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‘秋姬李’*PsMYB18*基因克隆与功能分析

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摘 要:【目的】克隆在采后‘秋姬李’果皮积累花色苷过程中高表达的MYB抑制因子*PsMYB18*基因,研究其序列特征、表达特点与功能。**【方法】**以‘秋姬李’为试材,采用qRT-PCR分析不同温度和光照处理条件下‘秋姬李’果皮中*PsMYB18*基因的转录水平,采用RT-PCR克隆*PsMYB18*基因,并通过烟草叶片瞬时表达试验分析*PsMYB18*的功能。**【结果】**qRT-PCR分析表明20 ℃和光照处理可促进‘秋姬李’果皮*PsMYB18*基因表达。*PsMYB18*基因的开放阅读框(ORF)为702 bp,编码233个氨基酸的蛋白。进化树分析表明PsMYB18与其他植物的花色苷合成抑制因子亲缘关系较近。序列比对结果表明其具有保守的R2R3结构域和抑制基序C1和C2。烟草瞬时表达试验表明,PsMYB18可抑制正调控因子PsMYB10.1和PsbHLH3的花色苷合成诱导功能。**【结论】**‘秋姬李’*PsMYB18*为花色苷合成抑制因子。

关键词:‘秋姬李’;*PsMYB18*;花色苷合成抑制因子

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Molecular cloning and function analysis of *PsMYB18* in ‘Akihime’ plum (*Prunus salicina* Lindl.)

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Abstract:【Objective】Temperature and light are important environmental factors that affect the biosynthesis and accumulation of anthocyanin in the fruit peel. Our previous study indicated that 20 ℃ temperature and light treatment (150 mol · m^{-2} · s^{-1}) could induce anthocyanin accumulation in the peel of postharvest ‘Akihime’ plums. Comparative transcriptomic analysis results demonstrated that the expression of a R2R3-MYB gene (designated as *PsMYB18*) was significantly upregulated in the fruit peel treated at 20 ℃+ light. The objective of the study was to isolate and elucidate the function of *PsMYB18* gene during anthocyanin accumulation in the peel of ‘Akihime’ plum.【Methods】The total RNA of the plum fruit peels was extracted using EZNA Plant RNA Kit (Omega Bio-tek, USA) according to manufacturer's instructions. Real-time quantitative PCR (qRT-PCR) was employed to determine the expression of *PsMYB18* in the peel of ‘Akihime’ plum fruits treated at 20 ℃ + light, 20 ℃+ dark, 30 ℃+ light and 30 ℃+ dark. First-strand cDNA was synthesized from 500 ng of total RNA using the PrimeScript RT reagent kit with gDNA Eraser (Takara, China). The qRT-PCR was performed using the Eppendorf Realplex4 real-time PCR system (Hamburg, Germany) in a total volume of 20 μL in each well containing 10 μL of 2×SYBR® Premix Ex Taq™ II (Tli RNaseH Plus, TaKaRa), 1 μL of cDNA (in 1:10 dilution), and 0.4 μL 10 μmol primers. The qPCR conditions were 30 s at 95 ℃, followed by 40 cycles of 5 s at 95 ℃, 15 s at 60 ℃, and 30 s at 72 ℃, followed by 60 ℃ to 95 ℃ melting curve detection. *Actin* gene was used as the reference. For gene cloning, total RNA was extracted from the fruit peel treated with 20 ℃

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and light. The first-strand cDNA was synthesized using First Strand cDNA Synthesis Kit (Thermo Scientific, USA). *PsMYB18* gene was isolated from the skin of 'Akihime' plums by reverse transcription polymerase chain reaction (RT-PCR). Phylogenetic tree was constructed using the MEGA 5.02 software to investigate the evolution relationship between *PsMYB18* and MYB proteins from other plant species. Multiple sequence alignments of PsMYB18 protein with MYB repressors from peach, apple, grape, poplar, *Medicago truncatula*, *Fragaria* × *ananassa* and *Antirrhinum majus* were performed using DNAMAN7.02. pCAMBIA1302 vector, which was linearized by restriction enzyme NcoI and BstEII was used to construct overexpression vectors. The coding regions of *PsMYB18*, *PsMYB10.1* and *PsbHLH3* were isolated using I-5™ 2×High-Fidelity Master Mix (MCLAB, San Francisco, CA) and then cloned into the pCAMBIA1302 vector using One Step Seamless Cloning kit (Aidlab, China). All constructs were introduced into *Agrobacterium tumefaciens* GV3101. Tobacco (*Nicotiana tabacum* L.) leaf transient expression assay was performed to verify the function of *PsMYB18* in anthocyanin biosynthesis. The Agrobacterium strain GV3101 containing overexpression vectors were grown at 28 ℃ in LB medium containing with antibiotics. Then *Agrobacterium* cells were harvested and resuspended in the infiltration buffer (10 mmol · L^{-1} MgCl2, 10 mmol · L^{-1}MES, pH 5.6, 100 μmol · L^{-1} acetosyringone) to a final OD_{600} of 0.8. The bacteria were incubated at room temperature for 3 h before infiltration. Digital photographs of anthocyanin development in tobacco leaves were taken at 7 days after infiltration.【Results】The qRT-PCR analysis revealed that the expression of *PsMYB18* in the fruit peel treated at 20 ℃ and light was significantly enhanced after 3 d of treatment and remained at a high level thereafter. However, no obvious expression upregulation of *PsMYB18* was detected in the fruit peel treated at 20 ℃ + dark, 30 ℃ + light and 30 ℃ + dark. These results were consistent with RNA-seq data. The cDNA sequence of *PsMYB18* was isolated from the peel of 'Akihime' fruits treated at 20 ℃ and light. *PsMYB18* contained open reading frame (ORF) of 702 bp in length, which was predicted to encode a protein of 539 amino acid residues. The results of phylogenetic analysis revealed that PsMYB18 and repressors of anthocyanin and proanthocyanidin biosynthesis, including FaMYB1, MtMYB2, PtMYB182, VvMYBC2-L3, VvMYBC2-L1 and PpMYB18, from other plants belonging to the same class. It had the closest relationship with PpMYB18 from peach. Multiple alignment of the deduced amino acid sequences of PsMYB18 and MYB repressors showed that PsMYB18 shared similarity of 96.57% with the PpMYB18 protein and contained the conserved R2 and R3 domains. It also had C1 and C2 repression motifs in the C-terminus. These results implicated that PsMYB18 could be a repressor of anthocyanin biosynthesis. To investigate the role of PsMYB18 in anthocyanin biosynthesis, the ORF of *PsMYB18* was amplified and ligated into the pCAMBIA1302 vector. Transient color assays in tobacco leaves showed that coinfiltration of *PsMYB18* with *PsbHLH3* could not induce anthocyanin accumulation. Furthermore, coinfiltration of *PsMYB10.1* with *PsbHLH3* resulted in red pigmentation after 7 days, while no pigmentation was observed when *PsMYB10.1* and *PsbHLH3* were co-infiltrated with *PsMYB18*.【Conclusion】In this study, *PsMYB18* was isolated and its expression was enhanced by the treatment at 20 ℃ and light. Phylogenetic and sequence analysis suggested that PpMYB18 was likely a repressor of flavonoid biosynthesis. Transient color assay results showed that PsMYB18 was a negative regulator of anthocyanin biosynthesis.

Key words: 'Akihime' plum; *PsMYB18*; Repressor of anthocyanin biosynthesis

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花色苷是一类有益于人体健康的天然色素[1]，也是决定李果实是否呈现消费者喜爱的鲜艳红色的关键[2]。花色苷合成和积累易受光照和温度等环境条件的影响[3-5]。成熟李果实不耐贮运，生产上常在未完全成熟时进行采收，以延长货架期[6]。因此，李果实常受光照不足和采收时成熟度过低等因素限制，着色不佳。阐明李果实花色苷合成的调控机制，对指导改进栽培或采后处理措施，促进李果实花色苷的合成与积累，改善色泽，提高营养价值与商品价值具有重要的意义。

花色苷合成和积累涉及到一系列合成酶和转运蛋白[7]。大量研究表明，花色苷的积累由R2R3-MYB转录因子、bHLH转录因子和WD40蛋白组成的MBW复合体调控，而MYB是控制花色苷积累的关键因子[8]。目前，已从许多果树（如苹果、桃、李、樱桃和柑橘）中鉴定出正调控花色苷积累的MYB转录因子，它们通过激活结构基因的转录诱导花色苷合成和积累[9-10]。此外，研究表明植物花色苷合成机制极为复杂，其他调控因子（如NAC、HY5、WRKY、bZIP和HD-Zip等）也可直接或通过与MYB互作调控结构基因的表达与花色苷合成[11-14]。近年来，研究人员发现MYB抑制因子也参与花色苷合成调控[15-17]。MYB抑制因子通过与激活因子竞争bHLH和/或结构基因启动子上的结合位点发挥抑制作用[18-20]。目前，已发现多种类型的类黄酮合成途径相关MYB抑制因子，如不包含抑制基序的R3-MYB[21-23]、AtMYBL2（包含部分R2结构域、完整R3结构域和抑制基序）[24]、包含R2R3结构域和抑制基序的R2R3-MYB[17, 20, 25]和CgRuby2^{Short}（仅包含部分R2和R3结构域，且无抑制基序）[26]。N端的R3结构域和C端的抑制基序是决定MYB抑制因子活性的关键因素[17, 20, 24, 27-28]。R2R3-MYB抑制因子在其C端具有C1 (lsrGIDPxT/NHR) 和C2 (pdLNLD/EL)两个保守的抑制基序[29]。Zhou等[17]从红肉桃中鉴定出一个在果肉中高表达的调控花色苷合成的R2R3-MYB抑制因子PpMYB18。他们认为PpMYB18与MYB激活因子共同作用维持花色苷和原花色苷积累的动态平衡，防止花色苷和原花色苷的过度积累。可见，MYB抑制因子也是错综复杂的花色苷合成调控系统的重要组成部分。MYB抑制因子的鉴定及其作用机制的解析有助于丰富和完善植物花色苷合成和积累的调控机制。

目前，李花色苷合成调控机制研究较少。研究人员先后从樱桃李、欧洲李和中国李中鉴定出了正调控果皮和果肉花色苷合成的MYB转录因子[2, 30-32]。然而，参与花色苷合成调控的MYB抑制因子尚未见报道。笔者通过比较转录组和荧光定量PCR分析发现了1个在20 ℃/光照处理诱导采后‘秋姬李’果皮花色苷积累过程中表达量升高的R2R3-MYB基因*PsMYB18*。进化树分析表明其可能为花色苷合成抑制因子，序列比对发现PsMYB18具有保守的R2R3结构域和抑制基序C1和C2。烟草叶片瞬时表达试验进一步验证了该基因对花色苷合成的抑制作用。该研究结果可为进一步剖析MYB抑制因子在李果实花色苷合成过程中的功能与作用机制提供参考。

1　材料和方法

1.1　试验材料与处理

本试验所用果实均为采自古田县的10年生李树的硬熟期（果皮由绿色转黄色）‘秋姬李’果实。果实采收后，运回实验室，挑选形状、颜色和大小一致的果实用于后续处理。将挑选好的果实分成4份，分别在20 ℃/光照、20 ℃/黑暗、30 ℃/光照和30 ℃/黑暗的条件下进行处理。所用光照强度均为150 $mol \cdot m^{-2} \cdot s^{-1}$，相对湿度为70%~80%。每个处理3次重复。于处理后0、3、6、8和12 d削取10个‘秋姬李’的果皮，切成大小相似的小片，并混合到一起，用液氮速冻后保存于−80 ℃冰箱。

1.2　*PsMYB18*的克隆与表达载体构建

采用EZNA Plant RNA Kit (Omega Bio-tek，USA)进行RNA提取，并采用ReverAid™ First Strand cDNA Synthesis Kit(Fermentas，USA)进行cDNA合成。根据转录组数据（NCBI的SRA数据库登录号为PRJNA509525）设计引物，采用Ⅰ-5™ 2× High-Fidelity Master Mix (MCLAB，San Francisco，CA)进行*PsMYB18*基因cDNA的分离。首先，以5'-ATGAGGAAACCCTGCTGCGATAAGC- 3' 和 5'-CTTCCACCTGGACGATGAATATTC-3'为引物进行*PsMYB18*基因片段扩增。随后，以5'- ACGGGGGACTCTTGACATGAGGAAACCCTGCTG - CGATAAGC- 3' 和 5'- ATTCGAGCTGGTCACCTCAAGAGAGGAAGGATATT 进行*PsMYB18*基因ORF序列扩增。目的片段经纯化后，采用One Step

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Seamless Cloning kit(Aidlab，中国)将目的片段连接到经NcoI和BstEII酶切的pCAMBIA1302载体，并转化大肠杆菌进行测序验证。

1.3 PsMYB18同源性分析

从NCBI数据库(http://www.ncbi.nlm.nih.gov/)中下载对应的其他物种的蛋白序列。利用DNAMAN7.02软件进行MYB的氨基酸序列比对，通过MEGA5.02软件构建Neighbor-Joining系统进化树。

1.4 *PsMYB18*的表达分析

采用带gDNA Eraser的PrimeScript RT试剂盒(Takara，中国)进行cDNA合成，总RNA用量为500 ng。采用Eppendorf RealPlex4 real-time PCR system (Hamburg，Germany)进行qRT-PCR分析，反应物体积为20 μL，包含10 μL 2×SYBR Premix Ex Taq™ Ⅱ (Tli RNaseH Plus，TaKaRa)，1 μL cDNA (1∶10稀释)，0.4 μL 10 μmol·L^{-1}引物。反应条件为95 ℃ 30 s；95 ℃ 5 s，60 ℃ 30 s，40个循环。*Actin*基因为内参。引物为PsMYB18-forward：5'-TCTTGTGGCCATGAGCTGCC-3'、PsMYB18-reverse：5'-TGTGGACGAGGCAAATTCCG-3'、Actin-forward：5'-AGGTTGTGGCTCCACCAGAG-3'和Actin-reverse：5'-CACTGACCCCACCTCAACGC-3'。基因表达量采用Fang[33]报道的方法计算。每个样品做3次技术重复和3次生物学重复。

1.5 烟草叶片瞬时表达实验

将*PsMYB18*表达载体以及参照上述方法构建的*PsMYB10.1*(GenBank登录号：MK105923)与*PsbHLH3* (GenBank登录号：MH383068)的表达载体分别转化农杆菌GV3101。分别挑取带有上述表达载体的农杆菌菌落于含有50 mg·L^{-1}利福平和50 mg·L^{-1}卡那霉素的YEB培养基中培养至OD_{600}值为0.8~1.0。5 000 r·min^{-1}离心5 min收集农杆菌，并采用缓冲液(10 mmol·L^{-1} $MgCl_2$,10 mmol·L^{-1} MES，100 μmol·L^{-1} AS)重悬至OD_{600}值为0.8。随后，在室温条件下静置3 h。烟草叶片瞬时颜色诱导实验参照Lin等[34]报道的方法。将上述农杆菌悬浮液单个或几个以1∶1混合后，用注射器注射烟草叶片。注射7 d后观察烟草叶片颜色并拍照。每个组合至少注射3枚叶片。

2 结果与分析

2.1 ‘秋姬李’果皮*PsMYB18*的发现与表达分析

为了研究温度和光照条件对采后李果皮花色苷积累的影响，在前期研究中采用不同条件(20 °C/光照、20 °C/黑暗、30 °C/光照和30 °C/黑暗)对采后‘秋姬李’进行处理，结果20 ℃/光照处理可诱导采后‘秋姬李’果皮积累花色苷，着紫红色，而20 ℃/无光以及30 ℃/光照或无光等条件下，‘秋姬李’果皮均不能积累花色苷(图1)。为了进一步探讨温度和光照调控采后‘秋姬李’果皮花色苷积累的分子机制，对不同处理条件下‘秋姬李’果皮的转录组(SRA数据库登录号PRJNA509525)进行比较分析，结果发现R2R3-MYB基因*PsMYB18*在经20 ℃和光照处理10 d后的‘秋姬李’果皮中的表达量显著高于30 ℃/光照、30 ℃/无光和20 ℃/无光处理的‘秋姬李’果皮(图2)。采用qRT-PCR对不同温度和光照处理条件下‘秋姬李’果皮中*PsMYB18*基因的转录水平进行分析，结果表明20 ℃/光照处理3 d后‘秋姬李’果皮中*PsMYB18*的表达量显著提高，并始终高于其他处理，而其他处理条件下的‘秋姬李’果皮中*PsMYB18*的表达量均未显著提高(图2)，与转录组分析结果一致。

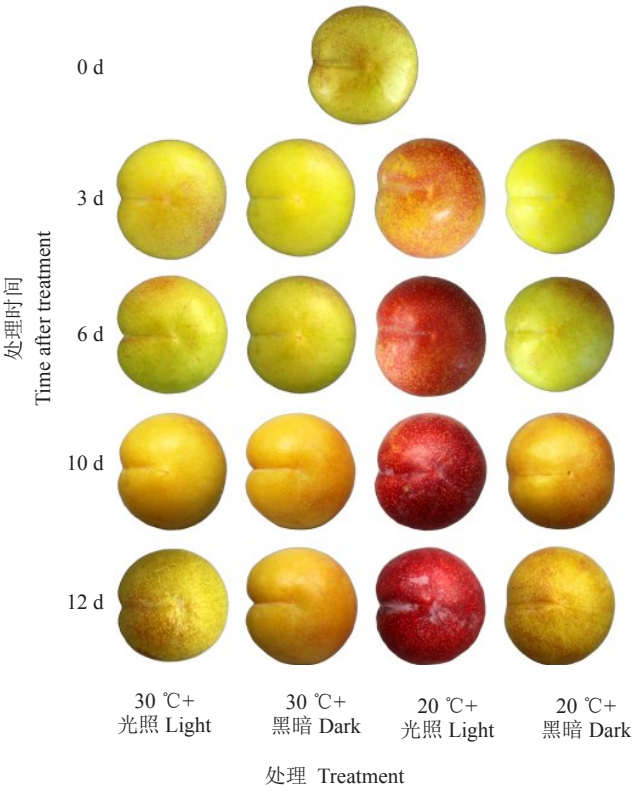

图1 温度和光照对采后‘秋姬李’果皮着色的影响

Fig. 1 Effects of temperature and light on pigmentation in the skin of postharvest ‘Akihime’ plum fruits

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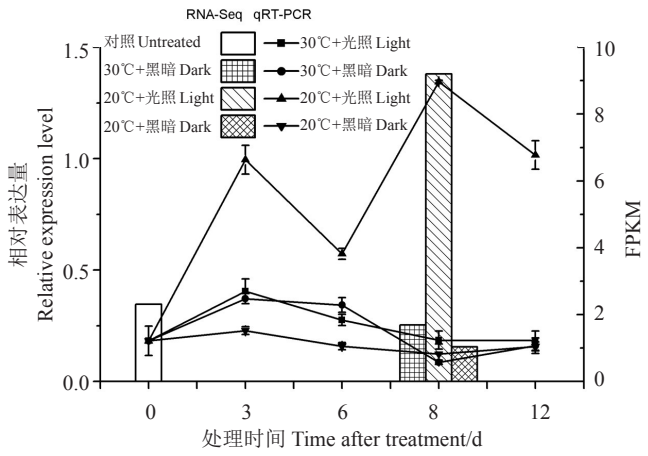

图 2　不同温度和光照处理条件下‘秋姬李’果皮*PsMYB18*的表达分析

Fig. 2 Expression analysis of *PsMYB18* in ‘Akihime’ plum fruit peel under different temperature and light treatments

2.2　‘秋姬李’果皮*PsMYB18*克隆与序列分析

为了进一步研究*PsMYB18*在温度和光照调控采后‘秋姬李’果皮积累花色苷过程中的作用，采用RT-PCR从‘秋姬李’果皮中分离得到了*PsMYB18*的ORF序列（GenBank登录号：MK284223），长度为702 bp，编码框编码233个氨基酸。利用MEGA5.02软件将‘秋姬李’PsMYB18蛋白序列与拟南芥、苹果、桃、梨、草莓和葡萄等物种的MYB蛋白序列进行比对，并构建进化树。结果表明，PsMYB18与FaMYB1、MtMYB2、PtMYB182、VvMYBC2- L3、VvMYBC2-L1和PpMYB18等花色苷和原花色素合成抑制因子属于同一个分支，且与PpMYB18的亲缘关系最近（图3）。序列比对分析结果表明，PsMYB18与花色苷合成抑制因子PpMYB18的序列相似性高达96.57%，含有保守的R2和R3结构域，且其C端含有与PpMYB18完全一致的保守抑制基序C1和C2，但无C3、C4和C5等基序（图4）。以上结果表明，*PsMYB18*可能是花色苷合成抑制因子。

2.3　*PsMYB18*的功能验证

为了进一步研究*PsMYB18*的功能，构建植物超表达载体，采用烟草叶片瞬时表达验证*PsMYB18*对花色苷合成的调控作用。如图5所示，烟草叶片注射*PsMYB18*或*PsMYB18+PsbHLH3*均不能诱导烟草叶片着色。注射花色苷合成正调控因子*PsMYB10.1+PsbHLH3*的烟草叶片呈红色，而注射*PsMYB10.1+PsbHLH3+PsMYB18*的烟草叶片保持绿色。以上结果表明*PsMYB18*对花色苷合成有抑制作用。

3　讨　论

MYB是一类在植物次生代谢等生物学过程中发挥重要作用的转录调控因子[35]。本研究从‘秋姬李’果皮中分离了在20 ℃/光照处理诱导‘秋姬李’果皮积累花色苷过程中高表达的*PsMYB18*基因。进化树分析结果表明，PsMYB18与其他植物的花色苷合成抑制因子亲缘关系较近，且序列比对分析显示有保守的抑制基序C1和C2，暗示PsMYB18可能负调控花色苷合成。烟草叶片瞬时表达实验进一步验证了PsMYB18对花色苷合成的抑制作用。

采后‘秋姬李’果皮中*PsMYB18*在20 ℃/光照处理条件下随花色苷积累而上调表达，而在30 ℃/光照、30 ℃/无光和20 ℃/无光处理等不能诱导采后‘秋姬李’果皮积累花色苷的处理条件下*PsMYB18*的表达量没有显著变化，这表明*PsMYB18*可能与20 ℃/光照处理诱导采后‘秋姬李’果皮积累花色苷有关。然而，系统进化分析结果表明，PsMYB18和其他植物的MYB抑制因子均属于R2R3-MYB家族的第四亚组，同属于该亚组的FaMYB1[25]、MtMYB2[20]、PtMYB182[28]、VvMYBC2- L1[16]、VvMYBC2-L3[36]和PpMYB18[17]均为花色苷和原花青素合成抑制因子。序列分析结果表明，PsMYB18与近缘物种桃PpMYB18的序列高度相似，且带有R2R3-MYB家族的第四亚组蛋白的特征结构域R2R3结构域和抑制基序C1和C2。这些结果表明PsMYB18可能为花色苷抑制因子。瞬时表达*PsMYB18*不能诱导烟草叶片积累花色苷，且*PsMYB18*与正调控因子*PsMYB10.1*和*PsbHLH3*共表达可抑制*PsMYB10.1*和*PsbHLH3*诱导烟草叶片积累花色苷。该结果进一步证明了PsMYB18对花色苷合成的负调控作用。类似地，*PtMYB182*[28]和*MtMYB2*[20]的超表达也可明显地抑制花色苷合成。有趣的是，*PsMYB18*的表达与采后‘秋姬李’果皮花色苷积累正相关。这与其他植物MYB抑制因子的表达模式完全相反。研究表明，拟南芥*AtMYBL2*[37]、矮牵牛花*PhMYB27*[38]和葡萄*VvMYB4-like*[39]等花色苷抑制因子的表达均与花色苷积累呈负相关。值得注意的是，草莓、苜蓿、杨树和桃等植物上也报道了MYB抑制因子的表达与花色苷积累呈正相关的情

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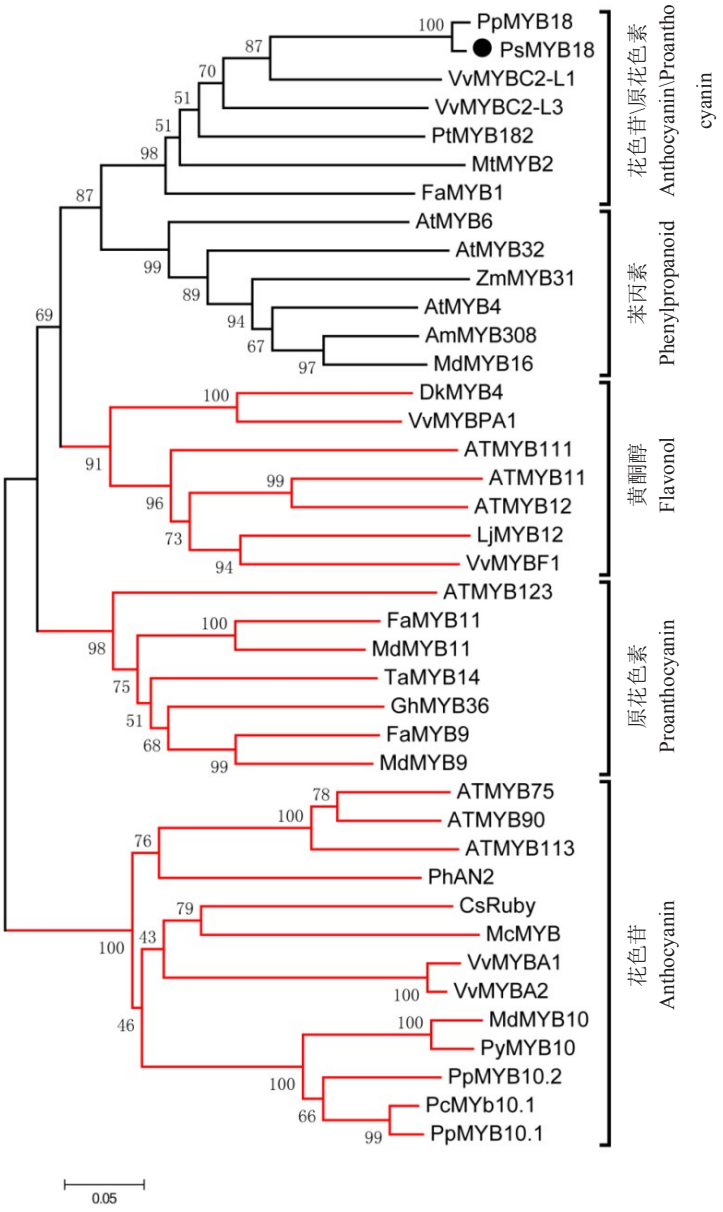

其他植物的 R2R3-MYB 序列包括 AtMYB6(Q38851)、ATMYB11(NP_191820)、ATMYB12(NP_182268)、ATMYB75(NP_176057)、ATMYB90(NP_176813)、ATMYB113(NP_176811)、AtMYB123(NP_198405)、AmMYB308(P81393)、CsRuby(NM_001288889)、DkMYB4(AB503701)、FaMYB1(AF401220)、FaMYB9(JQ989281)、FaMYB11(JQ989282)、GhMYB36(AF336284)、LjMYB12(AB334529)、McMYB(MH383068)、MdMYB9(DQ267900)、MdMYB10(EU518249)、MdMYB11(DQ074463)、MdMYB16(HM122617)、MtMYB2(XM_003616340)、PcMYB10.1(KP772281)、PhAN2(AB982128)、PpMYB10.1(XM_007216468)、PpMYB10.2(XM_007216161)、PpMYB18(KT159234)、PtMYB182(KP723392)、PyMYB10(GU253310)、TaMYB14(JN049641)、VvMYBF1(GQ423422)、VvMYBPA1(AM259485)、VvMYBC2- L3(KM046932)、VvMYBC2- L1(JX050227)、VvMYBA1(BAD18977)和 VvMYBA2(BAD18978)。红色线表示正调控因子，黑色线表示负调控因子。

Additional plant R2R3-MYB sequences include AtMYB6(Q38851), ATMYB11(NP_191820), ATMYB12(NP_182268), ATMYB75(NP_176057), ATMYB90(NP_176813), ATMYB113(NP_176811), AtMYB123(NP_198405), AmMYB308(P81393), CsRuby(NM_001288889), DkMYB4(AB503701), FaMYB1(AF401220), FaMYB9(JQ989281), FaMYB11(JQ989282), GhMYB36(AF336284), LjMYB12(AB334529), McMYB(MH383068), MdMYB9(DQ267900), MdMYB10(EU518249), MdMYB11(DQ074463), MdMYB16(HM122617), MtMYB2(XM_003616340), PcMYB10.1(KP772281), PhAN2(AB982128), PpMYB10.1(XM_007216468), PpMYB10.2(XM_007216161), PpMYB18(KT159234), PtMYB182(KP723392), PyMYB10(GU253310), TaMYB14(JN049641), VvMYBF1(GQ423422), VvMYBPA1(AM259485), VvMYBC2- L3(KM046932), VvMYBC2-L1(JX050227), VvMYBA1(BAD18977) and VvMYBA2(BAD18978). Red lines indicate positive regulators , black lines indicate negative regulators.

图 3 PsMYB18 与其他植物 R2R3-MYB 的系统进化树分析

Fig. 3 Phylogenetic tree of PsMYB18 and R2R3-MYB proteins from other plants

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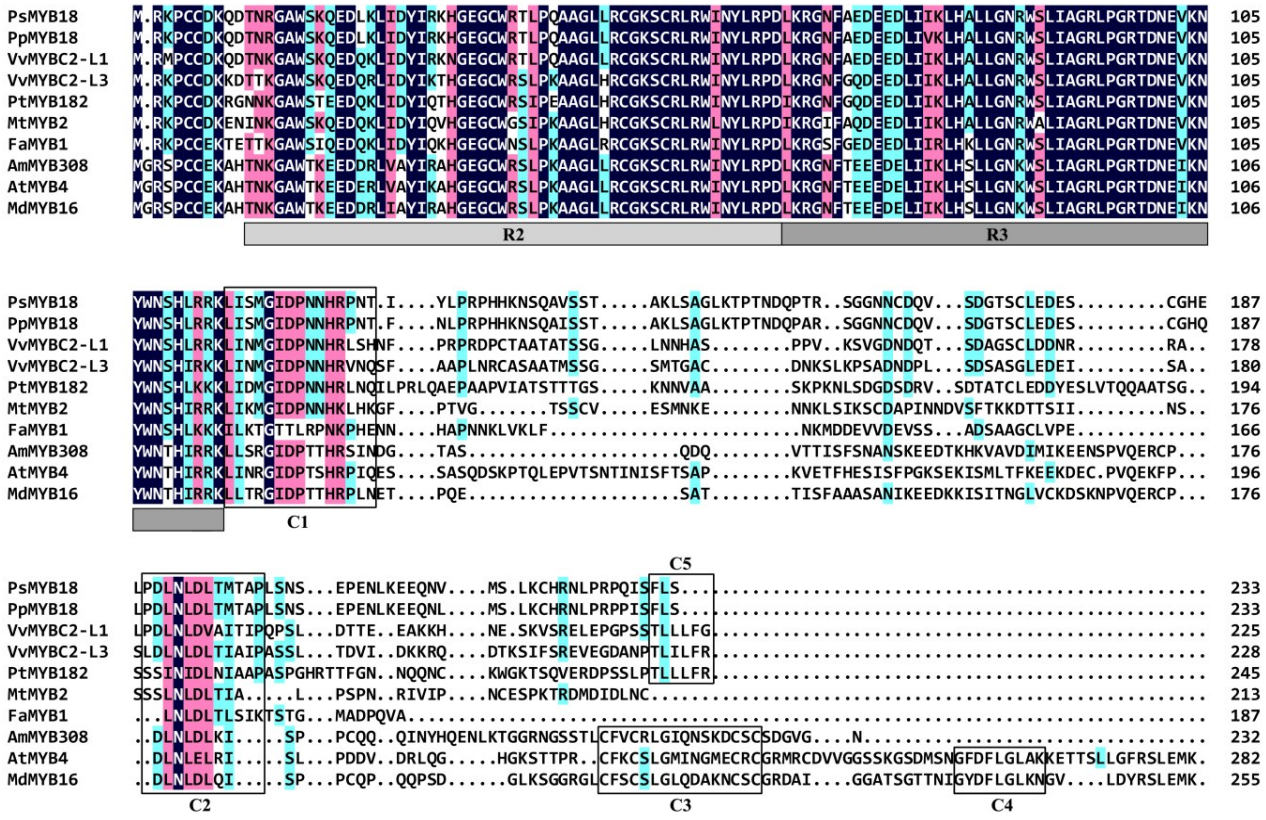

灰白色和灰色框表示 R2 和 R3 为保守结构域，C1~C5 为 C 端保守基序。

The R2 and R3 domains are indicated with greyish white and gray boxes，respectively. C1-C5 indicated conserved motifs in the C-terminus.

图 4　PsMYB18 与其他植物类黄酮相关 MYB 抑制因子的多重序列比对

Fig. 4　Multiple alignment of PsMYB18 with other known flavonoid-related MYB repressors in plants

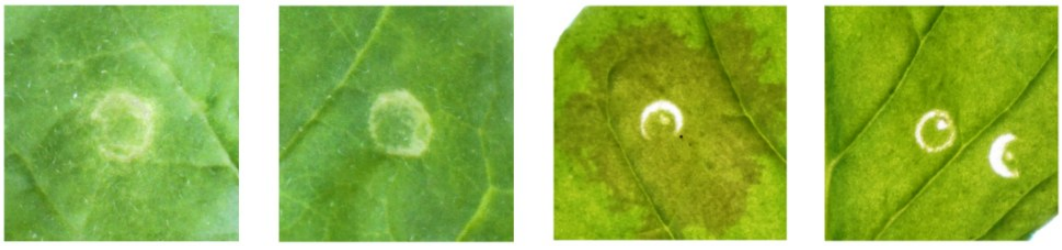

图 5　*PsMYB18* 基因的功能分析

Fig. 5　Functional analysis of the peach *PsMYB18* gene

况[25，40-41]。最近，Zhou 等[17]在桃上也发现了类似的情况。他们发现 *PsMYB18* 的同源基因 *PpMYB18* 在成熟红肉桃果肉中高表达，而在黄肉桃和白肉桃果肉中几乎不表达。他们通过双荧光素酶实验进一步研究发现 *PpMYB18* 的表达受花色苷和原花青素的正调控因子 PpMYB10.1 和 PpMYBPA1 诱导。花色苷和原花青素的 MYB 正调控因子诱导 MYB 抑制因子的情况在其他植物上已有报道[20，36]。Zhou 等[17]认为 MYB 正调控因子诱导抑制因子 *PpMYB18* 表达是桃果实维持花色苷和原花色苷积累的动态平衡的一种转录调节机制，用于防止花色苷和原花色苷的过度积累。PsMYB18 与桃 PpMYB18 序列高度相似，意味着二者可能通过相同的方式发挥作用。因此，笔者推测 *PsMYB18* 的表达与采后‘秋姬李’果皮花色苷积累呈正相关可能是由于其表达受花色苷合成正调控 MYB 诱导。但 *PsMYB18* 的表达是否受花色苷合成正调控 MYB 调控及其作用机制还有待进一步研究。此外，Zhou 等[17]并未通过果实基因沉默

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实验确定抑制*PpMYB18*基因表达是否可提高红肉桃中花色苷含量。因此，抑制*PsMYB18*的表达是否能够促进花色苷合成，提高李果实的花色苷含量也有待进一步探讨。

4 结 论

通过基于比较转录组分析结果克隆了R2R3-MYB基因*PsMYB18*，并对其表达模式、进化关系、序列特征和功能进行了分析，结果表明*PsMYB18*为花色苷合成抑制因子。该研究结果可为进一步剖析MYB抑制因子在李果实花色苷合成过程中的功能与作用机制提供参考。

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