randomized design, wild-type and T3 transgenic plants (>20 per line) were used for flowering-time and floral-phenotype recording. Statistical analyses were performed with SPSS statistics software (version 16.0; IBM Corp., Armonk, NY). Student's *t* test was used to detect the significance of flowering time differences.

Results

IDENTIFICATION OF *SOC1*-LIKE GENES IN MEI. The search for the homologs of arabidopsis AtSOC1 protein sequences from the mei genome (Zhang et al., 2012) identified three *PmSOC1*-like genes (*PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*). Each *PmSOC1*-like gene has a single long ORF encoding a putative protein of 214, 217, and 219 amino acids, respectively (Supplemental Fig. 1). The predicted molecular masses of *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3* are 24.6, 24.91, and 24.95 kDa, respectively; and the estimated isoelectric points are 9.46, 8.68, and 9.05. All three genes belong to the type II MADS-box proteins, as they harbor the strongly conserved MADS domain, a less-conserved K domain, and a highly variable C-terminal region (Supplemental Fig. 1). Structural analysis between the full-length cDNA and genomic DNA sequence revealed that *PmSOC1-1* and *PmSOC1-2* contained eight exons and seven introns, whereas *PmSOC1-3* consisted of seven exons and six introns. All locus regions of these genes displayed the consensus splice sites GT and AG. The start codon of *PmSOC1-3* is located in the first exon, whereas those of *PmSOC1-1* and *PmSOC1-2* are in the second exon (Supplemental Fig. 2). The lengths of first, third, fourth, fifth, and sixth exons within the coding region are the same in different plant species, and are: 182, 62, 100, 42, and 42 bp, respectively (Supplemental Fig. 2).

The sequence analysis using deduced amino acid sequences of *PmSOC1*-like genes revealed that *PmSOC1-1* shares a 68% sequence identity with arabidopsis SOC1. It shares an even higher sequence identity with SOC1 homolog genes in Rosaceae *Prunus* plants such as PaSOC1 [apricot (*Prunus armeniaca*); 98% identity], PpSOC1 [peach (*Prunus persica*); 95% identity], and PsSOC1 [plum (*Prunus salicina*); 95% identity]. However, *PmSOC1-2* and *PmSOC1-3* share a lower sequence identity with SOC1 orthologs in Rosaceae *Prunus* plants, for example, *PmSOC1-2* showed 59%, 57%, and 58% and *PmSOC1-3* showed 55%, 54%, and 55% identity to PaSOC1 (apricot), PpSOC1 (peach), and PsSOC1 (plum), respectively (Supplemental Fig. 1). Moreover, alignment of the deduced amino acid sequence of *PmSOC1*-like and other SOC1-like proteins revealed that they contain a well-conserved SOC1 motif at their C-terminus (Supplemental Fig. 1). This motif is only specific in the SOC1/TM3 clade of MADS-box genes from gymnosperms and angiosperms (Nakamura et al., 2005; Vandenbussche et al., 2003).

We constructed a phylogenetic tree to determine the evolutionary relationship between *PmSOC1*-like proteins and other

SOC1-like proteins (Fig. 1). The tree showed that three *PmSOC1*-like genes clustered in a dicotyledonous group which mostly included *SOC1* and its orthologs. On the other hand, the other *SOC1* orthologs such as *ZmSOC1* from maize and *TaSOC1* from wheat (*Triticum aestivum*) clustered in a monocotyledonous group. *PmSOC1-1* was closely related to arabidopsis *SOC1*, along with *SOC1*-like members from some woody perennial species. *PmSOC1-2* and *PmSOC1-3* formed distinct subgroups, which was more closely related to other less-studied arabidopsis *SOC1*-like genes. *PmSOC1-2* was grouped in phylogeny with *AtAGL71*, *AtAGL72*, and *AtAGL42*, whereas *PmSOC1-3* was grouped with *AtAGL14* and *AtAGL19*. Remarkably, each of the three *PmSOC1*-like genes, *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*, had their corresponding orthologous gene in peach (Fig. 1). Moreover, the distribution pattern of three *SOC1*-like genes in mei and peach are similar, that is, in each species, two genes were located on one chromosome, and the other gene was located on another chromosome (Wells et al., 2015; Xu et al., 2014). To be specific, in mei, *PmSOC1-1* and *PmSOC1-2* were located on chromosome 5, *PmSOC1-3* was located on chromosome 7; in peach, *PpMADS22* and *PpMADS60* were located on chromosome 2, and *PpMADS64* was located on chromosome 5. This similarity suggests that functions of *SOC1*-like genes in flowering are conserved in mei and peach.

TEMPORAL AND SPATIAL EXPRESSION PATTERNS OF *SOC1*-LIKE GENES. We used qRT-PCR to study the spatiotemporal expression patterns of the three *PmSOC1*-like genes (*PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*) in different organs of mei (Fig. 2). The results showed that in the adult tree, expression levels of the three *PmSOC1*-like genes were high in the leaf buds, flower buds, stems, and leaves, but relatively low in the reproductive organs (Fig. 2). In 1-month-old seedlings, *PmSOC1-1* and *PmSOC1-3* were expressed in roots, stems, and leaves, whereas *PmSOC1-2* was only expressed weakly in roots (Fig. 2). To determine whether the function of the three *PmSOC1*-like genes in transition of blossoms is as important as that of *AtSOC1* in *Arabidopsis*, we identified eight critical stages of mei bud differentiation (Fig. 3A) using the paraffin method. We detected the expression patterns of three *PmSOC1*-like genes during each differentiation stage (Fig. 3B). Throughout the bud differentiation process, all three genes presented a downward trend in terms of expression levels. They showed the highest expression profile in the undifferentiated stage (S1 stage), after which the expression levels of *PmSOC1-2* gradually decreased, and *PmSOC1-1* and *PmSOC1-3* expression profiles decreased less gradually. Once the flower bud differentiation commenced, expression levels started to decrease, suggesting that these three genes, especially *PmSOC1-2*, may play an important role in mei bud differentiation.

OVEREXPRESSION OF *SOC1*-LIKE GENES IN ARABIDOPSIS CAUSES EARLY FLOWERING. We analyzed the expression profiles of *PmSOC1*-like genes in transgenic arabidopsis using RT-PCR and qRT-PCR. Each *PmSOC1*-like gene was expressed in six transgenic arabidopsis lines (Fig. 4A). Real-time quantitative results showed that exogenous genes from different transgenic lines displayed various expression levels (Fig. 4B). The transgenic phenotype of 35S::*PmSOC1-3* was comparatively consistent, whereas the transgenic phenotypes of 35S::*PmSOC1-1* and 35S::*PmSOC1-2* exhibited both strong and weak phenotypes in terms of flowering time and the extent of phenotypic variation. The transgenic lines no. 1, no. 6, and no. 9 of 35S::*PmSOC1-1* transgenic line were strong phenotypes, whereas no. 13, no. 18, and no. 24 were weak phenotypes. The transgenic

Fig. 1. Phylogenetic analyses of PmSOCI1-like proteins from different plant species. The phylogenetic tree was generated by the neighbor-joining method after bootstrapping. Numbers at nodes indicate bootstrap values (1000 replicates). Dicots and monocots were in the same branch separately. A non-SOCI1-like protein, APETALA1 (AP1) from *Arabidopsis thaliana* was chosen as the outgroup. Accession numbers are as follows: *Prunus armeniaca* [PaSOCI1 (AGD88524)], *Prunus mume* [PmSOCI1-1 (AEQ20229), PmSOCI1-2 (KP938964), PmSOCI1-3 (KP938965)], *Prunus salicina* [PsOCI1 (AGD88523)], *Prunus xyedensis* [PyOCI1 (AEO20233)], *Spiraea cantoniensis* [ScSOCI1 (AEO20234)], *Photinia serratifolia* [PseOCI1 (AEO20232)], *Malus domestica* [MdSOCI1 (BAI49495)], *Fragaria vesca* [FvOCI1 (AEO20231)], *Rosa hybrida* [RhOCI1 (AEO20230)], *Populus tremuloides* [PTM5 (AF377868)], *Vitis vinifera* [VvOCI1 (ACZ26527)], *Eucalyptus grandis* [EgOCI1 (XP_010053874)], *Mangifera indica* [MiOCI1 (ADX97324)], *A. thaliana* [AtOCI1 (AEC10583)], AtAGL42 (AED97572), AtAGL71 (AED96138), AtAGL72 (AED96136), AtAGL14 (AEE83062), AtAGL19 (AEE84684), AtAP1 (CAA78909), *Triticum aestivum* [TaOCI1 (BAF56968)], and *Zea mays* [ZmOCI1 (ACG32892)]. *Prunus persica* (PpMADS22) is positioned on the peach genome map on scaffold 2: 20,600,270...20,606,939 reverse; PpMADS60 is positioned on the peach genome map on scaffold 2: 28,315,091...28,320,984 reverse; and PpMADS64 is positioned on the peach genome map on scaffold 5: 9,862,429...9,867,768 reverse).

lines no. 5, no. 8, and no. 10 of 35S::PmSOCI1-2 were strong phenotypes and no. 14, no. 19, and no. 28 were weak phenotypes.

The transgenic lines showing a strong phenotype of 35S::PmSOCI1-1 flowered early (5 rosette leaves) under LD conditions as compared with the wild-type *Arabidopsis* lines (13 rosette leaves) (Fig. 5IA). 35S::PmSOCI1-2 transgenic lines with strong phenotype showed the earliest flowering time among all transgenic lines (Table 1). Individual 35S::PmSOCI1-2 transgenic lines flowered even at three-rosette-leaves stage (Fig. 5IIA). The weak phenotype of 35S::PmSOCI1-2 could also flower with an average

of six rosette leaves. Under LD conditions, 35S::PmSOCI1-3 transgenic lines need 32.4–34.5 d to flower. This flowering time is slightly shorter than that of the wild-type lines, which takes ≈ 37.3 d on average to flower (Table 1). The above results suggest all three PmSOCI1-like genes can cause early flowering irrespective of their potencies.

OVEREXPRESSION OF PMSOCI1-1 AND PMSOCI1-2 IN ARABIDOPSIS AFFECTS FLORAL ORGAN DEVELOPMENT AND PLANT MORPHOGENESIS. Besides advanced flowering (Fig. 5IIIA–IIIG), the 35S::PmSOCI1-3 transgenic lines were not much different from the wild-type *Arabidopsis* (Fig. 5IVA–IVG). Analysis of qRT-PCR showed that PmSOCI1-3 transgenic lines had different expression profiles (Fig. 4B), suggesting that 35S::PmSOCI1-3 was stably expressed in transgenic lines.

When inducing early flowering in *Arabidopsis*, 35S::PmSOCI1-1 and 35S::PmSOCI1-2 transgenic lines showed weak and strong phenotypes in flowers, colors, and plant architecture. Except for the more significant petal refinement in 35S::PmSOCI1-2 transgenic lines, other variations in weak phenotypes of 35S::PmSOCI1-1 and 35S::PmSOCI1-2 transgenic lines remained the same as compared with the wild-type line. These included filamentous petals, upright petal entablature, which can barely spread into a cross (Fig. 5IB and IIB), elongated pistils, which extended to outer perianth (Fig. 5IC and IIC), leaf-like sepals (Fig. 5ID, IE, IID, and IIE), and petals and sepals not dropping off till the silique elongation period (Fig. 5IF and IIF). Their strong phenotypes were manifested in different variations. Besides all the variations showed in weak phenotypes, strong phenotypes of 35S::PmSOCI1-1 transgenic lines, there have been variations of petal color turning green as compared with the wild type (Fig. 5IH and II), whereas strong phenotypes of 35S::PmSOCI1-2 exhibited significant changes in plant morphogenesis, such as all lateral branches showing horizontal growth (Fig. 5IIG) so that the plant architecture of *Arabidopsis* completely changed. Moreover, 2–4 flowers connately formed terminal flower (TF) in part of the terminal inflorescence in 35S::PmSOCI1-2 strong phenotypes. The number of floral in TF was indefinite, and those flowers usually bloomed first (Fig. 5IIH and III).

Moreover, all strong phenotypes of 35S::PmSOCI1-1 and 35S::PmSOCI1-2 transgenic lines showed relatively high expression levels in qRT-PCR. For example, no.5 35S::PmSOCI1-2 transgenic line flowered early, and exhibited the most significant phenotypic variation with the highest expression level of transgenes in qRT-PCR (Fig. 4B), indicating a positive correlation between expression abundance of transgenes and phenotypic variation of transgenic plants.

Fig. 2. Expression analyses of *PmSOC1*-like genes in different organs of *Prunus mume* by real-time quantitative reverse transcription polymerase chain reaction. The level of expression was normalized to *P. mume PP2A* gene. Error bars represent standard errors for three replicate reactions.

PMSOC1-LIKE GENES CAN INDUCE THE EXPRESSION OF FLORAL MERISTEM IDENTITY GENES IN TRANSGENIC ARABIDOPSIS PLANTS. *SOC1* gene can integrate multiple flowering signaling pathways and promote flowering by upregulating its downstream floral meristem characteristic gene (Lee and Lee, 2010; Lee et al., 2008). We studied the expression profiles of *LFY*, *AP1*, *AGL24*, and *FUL* in strong-phenotype 6-, 12-, and 16-d-old transgenic arabidopsis seedlings, to verify whether the early flowering phenotype is caused by the upregulation of downstream floral meristem genes induced by *PmSOC1*-like genes.

According to our results, *LFY* maintained a significantly higher expression level in 35S::*PmSOC1*-2 transgenic arabidopsis line than the other two transgenic lines (35S::*PmSOC1*-1 and 35S::*PmSOC1*-3) (Fig. 6A). The expression profile of *AP1* showed a consistent trend with *LFY* (Fig. 6B). However, the expression profiles of *AGL24* that is functionally similar to *SOC1*, and *FUL*, which promote initiation and development of flowering (Liu et al., 2008; Michaels et al., 2003; Yu et al., 2002), showed a progressive rise along with the growth and development of plants (Fig. 6C and D).

Discussion

Flowering is a transition stage from vegetative to reproductive growth of plants, and is determined by internal and environmental factors. Woody perennials such as mei need to go through a long period of vegetative growth (juvenile period) before flowering, as compared with herbaceous plants such as arabidopsis. Understanding the flowering transition process in woody perennials is critically important in order to shorten the juvenile period and accelerate the cultivar improvement process. In the MADS-box gene family, the gene *SOC1* controls the flowering time. It can integrate a variety of flowering signals and has been intensively studied for years. Although

researchers have studied *SOC1* in the model plant arabidopsis, there have been few studies of *SOC1* homologous genes in woody perennials. In this study, we isolated three *SOC1*-like genes from mei and examined their expression patterns and functions.

PMSOC1-LIKE GENES BELONG TO THE SOC1/TM3 CLADE OF THE MADS-BOX TRANSCRIPT FACTORS. MADS-box gene is an important class of transcription factors widespread in eukaryotes. It plays an important role in plant growth regulation and signal transduction (Messenguy and Dubois, 2003; Ng and Yanofsky, 2001), especially in floral organ differentiation, flowering time, and related aspects of fruit development and maturation (Irish, 2003).

Phylogenetic analysis showed that MADS-box genes can be divided into two categories in eukaryotes, namely, type I and type II. In plants, the function of type I MADS-box genes remains unknown, and these genes only contain the conserved regions (Nam et al., 2004). The type II MADS-box genes are composed of domains with varying degrees of conservatism, including M (MADS-box), I (intervening), K (keratin), and C-terminal. Thus, it is also called MIKC-type MADS-box (Kaufmann et al., 2005). Dependent on differences of gene sequence and structure, MIKC-type MADS-box genes can be further categorized into MIKC* type and MIKC^C type (Alvarez-Buylla et al., 2000; Henschel et al., 2002). Currently, MIKC^C is the most known gene, and it has an essential role in plant growth and development. On the basis of the features of gene sequence, structure, and functions, MIKC^C can be further divided into 13 subfamilies, including AG-like, AGL2-like, AGL6-like, AGL12-like, AGL15-like, AGL17-like, DEF-GLO-like, FLC-like, GGM13-like, SQUA-like, STMADS11-like, TM8-like, and SOC1/TM3-like (Becker and Theissen, 2003). In the arabidopsis MADS-box family, the SOC1/TM3 subfamily includes six genes: *AGL14*, *AGL19*, *AGL20* (*SOC1*), *AGL42*, *AGL71*, and *AGL72*.

In our study, the three MADS-box genes separated from mei showed high homology with *SOC1/TM3* subfamily genes. They have typical MADS-box domain, K-box domain, and a highly conserved motif specific to *SOC1* on the C-terminal (Supplemental Fig. 1). These motifs play a significant role in the formation and transcriptional activation of protein complexes (Honma and Goto, 2001; Kramer and Irish, 1999). Therefore, they are included in the *SOC1/TM3* subfamily and are named *PmSOC1*-1, *PmSOC1*-2, and *PmSOC1*-3. Within the coding region, all *SOC1* orthologs consist of seven exons and six introns with exactly the same lengths of the first, third, fourth, fifth, and sixth exons (Supplemental Fig. 2). However, the total number of exons of those genes is different; for example, there are seven exons in *PmSOC1*-3, which differs from the eight exons in *PmSOC1*-1, *PmSOC1*-2, and *AtSOC1*. Moreover, the initiation codon of *PmSOC1*-3 is located in the first exon, whereas those of *PmSOC1*-1 and *PmSOC1*-2 are in the second exon. The region between the first exon and the initiation codon might be an important transcriptional regulatory position, because that of some other genes have distinct relationship to gene expression (Clancy and Hannah, 2002; Jeon et al., 2000; Jeong et al., 2006; Mun et al., 2002). Phylogenetic analysis suggested that *PmSOC1*-1, *PmSOC1*-2, and *PmSOC1*-3 genes might be orthologs of arabidopsis *SOC1*, *AGL42/71/72*, and *AGL14/19*, respectively. Previous studies revealed that there was a gene duplication that gave rise to the *SOC1* gene and *AGL42/71/72* genes (Dorca-Fornell et al., 2011). Thus, we

Fig. 3. Expression analyses of *PmSOCI*-like genes during *Prunus mume* floral bud differentiation. (A) Images of paraffin sections of floral bud at different developmental stages. To analyze the timing of floral bud formation, we examined paraffin sections that had been stained with safranin and fast green. Images of representative paraffin sections for floral bud at different developmental stages are shown. FP = flower primordium, SeP = sepal primordium, Se = sepal, PeP = petal primordium, StP = stamen primordium, Pe = petal, CaP = carpel primordium, Sty = style, An = anther, F = filament, and Ovu = ovule. (B) Relative expression of *PmSOCI*-like genes in the floral bud at different developmental stages. The level of expression was normalized to *P. mume* *PP2A* gene. The relative expression ratio of each sample is compared with the predifferentiation stage sample, and arbitrarily set to 1. S1 = predifferentiation stage, S2 = flower primordium differentiation stage, S3 = sepal differentiation stage, S4 = petal differentiation stage, S5 = stamen differentiation stage, S6 = pistil differentiation stage, S7 = pistil elongation stage, and S8 = ovule and anther formation stage.

Fig. 4. Expression analyses of *PmSOCI*-like genes in transgenic *Arabidopsis thaliana*. (A) The expression of an exogenous gene detected by reverse transcription polymerase chain reaction (RT-PCR). (B) The relative expression levels of an exogenous gene detected by real-time quantitative RT-PCR.

speculated that three *PmSOCI*-like genes have identical ancestral origin, but they differentiate during the evolutionary process, and eventually play different roles in development of

flowers and vegetative organs of mei. Moreover, comparing with the three genes (*AGL42/71/72*) in arabidopsis, mei and peach have only one ortholog (mei: *PmSOCI-2*, peach:

Fig. 5. Comparison of the phenotypes of wild-type, 35S::*PmSOCI-1*, 35S::*PmSOCI-2*, and 35S::*PmSOCI-3* transgenic *Arabidopsis thaliana*. (IA–II) Constitutive expression of 35S::*PmSOCI-1* transgenic *A. thaliana* plant. (IIA–III) Constitutive expression of 35S::*PmSOCI-2* transgenic *A. thaliana* plant; (IIIA–IIIG) constitutive expression of 35S::*PmSOCI-3* transgenic *A. thaliana* plant; (IVA–IVG) wild-type *A. thaliana* plants; (IA, IIA, and IIIA) 35S::*PmSOCI-1*, 35S::*PmSOCI-2*, and 35S::*PmSOCI-3* transgenic *A. thaliana* plants, early flowering in comparison with IVA at 30 d after sowing under long-day conditions; (IB and IIB) flower of 35S::*PmSOCI-1* and 35S::*PmSOCI-2* transgenic *A. thaliana*, respectively, with petals into filament-like structures; (IIIB, IIIC, IVB, and IVC) flower from 35S::*PmSOCI-3* transgenic and wild-type *A. thaliana*, respectively, with the normal petal; (IC and IIC) flower of 35S::*PmSOCI-1* and 35S::*PmSOCI-2* transgenic *A. thaliana*, respectively, with large carpel out of perianth; (IIID and IVD) flower from 35S::*PmSOCI-3* transgenic and wild-type *A. thaliana*, respectively, with the normal carpel; (ID, IE, IID, and IIE) flower of 35S::*PmSOCI-1* and 35S::*PmSOCI-2* transgenic *A. thaliana*, respectively, with sepals into leaf-like structures; (IIIG and IVG) flower from 35S::*PmSOCI-3* transgenic and wild-type *A. thaliana*, respectively, with the normal sepals; (IF and IIF) flower of 35S::*PmSOCI-1* and 35S::*PmSOCI-2* transgenic *A. thaliana*, respectively, with petals and sepals abscission inhibited at silique elongation stage; (IIIE and IVE) flower from 35S::*PmSOCI-3* transgenic and wild-type *A. thaliana*, respectively, with all organs falling from green silique at the same development stage with IF and IIF; (IIG) 35S::*PmSOCI-2* transgenic *A. thaliana* plants, horizontal growth of branches in comparison with IG, IIIF, and IVF; (IH and II) flower of 35S::*PmSOCI-1* transgenic *A. thaliana* with greenish sepals; (IIH and III) flower of 35S::*PmSOCI-2* transgenic *A. thaliana* with different morphologies of terminal flowers.

PpMADS60), and *AGL42*, *AGL71*, and *AGL72* were proved to be redundantly controlling flowering time in *Arabidopsis* (Dorca-Fornell et al., 2011). These data indicated that the duplication and diversity of *Arabidopsis* *SOCI*-like genes occurred after the divergence of *Arabidopsis* and *Prunus*.

THE EXPRESSION PATTERNS OF *PMSOCI*-LIKE GENES. So far, *MADS*-box genes in the *SOCI*/TM3 clade have been isolated from a wide range of plant species, including dicots, monocots, and gymnosperms. Most of these studies have investigated expression patterns of *SOCI*-like genes. The expression of *EgrMADS3* and *EgrMADS4*, two *SOCI*-like genes in the vegetative tissues (particularly apical shoots) of rose gum, was consistent with their probable roles as floral activators in this species (Watson and Brill, 2004). *VvMADS8*, which is a *SOCI*-like gene in grape, was most highly expressed in the axillary buds at the time when the inflorescence started to form in these buds, indicating that *VvMADS8* is a gene involved in the timing of flowering (Sreekantan and Thomas, 2006). *CISOCI-1* and *CISOCI-2*, which are two *SOCI*-like genes in chrysanthemum, were mainly expressed in leaves and shoot apices with the highest expression level after 7 d of SD

treatment, suggesting that these two *SOCI*-like genes might be involved in flower transition (Fu et al., 2013). Soybean *GmSOCI*-like gene was widely expressed at different levels in most organs with the highest expression in the shoot apex during the transition to flowering (Na et al., 2013). *CjMADS15*, which is an *SOCI*-like gene in Japanese cedar (*Cryptomeria japonica*), was expressed in all organs except pollen, and was especially expressed in needles, stems, and strobili (Katahata et al., 2014).

In our study, the expression of *PmSOCI-1*, *PmSOCI-2*, and *PmSOCI-3* was examined in vegetative and reproductive tissues and during floral bud differentiation development. Three *PmSOCI*-like genes were mainly expressed in vegetative organs such as leaves, stems, and buds, and at low expression levels in floral parts of the plants (Fig. 3). Furthermore, *PmSOCI-1* and *PmSOCI-2* were more highly expressed in leaves and stems of adult trees than in 1-month-old seedlings. In *Arabidopsis*, *SOCI* expression levels are also gradually increased during vegetative growth (Borner et al., 2000; Lee et al., 2000; Samach et al., 2000; Wang et al., 2009). The different expression levels of *PmSOCI-1* and *PmSOCI-2* in leaves and stems of adult and juvenile trees may reflect the maturity of the plants. During floral bud differentiation development, the expression level of *PmSOCI-2* was

decreased most significantly in three *PmSOCI*-like genes (Fig. 3B). These results indicated that the three *PmSOCI*-like genes might imply a general role in the transition from the vegetative to the reproductive phase in mei, especially *PmSOCI-2*.

Although *SOCI*-like genes share similar expressions as discussed above, there are studies that uncovered their different expression patterns. *Gerbera GhSOCI* was expressed in the inflorescence and organs derived from the inflorescence, but no expression was detected in the vegetative parts (Ruokolainen et al., 2011). Both *PTM5* in poplar and *DOSOCI* in dendrobium orchid are expressed in vascular tissues, implying that *SOCI*-like genes could similarly affect the life cycle of many flowering plants (Cseke et al., 2003; Ding et al., 2013). These results suggest that *SOCI*/TM3-like genes have various expression patterns, implying that the functions of these genes may be more diversified in the complex regulatory network of floral transition.

***PMSOCI-3* IS PRINCIPALLY ASSOCIATED WITH FLOWERING INDUCTION, WHEREAS *PMSOCI-1* AND *PMSOCI-2* ARE ASSOCIATED WITH FLORAL ORGAN DEVELOPMENT AND PLANT MORPHOGENESIS IN *ARABIDOPSIS*.** In *Arabidopsis* flowering process, *SOCI* was the first gene shown to be upregulated in

Table 1. Flowering phenotypes of transgenic *Arabidopsis thaliana*. Twenty transgenic plants of each line and 40 wild-type plants were used for observation.

Genotype	Wild type/transgenic line ^z	Rosette leaves [mean ± SE (no.)] ^y		Time to flowering [mean ± SE (d)]	
		LD	SD	LD	SD
Wild type	Col-0	13.5 ± 0.6 a ^x	30.8 ± 1.0 a	37.3 ± 1.2 a	85.0 ± 1.2 a
35S:: <i>PmSOC1-1</i>	No. 1△	5.5 ± 0.6 e	7.9 ± 1.4 f	26.3 ± 1.3 e	59.8 ± 1.7 e
	No. 6△	6.2 ± 0.8 e	8.6 ± 0.5 f	27.5 ± 1.2 cd	60.3 ± 2.2 e
	No. 9△	6.3 ± 1.5 e	9.0 ± 0.8 f	27.8 ± 0.5 de	61.8 ± 2.1 e
	No. 13☆	8.8 ± 1.0 cd	12.5 ± 1.0 de	32.8 ± 1.0 b	68.9 ± 0.9 d
	No. 18☆	9.0 ± 0.7 bcd	13.7 ± 0.9 d	33.2 ± 1.7 b	70.0 ± 0.8 d
35S:: <i>PmSOC1-2</i>	No. 24☆	8.5 ± 0.6 d	12.3 ± 1.0 de	32.0 ± 2.6 bc	69.3 ± 1.0 d
	No. 5△	3.3 ± 0.5 f	4.8 ± 1.0 g	19.7 ± 1.5 f	43.7 ± 1.2 f
	No. 8△	3.5 ± 0.6 f	5.5 ± 1.3 g	22.0 ± 2.0 f	44.6 ± 0.9 f
	No. 10△	3.5 ± 1.0 f	5.8 ± 0.5 g	22.3 ± 2.1 f	45.4 ± 0.5 f
	No. 14☆	5.8 ± 0.9 e	8.8 ± 1.0 f	27.8 ± 1.3 de	59.0 ± 1.4 e
35S:: <i>PmSOC1-3</i>	No. 19☆	6.3 ± 0.5 e	12.0 ± 0.8 e	28.7 ± 0.6 cd	59.3 ± 1.3 e
	No. 28☆	6.2 ± 1.5 e	10.9 ± 1.2 e	28.3 ± 1.0 d	60.4 ± 1.1 e
	No. 2	8.7 ± 0.4 d	17.8 ± 1.5 c	32.5 ± 1.3 b	78.0 ± 1.0 c
	No. 3	10.5 ± 1.3 bc	21.5 ± 1.3 b	34.0 ± 1.4 b	81.2 ± 0.9 b
	No. 5	9.3 ± 1.5 bcd	19.3 ± 0.5 c	32.4 ± 2.6 bc	78.5 ± 1.3 c
	No. 8	9.8 ± 2.1 bcd	21.3 ± 1.2 b	33.8 ± 1.0 b	79.7 ± 1.2 bc
	No. 11	11.0 ± 1.4 b	23.0 ± 1.4 b	34.5 ± 1.3 b	82.4 ± 1.3 b
	No. 12	9.0 ± 0.9 bcd	20.0 ± 2.8 bc	32.8 ± 2.5 b	78.3 ± 0.5 c

^zCol-0 = wild-type *A. thaliana* ecotype Columbia-0, △ = strong phenotype, ☆ = weak phenotype.

^yLD = long-day (16-h light/8-h dark) conditions, SD = short-day (8-h light/16-h dark) conditions.

^xDifferent lowercase letters in the same column indicate a significant difference at $P < 0.05$ according to Student's *t* test.

Fig. 6. Expression analyses of different flowering time genes in transgenic *Arabidopsis thaliana* seedlings grown for days 6, 12, and 16 in long-day conditions. Relative expression levels were normalized to *A. thaliana TUB2* expression. Error bars indicate the standard errors. (A) *LFY* expression, (B) *API* expression, (C) *AGL24* expression, and (D) *FUL* expression.

meristems to promote early flowering. Currently, the overexpression of *SOC1* homologous genes identified in other species consistently showed the early flowering phenotype in *Arabidopsis*. These species include *CISOC1-1* and *CISOC1-2* in chrysanthemum (Fu et al., 2013), *VvMADS8* in grape (Sreekantan and Thomas, 2006), *EgrSOC1* in lisianthus (Nakano et al., 2011), and *CsSL1* in sweet orange (Tan and Swain, 2007). The overexpression of *GmGAL1* in soybean and *DOSOC1* in

dendrobium orchid not only enables early flowering in wild-type *Arabidopsis* but also complements part of the late-flowering phenotype in *SOC1* mutants (Ding et al., 2013; Zhong et al., 2012). In our study, transgenic *Arabidopsis* lines presented early flowering phenotype under both LD and SD conditions, implying a conserved role of *SOC1*/TM3 gene family in promoting flowering.

In many species, *SOC1*-like genes have shown impacts on flower development, in addition to promoting flowering. Overexpression of *AtSOC1* in *Arabidopsis* resulted in greenish sepaloid petal and elongated pistil (Borner et al., 2000). Overexpressed *GhSOC1* in gerbera did not promote flowering, but led to changes in petal color and shape (Ruokolainen et al., 2011). Overexpression of *UNS* from petunia in *Arabidopsis* caused the appearance of trichomes in floral organs, which

should originally grow on leaves and stems, and further turned petals into a leaf-like structure (Ferrario et al., 2004). Overexpression of *DOSOC1* in dendrobium orchid influenced the normal development of floral organs, causing an unsound perianth on the top flower bud (Ding et al., 2013). Similarly, overexpression of *PmSOC1-1* and *PmSOC1-2* in *Arabidopsis* resulted in phenotypes such as filamentous petal and leaf-like sepal in our study (Fig. 5). Among them, overexpression of

PmSOC1-1 led to green petals and elongated pistils, which are consistent with phenotypes generated by overexpression of *AtSOC1* (Fig. 5IC and II). These phenotypes indicated that *SOC1*-like genes can regulate floral development apart from promoting flowering. Interestingly, the overexpression of *PmSOC1-2* changed the lateral branch angle of arabidopsis; thus, changing the architecture of arabidopsis (Fig. 5IIG). This phenotype has not yet been reported in any study of overexpression phenotypes of *SOC1* in other species. This phenotype suggested that overexpression of *SOC1*-like genes in mei may influence the expression of branch angle-related genes in arabidopsis, leading to the horizontal growth of branches. To further study the molecular mechanisms of increasing branch angle in transgenic arabidopsis, the expression of branch angle-related genes should be checked using qRT-PCR.

SOC1 genes promote flowering mainly by upregulating its downstream floral meristem genes. For instance, in the process of flowering development, heterodimers are formed by *SOC1* and *AGL24*, which activates *LFY* gene expression, thus promoting flowering (Lee et al., 2008). Similar mechanisms also appear between *FUL* and *SOC1*. Studies have found that *FUL* and *SOC1* can form a dimer in the cell nucleus and further bind to *LFY* promoter region, thus playing the same role. Therefore, redundancy dimers or higher-order molecular complexes can be formed between *SOC1*, *AGL24*, and *FUL* to ensure the activation of *LFY*, which subsequently activates other floral meristem genes (Balanza et al., 2014). Overexpression of *CISOC1* from chrysanthemum and *DOSOC1* from dendrobium orchid in arabidopsis can upregulate flowering genes such as *AGL24* and *LFY* (Ding et al., 2013; Fu et al., 2013). In our study, we used qRT-PCR to detect the downstream genes of *SOC1* including *LFY*, *AP1*, *AGL24*, and *FUL* in transgenic arabidopsis. The results showed that the introduction of *PmSOC1*-like genes led to different degrees of upregulations of *LFY*, *AP1*, *AGL24*, and *FUL* at various stages compared with the wild type (Fig. 6). The expression of *AGL24* and *FUL* displayed similar trends: both showed a gradual upward trend (from days 6, 12, and 16 after seed germination) suggesting that dimers may be formed between *SOC1* and *AGL24* or *FUL* to activate expression of *LFY* to promote flowering (Fig. 6C and D), and this can be further verified by *LFY* expression levels in transgenic arabidopsis (Fig. 6A). 35S::*PmSOC1-2* lines, the most notable early flowering ones, also presented the highest expression levels of *LFY* and *AP1*, revealing that the *PmSOC1-2* gene may effectively activate *LFY* gene expression by interacting with *AGL24* and *FUL*. However, we need more evidence to verify this hypothesis.

In recent years, studies have shown that *SOC1* also plays other functions in addition to integrating a variety of signals during the flowering process. For example, single *SOC1* mutant exhibited late-flowering phenotype, whereas double *SOC1-FUL* mutant showed traits of perennials, such as late flowering time, the formation of aerial rosettes, and repeated transition to vegetative growth. It is analyzed that *SOC1* and *FUL* might have inhibited plant transition from annuals to perennials (Melzer et al., 2008). In poplar, the specific expression of the *SOC1* homologous gene *PTM5* in vascular tissues suggests its involvement in vascular development (Cseke et al., 2003).

In conclusion, *PmSOC1*-like genes obtained from mei in this study contain typical MADS-box, K-box domain, and conserved motif, belonging to the *SOC1/TM3* subfamily of MADS-box gene family. Phylogenetic analysis showed that

PmSOC1-1 is more closely related to arabidopsis *SOC1*, *PmSOC1-2* is more closely related to *AGL71*, *AGL72*, and *AGL42*, and *PmSOC1-3* is more closely related to *AGL14* and *AGL19*. Through phenotypic analysis of transgenic arabidopsis, it is validated that all three genes promote flowering, of which *PmSOC1-2* is the most functionally significant one. In addition to early flowering, *PmSOC1-1* and *PmSOC1-2* can affect floral organ development. Moreover, overexpression of *PmSOC1-2* in transgenic arabidopsis can affect plant architecture, but this function has not been reported in any study of *SOC1* homologous genes. To conclude, three *PmSOC1*-like genes have identical ancestral origin and probably diversified their function during evolution. Our results provide a theoretical basis for the use of genetic engineering to shorten mei juvenile period and accelerate the cultivar improvement process. However, further studies on the upstream and downstream regulatory sequences of *PmSOC1*-like genes will provide deeper insights into mei flowering.

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PmSOC1-1	MVRGKTQMRRIENATSRQVTFSKRRSGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQTTIERYQKHTKDNLTNN	80
PmSOC1-2	MVRGKIEMKRIENATSRQVTFSKRRNGLLKKAFELSVLCDAQVSVIIFSQKGRLYEFSSSDMQETIKRYHKHAKAGQTN.	79
PmSOC1-3	MVRGKTQMKRIENAASQVTFSKRRNGLLKKAFELSVLCDAEVALIIFSTRGKLYEFSSSIGNTLDRYQKRVKQDGLGS	80
PaSOC1	MVRGKTQMRRIENATSRQVTFSKRRSGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQTTIERYQKHTKDNLTNN	80
PpSOC1	MVRGKTQMRRIENATSRQVTFSKRRSGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQTTIERYQKHTKDNHTSN	80
PsSOC1	MVRGKTQMRRIENATSRQVTFSKRRSGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQTTIERYQKHKADNHTNN	80
PySOC1	MVRGKTQMRRIENATSRQVTFSKRRSGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQTTIERYQKHTKDNHTNY	80
MdSOC1	MVRGKTQMRRIENATSRQVTFSKRRSGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQTTIERYQKHKAGNQTSN	80
ScSOC1	MVRGKTQMRRIENATSRQVTFSKRRSGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQTTIERYQKHKADNQTNN	80
RhSOC1	MVRGKTQMRRIENATSRQVTFSKRRSGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQTTIERYEKHKTQDNQANN	80
AtSOC1	MVRGKTQMKRIENATSRQVTFSKRRNGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSNMQDTIDRYLRHTKDRVST.	79
CcSOC1	MVRGKTQMRRIENATSRQVTFSKRRNGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQETIDRYRRHTKDAQPIN	80
CsSOC1	MVRGKTQMRRIENATSRQVTFSKRRNGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQETIERYLKHTKQTR..N	78
VvSOC1	MVRGKTQMRRIENATSRQVTFSKRRNGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQETIERYQRHTKDVHTNN	80
PTM5	MVRGKTQMRRIENATSRQVTFSKRRNGLLKKAFELSVLCDAEVALIIFSPRGKLYEFASSSMQETIERYRRHKENNTN.	79
ETL	MVRGKTQMKRIENDTSRQVTFSKRRNGLLKKAFELSVLCDAEVALIIFSPRGKLYEFSSSLCKTIEKYQTRAKDMEA..	78

MADS-box

PmSOC1-1	KSVSTDQNMQHLKQESSMMKQIELLEVSRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLREKGG	160
PmSOC1-2	KIEVEE.YVEQLKHESTAMAKKIEENLEASQKRLLGHLGDSVLELQIEITGQLERSVRKIRERKAHLFAEQMEQLRAKER	158
PmSOC1-3	KAV..QVDMHEGKDDTSSMAKKIDFIEASQKQLLGNCLESIEELQQTENQLERSLSKIRARKTQLLREQIENLKKEEK	158
PaSOC1	KSVSTDQNMQHLKQESSMMKQIEFLEISRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLREKGG	160
PpSOC1	KSVSTDQNMQHLKQESSMMKQIELLEVSRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIDQLREKGG	160
PsSOC1	KPVSTDQNMQHLKQESSMMKQIELLEVSRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLKEKGG	160
PySOC1	KSVSTDQNMQHLKQESSMMKQIELLEVSRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLREKEK	160
MdSOC1	KSSSNEQNMQHLKQKATIMMKQIELLEVSRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLREKEK	160
ScSOC1	KSVASEQNTQHLRQEASRMKQIEILEGSRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLREKEK	160
RhSOC1	KSVASEQNVQQLKHEATSMKQIEHLEVSRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLREKEK	160
AtSOC1	KPVS.EENMQHLKYEAMMMKQIELEASRRKLLGEGIGTCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLKQKEK	158
CcSOC1	KSVE..QNMQHLRHESANMVKQIELEVSRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIDKLKQKEK	158
CsSOC1	KQQPTEQNMQHLKHEAANMVKQIELEVSRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIDKLKQKEK	158
VvSOC1	YKTT.EHNMQQLKHEAANMAKKQIELEISRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLKEKEK	159
PTM5	KQPVE.QNMLQLKEEAASMIKKQIEHLEVSRRKLLGEGLGSCSIEELQIEEQQLERSVSNVRARKTQVFKEQIEQLKQKEK	158
ETL	KTAEIS..MQPSKGTLDMEKKQIEHLEISRRRLLGEGLGSCSIEELQQTENQLERSLTKIRARKNHLIREHIERLKAER	156

K-box

PmSOC1-1	ALAAENEKLEKCGRIQPRQA....SNEQRENLAFTSSPSSDVETELFIGLPERRKR	214
PmSOC1-2	LLEENAKLSEEFGAQPRLLQQQQLSVEEKGAVSYSLSPPSSSEVETELFIGPPVTRC	217
PmSOC1-3	NLFEQNAKLEKCGMQPLGPPSARKDEENCAVRQPRTPDM...EDVETDLVIGPPERRRSSQNP	219
PaSOC1	ALAAENERLEKCGRIQPRQA....SNEQRENLAFTSSPSSDVETELFIGLPERRKR	214
PpSOC1	ALAAENERLEKCGKIQPRKA....SNEQRENLAFTSSPSSDVETELFIGLPERRMKR	215
PsSOC1	ALAAENERLEKCGRIQPRQA....SNEQRENLAFTSSPSSDVETELFIGLPERRMKR	215
PySOC1	ALTAENERLEKCGSIQPRQA....SNEQRENLAFTSSPSSDVETELFIGLPEERRVKR	216
MdSOC1	LKAETARLVEKCGSFQPRKTLDERR....QNTTY.TDSSTSDVETELFIGLPESRA.RR	215
ScSOC1	MLAAENVRLMEKCGSIQMQAGAPQTSNEQREHLPY.ADSSPSSDVETELFIGMPERRAKR	221
RhSOC1	VLTAEQRLNEKCEAMQPRQP....VSEQRENLAFTSSPSSDVETELFIGLPERRSKH	215
AtSOC1	ALAAENEKLEKCGSHESEVSNKNQESTGRGDE....ESSPSSSEVETQLFIGLPCSSRK	214
CcSOC1	ALVAENARLCEKCG.IQLQQG....ANEHREISPY.EESNPSSDVETELFIGPPERRTKRLPPPN	217
CsSOC1	VLEAENTRLEKCGMENWQ...GSK...EQPENLTN.DDGASTSDVETELFIGPPERRARRLAIPPQ	219
VvSOC1	ALAAENARLCEKCG.VQPYQA....PNQENETLPS.AERSQNSDVSTDLFIGLPEGRAKRLLLGN	218
PTM5	LAAENARLSEDECG.AQSWPVSWEQRDDL.PREEQ...RESSISDVETELFIGPPETRTKRIPPRN	220
ETL	KLEEKRLKLEIECGKGLTPVS....SERPREEIR.AES...MDVETELFIGPPKR	205

K-box

SOC1 motif

Supplemental Fig. 1. Alignment of the amino acid sequences of *Prunus mume* SOC1-like genes and its orthologs from other plant species. Identical and similar amino acids among predicted protein sequences are shaded in black and gray, respectively. The regions of the MADS domain and K domain are underlined, and the specific SOC1 motif is boxed. The protein sequences included in the analysis have the corresponding protein names as follows: PaSOC1 (*Prunus armeniaca*), PpSOC1 (*Prunus persica*), PsSOC1 (*Prunus salicina*), PySOC1 (*Prunus yedoensis*), MdSOC1 (*Malus domestica*), ScSOC1 (*Spiraea cantoniensis*), RhSOC1 (*Rosa hybrida*), AtSOC1 (*Arabidopsis thaliana*), CcSOC1 (*Carya cathayensis*), CsSOC1 (*Citrus sinensis*), VvSOC1 (*Vitis vinifera*), PTM (*Populus tremuloides*), and ETL (*Eucalyptus globulus*).

Supplemental Fig. 2. Comparison of sequences and structures of *SOC1* homologs. Schematic presentation of intron/exon structure of *PmSOC1*-like genes compared with its orthologs. Introns are shown as lines and exons as boxes, with the open reading frame in black. Numbers in boxes represent the exon or intron length in base pairs. ATG translation initiation site and TGA/TAG translation stop site are marked.

Supplemental Table 1. Primers used for clone, quantitative real-time polymerase chain reaction (PCR), vector construction, and reverse transcription PCR (RT-PCR).

Purpose	Primer name	Primer sequence (5'–3')
Clone	GC-PmSOC1-1F	ATGGTGAGAGGAAAAACCCAGATGA
	GC-PmSOC1-1R	CTAGCGCTTCTTCTTTCTGGCAGT
	GC-PmSOC1-2F	ATGGTGAGAGGGAAGATTGAGATGA
	GC-PmSOC1-2R	TCAACAGCGCGTTACCGGC
	GC-PmSOC1-3F	ATGGTTAGGGGGAAGACTCA
	GC-PmSOC1-3R	CTATGGGTTTTGGCTACTTC
Quantitative real-time PCR	QP-PmSOC1-1F	TTTCAGTTCTTTGTGATGCTGAGG
	QP-PmSOC1-1R	CGGATTTGTGTGTGTAAGGTTG
	QP-PmSOC1-2F	TTTCAGTTCTCTGCGATGCTC
	QP-PmSOC1-2R	AATCTTGTGTTGTTTGGCCAG
	QP-PmSOC1-3F	AGCTCTCAGTTCTATGTGATGCTG
	QP-PmSOC1-3R	TTGATAACGGTCTAGTGTGTTGC
	PP2A-F	ATATAGCTGCTCAGTTCAACC
	PP2A-R	AAAAACAGTCACCACATTCTT
	TUB2-F	GGTAGATTCTGTTCCCTCGTGC
	TUB2-R	GCTCCTTCCGTGTAGTGTCC
	LFY-F	GAGGTAGTGGTTTGGGGACA
	LFY-R	TGTTTATGTAACTCGCTCCTGA
	AP1-F	CACCAAATCCAGCATCCTTAC
	AP1-R	GTTTCGAGATCATTCTCCTCA
	FUL-F	AGAAAGAACCAAGCTATGTTTCG
	FUL-R	GAGGAGGTTACGCAGTATTGAG
	AGL24-F	CGAAGACAAAACCAAGCAGC
	AGL24-R	ACACGGCTAAGTCCGGATTCT
Vector construction	PC-PmSOC1-1F	AGATCTATGGTGAGAGGAAAAACCCAGATGA Bgl II site
	PC-PmSOC1-1R	GGTGACCCTAGCGCTTCTTCTTTCTG BstE II
	PC-PmSOC1-2F	CAGATCTGATGGTGAGAGGGAAGATTGAGAT Bgl II site
	PC-PmSOC1-2R	GGTGACCCTCAACAGCGCGTTACCG BstE II
	PC-PmSOC1-3F	CAGATCTGATGGTTAGGGGGAAGACTCA Bgl II site
	PC-PmSOC1-3R	GGTGACCCTATGGGTTTTGGCTACTTC BstE II
RT-PCR	CaMV 35S-F	GGAAAGGCCATCGTTGAAGAT
	RT-PmSOC1-1R	CTAGCGCTTCTTCTTTCTGGCAGT
	RT-PmSOC1-2R	TCAACAGCGCGTTACCGGC
	RT-PmSOC1-3R	CTATGGGTTTTGGCTACTTC