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CLONING AND EXPRESSION OF FLORAL ORGAN IDENTITY GENES IN *PAEONIA OSTII* 'FENGDAN'

SHUANG ZHOU, CHAO MA, SHUANGCHENG GAO, DIANYUN HOU, PENG SONG, ZHENZHU GUO, YI ZHANG AND GUOAN SHI*

College of Agriculture/Mudan, Henan University of Science and Technology, Henan 471000, People's Republic of China
**Corresponding author's email: gashi1963@163.com*

Abstract

Tree peony is the most popular and important ornamental plant in China and has many different flower types. Although many studies about tree peony cultivation have been published, the regulatory mechanism of floral organ identity has not been explored. *Paeonia ostii* 'Fengdan' is a typical single-flower variety, and many important tree peony cultivars in China originated from homoploid hybridization between 'Fengdan' and other Paeonia species. Peony tea made from 'Fengdan' has already been introduced to the market, and the quality and price of peony tea are closely related to the flower type of 'Fengdan'. This research cloned six floral organ identity genes in 'Fengdan', namely, *PsAP1fd*, *PsAP2fd*, *PsAP3fd*, *PsPIfd*, *PsAGfd*, and *PsSEP1fd*. The bioinformatics analysis was performed, and results showed that all these genes encoded MADS-box proteins, except *PsAP2fd*, and *PsAP2fd* encoded an AP2 protein. Five MADS-box proteins encoded by these genes contained two conserved motifs: MADS-MEF2 and K-box domain. PsSEP1fd was hydrophilic and stable, whereas the other five proteins were hydrophilic and unstable. The results of qRT-PCR displayed that *PsAP1fd* was mostly observed in the petals and sepals, and *PsAP2fd* was mostly found in the petals. *PsAP3fd* had strong expressions in the stamens and petals. The highest expression level of *PsPIfd* was observed in the petals, followed by that in the stamens. The highest expression level of *PsAGfd* was observed in the pistils, followed by that in the stamens. *PsSEP1fd* was mainly expressed in the pistils. These results showed that the ABCE model was effective in the flower type formation of 'Fengdan'. Our work would help reveal the molecular mechanism underlying flower type formation in 'Fengdan' and promote quality control for peony tea products.

Key words: Cloning, Floral organ identity, Gene expression, MADS-box gene, *Paeonia ostii* 'Fengdan'.

Introduction

Flower development is strictly regulated. In 1991, the ABC model was proposed according to researches on *Arabidopsis* and *Antirrhinum* (Coen & Meyerowitz, 1991). The ABC model indicated that the flowers of *Arabidopsis* and *Antirrhinum* have four whorls, referred to as sepals, petals, stamens, and carpels, and flower organs are determined by A-, B-, and C-class genes (Coen & Meyerowitz, 1991). Sepal formation is controlled by A-class genes, while petal formation is regulated by A- and B-class genes. Stamen formation is controlled by B- and C-class genes, whereas the formation of carpel is determined by C-class genes (Coen & Meyerowitz, 1991). Furthermore, A- and C-class genes are antagonistic (Bowman *et al.*, 1991). The mutations of A-class gene resulted in sepals to change into pistils, as well as petals to change into stamens. B-class gene mutations resulted in petals and stamens to change into sepals and pistils, respectively. The mutations of C-class gene promote the transition of stamens into petals and transition of pistils into sepals (Bowman *et al.*, 1991; Coen & Meyerowitz, 1991; Weigel & Meyerowitz, 1994). *APETALA1* (*AP1*) and *AP2* in *Arabidopsis* are A-class genes, whereas *AP3* and *PISTILLATA* (*PI*) in *Arabidopsis*, *GLOBOSA* (*GLO*) and *DEFICIENS* (*DEF*) in *Antirrhinum*, *pMADS1*, *pMADS2*/*FBP3* (*FLORAL BINDING PROTEIN3*) and *FBP1* in petunia are B-class genes (Riechmann & Meyerowitz, 1998; Eckardt, 2003; Pařenicová *et al.*, 2003). *PLENA* in snapdragon, *AGAMOUS* (*AG*) in *Arabidopsis*, and *pMADS3* in petunia are C-class genes (Eckardt, 2003).

ABC model had been comprehensively studied and extended to ABCDE model. *FBP7* and *FBP11* in petunia (Angenent *et al.*, 1995), *SHATTERPROOF1* (*SHP1*), *SHP2*, *SEEDSTICK* (*STK*) in *Arabidopsis* (Favaro *et al.*, 2003) are all D-class genes, which determine ovule development and have redundant effects similar to C-class genes (Colombo *et al.*, 1995; Jack, 2004).

The discovery of E-class genes is a significant improvement for the ABC model. These genes were first found in tomato *MADS box gene no.5* (*TM5*) (Pnueli *et al.*, 1994), as well as petunia *FBP2* (Angenent *et al.*, 1994; Ferrario *et al.*, 2003). Some researchers attempted to change the expression levels of *ABC* genes to induce the transition of leaves into floral organs, but they were unsuccessful (Mizukami & Ma, 1992; Krizek & Meyerowitz, 1996; Pelaz *et al.*, 2000). *ABC* genes are significant to floral organs formation, and another class of floral organ identity genes are also essential to the transition of vegetative organs into floral organs. In *Arabidopsis*, the formation of the complexes of ABC proteins and SEP proteins is sufficient for converting vegetative organs into floral organs (Pelaz *et al.*, 2000; Honma & Goto, 2001; Pelaz *et al.*, 2001b). Therefore, the ABCE model was updated according to the ABC model. The ABCE model indicated that A+E regulates sepal formation (Pelaz *et al.*, 2001a), A+B+E regulates petal formation, B+C+E regulates stamen formation (Honma & Goto, 2001; Pelaz *et al.*, 2001b; Ferrario *et al.*, 2003), C+E regulates pistil formation (Fan *et al.*, 1997; Pelaz *et al.*, 2000).

Tree peony (*Paeonia suffruticosa* Andrews) has a long history of cultivation in China, which belongs to section *Moutan* DC of the genus *Paeonia* and family Paeoniaceae. It is called "king of flowers" for its beautiful and bright colors and large and diverse flowers. *Paeonia ostii* 'Fengdan' is a popular ornamental, medicinal and oil-seed tree peony variety in China (Liu *et al.*, 2019). In 2011, the seeds of 'Fengdan' were identified as novel

sources of edible plant oil in China. Seed oil extracted from 'Fengdan' was found to be rich in unsaturated fatty acids, especially the proportion of α-linolenic acid in peony seed oil is extremely high (Li *et al.*, 2015). In 2013, the flowers of 'Fengdan' were identified as a new food resource in China and found to be rich in flavonoids (Zhang *et al.*, 2017). 'Fengdan' was used in genetic map construction and QTL analysis (Guo *et al.*, 2017; Zhang *et al.*, 2019). The callus, direct somatic embryogenesis, and shoot organogenesis were induced in 'Fengdan' (Du *et al.*, 2020; Ren *et al.*, 2020). Some peony MADS-box genes were identified, and the expression patterns of these genes were analyzed, which are involved in flower organ formation (Wang *et al.*, 2019). However, the regulation mechanism of flower organ formation in tree peony has not been fully clarified. The flower of *P. ostii* 'Fengdan' has typical four whorls: sepals, petals, stamens, and pistils, and many important peony cultivars in China originated from homoploid hybridization between *P. ostii* and other Paeonia species. Consequently, studies on 'Fengdan' will increase the understanding of floral organ identity in tree peony and provide basic knowledge of cultivar breeding.

This research used RT-PCR to clone *PsAP1fd* and *PsAP2fd* (A-class genes); *PsAP3fd* and *PsPIfd* (B-class genes); *PsAGfd* (C-class gene); and *PsSEP1fd* (E-class gene) in 'Fengdan'. These genes are involved in flower type formation. Bioinformatics analysis and expression patterns of these genes in different flower organs were carried out. This research will serve as a foundation for the study of the mechanism of flower type formation in tree peony.

Materials and methods

Plant materials: All the plants were grown in Henan University of Science and Technology, Luoyang, Henan Province, China. 'Fengdan' flowers at the bloom stage were collected in April 2017. The flowers were divided into sepals, petals, stamens, and pistils for further analysis.

RNA extraction and reverse transcription: A MiniBEST Plant RNA Extraction Kit (TaKaRa, Japan) was used for RNA Extraction of 'Fengdan'. Then, the samples were measured using a Multiskan Go microplate spectrophotometer (Thermo Scientific, USA). The A_{260}/A_{280} values ranged from 1.8 to 2.0. From each sample, 1000 ng of RNA of each sample was used as the template and a PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan) was used for reverse transcription.

Isolation of genes: According to transcriptome sequencing performed in our laboratory (unpublished), Primer Premier 5.0 (Premier Biosoft, Palo Alto, USA) was used for primer pair design (Table 1). PCR reactions were carried out using *TaKaRa Ex Taq*® (TaKaRa, Japan). PCR reactions were performed using three-step cycling conditions: *PsAP1fd*, *PsAP3fd*, *PsPIfd*, *PsAGfd*, and *PsSEP1fd*: 94℃ for 5 min, then 35 cycles of 94℃ for 30 s, 52℃ for 30 s, and 72℃ for 1 min with a final extension of 72℃ for 10 min; *PsAP2fd*: 94℃ for 5 min, 35 cycles of 94℃ for 30 s, 52℃ for 30 s and 72℃ for 2 min with a final extension of 72℃ for 10 min. Then, 1.5% agarose gel electrophoresis was used for the detection of PCR products. A gel extraction kit (CoWin Biosciences, China) was used in purifying the incised gels. PMD18-T vector, as well as *E.coli* DH5α Competent Cells (TaKaRa, Japan) were used in cloning the extracted products. Recombinant plasmids were selected, then sequenced by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China).

Sequence analysis: DNAMAN6.0 software was used in analyzing *PsAP1fd*, *PsAP2fd*, *PsAP3fd*, *PsPIfd*, *PsAGfd*, and *PsSEP1fd* sequences. ORF search was carried out according to the NCBI ORF Finder (https://www.ncbi.nlm.nih.gov/orffinder/), as well as conserved domain analysis performed using NCBI Conserved Domains Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi?). The properties of these proteins were analyzed on ExPASy (http://us.expasy.org/tools/protparam.html). MEME (http://meme-suite.org/tools/meme) was used in identifying the conserved protein motifs of the proteins. Homology search was investigated according to NCBI-BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Gene expression analysis: The expression patterns of *PsAP1fd*, *PsAP2fd*, *PsAP3fd*, *PsPIfd*, *PsAGfd*, and *PsSEP1fd* were detected through qRT-PCR. LightCycler 96 (Roche, Germany) was used. *P. suffruticosa GAPDH* (according to transcriptome sequencing performed by our laboratory) was used as the internal control. Primers of qRT-PCR were designed as above (Table 2). The TB Green™*Premix Ex Taq*™II (Tli RNaseH Plus) (TaKaRa, Japan) was used for qRT-PCR. The amplification was performed as this conditions: 94℃ for 30 s, 40 cycles of 94℃ for 5 s, and 60℃ for 30 s. The relative expression levels of the detected genes were computed according to the methods described by Schmittgen and Livak (Schmittgen & Livak, 2008). The expression level in the petals was used as the control. Triplicate reactions were analyzed. Statistical analysis was carried out using Microsoft Excel, and data were analyzed using one-way ANOVA test.

Results

Morphological description of flower in 'Fengdan': *P. ostii* 'Fengdan' is a typical single-flower variety, with only two whorls of petals, which are broad and flat (Fig. 1a). The sepals, petals, stamens, and pistils of 'Fengdan' developed normally (Fig. 1b) and were thus good materials for studying the mechanism of flower type formation in tree peony.

Isolation of floral organ identity genes in 'Fengdan': The full-length cDNAs of *PsAP1fd*, *PsAP2fd*, *PsAP3fd*, *PsPIfd*, *PsAGfd* and *PsSEP1fd* were successfully identified from 'Fengdan' through RT-PCR (Table 3). The cDNAs of *PsAP1fd*, *PsAP2fd*, *PsAP3fd*, *PsPIfd*, *PsAGfd* and *PsSEP1fd* were 799, 1697, 815, 791, 925, and 869 bp, respectively, containing ORFs of 729, 1533, 666, 639, 777, and 735 bp, respectively, and encoding proteins of 242, 510, 221, 212, 258, and 244 aa, respectively.

The isolated genes were deposited in the GenBank, and the accession numbers were MT822685 (*PsAP1fd*), MT822686 (*PsAP2fd*), MT822687 (*PsAP3fd*), MT822688 ((*PsPIfd*), MT822689 (*PsAGfd*), and MT822690 (*PsSEP1fd*; Table 3).

Table 1. Primer sequences used for floral organ identity genes isolation in 'Fengdan'.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
PsAP1fd	TTGTCTGTTTGGGTGGTGGGA	CATAACAGTCCGAAGGAGTGC
PsAP2fd	GAGTCTCATAGAGTAATCAGC	GAAGAAAGAATCTCACAAGC
PsAP3fd	CCATTGGAGGTGATTGCTA	ATTGGACCATGGGTTGAGTTG
PsPIfd	TTGTGGCTAGACTTGAAGAGA	TCACACAAACCAAGTTCAT
PsAGfd	CCTGCTCAGATTTTGTGGGA	CCGCAGAATTTGATGACAG
PsSEP1fd	AGATCAGCTGGTTCCCAAGAG	GTTACAAATTCCAAGCAAGC

Table 2. Gene-specific primer sequences for detection by qRT-PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	TGTTCACTCCATCACTGCTAC	ACATCCACAGTAGGAACACGA
PsAP1fd	GGAGAACCAACAGAAATGAG	ATACACCAAAGCACCCAAG
PsAP2fd	TATACAAGTGAGGCAAACG	GAGATGGAACAATGTGAAG
PsAP3fd	GGAGAATGAGGGAGACTATG	CATGACTCAAGAGAGGTGC
PsPIfd	ATGGAATTTCCCAAGAGGC	GGAAGGCGTAAGGAATCAG
PsAGfd	CAAATGAACTTGATGCCAG	ATTGAAGAGCGATTTGGTC
PsSEP1fd	GTTCAGACCAAATGACGGC	ACTCAGAGCATCCATCCAGG

Table 3. Gene sequences of floral organ identity genes in 'Fengdan'.

Gene	Full length (bp)	ORF (bp)	5'-UTR (bp)	3'-UTR (bp)	Amimo acid	Accession number
PsAP1fd	799	729	35	35	242	MT822685
PsAP2fd	1697	1533	51	113	510	MT822686
PsAP3fd	815	666	36	113	221	MT822687
PsPIfd	791	639	28	124	212	MT822688
PsAGfd	925	777	113	35	258	MT822689
PsSEP1fd	869	735	79	55	244	MT822690

Table 4. Physical and chemical parameters of proteins related to floral organ identity in 'Fengdan'.

Protein	Formula	Molecular weight	Theoretical pI	Instability index	GRAVY value
PsAP1fd	$C_{1217}H_{1976}N_{360}O_{375}S_{11}$	28003.92	9.01	50.47	-0.788
PsAP2fd	$C_{2461}H_{3794}N_{740}O_{793}S_{18}$	57012.76	6.66	51.93	-0.911
PsAP3fd	$C_{1122}H_{1803}N_{325}O_{341}S_{12}$	25686.35	9.30	43.17	-0.822
PsPIfd	$C_{1068}H_{1745}N_{319}O_{334}S_{10}$	24719.14	8.65	48.67	-0.850
PsAGfd	$C_{1268}H_{2057}N_{391}O_{403}S_{10}$	29548.26	9.47	52.98	-0.914
PsSEP1fd	$C_{1220}H_{1960}N_{354}O_{375}S_{10}$	27907.73	8.78	29.76	-0.654

Sequence analysis of floral organ identity genes in 'Fengdan': Conserved domain analysis confirmed that all proteins encoded by the genes contained MADS-MEF2-like and K-box domain, except PsAP2fd (Fig. 2). PsAP2fd contained two typical AP2 domains, which belong to the AP2 family (Fig. 2b). The Molecular weight varied from 24.72 KDa to 57.01 KDa, and the theoretical pI varied from 6.66 to 9.47. Only the instability index of PsSEP1fd was less than 40, and it was stable. The other five proteins detected were considered unstable. The GRAVY values of the proteins were all less than 0, and thus they were all predicted to be hydrophilic (Table 4).

BLAST analysis showed that PsAP1fd shared 75.20-99.59% identity with AP1 from *Paeonia suffruticosa*, *Paeonia lactiflora*, *Vitis riparia*, *Herrania umbratica*, *Durio zibethinus*, and *Rhamnella rubrinervis*. PsAP2fd shared 66.67-100.00% identity with AP2 from *Paeonia suffruticosa*, *Paeonia lactiflora*, *Vitis vinifera*, *Vitis riparia*, *Theobroma cacao*, *Nyssa sinensis*, and *Durio zibethinus*. PsAP3fd shared 70.97-100.00% identity with AP3 from *Paeonia suffruticosa*, *Paeonia lactiflora*, *Vitis vinifera*, *Cephalotus follicularis*, *Nyssa sinensis*, and *Mercurialis annua*. PsPIfd shared 71.70-99.06% identity with PI from *Paeonia suffruticosa*, *Paeonia lactiflora*, *Mercurialis annua*, *Vitis vinifera*, *Vitis riparia*, *Manihot esculenta*, and *Jatropha curcas*. PsAGfd shared 78.93-97.29% identity with AG from *Paeonia suffruticosa*, *Cercidiphyllum japonicum*, *Vitis riparia*, *Prunus serotina*, *Manihot esculenta*, and *Tripterygium wilfordii*. PsSEP1fd shared 77.46-97.13% identity with SEP1 from *Paeonia lactiflora*, *Carica papaya*, *Vitis riparia*, *Vitis vinifera*, *Theobroma cacao*, and *Durio zibethinus* (Table 5).

Conserved domain analysis showed that the proteins detected were MADS-box proteins, except PsAP2fd, which belongs to the AP2 family (Fig. 2). To examine the common feature of 'Fengdan' MADS-box proteins, the MEME suite was used in identifying their conserved motifs and sequence logos. Five conserved motifs (called Motif 1-5) were identified, and only three motifs were usable (the E-values of Motif 4 and Motif 5 were larger than 0.05; Fig. 3b). The motifs were then matched to two different domains. Motif 1 and 3 at the N-terminus were in the MADS domain, and Motif 2 was in the K-box domain. A less-well-conserved I (intervening) domain, as well as a variable C-terminal region were found in these proteins (Fig. 3a).

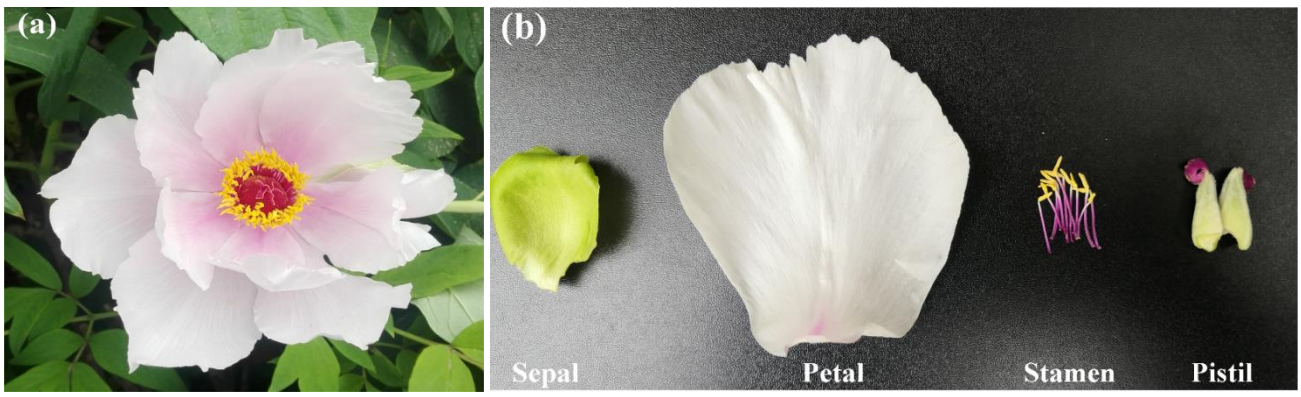

Fig. 1. Phenotype of *P. ostii* 'Fengdan'. (a) The flower of 'Fengdan'. (b) Different floral organs of 'Fengdan'.

Table 5. Comparisons of deduced floral organ identity proteins in 'Fengdan' with other plants.

Protein	Species	GenBank accession no.	Indentity (%)
PsAP1fd	*Paeonia suffruticosa*	AJO68022.1	99.59
	Paeonia lactiflora	AGH61290.1	97.11
	Vitis riparia	XP_034699499.1	81.74
	Herrania umbratica	XP_021292999.1	78.19
	Durio zibethinus	XP_022776709.1	77.78
	Rhamnella rubrinervis	KAF3431618.1	75.20
PsAP2fd	*Paeonia suffruticosa*	AEK33829.1	100.00
	Paeonia lactiflora	AGI61068.1	96.75
	Vitis vinifera	NP_001267881.1	71.80
	Vitis riparia	XP_034691428.1	71.61
	Theobroma cacao	XP_007047337.2	68.33
	Nyssa sinensis	KAA8547120.1	68.27
	Durio zibethinus	XP_022740198.1	66.67
PsAP3fd	*Paeonia suffruticosa*	AEK33828.1	100.00
	Paeonia lactiflora	AGH61291.1	91.86
	Vitis vinifera	NP_001267937.1	73.27
	Cephalotus follicularis	GAV72187.1	72.35
	Nyssa sinensis	KAA8531940.1	71.69
	Mercurialis annua	QER90709.1	70.97
PsPIfd	*Paeonia suffruticosa*	QCQ84555.1	99.06
	Paeonia lactiflora	AGH61293.1	96.23
	Mercurialis annua	ALK01328.2	76.89
	Vitis vinifera	NP_001267875.1	76.53
	Vitis riparia	XP_034676852.1	76.06
	Manihot esculenta	XP_021601944.1	73.71
	Jatropha curcas	XP_012078322.1	71.70
PsAGfd	*Paeonia suffruticosa*	AGS12611.1	97.29
	Cercidiphyllum japonicum	ASY97759.1	83.56
	Vitis riparia	XP_034696943.1	81.70
	Prunus serotina	ACH72974.1	80.69
	Manihot esculenta	XP_021599035.1	79.57
	Tripterygium wilfordii	KAF5736281.1	78.93
PsSEP1fd	*Paeonia lactiflora*	AQM56645.1	97.13
	Carica papaya	ACD39982.1	79.67
	Vitis riparia	XP_034705766.1	79.51
	Vitis vinifera	NP_001268109.1	79.18
	Theobroma cacao	XP_007032865.1	78.28
	Durio zibethinus	XP_022727577.1	77.46

Fig. 2. ORF sequences of *PsAP1fd* (a), *PsAP2fd* (b), *PsAP3fd* (c), *PsPIfd* (d), *PsAGfd* (e), *PsSEP1fd* (f) and their deduced amino acid sequences. Yellow: MADS-MEF2-like domain; Blue: K-box domain; Green: AP2 domain.

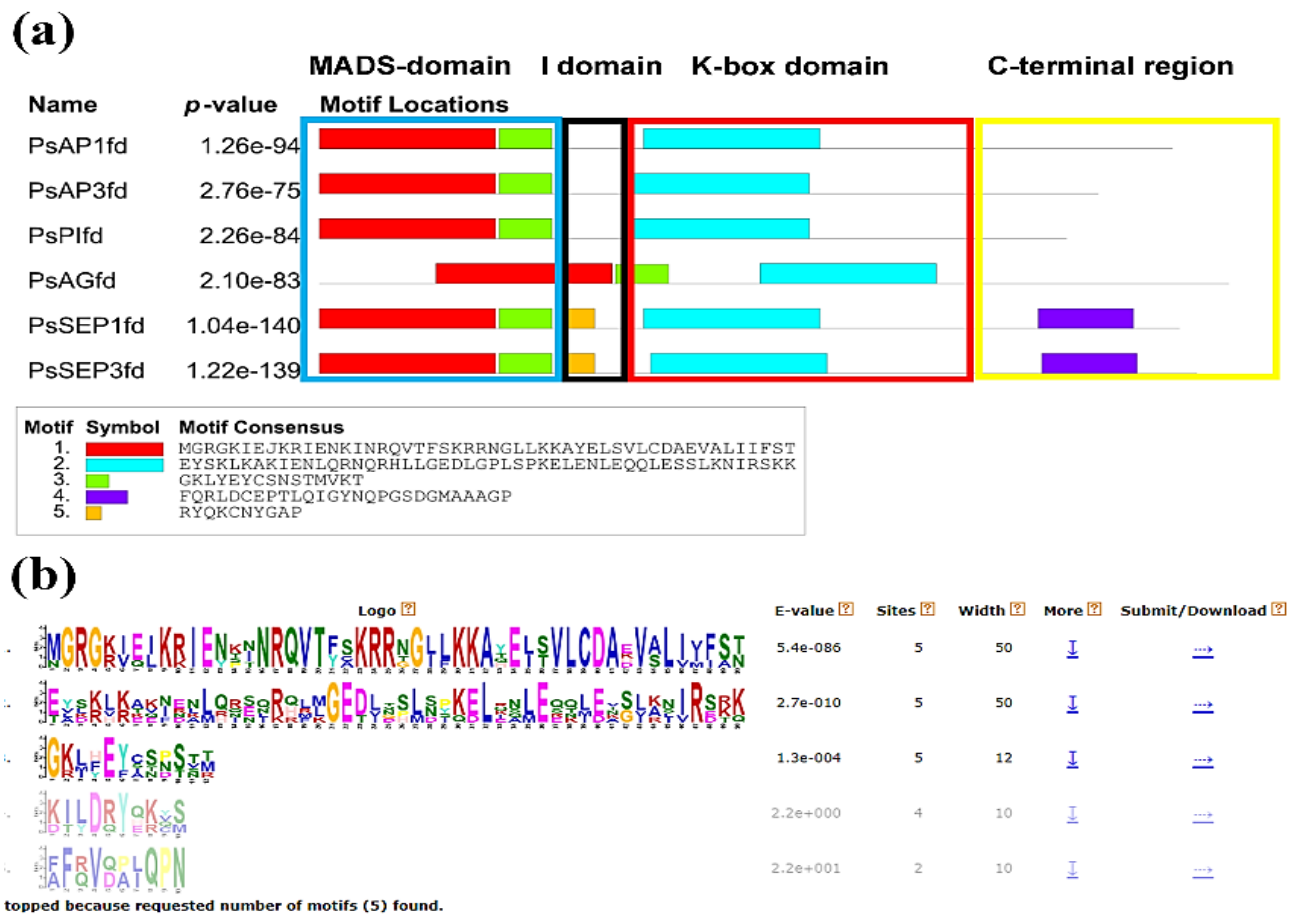

Fig. 3. Distribution of conserved motifs in MADS-box proteins in 'Fengdan'. (a) Motif distribution in each MADS-box protein in 'Fengdan'. Motif 1 and 3 were in MADS domain at N-terminus, followed by Motif 2 in K-box domain. A less-well-conserved I domain and a variable C-terminal region were also found. (b) Only 3 motifs were usable because the E-values of Motif 4 and Motif 5 were larger than 0.05.

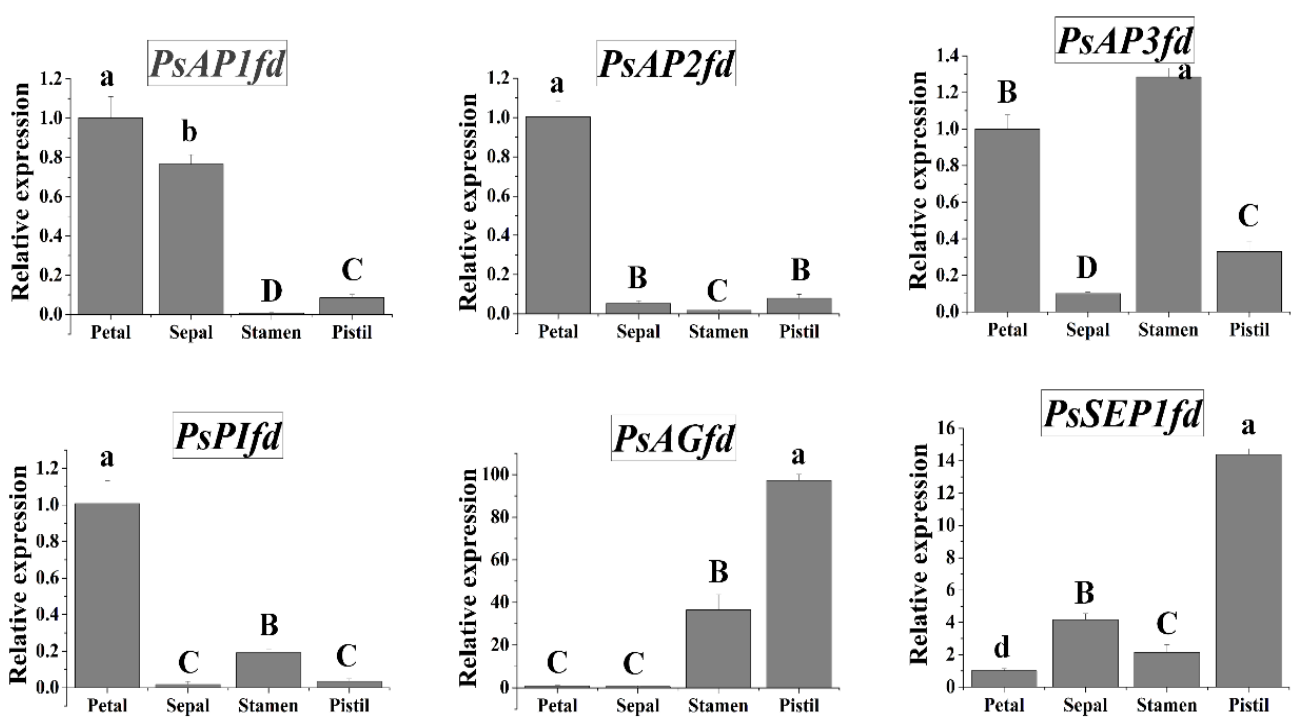

Fig. 4. Expression analysis of floral organ identity genes in the different floral organs of 'Fengdan'. The values are mean ± SD and the bars with different letters indicate significant differences at $p<0.05$ (lower case letters) or $p<0.01$ (capital letters), respectively (based on the one-way ANOVA test).

Expression patterns of floral organ identity genes in 'Fengdan': The expression patterns of these genes were investigated through qRT-RCR. The expression levels of the genes considerably varied among different floral organs. *PsAP1fd* was predominantly observed in the sepals and petals, as well as the highest expression level of *PsAP2fd* was found in the petals. By contrast, *PsAP3fd* had strong expressions in the petals and stamens, but had low expressions in the sepals and pistils. The highest expression level of *PsPIfd* was observed in the petals, followed by the stamens, but the gene was hardly detected in the sepals and pistils. *PsAGfd* was predominantly expressed in the stamens and pistils, and its highest expression level was found in the pistils. The expressions of *PsAGfd* were hardly detected in the sepals and petals. *PsSEP1fd* was observed in all the whorls of the flower organs, and the highest expression level of *PsSEP1fd* was found in the pistils, followed by the sepals (Fig. 4).

Discussion

MADS-box genes encoded transcriptional regulators that are active in diverse plant development processes, such as floral transition, flowering time regulation, and floral organ identify (Becker & Theißen, 2003; Dornelas *et al.*, 2011). Our results showed that in 'Fengdan', the floral organ identity genes examined were all MADS-box family, except *PsAP2fd*, which belongs to the AP2 family. This result is consistent with previous research (Pařenicová *et al.*, 2003; Wang *et al.*, 2019). The proteins encoded by these MADS-box genes had two conserved domains: which were MADS-box and K-box domain, respectively. A less-well-conserved I domain, as well as a variable C-terminal region were also found. Therefore, the proteins were MIKC-type proteins (type II MADS-domain proteins). The MADS-box domain is important to many functions, such as DNA binding, nuclear localization, accessory factor binding and dimerization (Theissen *et al.*, 2000; Ng & Yanofsky, 2001; Immink *et al.*, 2002). The K-box domain is important for dimerization, while the I domain is involved in regulatory determinant for the selective formation of DNA-binding dimers, as well as the C-terminal region is associated with functional specificity, formation of ternary or quaternary protein complexes, and transcriptional activation (Riechmann & Meyerowitz, 1997; Egea-Cortines *et al.*, 1999; Honma & Goto, 2001; Lamb & Irish, 2003). The genomic DNA sequence and coding sequence of the B-class genes *PsTM6* from 23 different tree peony cultivars were obtained and analyzed, and the results showed that the electronic charge and polarity of PsTM6 paralogs varied because of amino acid substitution leading to functional differentiation, which significantly affected stamen petalody and caused variations in flower shapes in tree peony (Shu *et al.*, 2012). Different selection forces generated the different regions of *PsTM6*, especially in the K-box domain (Shu *et al.*, 2012).

The ABCE model is closely related to flower type formation, and this relationship has been confirmed in many species (Wagner *et al.*, 1999; Lenhard *et al.*, 2001; Lohmann *et al.*, 2001; Krizek & Fletcher, 2005). Previous research showed that the MADS-box proteins related to flower organ identity often function as complexes: A+E regulates sepal development (Pelaz *et al.*, 2001a), A+B+E regulates petal formation, B+C+E regulates stamen development (Honma & Goto, 2001; Pelaz *et al.*, 2001b; Ferrario *et al.*, 2003), C+E regulates pistil development (Fan *et al.*, 1997; Pelaz *et al.*, 2000). Furthermore, protein complexes comprising AG, SEP, STK or AG, SEP, SHP both control ovule development in *Arabidopsis* (Favaro *et al.*, 2003). Flower type is a valuable ornamental characteristic, and tree peony has 10 flower types, including lotus, crown, chrysanthemum, rose, globular, and crown-proliferation (Wang & Yuan, 2003). Increase in petals, stamen petalody, pistil petalody, and flower overlapping generate different flower types in tree peony (Wang & Yuan, 2003). Diverse flower types are one of the most significant characteristics for cultivar classification (Wang & Yuan, 2003; Shu *et al.*, 2012). Nevertheless, the molecular mechanism underlying floral organ identity in tree peony remains unclear. Some *ABCE* genes in tree peony and herbaceous peony have been studied (Shu *et al.*, 2012; Ge *et al.*, 2014; Gong *et al.*, 2017; Wang *et al.*, 2019), but further functional research is needed. Ge *et al.* isolated *ABE* genes in herbaceous peony and detected their expression patterns in the three cultivars, which had different flower types. The results suggested that the expression levels of A- and E-class genes increased, while these of B-class genes reduced with the depth of stamen petaloidy. This study focused on stamen petaloidy rather than on floral organ identity (Ge *et al.*, 2014). According to our study in 'Fengdan', *PsAP1fd* (A-class gene) and *PsSEP1fd* (E-class gene) had strong expressions in the sepals. *PsAP1fd*, *PsAP2fd* (A-class genes), as well as *PsAP3fd*, *PsPIfd* (B-class genes) had high expressions in the petals. *PsAP3fd*, *PsPIfd* (B-class genes), as well as *PsAGfd* (C-class gene) had strong expressions in the stamens. *PsSEP1fd* (E-class gene) had normal expression. The highest expression levels of *PsAGfd* (C-class gene) and *PsSEP1fd* (E-class gene) were detected in the pistils. These results were consistent with those of the ABCE model and previous studies (Wang *et al.*, 2019).

In *Arabidopsis*, there are 4 E-class genes named *SEP1*, *SEP2*, *SEP3* and *SEP4*, respectively, which have specific expression levels in diverse flower organs (Pelaz *et al.*, 2000; Honma & Goto, 2001; Pelaz *et al.*, 2001a, 2001b; Ditta *et al.*, 2004). In tree peony 'Ziluo Lan', *SEP1* is primarily expressed in the sepals, stamens, and pistils; *SEP3* is detected in all the whorls of the flower organs; *SEP4* has high expression levels in the sepals, as well as stamens (Wang *et al.*, 2019). *PlSEP3* had extremely high expression in the sepals of *P. lactiflora* 'Hangshao' (Ge *et al.*, 2014). In our study, the highest expression level of *PsSEP1fd* was found in the pistils, followed by the sepals. These results indicated that *ABC* genes play the same role in flower organ formation in 'Fengdan' and the expression patterns of E-class genes varied among cultivars, exhibiting a significant role in flower type formation.

In 2013, the flowers of 'Fengdan' were identified as new food resource in China and were found to contain abundant flavonoids (Zhang *et al.*, 2017). Peony tea made by 'Fengdan' has been introduced to the market, and the quality and price of peony tea are closely related to the

flower type of 'Fengdan'. This research will serve as a foundation for the mechanism of the flower type formation in tree peony, as well as promotion of quality control for peony tea products.

Conclusion

Genes in the four floral organs of *P. ostii* 'Fengdan' were isolated through RT-PCR and identified: *PsAP1fd* and *PsAP2fd* (A-class genes); *PsAP3fd* and *PsPIfd* (B-class genes); *PsAGfd* (C-class gene); and *PsSEP1fd* (E-class genes). They are all MADS-box family except *PsAP2fd*, which belongs to the AP2 family. The six genes played different roles during floral organ development. *PsAP1fd* was primarily observed in the sepals and petals. *PsAP2fd* was primarily found in the petals. *PsAP3fd* was significantly expressed in the petals, as well as stamens. The highest expression level of *PsPIfd* was observed in the petals, followed by the stamens. *PsAGfd* was predominantly detected in the stamens and pistils, and its highest expression levels were found in the pistils. *PsSEP1fd* was mainly expressed in the pistils.

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