PmSOC1s and PmDAMs participate in flower bud dormancy of *Prunus mume* by forming protein complexes and responding to ABA

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Summary

Flowering time is an important agronomic trait, which can affect the fruit-bearing and ornamental value of plants. Being a perennial woody horticultural plant in the temperate zone, the flowering time of *Prunus mume* is deeply affected by the dormancy duration. It is well known that MADS-box transcription factors play vital roles in the flowering time and dormancy processes, such as *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)* and *Dormancy Associated MADS-box (DAM)* genes. Here, the expression patterns of *PmSOC1s* and *PmDAMs* and their relationships with the ABA content in flower bud dormancy were analyzed. They had a similar expression trend, which gradually decreased in the process and positively correlated with the content of ABA. Besides, several ABA-responsiveness elements were predicted in promoter sequences of *PmSOC1s* and *PmDAMs*. The expression level of *PmSOC1s* and *PmDAMs* under ABA treatment was detected by qRT-PCR. Results showed that *PmSOC1-2*, *PmSOC1-3* and *PmDAM6* were highly sensitive to ABA. Moreover, *PmSOC1-1*, *PmSOC1-2*, *PmSOC1-3* were detected in the nucleus of the cells in the *Nicotiana benthamiana* leaves through transient transformation. Furthermore, the results of yeast two-hybrid showed that there was a strong interaction between *PmSOC1-2* and *PmDAM6*. Our results indicated that *PmSOC1s* and *PmDAMs* participated in flower bud dormancy by forming protein complexes, and were regulated by ABA, which laid a foundation for elucidating the common mechanism of flowering and dormancy of *P. mume*.

Keywords

flower bud dormancy, flowering time, interaction, *Prunus mume*, transcription factors

Introduction

Mei (*Prunus mume*) is an important fruit and ornamental crop in the Rosaceae family, which has been domesticated in East Asia for about 3,000 years. After the long-term domestication and cultivation, it gained some unique ornamental characteristics, such as colorful corollas, pleasant fragrance,

Significance of this study

What is already known on this subject?

- For perennial plants growing in temperate zones, the flowering time is affected by the duration of dormancy. Studies indicate that flowering time and dormancy processes have a common mechanism. Some MADS-box transcription factors participate in the flowering time process, dormancy process, or both. Among them, SOC1 is a famous flowering promoter, while DAM is a typical dormancy related transcription factor. Meanwhile, it is reported that SOC1 is also involved in dormancy.

What are the new findings?

- During the flower bud dormancy process, the expression trends of *PmSOC1s* and *PmDAMs* were similar and positively correlated with ABA content. Several ABA-responsiveness elements (ABREs) were found in their promoters. ABA regulates their expression. Moreover, *PmSOC1s* were detected in the nucleus of the cells, possibly dimerizing with *PmDAMs*. The interaction between *PmSOC1-2* and *PmDAM6* was the strongest.

What is the expected impact on horticulture?

- This study lays a foundation for revealing the common molecular mechanism of flower bud dormancy and flowering times. This provides a theoretical basis for cultivating new varieties of *Prunus mume* with different flowering times by genetic engineering breeding measures.

and varying flower patterns. It originates in China, spreads widely in temperate or boreal zones, and blooms in winter or early spring. Its flowering time is affected by the duration of flower bud dormancy. Bud dormancy is a self-protection mechanism to survive from abiotic stresses, such as the short photoperiod and low temperature during autumn and winter (Maurya and Bhalerao, 2017). Absciscic acid plays an important role in regulating plant physiological activities during bud dormancy. The short photoperiod can induce the accumulation of ABA, which can initiate the establishment of dormancy (Ruttink et al., 2007; Singh et al., 2018; Wang et

al., 2015). Therefore, it is very valuable to study the molecular mechanism of its flowering time and dormancy. A long-lasting hypothesis has been put forward that flowering and dormancy share a common mechanism, suggesting the roles of several MADS-box genes (Brunner et al., 2014; Chouard, 1960; Horvath, 2009; Horvath et al., 2003; Subbaraj et al., 2010).

MADS-box transcription factors encode important transcriptional regulators. All members of the MADS-box gene family contain a conserved MADS domain consisting of approximately 60 amino acids. They have diverse roles in various developmental processes in plants, such as fruit development and ripening, seed development and maturity, vegetative growth, flowering initiation, floral organ morphogenesis, flowering process, and dormancy (Smaczniak et al., 2012). MADS-box gene family consisted of several subfamilies, such as SEP, AP1/FUL, AG, TM8, AP3/PI, FLC, SVP, SOC1 subfamilies, and so on.

SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), a member of the MADS-box gene family, is known as a flowering pathway integrator of the photoperiod, aging, gibberellin, autonomous, and vernalization pathways (Jung et al., 2012; Lee and Lee, 2010; Moon et al., 2003; Seo et al., 2009). It promotes flowering in many woody plants, such as pear (*Pyrus bretschneideri*) (Liu et al., 2019), loquat (*Eriobotrya japonica*) (Jiang et al., 2019), mango (*Mangifera indica*) (Wei et al., 2016), black bamboo (*Phyllostachys violascens*) (Liu et al., 2016a, b), Japanese cedar (*Cryptomeria japonica*) (Katahata et al., 2014), kiwifruit (*Actinidia* spp.) (Voogd et al., 2015) and mei (*P. mume*) (Li et al., 2016). Another *Dormancy Associated MADS-box (DAM)* gene, which belongs to the *SVP* subfamily of the *MADS-box* gene family, is crucial for bud dormancy. It is widely present in perennial woody plants, such as mei (*P. mume*), black cottonwood (*Populus trichocarpa*), apple (*Malus domestica*), and so on (Kumar et al., 2016; Leseberg et al., 2006; Wu et al., 2017; Zhao et al., 2018a). It was originally discovered in the *evg* (evergrowing) mutant of peach (*Prunus persica*) (Bielenberg et al., 2008; Li et al., 2009). In *Arabidopsis*, the *SVP* subfamily has two members: *AtSVP* and *AtAGL24*. Their sequences are highly similar, but their functions are opposite: *AtSVP* is a flowering inhibitor, while *AtAGL24* facilitates flowering (Hartmann et al., 2000; Michaels et al., 2003; Yu et al., 2002). *AtAGL24* can upregulate *AtSOC1* and interacts with *AtSOC1*, forming a positive-feedback loop (Lee et al., 2008; Liu et al., 2008; Michaels et al., 2003; Yu et al., 2002). In *P. mume*, eight members in the *SVP* subfamily are divided into two groups: *PmSVPs* and *PmDAMs*, which are homologous to *AtSVP* and *AtAGL24*, respectively (Li et al., 2017). Overexpression of *PmSVP1* in *Arabidopsis* delayed flowering, and down-regulated *AtSOC1* (Li et al., 2017). However, the constitutive expression of *PmDAM6* in poplar led to growth cessation and terminal bud set, which indicated that it was a growth suppressor and dormancy promoter (Sasaki et al., 2011). It is illustrated that the function of *PmDAM6* is unlike that of its homologous *AtAGL24*, but similar to that of *AtSVP*.

Dormancy is distinguished into three types: paradormancy (growth suppression resulting from another organization of the plant), endodormancy (inhibition existing in the dormancy organization itself), and ecodormancy (caused by threatening environmental factors) (Lang, 1987). When the chilling requirement is satisfied and the environmental factors are suitable, the seasonal dormancy is released, resulting in floral bloom (Yamane, 2014). It is reported that MADS-box genes play a key role in the flowering and dor-

mancy processes. Eighty MADS-box genes have been identified in the genome of *P. mume* (Xu et al., 2014). *PmSOC1s* and *PmDAMs* are important members of the MADS-box gene family. *PmSOC1* has three members: *PmSOC1-1*, *PmSOC1-2*, and *PmSOC1-3*. All of them can induce early flowering, *PmSOC1-3* is the most significant one (Li et al., 2016). *PmDAM* contains six members, which respond to seasonal changes. They are divided into two groups according to their expression patterns: *PmDAM1, 2, 3* and *PmDAM4, 5, 6* (Zhao et al., 2018a). To further understand the molecular mechanism of flowering and dormancy in *P. mume*, in this study, we analyzed the expression patterns of *PmSOC1s* and *PmDAMs* and their relationship with ABA content in the flower bud dormancy process. We predicted their promoter *cis*-elements composition and detected their response to exogenous ABA and explored the interactions between three *PmSOC1s* and six *PmDAMs* proteins in yeast. The inner inducing model between ABA and *PmSOC1* and *PmDAM* proteins in the flower bud dormancy process may provide a new perspective for dormancy mechanism.

Materials and methods

Plant materials

The *P. mume* tree was grown in the nursery garden of Beijing Forestry University (40°07'N, 116°11'E). Flower buds for ABA determination and transcriptome sequencing were sampled once a week from November to March of the next year. The status of samples was determined by the result of bud break percentage (Zhang et al., 2018). The flower buds used for gene cloning were collected in July because the expression level of *PmSOC1s* was high at this stage (Li et al., 2016). The samples were quickly frozen in liquid nitrogen after collection and then stored at -80°C.

Isolation of *PmSOC1* and *PmDAMs*

Three *PmSOC1s* and six *PmDAMs* in *P. mume* have been identified in previous studies (Li et al., 2016; Zhao et al., 2018a), and the plasmids of *PmDAMs* were obtained from the previous experiment (Zhao et al., 2018a). *PmSOC1s* genes were cloned by specific primers (Supplemental Table S1), which were designed by Oligo 7 based on the genome data (Xu et al., 2014; Zhang et al., 2012). Firstly, the total RNA was isolated by EASYspin Plus Plant RNA Kit (Aidlab, China). According to its operation manual, 2 µg total RNA was reverse transcribed into cDNA with TIANScript First Strand cDNA Synthesis Kit (Tiangen, China). The volume of the PCR reaction system and PCR program was set according to previous reports (Zhou et al., 2017). Then, the target sequences were separated from the gel by Gel Extraction Kit (Tiangen, China), and linked to the TOPO cloning vector (CloneSmarter, U.S.A.) and then transferred into *Escherichia coli* 'DH5α' (Tiangen, China). The positive colonies were sequenced by Sangon Biological Engineering Co., Ltd. (China). Finally, plasmids were extracted by Plasmid Miniprep Kit (Tiangen, China) and stored at -20°C for later use.

Promoter *cis*-element prediction of *PmSOC1s* and *PmDAMs*

The 2,000 bp length sequences of the *PmSOC1s* and *PmDAMs* promoters were obtained from the *P. mume* genome data (<http://prunusmumegenome.bjfu.edu.cn/>). The promoter *cis*-elements were predicted on the PlantCARE website (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

ABA treatment of flower bud

To detect the expression of *PmSOC1s* and *PmDAMs* in response to ABA, 100 mg L⁻¹ ABA was used to treat the new branches with flower buds in dormancy (between EDII and EDIII). The flower buds were sampled after 0, 2, 4, 8, 12, 24, 48, and 72 h, frozen in liquid nitrogen, then stored in -80°C until RNA extraction.

RNA extraction and qRT-PCR analysis of *PmSOC1s* and *PmDAMs*

EASYspin Plus Plant RNA Kit (Aidlab, Beijing, China) was used to extract the total RNA. TIANScript First Strand cDNA Synthesis Kit (Tiangen, Beijing, China) was employed to synthesize the first-strand cDNA. The PikoReal real-time PCR system (Thermo Fisher Scientific, CA, U.S.A.) was applied to carry out the qRT-PCR experiment. The 10 µL qRT-PCR reaction system contained 0.2 µL of each primer (Supplemental Table S1), 5 µL of SYBR Premix ExTaq II (Takara, Dalian, China), and 1 µL of cDNA. The reaction condition was 95°C for 30 s, 40 cycles of 95°C for 5 s, and 60°C for 30 s, and end in 20°C. Each reaction was performed three times. The protein phosphatase 2A (PP2A) gene of *P. mume* was used as the reference gene for normalization (Wang et al., 2014). The relative expression level of genes was calculated by the 2^{-ΔΔCt} method (Schmittgen and Livak, 2008).

PmSOC1 subcellular localization analysis

First, the subcellular localization of the PmSOC1 proteins was predicted on the Plant-mPloc websites (<http://www.cs-bio.sjtu.edu.cn/bioinf/Cell-mPloc-2/>) (Chou and Shen, 2010). Then, 35S::PmSOC1-GFP vectors were constructed by *Spe* I and *Kpn* I sites via In-Fusion HD Cloning Kit (TaKaRa) with specific primers (Supplemental Table S1). The vectors were transformed into *Agrobacterium tumefaciens* 'GV3101', and further introduced into the leaves of *N. benthamiana* following the reported methods (Zhao et al., 2018a). The subcellular localization of PmSOC1s proteins was detected via Leica TCS SP8 confocal laser scanning microscope with the parameter settings: 488 nm for GFP and 405 nm for DAPI.

Yeast Two-Hybrid Assay

The recombinant plasmids of *PmDAMs* and prey vector pGADT7, *PmDAMs* and bait vector pGBKT7 were used as prepared in the previous report (Zhao et al., 2018b). Three *PmSOC1* genes were cloned into the cleaved sites *Eco*RI and *Bam* HI of the bait vector pGBKT7 and prey vector pGADT7

(Clontech, MountainView, CA, U.S.A.) via In-Fusion HD Cloning Kit System (Clontech). Primers are shown in Supplemental Table S1. The bait and prey plasmids were transformed into *Saccharomyces cerevisiae* strain 'Y2Hgold' and 'Y187' (Clontech) via the Yeast maker Yeast Transformation System 2 (Clontech), and the transformants were cultured on SD plates lacked Trp and Leu. Autoactivation and toxicity of Y2Hgold bait strains were checked before the interaction experiment; meanwhile, the mating experiment was performed as reported previously (Zhou et al., 2017). At last, the transformed yeast cells were cultured on the solid SD/-Trp/-Leu and SD/-Leu/-Trp/-His/-Ade/X-α-Gal/AbA medium for 3–5 days at 30°C. Each protein-protein reaction was done three times.

Results and discussion

Expression profiles of *PmSOC1s* and *PmDAMs* and their relationship with ABA content in flower bud dormancy

To figure out the relationship between *PmSOC1s* and flower bud dormancy in *P. mume*, we analyzed the expression patterns of *PmSOC1s* and *PmDAMs* (the notable dormancy related genes) and estimated the relationship between their expression level and endogenous ABA accumulation. The transcriptome data and ABA content were measured in our previous study; the flower bud dormancy process was divided into four nodes: endodormancy I (EDI), endodormancy II (EDII), endodormancy III (EDIII), and natural flush stage (NF) (Zhang et al., 2018). As shown in Figure 1, during the flower bud dormancy process, *PmSOC1s* and *PmDAMs* had a similar expression trend, which diminished progressively and maintained at a very low level until the dormancy release. Meanwhile, their expression trend was consistent with that of ABA content. This result indicated that the expression level of *PmSOC1s* and *PmDAMs* was suppressed in the flower bud dormancy process, and maybe positively correlated with the content of ABA.

DAM gene is critical in flower bud dormancy of perennial plants (Li et al., 2009; Sasaki et al., 2011; Zhao et al., 2018a). Six *PmDAM* genes are the homologous genes of *AtAGL24* in the SVP branch of the phylogenetic tree (Li et al., 2017), which are divided into two groups based on the expression patterns in the flower bud developmental process. Group one includes *PmDAM1*, *PmDAM2* and *PmDAM3*, which may be more responsible for the flower bud development than dormancy. Group two contains *PmDAM4*, *PmDAM5*, and *PmDAM6*, which primarily oversee the flower bud dorman-

FIGURE 1. Expression profiles of *PmSOC1s* and *PmDAMs* and their relationship with ABA content during flower bud dormancy. The flower bud dormancy process of *P. mume* was divided into four different stages (EDI, EDII, EDIII, and NF).

FIGURE 2. The phytohormone and stress response promoter *cis*-elements in *PmSOC1* (A) and *PmDAMs* (B). The genes are represented by different colors in each graphic.

cy (Sasaki et al., 2011; Zhao et al., 2018a). Overexpression of *PmDAM6* in both poplar and apple led to shoot growth inhibition, early bud set, and bud break repression (Sasaki et al., 2011; Yamane et al., 2019). The expression trends of *PmSOC1s* were similar to that of *PmDAMs*. Therefore, we proposed that *PmSOC1s* may participate in the flower bud dormancy process by interacting with *PmDAMs*.

The phytohormone and stress response *cis*-elements in the promoter of *PmSOC1s* and *PmDAMs*

Gene expression level was affected by its promoter *cis*-elements. To further understand the influencing factors of the gene expression level of *PmSOC1s* and *PmDAMs*, the promoter *cis*-elements composition of *PmSOC1s* and *PmDAMs* were predicted by PlantCARE with a length of 2,000 bp. Several phytohormone and stress-response *cis*-elements were identified (Figure 2; Supplemental Table S2). In this study, the phytohormone response *cis*-elements include ABA-responsive element ABRE, IAA-responsive element TGA-element and AuxRR-core, GA-responsive element P-box and GARE-motif, MeJA-responsive elements TGACG-motif and CGTCA-motif, and SA-responsive element TCA-element. The stress-response *cis*-elements contain defense and stress responsiveness TC-rich repeats, low-temperature responsive elements LTR, drought induction element MBS, anoxic specific induction element GC-motif, anaerobic induction element ARE, and wound-responsive element WUN-motif. All three *PmSOC1s* genes contained ABA-/MeJA-/SA-responsive elements and ARE anaerobic induction element. But, the defense and stress-responsive elements and low-temperature responsive elements only existed in the promoter of *PmSOC1-2*. In the promoters of *PmDAMs*, only MeJA-responsive elements and ARE anaerobic induction elements were included in all the members (Supplemental Table S2). Besides, except for *PmDAM2*, the other five *PmDAMs* genes contained several ABA-responsive elements. However, the IAA-responsive elements were contained only in the promoter of *PmDAM1*. The GA-responsive elements were included in the promoter of *PmDAM2* and *PmDAM3*. Moreover, as shown in Supplemental Table S2, the low-temperature responsive elements and the drought induction elements were included in *PmDAM2*, 4, 5 and *PmDAM1*, 4, 6, respectively.

Among them, the ABA-responsiveness elements and the MeJA-responsive elements were the most abundant (Figure 2). In the promoter of *PmSOC1s*, there were 21 ABA-responsive elements and 14 MeJA-responsive elements (Supplemental Table S2). The number of ABA-responsive elements in *PmSOC1-2* was the largest (12),

followed by *PmSOC1-3* (8) and *PmSOC1-1* (1). In the promoters of *PmDAMs*, 22 ABA-responsive elements and 26 MeJA-responsive elements were counted (Supplemental Table S2). Except for one ABA-responsive element in *PmDAM1*, the other 21 ABA-responsive elements were evenly distributed in *PmDAM3*, 4, 5, 6 (Figure 2).

Abscisic acid, as a dormancy trigger phytohormone, plays an important role in the dormancy process. Its content decreased gradually with the dormancy released (Zhang et al., 2018). The expression pattern of *PmSOC1s* and *PmDAMs* in the dormancy process was positively correlated with the content of ABA (Figure 1). Through analysis, several ABA-responsive elements ABRE were found in the promoters of *PmSOC1s* and *PmDAMs*. In recent research, the expression of *SOC1* in *Arabidopsis* was regulated by ABA-responsive element (ABRE)-binding factors, AtABF3 and AtABF4, with the NF-YC complex (Hwang et al., 2019). In *Pyrus pyrifolia*, two *DAM* genes were regulated by ABA-responsive element (ABRE)-binding factors by binding directly to their promoters. One is *PpyDAM3*, whose expression was activated by PpyABF3 (Yang et al., 2020), another is *PpDAM1*, whose expression was negatively regulated by PpAREB1 (Tuan et al., 2017). Therefore, we speculated that *PmSOC1s* and *PmDAMs* may be regulated by ABA.

Expression patterns of *PmSOC1s* and *PmDAMs* under ABA treatment

In order to understand the response of *PmSOC1s* and *PmDAMs* genes to ABA, the expression of *PmSOC1s* and *PmDAMs* was analyzed after ABA treatment. As shown in Figure 3, the expression levels of the three *PmSOC1s* genes fluctuated while under ABA stress. The expression trend of *PmSOC1-2* and *PmSOC1-3* was similar, which descended gradually from 0–4 h, then bounced back partly at 8 h, and decreased at 12 h, but increased continuously until 72 h. However, from 0–4 h, the expression pattern of *PmSOC1-1* was different from the previous two genes, it had negligible change. But it was similar to them from 8–72 h. All the *PmDAMs* genes showed obvious changes in their expression trend under ABA treatment, except for *PmDAM5*, whose expression was too low to be detected by qRT-PCR. According to their expression trends, *PmDAM1*, *PmDAM2* and *PmDAM3* can be grouped. Their expression levels fluctuated in a small range from 0–12 h after spraying ABA, but began to rise significantly at 24 h, reached a peak at 48 h, and finally dropped at 72 h. Another team consisted of *PmDAM4* and *PmDAM6*, whose expression profiles were only similar from 12–72 h. At 12 h, their expression decreased to a low level, then raised

at 24 h. After that, there was a small decline (48 h) and a soft rise (72 h). The difference was during 0–8 h, the expression of *PmDAM4* fluctuated slightly, but the expression of *PmDAM6* rose gradually.

In summary, the three *PmSOC1s* and six *PmDAMs* genes had diverse responses to ABA. After the exogenous ABA treatment (0–72 h), the expression of these genes was induced at different patterns (Figure 3). Among them, the response

time of *PmSOC1-1* to ABA was relatively long, the expression of *PmSOC1-1* changed significantly until 8 h after spraying. *PmSOC1-2* and *PmSOC1-3* were sensitive to ABA, they responded quickly after treatment. The expression of *PmDAM5* was hardly detectable, suggested to be negatively regulated by ABA. The response of *PmDAM1*, 2, 3 and *PmDAM4* to ABA was slow; their expression fluctuated obviously till 24 h and 12 h, respectively. Nevertheless, the sensitivity of *PmDAM6*

FIGURE 3. The expression profiles of *PmSOC1s* and *PmDAMs* under ABA treatment.

FIGURE 4. Subcellular localization of three PmSOC1s. The PmSOC1-GFP fusion proteins were expressed in *N. benthamiana* leaves and observed under the confocal microscope. The green, blue, and red fluorescent presented protein, nucleus, and chloroplast positions individually. The merge pictures were made of GFP, DAPI and chloroplast pictures. Scale bar = 50 μ m.

to ABA is high, which is the same as that of *PmSOC1-2* and *PmSOC1-3*. This may be closely related to the number and location of ABA-responsive elements in their promoters. The promoters of *PmSOC1-2, 3* and *PmDAM6* contain more ABA-responsive elements (Figure 2).

Subcellular localization analysis of PmSOC1s

For the location information of PmSOC1s proteins in the cell, firstly we predicted on the Plant-mPloc website. The results showed that the three proteins were in the nucleus. Then we transiently transfected *35S::PmSOC1-GFP* in the *N. benthamiana* leaves; the results are shown in Figure 4. The GFP fluorescence signals were detected clearly in the nucleus, with the nucleus marker, 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI), under the confocal microscope. The experiment results confirmed the predicted results. The results were consistent with the previous studies about two woody species, *Eriobotrya japonica* (Jiang et al., 2019) and *Phyllostachys violascens* (Liu et al., 2016).

Protein-protein interactions of PmSOC1s and PmDAMs

DAM genes are a class of significant dormancy related genes, which have been studied in many perennial fruit plants, including peach, pear, apple, kiwifruit, and sweet cherry (Kumar et al., 2016; Li et al., 2009; Niu et al., 2016; Wang et al., 2020; Wu et al., 2012). To clarify whether *PmSOC1s* were involved in flower bud dormancy of *P. mume*, the protein-protein interaction relationships between PmSOC1s and PmDAMs have been analyzed through yeast two-hybrid assays. No autoactivation activity and toxicity were detected in all the nine bait proteins. PmSOC1-1, PmSOC1-2 and PmSOC1-3 could not form homologous dimers. Consequently, PmSOC1-1 could dimerize with PmDAM1 only, PmSOC1-2 could dimerize with all the PmDAMs except PmDAM3, and PmSOC1-3 could not form heterodimers with all the six PmDAMs proteins (Figure 5). Under different combina-

tions of bait and prey, the relationships between PmSOC1-1 and PmDAM1, PmSOC1-2 and PmDAM1, PmSOC1-2 and PmDAM6 were all strong. However, the interactions between PmSOC1-2 and PmDAM2/PmDAM4/PmDAM5 appeared weak. Among them, the interactions of PmSOC1-2 and PmDAM6 were the strongest, followed by that of PmSOC1-2 and PmDAM1, and PmSOC1-1 and PmDAM1.

It was reported in apricot that *ParSOC1* was associated with winter chilling requirements and dormancy break (Trainin et al., 2013). Moreover, ectopic expression of *AcSOC1* in kiwifruit can shorten dormancy duration without chilling stress, although it cannot influence dormancy establishment (Voogd et al., 2015). At the same time, the dormancy-related protein AcSVP could interact with AcSOC1 (Voogd et al., 2015; Wu et al., 2012). Similar studies had been investigated in sweet cherries. The protein interaction between PavDAM1/5 and PavSOC1 had been detected *in vivo* and *in vitro*, suggesting their collective participation in the bud dormancy and flower development of sweet cherries (Wang et al., 2020). Hence, for further understanding the possible role of *PmSOC1s* in the flower bud dormancy process, we studied the relationships of PmSOC1s and PmDAMs by Yeast Two-Hybrid Assay.

PmSOC1-1 and PmSOC1-2 can interact with one (PmDAM1) and five PmDAMs (PmDAM1, 2, 4, 5, 6), respectively. But PmSOC1-3 can hardly interact with PmDAMs. The interaction between PmSOC1-2 and PmDAM6 was the strongest. Surprisingly, this result is partly different from the previous study for *P. mume* 'Nanko' (Kitamura et al., 2016). In *P. mume* 'Nanko', the interaction between PmDAM6 and PmSOC1 (Unigene ID: PmC002566, 99% identity with *PmSOC1-1* in this study) was detected, and a partial-length (amino acid positions: +1 to +176) sequence of *PmDAM6* was used to construct bait vector (Kitamura et al., 2016). The difference is that the full-length cDNA sequence of *PmDAM6* was used to construct both bait and prey vectors to interact with PmSOC1s in this study.

FIGURE 5. Protein-protein interactions between PmSOC1s and PmDAMs. T7-53/T7-T and T7-Lam/T7-T were positive control and negative control, respectively. The symbol (+) represents the capacity of the reaction. More symbols (+) mean a stronger reaction.

By synthesizing all the results in this study and combining the protein interaction result of *PmDAMs* from our previous study (Zhao et al., 2018a), we concluded a hypothetical model between ABA and PmSOC1 and PmDAM proteins in the flower bud dormancy process in Figure 6. There are several ABA-responsive elements in the promoters of *PmSOC1s* and *PmDAMs*, and their expression can be induced by exogenous ABA. During flower bud dormancy, the expression trend of *PmSOC1s* and *PmDAMs* was similar, and they were positively correlated with the endogenous ABA content. Therefore, we speculated that with the change of environmental condi-

tions (low temperature and short photoperiod) in autumn, a large amount of ABA was accumulated in the flower buds of *P. mume*, which maintained the expression of *PmSOC1* and *PmDAM* genes. The protein-complexes of PmSOC1-2 and PmDAM6, PmDAM1 and PmSOC1-1 formed to regulate the flower bud dormancy. But, how the protein-complexes work in dormancy regulation is still unclear, and further research is needed. Accordingly, the function of protein-complexes formed with PmSOC1s is indeed an interesting issue to further investigate the common mechanism of the flower bud dormancy and flowering processes in *P. mume*.

FIGURE 6. The hypothetical model between PmSOC1s and PmDAMs and ABA in the flower bud dormancy of *P. mume*.

Conclusion

In summary, the results suggested that the dormancy related phytohormone ABA can regulate the expression of *PmSOC1s* and *PmDAMs*. Among the three *PmSOC1s* genes, *PmSOC1-2* was the most likely candidate gene for flower bud dormancy. This provides a theoretical basis for developing new varieties of *P. mume* with a wide flowering period.

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL TABLE S1. Related to experimental procedures. Primers used.

Name	Sequence 5'–3'	Experiment
RT- PmSOC1-1F	TTTCAGTTCTTTGTGATGCTGAGG	qRT-PCR
RT- PmSOC1-1R	CGGATTTGTTGTTGGTAAGGTTG	
RT- PmSOC1-2F	TTTCAGTTCTCTGCGATGCTC	
RT- PmSOC1-2R	AATCTTGTTGGTTTGCCAG	
RT- PmSOC1-3F	AGCTCTCAGTTCTATGTGATGCTG	
RT- PmSOC1-3R	TTGATAACGGTCTAGTGTGTTGC	
RT- PmDAM1-F	AGTATGAAGGATGTTATTCAA	
RT- PmDAM1-R	CTTAAGTTCCTTGCTCAATCT	
RT- PmDAM2-F	AACCAGCTACGGCAGAGGATG	
RT- PmDAM2-R	AGATTCAGATGACATGCCTT	
RT- PmDAM3-F	TCGGATTGAGCAAGGAAGTGG	
RT- PmDAM3-R	CATTCTCAGTTCTTCCTTTGT	
RT- PmDAM4-F	ACCCTTGTCGGTGTGATGGAA	
RT- PmDAM4-R	ATCACCATCTGATTGTTGCCT	
RT- PmDAM5-F	AGGCTGAATAATAATATTGAA	
RT- PmDAM5-R	TTAACGCCCCAGTTTGAGAGA	
RT- PmDAM6-F	AACCAACAACCAAGTTAAGGCATA	
RT- PmDAM6-R	CAATTACGGCAGATTCAGATGA	
PmSOC1-1F	ATGGTGAGAGGAAAAACCCAGATGA	Clone
PmSOC1-1R	CTAGCGCTTTCTTCTTTCTGGCAGT	
PmSOC1-2F	ATGGTGAGAGGGAAGATTGAGATGA	
PmSOC1-2R	TCAACAGCGCGTTACCGGC	
PmSOC1-3F	ATGGTTAGGGGGAAGACTCA	
PmSOC1-3R	CTATGGGTTTTGGCTACTTC	
SL-PmSOC1-1F	GTCGACATTTAAATACTAGTATGGTGAGAGGAAAAACCCAGATGA	Subcellular localization
SL-PmSOC1-1R	CCCTTGCTCACCATGGTACCCTAGCGCTTTCTTCTTTCTGGCAGT	
SL-PmSOC1-2F	GTCGACATTTAAATACTAGTATGGTGAGAGGGAAGATTGAGATGA	
SL-PmSOC1-2R	CCCTTGCTCACCATGGTACCTCAACAGCGCGTTACCGGCGGTCC	
SL-PmSOC1-3F	GTCGACATTTAAATACTAGTATGGTTAGGGGGAAGACTCA	
SL-PmSOC1-3R	CCTTGCTCACCATGGTACCCTATGGGTTTTGGCTACTTCT	
BK-SOC1-1F	CATGGAGGCCGAATTCATGGTGAGAGGAAAAACCCA	Y2H assay pGBKT7 construction
BK-SOC1-1R	GCAGGTCGACGGATCCCTAGCGCTTTCTTCTTTCTG	
BK-SOC1-2F	CATGGAGGCCGAATTCATGGTGAGAGGGAAGATTGA	
BK-SOC1-2R	GCAGGTCGACGGATCCTCAACAGCGCGTTACCG	
BK-SOC1-3F	CATGGAGGCCGAATTCATGGTTAGGGGGAAGACTCA	
BK-SOC1-3R	GCAGGTCGACGGATCCCTATGGGTTTTGGCTACTTC	
AD-SOC1-1F	GGAGGCCAGTGAATTCATGGTGAGAGGAAAAACCCA	Y2H assay pGADT7 construction
AD-SOC1-1R	CGAGCTCGATGGATCCCTAGCGCTTTCTTCTTTCTG	
AD-SOC1-2F	GGAGGCCAGTGAATTCATGGTGAGAGGGAAGATTGA	
AD-SOC1-2R	CGAGCTCGATGGATCCTCAACAGCGCGTTACCG	
AD-SOC1-3F	GGAGGCCAGTGAATTCATGGTTAGGGGGAAGACTCA	
AD-SOC1-3R	CGAGCTCGATGGATCCCTATGGGTTTTGGCTACTTC	

SUPPLEMENTAL TABLE S2. The phytohormone and stress response *cis*-elements in the promoter of *PmSOC1s* and *PmDAMs*.

Gene	Site name	Sequence	Number	Function
<i>PmSOC1-1</i>	ABRE	ACGTG	1	ABA-responsiveness
	P-box	CCTTTTG	2	GA-responsiveness
	TGACG-motif	TGACG	1	MeJA-responsiveness
	CGTCA-motif	CGTCA	1	
	TCA-element	CCATCTTTTT	1	SA-responsiveness
	ARE	AAACCA	3	Anaerobic induction
<i>PmSOC1-2</i>	ABRE	GACACGTGGC	2	ABA-responsiveness
		CACGTG	3	
		ACGTG	6	
		GCAACGTGTC	1	
	TGACG-motif	TGACG	2	MeJA-responsiveness
	CGTCA-motif	CGTCA	2	
	TCA-element	CCATCTTTTT	2	SA-responsiveness
	TC-rich repeats	GTTTTCTTAC	1	Defense and stress responsiveness
	LTR	CCGAAA	3	Low-temperature responsiveness
	ARE	AAACCA	1	Anaerobic induction
	ABRE	CGCACGTGTC	1	ABA-responsiveness
		CACGTG	2	
<i>PmSOC1-3</i>		ACGTG	4	
		GACACGTGGC	1	
	TGACG-motif	TGACG	4	MeJA-responsiveness
	CGTCA-motif	CGTCA	4	
	TCA-element	CCATCTTTTT	1	SA-responsiveness
		TCAGAAGAGG	1	
	ARE	AAACCA	3	Anaerobic induction
	ABRE	ACGTG	1	ABA-responsiveness
	TGA-element	AACGAC	2	IAA-responsiveness
	AuxRR-core	GGTCCAT	1	
	CGTCA-motif	CGTCA	2	MeJA-responsiveness
	TGACG-motif	TGACG	2	
<i>PmDAM1</i>	MBS	CAACTG	1	Drought induction
	GC-motif	CCCCCG	1	Anoxic specific induction
	ARE	AAACCA	3	Anaerobic induction
	GARE-motif	TCTGTTG	1	GA-responsiveness
	P-box	CCTTTTG	1	
	TGACG-motif	TGACG	3	MeJA-responsiveness
	CGTCA-motif	CGTCA	3	
	LTR	CCGAAA	2	Low-temperature responsiveness
	ARE	AAACCA	4	Anaerobic induction
	ABRE	ACGTG	5	ABA-responsiveness
<i>PmDAM2</i>	ABRE4	CACGTA	1	
	P-box	CCTTTTG	1	GA-responsiveness
	TCA-element	CCATCTTTTT	1	SA-responsiveness
	CGTCA-motif	CGTCA	5	MeJA-responsiveness
	TGACG-motif	TGACG	5	
	ARE	AAACCA	3	Anaerobic induction
	ABRE	ACGTG	3	ABA-responsiveness
		GACACGTGGC	1	
	ABRE4	CACGTA	1	
	TGACG-motif	TGACG	1	MeJA-responsiveness
<i>PmDAM3</i>	CGTCA-motif	CGTCA	1	
	LTR	CCGAAA	5	Low-temperature responsiveness
	ARE	AAACCA	2	Anaerobic induction
	MBS	CAACTG	2	Drought induction
<i>PmDAM4</i>				

SUPPLEMENTAL TABLE S2. Continued.

Gene	Site name	Sequence	Number	Function
<i>PmDAM5</i>	ABRE	ACGTG	3	ABA-responsiveness
		GCAACGTGTC	1	
		CACGTG	1	
	TGACG-motif	TGACG	1	MeJA-responsiveness
	CGTCA-motif	CGTCA	1	
	LTR	CCGAAA	1	Low-temperature responsiveness
<i>PmDAM6</i>	ARE	AAACCA	2	Anaerobic induction
	ABRE	ACGTG	3	ABA-responsiveness
		GCAACGTGTC	1	
		CACGTG	1	
		TACGTGTC	1	
	ABRE4	CACGTA	2	MeJA-responsiveness
	CGTCA-motif	CGTCA	1	
	TGACG-motif	TGACG	1	
	ARE	AAACCA	1	Anaerobic induction
	GC-motif	CCCCCG	1	Anoxic specific induction
	MBS	CAACTG	1	Drought induction
	WUN-motif	AAATTCCT	1	Wound-responsiveness

ABA: abscisic acid; IAA: auxin; GA: gibberellin; SA: salicylic acid; MeJA: methyl jasmonate.