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甜樱桃成花相关MADS-box基因的克隆及表达分析

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摘 要:【目的】克隆甜樱桃中可能参与成花途径的MADS基因,分析其基本信息,研究其在不同组织中的表达情况。【方法】以甜樱桃'萨米特'('Summit')为试材,结合桃基因组分析,克隆得到14个可能参与成花的MADS基因,分别命名为*PaSOC1*、*PaAG*、*PaAP1*、*PaAP1-2*、*PaAP3*、*PaPI*、*PaSVP*、*PaAGL24*、*PaSEP1*、*PaSEP2*、*PaSEP3*、*PaSEP4*、*PaSEP5*、*PaFLC*,通过DNAMAN、SMART、protein BLAST和MEGA5等软件分析14个甜樱桃的MADS基因结构、氨基酸结构域及其进化关系,RT-PCR检测其在樱桃根、叶芽、叶、花芽、花、韧皮部中的表达模式。【结果】14个甜樱桃MADS成员大小为612~765 bp,均含有典型的MADS结构域和K-box结构域,含有6~8个内含子。分属7个亚组,*PaSEP1*、*PaSEP2*、*PaSEP3*、*PaSEP4*、*PaSEP5*属于SEP亚组,*PaAP1*、*PaAP1-2*属于AP1亚组,*PaAG*属于AG亚组,*PaSOC1*属于SOC1亚组,*PaAP3*和*PaPI*属于AP3/PI亚组,*PaSVP*属于SVP亚组,*PaAGL24*属于AGL24亚组,*PaFLC*并未聚到FLC亚组中。RT-PCR分析显示,14个MADS基因在花芽或花中均有不同程度的表达,此外,除*PaAP3*、*PaSEP1*、*PaSEP4*及*PaSEP5*之外,其他几个基因在韧皮部中也有不同程度的表达。【结论】获得的甜樱桃MADS-box基因结构高度保守,参与调控成花及花发育过程。

关键词:甜樱桃;MADS-box家族基因;信息学分析;表达分析

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Isolation and expression analysis of MADS-box gene related to flowering regulation in sweet cherry

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Abstract:【Objective】It has been known that MADS-box gene family members participate in flowering-regulating pathway of higher plants. However, there are very few reports and evidence of MADS-box family genes functioning on cherry flowering. This study aims to clone and characterize MADS-box family genes of sweet cherry (*Prunus avium*), and analyze the spatial-temporal gene expression of them in different cherry tissues. This research will provide important evidences for further understanding the effects of MADS-box family transcription factors in cherry flowering.【Methods】Here we first used sweet cherry 'Summit' as material, referring to peach genome information as sweet cherry genome is still unknown, we cloned and characterized 14 MADS-box genes that might be related to flowering regulation, named *PaSOC1*, *PaAG*, *PaAP1*, *PaAP1-2*, *PaAP3*, *PaPI*, *PaSVP*, *PaAGL24*, *PaSEP1*, *PaSEP2*, *PaSEP3*, *PaSEP4*, *PaSEP5* and *PaFLC*. Besides, the bioinformatics software of DNAMAN, SMART and protein BLAST were used to analyze the features of the 14 MADS genes. Additionally, MEGA5 was also used for a cluster analysis of these 14 MADS-box genes and other 132 MADS-box genes. And the spatial-temporal RT-PCR

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gene expression analysis of these 14 genes were conducted in the tissues of root, leaf bud, leaf, flower bud, flower and phloem in sweet cherry.【Results】14 full-length MADS-box genes (*PaSOC1*, *PaAG*, *PaAP1*, *PaAP1-2*, *PaAP3*, *PaPI*, *PaSVP*, *PaAGL24*, *PaSEP1*, *PaSEP2*, *PaSEP3*, *PaSEP4*, *PaSEP5* and *PaFLC*) were 690, 732, 753, 768, 615, 633, 678, 696, 735, 723, 723, 735, 756 and 648 bp, respectively, and the molecular weight were 26, 28, 29, 29, 24, 24, 26, 26, 28, 28, 28, 28, 29 and 24 ku, respectively. Except the isoionic point of *PaAGL24* was acidic, the other 13 genes that *PaSOC1*, *PaAG*, *PaAP1*, *PaAP1-2*, *PaAP3*, *PaPI*, *PaSVP*, *PaSEP1*, *PaSEP2*, *PaSEP3*, *PaSEP4*, *PaSEP5* and *PaFLC* were alkaline. The structural analysis results showed that all these 14 genes contained MADS-box domain and K-box domain. Among them, *PaAG*, *PaAGL24*, *PaAP3*, *PaPI*, *PaSVP*, *PaFLC* contained 6 introns, *PaSOC1*, *PaAP1*, *PaAP1-2*, *PaSEP1*, *PaSEP2*, *PaSEP4*, *PaSEP5* contained 7 introns and *PaSEP3* contained 8 introns. In addition, it was interesting that the length and distribution of introns of those genes presented evolutional regularity, that is, the genes with high homology showed the same distribution of introns. Nevertheless, the result of phylogenetic analysis revealed that the 14 cherry MADS-box genes could be manually divided into 7 subclasses. The details were descripted as following: *PaSEP1*, *PaSEP2*, *PaSEP3*, *PaSEP4* and *PaSEP5* belonged to SEP subclass. *PaAP1* and *PaAP1-2* belonged to AP1 subclass. *PaAG* belonged to AG subclass. *PaSOC1* belonged to SOC1 subclass. *PaAP3* and *PaPI* belonged to AP3/PI subclass. *PaSVP* belonged to SVP subclass. *PaAGL24* belonged to AGL24 subclass. While *PaFLC* was extremely special, because that subclass with FLC of woody plant pear neither belonged to a subclass with FLC of herbaceous plant *Arabidopsis* nor belonged to a subclass with FLC of vine plant *Vitis vinifera*. The results allowed us to speculate that except for the difference in verbalization and dormancy there were also some differences in the function of FLC genes in different plant species. Comprehensively, RT-PCR results confirmed that all MAD-box family genes performed their own expression pattern in all tissues. The detailed were listed as follows. *PaSOC1* was expressed in the tissues of cherry root, leaf bud, leaf, flower bud and phloem. *PaAG* was expressed in cherry leaf, flower bud, flower and phloem. Furthermore, *PaAP1* was mainly expressed in cherry flower bud, and were weakly expressed in leaf, flower and phloem, while *PaAP1-2* was expressed in various cherry tissues. *PaAP3* was expressed in cherry root and were weakly expressed in flower. *PaPI* was expressed in cherry leaf bud, leaf, flower bud flower and phloem. *PaSVP* was expressed in cherry root, leaf bud, leaf, flower bud and phloem. *PaAGL24* was expressed in cherry leaf bud, leaf, flower bud and phloem. *PaSEP1* was expressed in cherry flower bud and flower. *PaSEP2* was expressed in cherry leaf bud, flower bud, flower and phloem. *PaSEP3* was expressed in cherry leaf bud, flower bud, flower and phloem. *PaSEP4* was expressed in cherry flower bud and flower. *PaSEP5* was expressed in cherry flower bud and flower. *PaFLC* was expressed in cherry leaf, flower bud and phloem. Except for *PaAP3*, *PaSEP1*, *PaSEP4* and *PaSEP5*, the rest genes were also expressed in phloem. These results had been never reported in previous researches, which allowed us to speculate that the specify and universality of phloem-derived gene expression pattern might be a unique character of woody plants. In addition, these results suggested that MADS-box genes were widely involved in sweet cherry growth and development process.【Conclusion】14 PaMADS-box family genes obtained in sweet cherry were highly and structurally conserved. They were involved in regulation of flowering and flower development processes in sweet cherry.

Key words: Sweet cherry; MADS-box genes; Bioinformatics; Expression analysis

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樱桃生产中常通过前期修剪、环剥、喷施生长调节剂、采用早花砧木等方法缩短树体童期，增加成花量，进而使果品提前上市，提高果农经济效益，因此，成花调控研究对辅助樱桃生产具有重要意义。植物开花受到多个基因共同协调调控。在拟南芥中，开花时间受多种途径影响，其中春化途径、光周期途径、赤霉素途径和自主调控途径研究较为详细[1]。MADS-box基因*FLC*（*FLOWERING LOCUSC*）和*SVP*（*SHORT VEGETATIVE PHASE*）在春化途径和自主调控途径中起着至关重要的作用[2-3]，*flc*缺失突变可以导致植株提前开花[4]，证实该类基因在从营养生长转向生殖生长过程中起负调控作用。自主途径不仅能够抑制*FLC*和*SVP*基因表达，而且还能够上调MADS-box基因*AGL24*（*AGAMOUS-LIKE 24*）表达[5]。拟南芥作为长日照植物，长日照条件下将诱导*CO*（*CONSTANS*）基因表达，*CO*突变会导致植物推迟开花，*CO*通过结合光信号和生物钟信号来调节*FT*（*FLOWERING LOCUS T*）基因以及MADS-box基因*SOC1*（*SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1*），*SOC1*不仅能够介导4个途径的花时基因信号，也会作用于下游部位的花分生组织特异基因，从而诱导植物开花[6-7]。此外，在分生组织中，FT蛋白与FLOWERING LOCUS D（FD）蛋白结合，启动*AP1*（*APETALA1*）和*LFY*（*LEAFY*）基因表达，进而调控花发育[8-10]。

MADS-box基因除了能够调控开花时间外，在花器官形成、花粉发育等方面也具有重要的调节作用。根据在成花不同阶段所起的作用，MADS-box基因分为ABCDE五大类，拟南芥中行使A类功能的MADS-box基因是*AP1*和*FUL*（*FRUITFULL*，之前命名为*AGL8*），主要负责花萼和花瓣的发育[11-13]；行使B类功能的基因是*AP3*（*APETALA3*）和*PI*（*PISTILLATA*），表达部位集中在花瓣和雄蕊中[14-15]；C类功能基因主要是*AG*（*AGAMOUS*），负责调控雄蕊和心皮发育[16]；D类功能的基因为*AGL11*（*AGAMOUS-LIKE 11*），后命名为*STK*（*SEEDSTICK*），在胚珠中特异表达[17]；E类功能的基因是*SEP 1/2/3/4*（*SEPALLATA1/2/3/4*）（曾命名*AGL2*，*4*，*9*和*3*），负责调控四轮花器官的形成[18-19]。

目前，关于樱桃MADS-box基因的研究报道很少，笔者课题组前期对甜樱桃（*Prunus avium*）*AP1*进行研究，发现*PaAP1*能够有效促进拟南芥早花[20]，对于其他成员还未做深入研究，笔者以3 a（年）树龄甜樱桃‘萨米特’（‘Summit’）为试材，拟克隆甜樱桃中*SOC1*、*AG*、*AP1*、*AP3*、*PI*、*SVP*、*AGL24*、*SEP1/2/3/4*、*FLC*、*FUL*等可能参与成花的MADS-box同源基因，对其进行序列信息学分析，并检测其在不同组织中的表达模式，探讨其可能参与的樱桃成花调控机制，为深入研究樱桃成花作用机制提供理论依据。

1 材料和方法

1.1 材料

2016年4月在北京市农林科学院林业果树研究所樱桃圃采集甜樱桃‘萨米特’的花，2016年8月采集其根（砧木）、韧皮部、叶、叶芽、花芽等部位。所有材料使用液氮速冻，-80 ℃保存备用。

樱桃总RNA的提取采用EASYspin植物RNA快速提取试剂盒（北京博迈德生物科技有限公司），利用SuperScript Ⅲ Reverse Transcriptase试剂盒（Invitrogen）合成第1链cDNA，樱桃基因组DNA的提取采用改良CTAB法进行，大肠杆菌感受态为Trans 5α菌株（北京全式金生物技术有限公司），克隆载体为pMD19-T（TaKaRa），由生工生物工程（上海）股份有限公司合成引物，测序由北京擎科新业生物技术有限公司完成。

1.2 MADS-box基因克隆及序列分析

根据GenBank中拟南芥、葡萄、梨等物种的*SOC1*、*AG*、*AP1*、*AP3*、*PI*、*SVP*、*AGL24*、*SEP1*/2/3/4、*FLC*、*FUL*基因序列结合桃基因组（https://www.rosaceae.org/）进行基因比对分析，将筛选出的DNA片段利用FGENESH（http://www.softberry.com/berry.phtml? topic=fgenesh&group=programs&subgroup=gfind）进行分析，根据其结果设计特异引物（表1）。

以‘萨米特’花芽和花的混合样品cDNA为模板进行PCR扩增，扩增程序为94 ℃ 5 min，94 ℃ 30 s，各自退火（表1）30 s，72 ℃ 1 min，72 ℃ 10 min。1.5%（ω）琼脂糖凝胶检测，切胶回收，连接克隆载体转化大肠杆菌，PCR鉴定，将阳性克隆送公司测序。

开放阅读框（ORF）和氨基酸序列分析利用BLAST在NCBI（http://www. ncbi. nlm.nih. gov/）和SMART（http://smart.embl.de/）中进行。使用ProtParam（http://www.expasy.org /tools/pi_tool.html）计算蛋白分子质量及等电点。

1.3 内含子分析

将克隆得到的甜樱桃MADS-box基因与之前根

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表1　引物序列

Table 1　Primers sequences

基因名称 Gene name	上游引物 Forward primer (5'-3')	下游引物 Reverse primer (5'-3')	退火温度 Annealing temperature/℃	用途 Purpose
PaSOC1	CTTCTGTTAAAAATGGTGAGAGG	TCTGAGGAGAAGCTAGCGCT	52	全长克隆 Full-length cloning
PaAG	TGATTCAGCTTGCAACTATGG	CCAAGCACATTAAACTAATTGAAG	50	全长克隆 Full-length cloning
PaAP1	ATGGGAAGGGGTAGGGTTCAG	TCAAGCAGCAAAGCATCCGA	57	全长克隆 Full-length cloning
PaAP1-2	CTAGTAGTATATATTATGGGGAGGGGA	CATGAATTCTTCTATTCATTAAGGTG	50	全长克隆 Full-length cloning
PaAP3	ATGGCGAGGGGTAAGATCCA	GCATTGGCTATCCTGCAGCC	59	全长克隆 Full-length cloning
PaPI	CGAAAATTGAGAGATATGGGGA	GACAGATGAAGATATAGATGCATTACA	52	全长克隆 Full-length cloning
PaSVP	GAAGATCGATCGATGGCGAG	GCATCATATCAAGAGTATTGATTAGAC	51	全长克隆 Full-length cloning
PaAGL24	GGAAACGGTGAAAATGATGAG	AGTAGCTCACCCCAGTTTGAG	52	全长克隆 Full-length cloning
PaSEP1	TTTTAGCTGGCAAGGACATG	TGATATGAGGATTTATCTCACAG	50	全长克隆 Full-length cloning
PaSEP2	ATGGGGAGGGGGAGAGTGGA	TCATGGCAACCATCCTGCCAT	60	全长克隆 Full-length cloning
PaSEP3	ATGGGAAGAGGGAAGGTAGAG	TCGCTCCAAGATTCAGAGCAC	56	全长克隆 Full-length cloning
PaSEP4	ATGGGGAGGGGGAGAGTGGA	GATCAGACTTTCAAAGAACCCATCCCT	59	全长克隆 Full-length cloning
PaSEP5	GAAAGAGATATGGGAAGAGGTAGA	TCAAAGTCAAAGCATCCACC	53	全长克隆 Full-length cloning
PaFLC	ATGGGACGAGGGAAGGTGCA	TCAGAACAAATGGAGCATCGTC	57	全长克隆 Full-length cloning
PaACTIN	CTCCTCTCAACCCTAAGGCTAACAG	CAGTTGTACGACCACTGGCATACAG	60	全长克隆 Full-length cloning
PaSOC1 P1	ATGCAGACAACCATAGAACG	CTCCTCTCCAACTGTTGCTCA	56	内含子验证 Verification of intron
PaSOC1 P2	AGGGTCTAGGATCATGCACT	TCTGAGGAGAAGCTAGCGCT	56	内含子验证 Verification of intron
PaAG P1	TGATTCAGCTTGCAACTATGG	GCATGCCTTCTTGTACCTCTC	52	内含子验证 Verification of intron
PaAG P2	GAGAGGTACAAGAAGGCATGC	CCAAGCACATTAAACTAATTGAAG	52	内含子验证 Verification of intron
PaAP1 P1	ATGGGAAGGGGTAGGGTTCAG	AGAGCGGTCTCAAGCTGGTG	60	内含子验证 Verification of intron
PaAP1 P2	GATCTCGATTCGTTGACTCTGA	TCAAGCAGCAAAGCATCCGA	60	内含子验证 Verification of intron
PaAP1-2 P1	CTAGTAGTATATATTATGGGGAGGGGA	TGCTCCAAATTCTGAAGCTC	58	内含子验证 Verification of intron
PaAP1-2 P2	GAGCTTCAGAATTTGGAGCA	CATGAATTCTTCTATTCATTAAGGTG	56	内含子验证 Verification of intron
PaAP3 P1	ATGGCGAGGGGTAAGATCCA	CTCAACACCACGCAGTTCATC	60	内含子验证 Verification of intron
PaAP3 P2	GATGAACTGCGTGGTGTTGAG	GCATTGGCTATCCTGCAGCC	60	内含子验证 Verification of intron
PaPI P1	CGAAAATTGAGAGATATGGGGA	GCTTGTCCCGGTTACTTGCA	60	内含子验证 Verification of intron
PaPI P2	CAAGTAACCGGGACAAGCAG	GACAGATGAAGATATAGATGCATTACA	57	内含子验证 Verification of intron
PaSVP P1	GAAGATCGATCGATGGCGAG	TCCTTGCTCAACGCAGAGTAG	60	内含子验证 Verification of intron
PaSVP P2	CTACTCTGCGTTGAGCAAGGA	GCATCATATCAAGAGTATTGATTAGAC	60	内含子验证 Verification of intron
PaAGL24 P1	GGAAACGGTGAAAATGATGAG	CTCAACAGACGGTTCGTCAG	53	内含子验证 Verification of intron
PaAGL24 P2	CTGACGAACCGTCTGTTGAG	AGTAGCTCACCCCAGTTTGAG	53	内含子验证 Verification of intron
PaSEP1 P1	TTTTAGCTGGCAAGGACATG	CTTGACCATAACTGCACTTCTG	55	内含子验证 Verification of intron
PaSEP1 P2	CAGAAGTGCAGTTATGGTCAAG	TGATATGAGGATTTATCTCACAG	55	内含子验证 Verification of intron
PaSEP2 P1	ATGGGGAGGGGGAGAGTGGA	GCTCCTTGGTGCCTAAATGAC	60	内含子验证 Verification of intron
PaSEP2 P2	GTCATTTAGGCACCAAGGAGC	TCATGGCAACCATCCTGCCAT	60	内含子验证 Verification of intron
PaSEP3 P1	ATGGGAAGAGGGAAGGTAGAG	GTTGCACTTCTGGTACCTCTCAAG	60	内含子验证 Verification of intron
PaSEP3 P2	CTTGAGAGGTACCAGAAGTGCAAC	TCGCTCCAAGATTCAGAGCAC	60	内含子验证 Verification of intron
PaSEP4 P1	ATGGGGAGGGGGAGAGTGGA	GCAGGAATGTTGGTATCGTTCGA	62	内含子验证 Verification of intron
PaSEP4 P2	TCGAACGATACCAACATTCCTGC	GATCAGACTTTCAAAGAACCCATCCCT	63	内含子验证 Verification of intron
PaSEP5 P1	GAAAGAGATATGGGAAGAGGTAGA	GTCTCATTGACTGGTCTGTTGG	54	内含子验证 Verification of intron
PaSEP5 P2	CCAACAGACCAGTCAATGAGAC	TCAAAGTCAAAGCATCCACC	54	内含子验证 Verification of intron
PaFLC P1	ATGGGACGAGGGAAGGTGCA	GTATCTGGTAACGCTCAAGAACCT	58	内含子验证 Verification of intron
PaFLC P2	AGGTTCTTGAGCGTTACCAGATAC	GGTGAGCTCTGTCACATCCAGA	58	内含子验证 Verification of intron
PaFLC P3	TCTGGATGTGACAGAGCTCACC	TCAGAACAAATGGAGCATCGTC	60	内含子验证 Verification of intron

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据桃基因组获得的DNA片段进行比对，分析其内含子分布，根据其分布设计跨内含子特异引物（表1），以‘萨米特’基因组DNA为模板，进行PCR扩增验证，并根据外显子和内含子序列大小等比例作图。

1.4 聚类分析

收集GenBank中拟南芥、葡萄、草莓等MADS-box基因，剔除重复序列（剩余132个基因），利用MEGA 5[21]邻接法[22]作图，进化距离根据JJT法计算[23]，γ参数设为0.9。进行1 000次置信度重复[24]。制图完成后利用Photoshop对不同亚族进行标注。

1.5 RT-PCR表达模式分析

以樱桃根、叶芽、叶、花芽、花、韧皮部cDNA为模板，根据表1引物对各组织中表达量进行鉴定，检测PCR循环数设为35个循环，内参选用樱桃*Actin*，检测PCR循环数为32个，2%（ω）琼脂糖凝胶电泳，利用ImageJ软件对电泳条带亮度进行分析，Excel作图。

2 结果与分析

2.1 樱桃成花相关MADS-box基因的克隆及分析

通过比对，从桃基因组中共分析出24个MADS-box基因序列，经比对筛选，去除重复序列后剩余15个基因序列，以甜樱桃混合cDNA为模板，利用各个MADS-box基因特异引物进行PCR扩增，获得全长为615~768 bp的14个MADS-box基因（图1），经NCBI比对分析，根据其同源性以及诱饵基因分别将这14个甜樱桃MADS-box基因命名为*PaSOC1*、*PaAG*、*PaAP1*、*PaAP1-2*、*PaAP3*、*PaPI*、*PaSVP*、*PaAGL24*、*PaSEP1*、*PaSEP2*、*PaSEP3*、*PaSEP4*、*PaSEP5*、*PaFLC*。编码氨基酸范围为204~255个，分子质量为24~29 ku，除了*PaAGL24*等电点呈酸性外，其余13个成员等电点均呈碱性（表2）。

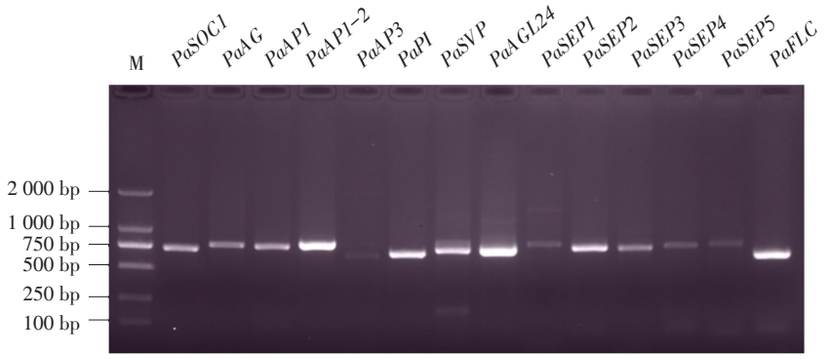

图1 甜樱桃成花相关MADS-box基因克隆

Fig. 1 Isolation of MADS-box gene in sweet cherry

2.2 内含子分析

经比对分析和PCR验证发现，*PaAG*、*PaAGL24*、*PaAP3*、*PaPI*、*PaSVP*、*PaFLC*含有6个内含子，*PaSOC1*、*PaAP1*、*PaAP1-2*、*PaSEP1*、*PaSEP2*、*PaSEP4*、*PaSEP5*含有7个内含子，*PaSEP3*含有8个内含子（图2）。MEGA 5.0聚类分析可见，内含子长度以及分布具有一定的进化规律，同源性相近的基因之间，内含子分布情况比较相近。

2.3 结构分析

利用DNAMAN比对和在线软件SMART分析14个甜樱桃MADS-box基因氨基酸的保守结构

表2 樱桃成花相关MADS-box基因

Table 2 The MADS-box genes related to flowering in sweet cherry

基因名称 Gene name	开放阅读框长度 ORF length/bp	编码蛋白数 Protein number/aa	分子质量 Molecular weight/ku	等电点 Isoelectric point
PaSOC1	690	229	26	9.57
PaAG	732	243	28	9.44
PaAP1	753	250	29	8.67
PaAP1-2	768	255	29	8.64
PaAP3	615	204	24	9.81
PaPI	633	210	24	8.72
PaSVP	678	225	26	6.26
PaAGL24	696	231	26	5.30
PaSEP1	735	244	28	8.58
PaSEP2	723	240	28	8.79
PaSEP3	723	240	28	8.23
PaSEP4	735	244	28	9.08
PaSEP5	756	251	29	8.62
PaFLC	648	215	24	8.60

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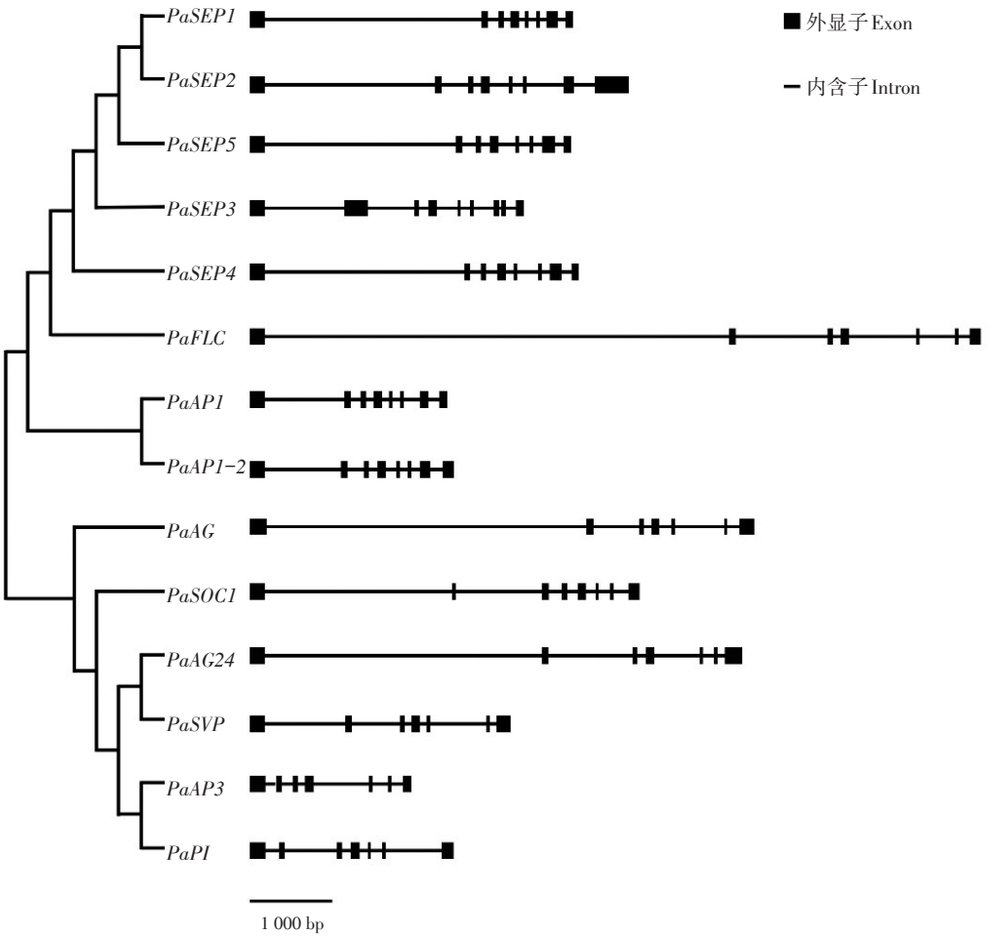

图2　*PaMADS*基因内含子和外显子结构

Fig. 2　Intron-exon structures of *PaMADS* genes

域，结果发现，每个成员均含有保守的MADS结构域和K-box结构域，除了*PaAG*外，其他成员的MADS结构域均位于N端末端，C端保守性不强（图3、图4）。

2.4　聚类分析

结合NCBI GenBank中筛选出的122个拟南芥以及10个其他物种的MADS-box家族成员，利用MAGE 5.0对14个樱桃MADS-box基因进行聚类分析，结果显示樱桃14个MADS-box成员分为7个亚组，其中，*PaSEP1*、*PaSEP2*、*PaSEP3*、*PaSEP4*、*PaSEP5*属于SEP亚组，*PaAP1*、*PaAP1-2*属于AP1亚组，*PaAG*属于AG亚组，*PaSOC1*属于SOC1亚组，*PaAP3*、*PaPI*属于AP3/PI亚组，*PaSVP*属于SVP亚组，*PaAGL24*属于AGL24亚组，*PaFLC*并未聚到FLC亚组，而是和梨FLC一起聚到邻近组中（图5）。

2.5　表达模式分析

经RT-PCR分析发现，*PaSOC1*在根、叶芽、叶、花芽、韧皮部中均有不同程度表达，在花中不表达；*PaAG*只在叶和花中表达量略高，在花芽和韧皮部中有微量表达；*PaAP1*在花芽中大量表达，在叶芽、叶、花和韧皮部中有微量表达；*PaAP1-2*在各个组织中均有不同程度的表达；*PaAP3*在除了根中表达外，在花中也有微量表达；*PaPI*在叶芽、花芽、花中表达量较高，在韧皮部有微量表达；除花以外，*PaSVP*在其他各组织中均有表达；*PaAGL24*在叶芽、叶、花芽、韧皮部中有较高表达；*PaSEP1*和*PaSEP4*的表达模式较为相近，均在花芽和花中有微量表达；*PaSEP2*与*PaSEP3*表达模式较为一致，都是在叶芽、花芽、花和韧皮部中不同程度表达；*PaSEP5*在叶、花芽、花和韧皮部有不同程度的轻微表达；*PaFLC*在叶、花芽和韧皮部中表达（图6）。

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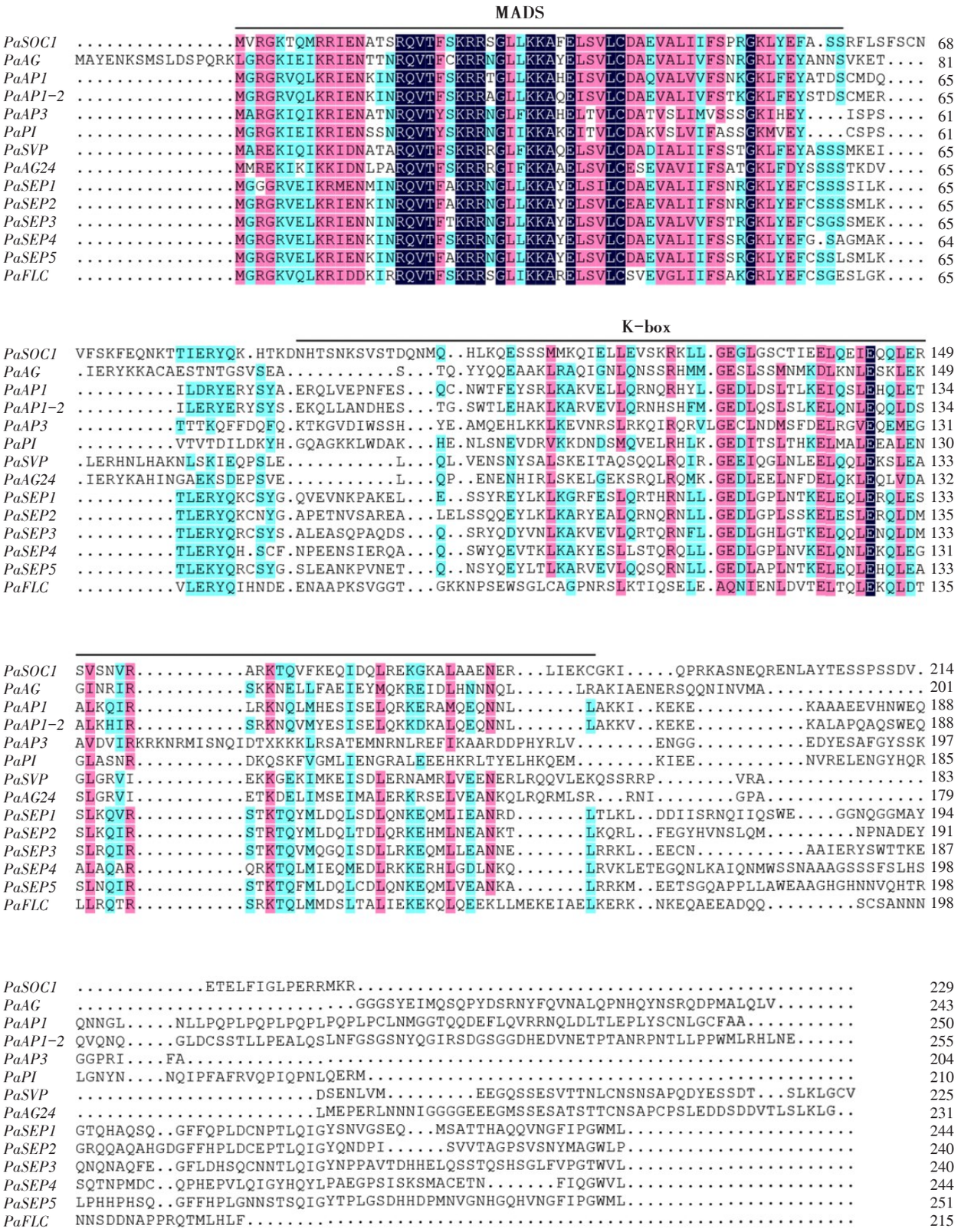

图 3 PaMADS 基因氨基酸序列多重比较

Fig. 3 Sequences alignment of PaMADS proteins

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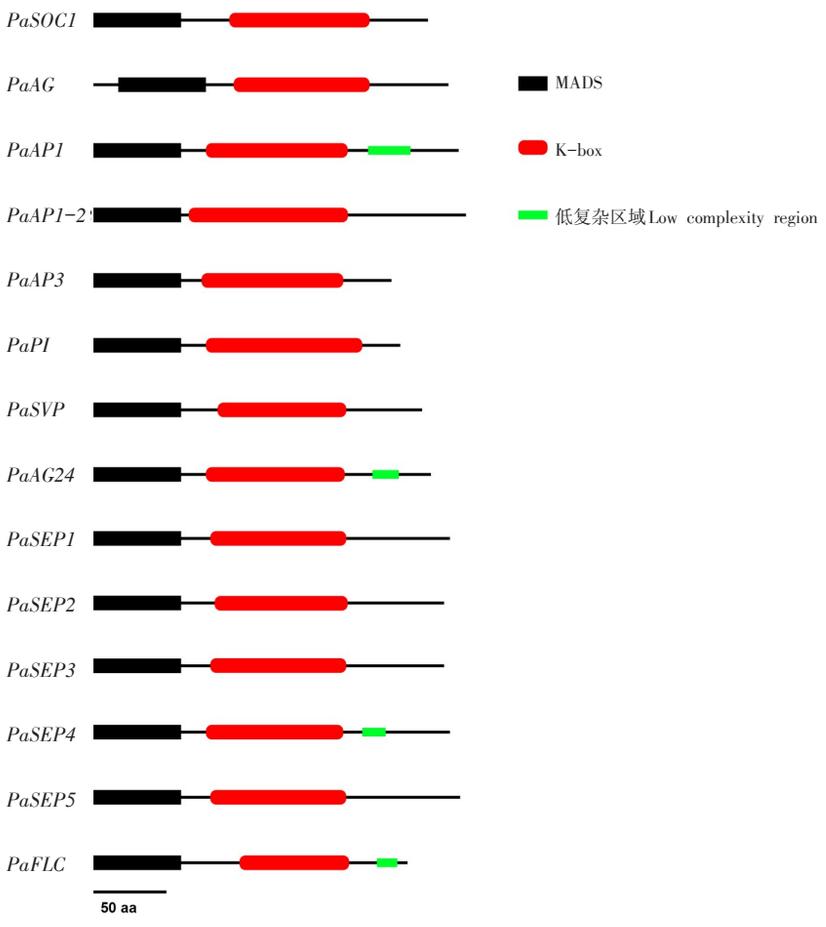

图4　*PaMADS*基因保守元件分布

Fig. 4　Distribution of the identified motifs in *PaMADS* genes

3　讨　论

在植物生长发育过程中，MADS-box基因这类具有典型MADS结构域的转录因子在调控根的营养吸收、叶片的发育、春化、分生组织分化、果实成熟、胚的发育等方面起作用[25-26]，尤其在开花时间和花器官的发育中，MADS-box基因家族的*SOC1*、*AG*、*AP1*、*AP3*、*PI*、*SVP*、*AGL24*、*SEP1*、*SEP2*、*SEP3*、*SEP4*、*FLC*等成员在不同时期扮演着重要角色[4-7,11-12,14-19]。甜樱桃中存在1个*SOC1*基因、1个*AG*基因、2个*AP1*基因（*PaAP1*、*PaAP1-2*）、1个*AP3*基因、1个*PI*基因、1个*SVP*基因、1个*AGL24*基因、5个*SEP*基因（*PaSEP1*、*PaSEP2*、*PaSEP3*、*PaSEP4*、*PaSEP5*）和1个*FLC*基因。

植物中MIKC类的MADS-box转录因子的结构研究比较清楚，通常含有4个结构域，分别为MADS-box（M）、Intervening domain（I）、Kertain-like domain（K）和C-terminal domain（C）[27-28]。M区具有结合DNA、蛋白质二聚化以及与其他因子结合的功能[29]；I区位于M区与K区之间，长约30 aa，保守性较低，可以促进二聚体的转录因子与DNA结合[30]；K区约有70个氨基酸，其二级结构为3个α螺旋（K1、K2和K3）组成的卷曲-卷曲（coiled-coil）结构，参与介导蛋白-蛋白的相互作用[31]；C区位于在K区下游，在序列和长度上都最具变化，在不同类的MADS-box基因中常含有一些能够在蛋白复合体的形成和转录激活中起重要作用的保守基序（motif）[32-34]。经预测，甜樱桃*PaSOC1*、*PaAG*、*PaAP1*、*PaAP1-2*、*PaAP3*、*PaPI*、*PaSVP*、*PaAGL24*、*PaSEP1*、*PaSEP2*、*PaSEP3*、*PaSEP4*、*PaSEP5*、*PaFLC*这14个基因都具有典型的M和K结构域，而I区及C区相对保守性较低，说明这些MADS-box基因在不同植物物种间还是相对保守的。

不同植物中存在数量不等的MADS-box基因。

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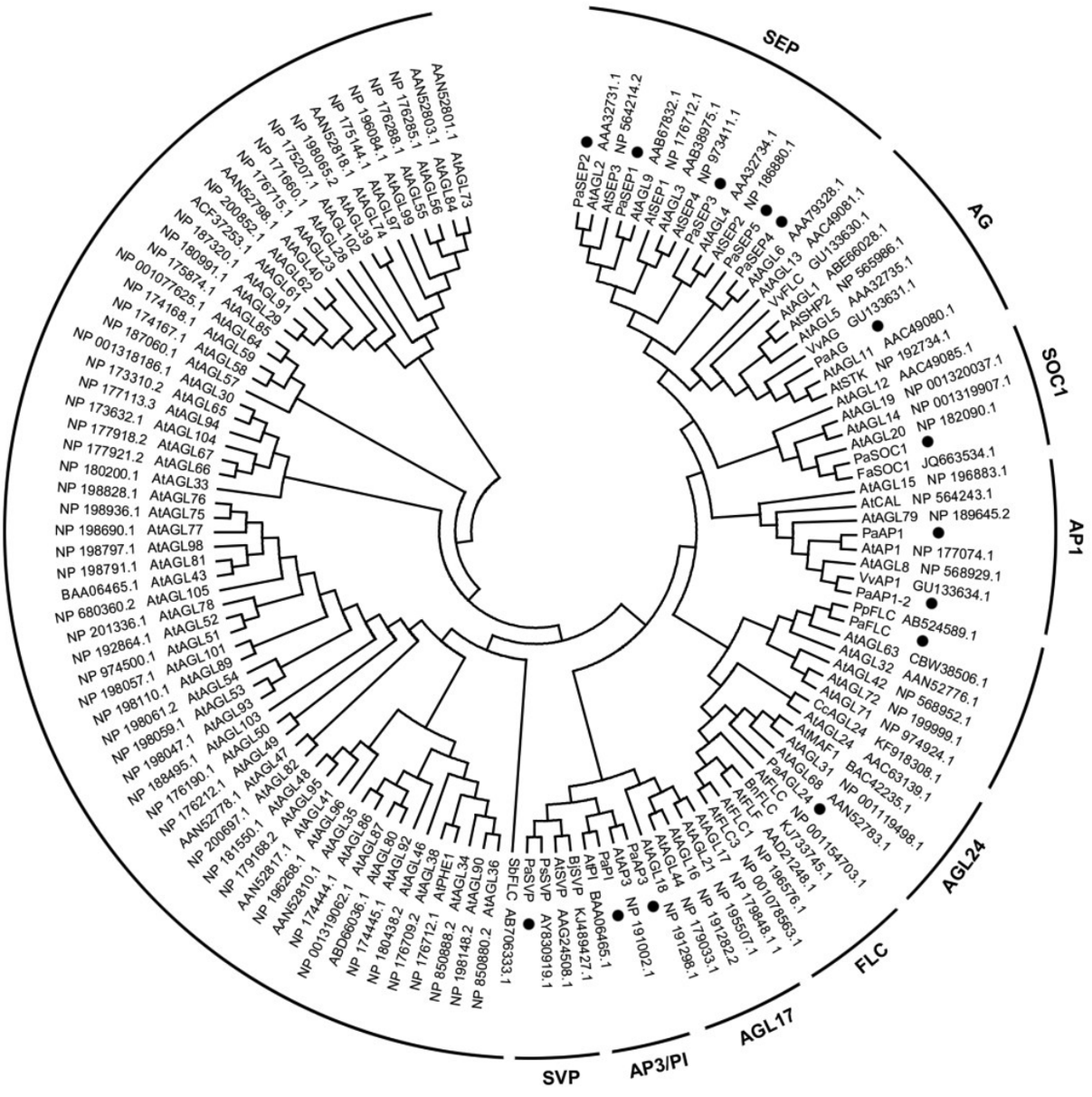

图 5 甜樱桃、葡萄、梨和拟南芥 MADS-box 基因聚类分析

Fig. 5 Phylogenetic relationships of MADS-box genes from sweet cherry, grapevine (*Vitis vinifera*), pear (*Pyrus pyrifolia*) and *Arabidopsis*

分子系统发育分析表明，MIKC类的MADS-box基因分为12个主要亚组，即AG、AGL6、AGL12、APETALA3（AP3）/PISTILLATA（PI）- like（B）、GGM13（Bs）、TM3、STMADS11、AGL2（SEP）、AGL17、APETALA1（AP1）/SQUA、AGL15和FLC[35]。聚类分析显示，甜樱桃14个MADS-box基因分属于不同亚组，其中PaFLC聚类比较特别，并未与草本的拟南芥FLC聚在一起，也未和藤本科植物葡萄FLC聚类一组，而是与木本的梨聚在一起，推测不同科植物之间因春化作用以及休眠模式不同，FLC基因在功能上已经发生了明显的分化，结构间也存在一定差异。

进一步对14个甜樱桃MADS-box基因表达模式分析的结果显示，*PaSOC1*、*PaSVP*及*PaFLC*表达模式一致，这与前人报道的SVP能够与FLC以二聚体形式直接结合*SOC1*启动子调节*SOC1*表达的结果[36-39]类似，但*PaFLC*在根和叶芽中的表达量与*PaSOC1*、*PaSVP*相比较低，推测由其他调控因素导

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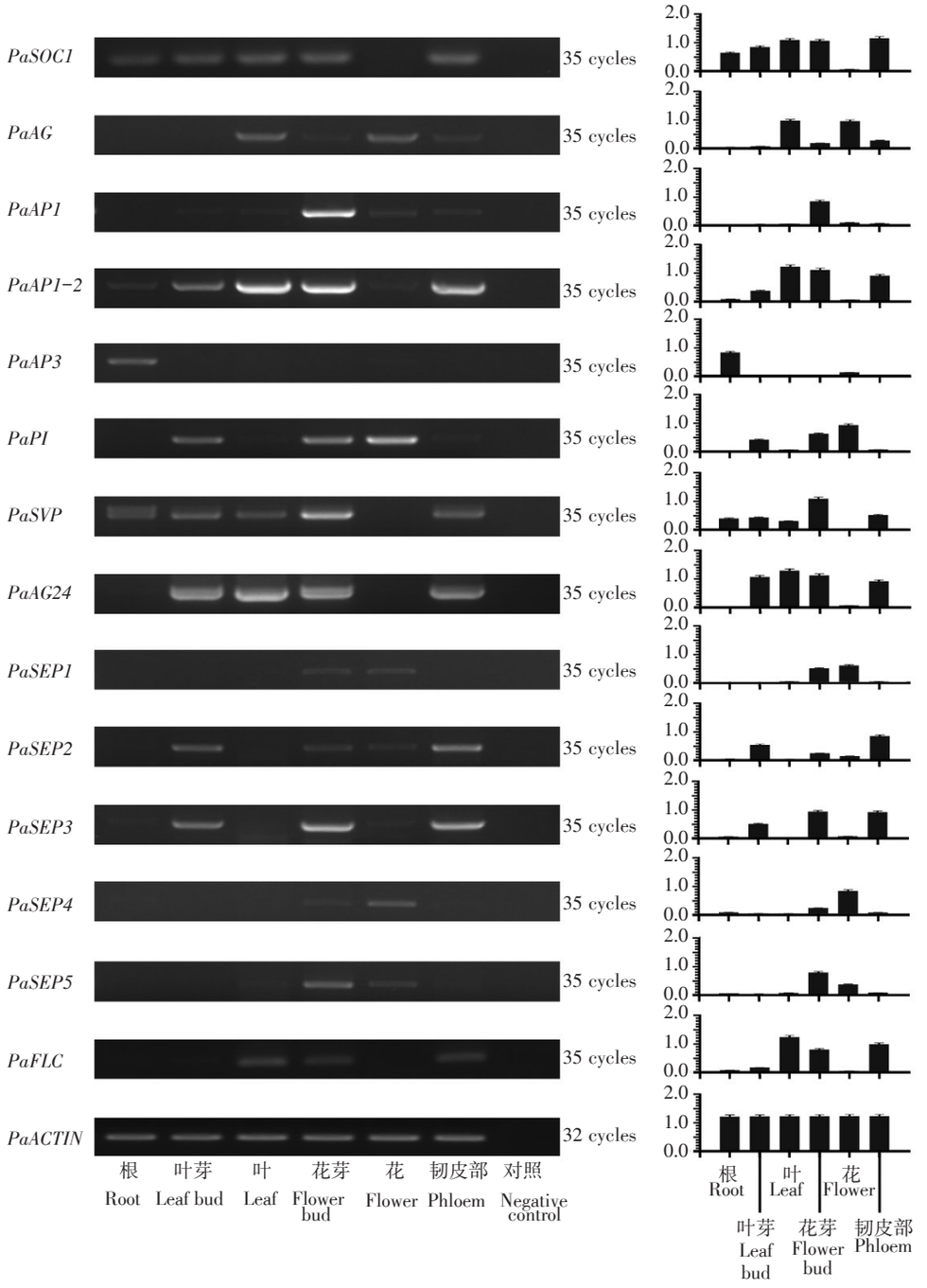

图6　*PaMADS*基因在不同樱桃组织中的表达分析

Fig. 6　Expression analysis of *PaMADS* genes in different tissues of cherry

致。

甜樱桃中存在2个行使A类功能的*AP1*（*PaAP1*、*PaAP1-2*），2者表达模式存在差异，推测不同时期2者起不同作用。笔者课题组前期研究发现，超表达*PaAP1*能够有效促进拟南芥提早开花[20]，*PaAP1-2*表达模式与*PaAGL24*比较相近，其具体功能还有待进一步研究。行使B类功能的*AP3*和*PI*负责花瓣和雄蕊发育[14-15]，*PaPI*的表达模式比较符合前期研究，*PaAP3*在花中微弱表达，但在根中大量表达，说明*PaAP3*可能参与樱桃根发育调控，但是其作用机制还需另做详细研究。E类基因SEP家族*PaSEP1*、*PaSEP4*、*PaSEP5*表达模式相近，而*PaSEP2*、*PaSEP3*表达模式相近，E类基因负责调控四轮花器官的形成[18-19]，并且拟南芥4个SEP-like基

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因在花的发育过程中具有明显功能冗余情况，单独缺失突变不会对表型产生影响[18, 40]，因此，推测*PaSEP1*、*PaSEP4*、*PaSEP5*以及*PaSEP2*、*PaSEP3*存在功能互补的作用。

除此之外，*PaSOC1*、*PaAG*、*PaAP1*、*PaAP1-2*、*PaSVP*、*PaAGL24*、*PaSEP2*、*PaSEP3*、*PaFLC*等MADS-box基因除了在花芽和花等组织表达外，还在韧皮部中大量表达，在以往研究中未见相关报道，该表达模式是否属于木本植物独有的特性有待进一步研究。

樱桃作为多年生木本蔷薇科植物，具有明显不同于其他果树的成花特征，花芽于每年5—6月开始形成，且具有独特的“侧芽成花，顶芽成枝”的花束状果枝，此外，甜樱桃成花还受到砧木影响，早花砧木能促使接穗提前1~2 a进入盛花期，成花机制较为复杂，但到目前为止，樱桃成花机制研究却远远落后于苹果、葡萄等其他果树。笔者对14个甜樱桃成花相关MADS-box基因基本信息、保守结构域、进化关系和组织表达特异性等方面进行了详细的分析，为进一步探讨甜樱桃MADS-box基因在甜樱桃成花发育以及其他器官发育过程的作用机制奠定了相关的理论基础。

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