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).
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)], DmSOC1 [dendrobiyum orchid (Dendrobium)], GmSOC1 [soybean (Glycine max)], EgrSOC1 [lilac (Syringa vulgaris)], ZmmADS1 [maize (Zea mays)], TrcMADS1 [trillium (Trillium camtschatcense)], CISOCl [chrysanthemum (Chrysanthemum luminiflorum)], HvSOC1 [barley (Hordeum vulgare)], PTM5 [poplar (Populus tremuloides)], ET1 [texasian blue gum (Eucalyptus globulus)], and VvMADS8 [grape (Vitis vinifera)] (Cseke et al., 2003; Decrooq et al., 1999; Ding et al., 2013; Ferrari 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; Papaphthimio 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. 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 (EgrMADS 3 and EgrMADS 4) 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
The amplified products were separated in 1.0% agarose gels, 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 μL containing 1 μL cDNA, 0.8 U Taq DNA polymerase (Promega), 0.5 μM of each primer, 1× 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 nos. 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 predifferentiation 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 (≈45 d after full bloom, ≈10 mm diameter), and mature fruit (≈95 d after full bloom, ≈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-μL quantitative RT-PCR mixture contained 1 μL of diluted cDNA, 200 nm of each primer, and 10 μL 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−ΔΔCt 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–BstE 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 BstE 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 aridopsis 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 aridopsis 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 aridopsis 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, AP1, 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, AP1, 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 leaves was counted when the first flower opened. According to Student’s t test was used to detect the significance of flowering time differences.

Results

Identification of SOCl-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 PmSOC1-1 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, second, 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
lines no. 5, no. 8, and no. 10 of 35S::PmSOC1-2 were strong phenotypes and no. 14, no. 19, and no. 28 were weak phenotypes. The transgenic lines showing a strong phenotype of 35S::PmSOC1-1 flowered early (5 rosette leaves) under LD conditions as compared with the wild-type arabidopsis lines (13 rosette leaves) (Fig. 5IA). 35S::PmSOC1-2 transgenic lines with strong phenotype showed the earliest flowering time among all transgenic lines (Table 1). Individual 35S::PmSOC1-2 transgenic lines flowered even at three-rosette-leaves stage (Fig. 5IA). The weak phenotype of 35S::PmSOC1-2 could also flower with an average of six rosette leaves. Under LD conditions, 35S::PmSOC1-3 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 PmSOC1-like genes can cause early flowering irrespective of their potencies.

**OVEREXPRESSION OF PMSOC1-1 AND PMSOC1-2 IN ARABIDOPSIS AFFECTS FLORAL ORGAN DEVELOPMENT AND PLANT MORPHOGENESIS.** Besides advanced flowering (Fig. 5IIA–IIIG), the 35S::PmSOC1-3 transgenic lines were not much different from the wild-type arabidopsis (Fig. 5IVA–IVG). Analysis of qRT-PCR showed that PmSOC1-3 transgenic lines had different expression profiles (Fig. 4B), suggesting that 35S::PmSOC1-3 was stably expressed in transgenic lines.

When inducing early flowering in arabidopsis, 35S::PmSOC1-1 and 35S::PmSOC1-2 transgenic lines showed weak and strong phenotypes in flowers, colors, and plant architecture. Except for the more significant petal refinement in 35S::PmSOC1-2 transgenic lines, other variations in weak phenotypes of 35S::PmSOC1-1 and 35S::PmSOC1-2 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 1IB), elongated pistils, which extended to outer perianth (Fig. 5IC and 1IC), leaf-like sepals (Fig. 5ID, IE, IIE, and IIF), 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::PmSOC1-1 transgenic lines, there have been variations of petal color turning green as compared with the wild type (Fig. 5IH and IIF), whereas strong phenotypes of 35S::PmSOC1-2 exhibited significant changes in plant morphogenesis, such as all lateral branches showing horizontal growth (Fig. 5IG) so that the plant architecture of arabidopsis completely changed. Moreover, 2–4 flowers consecutively formed terminal flower (TF) in part of the terminal inflorescence in 35S::PmSOC1-2 strong phenotypes. The number of floral in TF was indefinite, and those flowers usually bloomed first (Fig. 5IH and III).

Moreover, all strong phenotypes of 35S::PmSOC1-1 and 35S::PmSOC1-2 transgenic lines showed relatively high expression levels in qRT-PCR. For example, no. 5 35S::PmSOC1-2 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.
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 3SS::PmSOC1-2 transgenic Arabidopsis line than the other two transgenic lines (3SS::PmSOC1-1 and 3SS::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). Depending 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, GMM13-like, SQUA-like, STMADS11-like, TMS-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 (Supplementary 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
speculated that three *PmSOC1*-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: *PmSOC1*-2, peach:
be redundantly controlling flowering time in arabidopsis. occurred after the divergence of arabidopsis and gymnosperms. Most of these studies have investigated expression patterns of SOC1-like genes in the reproductive phase in mei, especially involving in flower transition (Fu et al., 2013). Soybean GmSOC1-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). CjMADS13, which is an SOC1-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 PmSOC1-1, PmSOC1-2, and PmSOC1-3 was examined in vegetative and reproductive tissues and during floral bud differentiation development. Three PmSOC1-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, PmSOC1-1 and PmSOC1-2 were more highly expressed in leaves and stems of adult trees than in 1-month-old seedlings. In arabidopsis, SOC1 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 PmSOC1-1 and PmSOC1-2 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 PmSOC1-2 was decreased most significantly in three PmSOC1-like genes (Fig. 3B). These results indicated that the three PmSOC1-like genes might imply a general role in the transition from the vegetative to the reproductive phase in mei, especially PmSOC1-2.

Although SOC1-like genes share similar expressions as discussed above, there are studies that uncovered their different expression patterns. Gerbera GhSOC1 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 DOSOC1 in dendrobiom orchid are expressed in vascular tissues, implying that SOC1-like genes could similarly affect the life cycle of many flowering plants (Cscke et al., 2003; Ding et al., 2013). These results suggest that SOC1/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.

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 SOC1-like genes occurred after the divergence of arabidopsis and Prunus.

**The expression patterns of PMSOC1-like genes.** So far, MADS-box genes in the SOC1/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 SOC1-like genes. The expression of EgrMADS3 and EgrMADS4, two SOC1-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 SOC1-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). CjSOC1-1 and CjSOC1-2, which are two SOC1-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 SOC1-like genes might be involved in flower transition (Fu et al., 2013). Soybean GmSOC1-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). CjMADS13, which is an SOC1-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).

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meristems to promote early flowering. Currently, the over-expression of SOC1 homologous genes identified in other species consistently showed the early flowering phenotype in arabidopsis. These species include ClSOC1-1 and ClSOC1-2 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

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 | Rosette leaves [mean ± SE (no.)] | Time to flowering [mean ± SE (d)] |
|------------|--------------------------|----------------------------------|-----------------------------------|
|            |                          | LD | SD | LD | SD |
| Wild type  | Col-0                    | 13.5 ± 0.6 a | 30.8 ± 1.0 a | 37.3 ± 1.2 a | 85.0 ± 1.2 a |
| 35S::PmSOC1-1 | 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 |
|            | No. 24☆                | 8.5 ± 0.6 d | 12.3 ± 1.0 de | 32.0 ± 2.6 bc | 69.3 ± 1.0 d |
| 35S::PmSOC1-2 | 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 |
|            | 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 |
| 35S::PmSOC1-3 | 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 |

Col-0 = wild-type A. thaliana ecotype Columbia-0, △ = strong phenotype, ☆ = weak phenotype. LD = long-day (16-h light/8-h dark) conditions, SD = short-day (8-h light/16-h dark) conditions. Different 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) AP1 expression, (C) AGL24 expression, and (D) FUL expression.
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. 5IHG). 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 (Balanzà et al., 2014). Overexpression of CISO1 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). 3SS::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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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*), PTM5 (*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  | CTAAGGCGCTTCTTCTTTCTTGCGAGT             |
|                              | GC-PmSOC1-2F  | ATGGTGAGAGGAAAGATTGAGATGA              |
|                              | GC-PmSOC1-2R  | TCAACAGGCGGTTACCCGC                    |
|                              | GC-PmSOC1-3F  | ATGGTTAGGGGGAAGACTCA                   |
|                              | GC-PmSOC1-3R  | CATGGGCTTTGCGACTTC                     |
| **Quantitative real-time PCR**| QP-PmSOC1-1F  | TTTCAGTTCTTTGATGAGGAGG                 |
|                              | QP-PmSOC1-1R  | CGGATTGTGGTTGAGTAAGGTTG               |
|                              | QP-PmSOC1-2F  | TTTCAGTTCTGCTGAGTCTC                  |
|                              | QP-PmSOC1-2R  | AATCTGTTTGGTTGCCAG                     |
|                              | QP-PmSOC1-3F  | AGCTCTCAGTTCTATGTAGCTG                |
|                              | QP-PmSOC1-3R  | TTGATAACGGTCTAGTGTGTTG                |
|                              | PP2A-F        | ATATAGCTGCTACAGTCAA                    |
|                              | PP2A-R        | GGAACAGTACCCGATTCTT                   |
|                              | TUB2-F        | GGCTCGTCGGTCTAGTCTC                   |
|                              | TUB2-R        | GCTCCCTCGCTAGTCTGCC                   |
|                              | LFY-F         | GAGGAGCAGTGCTGCTGCA                    |
|                              | LFY-R         | GAAAGAAAACGGACTATGGTG                 |
|                              | AP1-F         | CACAAATCCAGCTCTACAA                   |
|                              | AP1-R         | GTTCAGATGTTGTTGGAGA                    |
|                              | FUL-F         | AGAAAGAACCAGCTATGGTG                  |
|                              | FUL-R         | GAGGAGTTACGCAGTATGGAG                 |
|                              | AGL24-F       | CGAAGACAAGAACGACAGC                   |
|                              | AGL24-R       | ACACGGCTAAATGCACGATTC                 |
| **Vector construction**      | PC-PmSOC1-1F  | AGATCTATGGTGAGGAAAACCCAGATGA Bgl II site |
|                              | PC-PmSOC1-1R  | GGTGACCGCTAGCCTTTCTTTCTTG              |
|                              | PC-PmSOC1-2F  | CAGATGCTGATGGTAGAGGАAGATTGAGAT       |
|                              | PC-PmSOC1-2R  | GTTCGACCTCAACAGCGGTTACC               |
|                              | PC-PmSOC1-3F  | CAGATCTGATGGTACGGAGGАAGACTCA          |
|                              | PC-PmSOC1-3R  | GGТGАCССGАTAАGГТТСGCTААСТС               |
| **RT-PCR**                   | CaMV 35S-F    | GGAAGACCATCGTTGAAG                    |
|                              | RT-PmSOC1-1R  | CTAGCGCTTTCTTCTTTCTTGCGAGT            |
|                              | RT-PmSOC1-2R  | TCAACAGGCGGTTACCAGC                   |
|                              | RT-PmSOC1-3R  | CATGGGCTTTGGCTACTTC                   |