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刺葡萄R2R3-MYB转录因子*VdMYB14*调控类黄酮合成功能解析

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摘 要【目的】类黄酮作为葡萄果实中一类重要次生代谢物质,进一步研究其合成调控机制对于提高果实品质具有重要意义。**【方法】**结合前期研究基础,以会同黑果刺葡萄(*Vitis davidii* '1338')为试材,通过qRT-PCR分析*VdMYB14*在6个果实不同发育阶段果皮中的表达水平变化。利用MEGA软件构建系统发育树,分析VdMYB14蛋白与其他类黄酮相关MYB蛋白的系统发育关系。利用亚细胞定位技术分析VdMYB14在细胞中的位置。在烟草中异源表达*VdMYB14*基因,验证其对类黄酮合成的调控功能。**【结果】**VdMYB14蛋白与苹果花色苷合成负调控因子MdMYB111同源度较高,亚细胞定位发现VdMYB14定位在细胞核中。与野生型相比,*VdMYB14*转基因烟草株系的花中原花色素含量增加,花色苷积累减少。过表达*VdMYB14*烟草花中,原花色素合成关键基因*NtLAR*和*NtANR*的表达量显著上调,而花色苷合成关键基因*NtUFGT*的表达量显著下调。**【结论】***VdMYB14*基因能够促进原花色素合成途径关键基因的表达,抑制花色苷合成关键基因的表达,导致类黄酮前体物质倾向于原花色素合成途径,从而抑制花色苷合成,促进原花色素积累。

关键词:葡萄;花色苷;原花色素;R2R3-MYB转录因子;*VdMYB14*

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Functional analysis of *Vitis davidii* R2R3-MYB transcription factor *VdMYB14* in the regulation of flavonoid biosynthesis

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Abstract:【Objective】Flavonoids are important secondary metabolites in grapes and are associated with the sensory characteristics of red wine such as color and astringency. The flavonoid pathway is regulated by the MBW transcription complex at the transcriptional level. In this complex, the MYB transcription factor is a decisive regulator that positively or negatively regulates various structural genes in the flavonoid pathway to maintain the balance in flavonoid content in different plant organs. Clustering analysis and functional enrichment analysis of differentially expressed genes were carried out based on the transcriptome data of grapes and the *MYB* gene *VdMYB14* was selected for participation in the regulation of anthocyanin synthesis. In this study, we analyzed the structure and function of the MYB transcription factor to further elucidate its role in regulating anthocyanin synthesis in grapes. This analysis coul contribute to in-depth understanding of the mechanism of flavonoid synthesis and improving fruit quality.【Methods】Berries skins of huitong black spine grapes (*Vitis davidii* '1338') in 6 developmental stages after flowering were used as experimental materials. qRT-PCR were used to analyze the expression of the *VdMYB14*. The plant total RNA rapid extraction kit was used to extract the total RNA

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from the samples. The PrimeScript™ RT kit with DNAase was used to remove contaminated genomic DNA and to carry out reverse transcription for cDNA synthesis. The plant anthocyanin content measurement kit (Solarbio) and plant proanthocyanidin content measurement kit (Solarbio) were used to measure anthocyanin and proanthocyanidin content, respectively. The MEGA software was used to analyze the phylogenetic relationship between the VdMYB14 protein and the other flavonoid-related MYB. Homologous recombination was used to insert the *VdMYB14* gene in the PBI121 vector and tobacco leaves were transfected. The expression position of the VdMYB14 protein in the cells was observed. Heterologous expression was carried out in the tobacco to validate whether gene *VdMYB14* could regulate anthocyanin synthesis.【Results】qRT-PCR results showed that the gene *VdMYB14* expression level increased in the early stage and reached its peak 40 days after flowering. Subsequently, its expression level decreased, which wass consistent with the previous transcriptome results. The subcellular localization results showed that the VdMYB14 was mainly localized in the nucleus and only a few VdMYB14 were localized in the cytoplasm. The phylogenetic tree results showed that the protein VdMYB14 was highly homologous to the VvMYB14 and the VvMYB15 from grapes (*Vitis vinifera* L.). Furthermore, these three genes and the MdMYB111 from apples were located at the same branch, indicating that the VdMYB14 and theMdMYB111 had a similar function. In the identification of the petal color of tobacco, the degree of petal color of the transgenic lines was significantly lighter than that of the wild type, and there were differences between different transgenic lines. Measurements of anthocyanin content in the transgenic tobacco showed that anthocyanin content in the petals of transgenic tobacco was significantly lower than that in the wild-type plants. However, the proanthocyanidin measurement results showed that the proanthocyanidin content in the petals of the transgenic tobacco was higher than that in the wild-type. The overexpression of the gene *VdMYB14* in tobacco affected the expression of the structural genes in the flavonoid pathway. Compared with the wild-type, the expression levels of the *NtCHI*, *NtDFR*, *NtLAR* and *NtANR* in the *VdMYB14*-overexpressing tobacco petals were significantly upregulated. Among them, the difference in the expression levels of *NtLAR* and *NtANR* was the highest although there were no significant changes in the expression levels of the *NtCHI*, *NtCHS* and *NtF3H*. However, the *NtUFGT* expression in the transgenic tobacco petals was lower than that in the wild-type petals. However, in the *VdMYB14*-overexpressing tobacco, the expression levels of the *NtANR* and *NtLAR* were significantly upregulated, and the expression level of the structural gene *NtUFGT* of the anthocyanin synthesis pathway decreased. This would cause more leucoanthocyanidin and anthocyanidin to enter the proanthocyanidin pathway, thereby inhibiting the synthesis of anthocyanin. This showed that the *VdMYB14* gene might regulate the transfer of the anthocyanin pathway to proanthocyanidin pathway, thereby inhibiting anthocyanin synthesis and affecting the the petal color of tobacco. In this study, the expression level of the *VdMYB14* was high in the early stage of grape berry development. As the fruit entered the veraison, its expression gradually decreased. This indicated that the gene *VdMYB14* might inhibit the accumulation of anthocyanins in the early stage of berry development.【Conclusion】This study demonstrated the effect of the grape *VdMYB14* in regulating anthocyanin and proanthocyanidin synthesis. VdMYB14 overexpression in tobacco significantly affected the expression of structural genes in the flavonoid pathway, particularly key structural genes in the proanthocyanidin pathway and anthocyanin pathway, causing intermediate products from the anthocyanin pathway to enter the proanthocyanidin pathway.

Key words: Grapevine; Anthocyanin; Proanthocyanidin; R2R3-MYB transcription factor; *VdMYB14*

葡萄果实类黄酮主要包括花色苷和原花色素。花色苷与果实颜色密切相关，果皮中花色苷的含量和组分决定了果实颜色[1]。原花色素赋予水果的涩味和苦味，是影响水果品质的重要因素之一[2]。此外，花色苷和原花色素广泛存在于葡萄的茎、花、果实和种子等器官[3]，能够提高植物抵御紫外线和病原体等胁迫的能力[4-5]。作为葡萄果实中重要的代谢物质，花色苷和原花色素不仅对葡萄的抗逆性有重要作用，而且在人体抗氧化、抗突变及抗肿瘤等方面也有一定的药理活性[6-7]。因此，科研人员在植物花色苷和原花色素的合成和调控等方面开展了广泛的研究。

类黄酮途径有两个重要分支，分别为花色苷和原花色素途径。在类黄酮途径的上游，共同的前体物质由查尔酮异构酶（CHI）、类黄酮 3’-羟化酶（F3’H）、二氢黄酮醇 4-还原酶（DFR）、无色花色素双加氧酶（ANS）等酶催化完成。UDP-葡萄糖类黄酮 3-*O*-葡萄糖基转移酶（UFGT）是花色苷合成的关键酶。无色花色素还原酶（LAR）和花色素还原酶（ANR）是原花色素合成途径的关键酶，使无色花青素和花色素转向原花色素途径，催化原花色素的合成[8-10]。类黄酮途径在转录水平上受 MBW 转录复合体（MYB-bHLH-WD40）的调控[11]。其中，MYB 转录因子是 MBW 复合体中决定性的调节因子[12]，含有 1~4 个不完全重复单元（R），对类黄酮途径中结构基因进行正向或负向调节，维持不同植物器官中类黄酮含量平衡[13]。苹果 *MdMYB1*、*MdMYB10*[14-15]、桃 *PpMYB10*[16]、葡萄 *VvMYBA1*、*VvMYBA2*、*VvMYBPA2*[17-18]等，属于正调控 MYB 因子，提高类黄酮途径中结构基因转录水平，促进花色苷和原花色素合成。拟南芥 *AtMYB123*[19]、草莓 FaMYB1[20]、桃 PpMYB18[21]、葡萄 *VvMYBC2-L3*、*VvMYBC2-L2*[22-23]、苹果 *MdMYB16*、*MdMYB15L*[24-25]等为负调控 MYB 因子，通过与 MBW 复合体竞争 bHLH 转录因子，阻遏复合体的形成，或直接结合到结构基因启动子，抑制结构基因的表达，从而抑制花色苷的合成。

基于前期刺葡萄果皮转录组数据，通过差异表达基因聚类与功能富集分析等方法，筛选到一个 MYB 基因（*VdMYB14*），其表达模式与果实中花色苷的积累趋势呈负相关[26]，推测其在花色苷合成中可能发挥负调控作用。笔者通过分析该 MYB 转录因子的结构和功能，进一步明确其在葡萄中对花色苷和原花色素合成的调控作用，对开展葡萄果实中类黄酮物质的合成调控研究具有重要意义。

1　材料和方法

1.1　实验材料

以中国农业科学院郑州果树研究所国家果树种质郑州葡萄圃的湖南会同黑果刺葡萄（*Vitis davidii* ‘1338’）为试材，分别于花后 20、40、60、80、100 和 120 d 采集浆果，每次取 3 穗果实，每穗 20 粒浆果混匀，3 次生物学重复。剥取新鲜果皮液氮冷冻后置于−80 ℃冰箱保存备用。

烟草‘NC 89’（*Nicotiana tabacum*）和本氏烟草（*Nicotiana benthamiana*）种植于 16 h 光照、8 h 黑暗的温室中。

1.2　葡萄果皮中花色苷含量检测

花色苷提取：取 0.5 g 冷冻研磨的果皮粉于 8 mL 2%的甲酸甲醇溶液中，混匀后，超声提取 10 min，200 r·min^{-1} 摇床避光震荡 30 min，然后 4 ℃、12 000 r·min^{-1} 离心 10 min，收集上清液。上述步骤 2 次重复，收集 3 次提取的上清液于旋转蒸发器中旋转蒸干，用 10 mL 0.1%盐酸甲醇溶液定容，经 0.22 μm 有机滤膜过滤后于 10 mL 离心管避光保存。每个样品 3 次重复。花色苷含量测定参照翦祎等[27]的方法。

1.3　RNA提取、反转录和荧光定量PCR

使用植物总 RNA 快速提取试剂盒（华越洋，北京）提取样品的总 RNA，PrimeScript™ RT with gDNA Eraser 试剂盒（TaKaRa，大连）去除基因组 DNA 污染及反转录合成 cDNA，具体合成方法参照试剂盒说明书。反转录得到的 cDNA 保存于−20 ℃冰箱。使用 SYBR Green Master Mix 试剂盒（Roche，巴塞尔）进行 qRT-PCR，设置 3 次生物学重复。参考 Pérez-Díaz 等[13]的报道选择内参基因，利用 $2^{-\triangle\triangle Ct}$ 法计算基因相对表达量。表 1 中列出了用于 qRT-PCR 的引物序列。

1.4　基因克隆及系统发育分析

参考葡萄基因组中目的基因的 CDS 序列，使用 Premier 5.0 软件设计特异性引物，（上游引物 5’-ATGGGGAGAGCTCCATGTTGT-3’；下游引物 5’-TCATATTTCTGATAATTCATGCAACTCC-3’），并由生工生物工程（上海）股份有限公司合成。使

用高保真酶 KOD-Plus-Neo（Toyobo，北京）进行 PCR 扩增，反应体系和程序均按照说明书步骤进行，PCR 产物回收并连接至 T 载体，转化至大肠杆菌感受态后，挑取单克隆送至上海生物工程公司测序验证。从 UniProt 数据库（https://www.uniprot.org/）下载类黄酮物质合成的相关 MYB 蛋白序列，使用 MEGA 7.0 软件构建系统发育树，进行系统发育分析。

表1 qPCR引物列表[13]

Table 1 qPCR primers list[13]

基因名称 Gene name	引物名称 Primer name	长度 Length/bp	引物序列(5'-3') Primer sequences (5'-3')	退火温度 Annealing temperature/℃
NtActin	NtActin-F	20	AATGATCGGAATGGAAGCTG	56
	NtActin-R	20	TGGTACCACCACTGAGGACA	
NtCHS	NtCHS-F	20	AGAAAAGCCTTGTGGAAGCA	56
	NtCHS-R	20	CTTGGTCCAAAATTGCAGG	
NtCHI	NtCHI-F	20	GAAATCCTCCGATCCAGTGA	56
	NtCHI-R	20	CAACGTTGACAACATCAGGC	
NtF3H	NtF3H-F	20	ACAGGGTGAAGTGGTCCAAG	56
	NtF3H-R	20	CCTTGGTTAAGGCCTCCTTC	
NtDFR	NtDFR-F	20	TCCCATCATGCGATCATCTA	57
	NtDFR-R	20	ATGGCTTCTTTGTCACGTCC	
NtANS	NtANS-F	20	TGGCGTTGAAGCTCATACTG	56
	NtANS-R	20	GGAATTAGGCACACACTTTGC	
NtFLS	NtFLS-F	20	GAACTTGAAGGGAAAAGGGG	56
	NtFLS-R	20	TCCCTGTAGGAGGGAGGATT	
NtLAR	NtLAR-F	20	TCAAGGTCCTTTACGCCATC	58
	NtLAR-R	20	ACGAACCTGCTTCTCTTTGG	
NtANR	NtANR-F	20	CATTTGACTTTCCCAAACGC	58
	NtANR-R	20	ATTGGGCTTTTGAGTTGTGC	
NtUFGT	NtUFGT-F	20	GAGTGCATTGGATGCCTTTT	56
	NtUFGT-R	20	CCAGCTCCATTAGGTCCTTG	
VdMYB14	VdMYB14-F	20	GTCATCCAACAAATCCGGCG	56
	VdMYB14-R	20	ACGCGGAGAGAATGGAAACT	

1.5 亚细胞定位及烟草遗传转化

在载体 PBI121-35S-GFP 选择酶切位点（*Bam* HⅠ和 *Xba*Ⅰ）进行双酶切，在酶切位点处设计特异性引物 VdMYB14-PBI121-F 和 VdMYB14-PBI121-R，使用莫奈公司的无缝克隆试剂盒，将目的基因构建到 PBI121 载体克隆位点，PCR 鉴定为阳性后保存。以 PBI121-35S-GFP 空载为对照，参照欧阳梦真等[28]的方法进行烟草下表皮注射和亚细胞定位观察。采用同源重组方法将 *VdMYB14* 基因构建到 PBI121-35S 载体并进行阳性鉴定，参考徐琳等[29]的方法进行叶盘法转化烟草和植株再生，转基因烟草经抗生素筛选和 PCR 鉴定获得阳性植株，移栽到温室，观察表型变化。

1.6 烟草花中花色苷和原花青素含量检测

取完全盛开的烟草花瓣，在液氮中研磨之后，使用植物花色苷含量检测试剂盒（Solarbio）和植物原花青素含量检测试剂（Solarbio），提取花色苷和原花青素并检测含量，所有样品设置 3 个独立的生物学重复。

1.7 数据分析

数据统计使用单因素方差分析对每个变量进行 Tukey's 检验（$p < 0.05$），数据以平均值±标准差（SD）表示。

2 结果与分析

2.1 果皮总花色苷含量测定及 *VdMYB14* 基因表达分析

果皮中总花色苷在果实发育前期含量低，花后 40 d 内基本没有花色苷积累。随着果实生长发育，花色苷含量逐渐升高，花后 60 d 有少量花色苷积累，花后 80 d 花色苷开始大量积累，花后 120 d 花色苷含量达到最大值。而 *VdMYB14* 基因在早期的表达水平较高，从花后 80 d 开始，表达水平逐渐降低，与花色苷的积累趋势相反（图 1）。

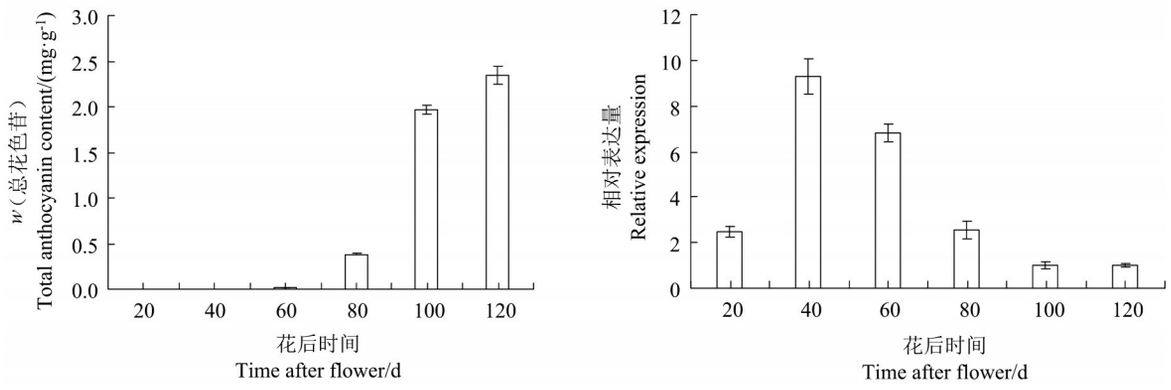


图1　果皮中总花色苷含量变化及*VdMYB14*表达分析

Fig. 1　Change of total anthocyanin content in berry skin and analysis of *VdMYB14* expression

2.2　果皮中*VdMYB14*基因的克隆

与参考序列相比，刺葡萄*VdMYB14*基因的CDS区的47、362、401、427、577、584和683位有7个碱基变化，在518~523区间有连续6个碱基（ACCACC）缺失（图2-a）。蛋白质序列中134、143、193和195位有4个氨基酸差异，在173位有2个氨基酸（TT）缺失（图2-b）。

a. 核酸序列分析；b. 蛋白序列分析。

a. Nucleic acid sequence analysis; b. Protein sequence analysis.

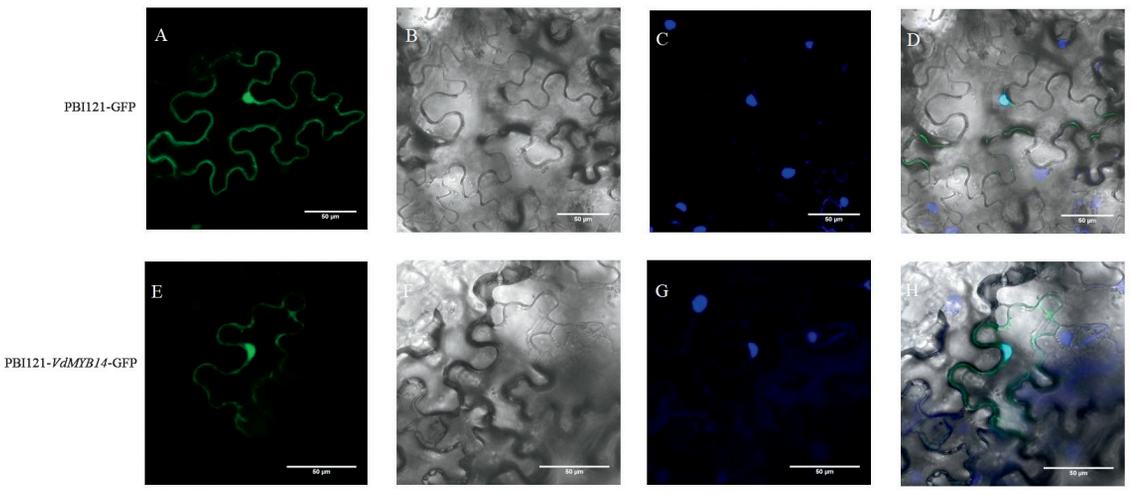
图2　基因克隆结果分析

Fig. 2　Analysis of gene cloning results

2.3　VdMYB14亚细胞定位及系统发育分析

将PBI121-*VdMYB14*-GFP转化烟草叶片中，转化PBI121-GFP空载体为对照，激光共聚焦显微镜下观察，PBI121-GFP在细胞核和细胞质均能发出的绿色荧光，细胞核中的绿色荧光与DAPI激发的蓝色荧光重合，说明转化试验方法满足试验要求。转化PBI121-*VdMYB14*-GFP的烟草叶片荧光信号主要集中在细胞核中，且定位于细胞核的荧光与DAPI发的蓝色荧光重合（图3），这表明VdMYB14主要在细胞核中表达，为转录因子。

使用MEGA软件，采用邻接法（Neighbor-Joining）构建系统发育树（图4）。系统发育树有两个明显分支，苹果MdMYB10、葡萄VvMYBA2等MYB转录因子分支与草莓FaMYB1、葡萄VvMYBC2-L3等MYB转录因子分支。刺葡萄VdMYB14与欧亚种葡萄VvMYB14、VvMYB15具有很高同源度。并且VdMYB14与苹果MdMYB111具有较高的同源度，推测VdMYB14与MdMYB111在花色苷合



A~D. 转入 PBI121-GFP 空载体的烟草细胞；D~H. 转入 VdMYB14-GFP 融合蛋白的烟草细胞；A、E 为荧光激发图，B、F 为明场图；C、G 为 DAPI 染色图；D、H 为叠加图。

A-D. Tobacco subleaf epidermis cells carrying empty PBI121-GFP vector; D-H. Tobacco subleaf epidermis cells carrying carrying VdMYB14-GFP fusion protein; A and E were fluorescence excitation field; B and F were shot in bright field; C and G were shot in DAPI staining; D and H were overlays field.

图3 VdMYB14蛋白在烟草表皮细胞中的亚细胞定位

Fig. 3 Subcellular localization of VdMYB14 in tobacco epidermal cells

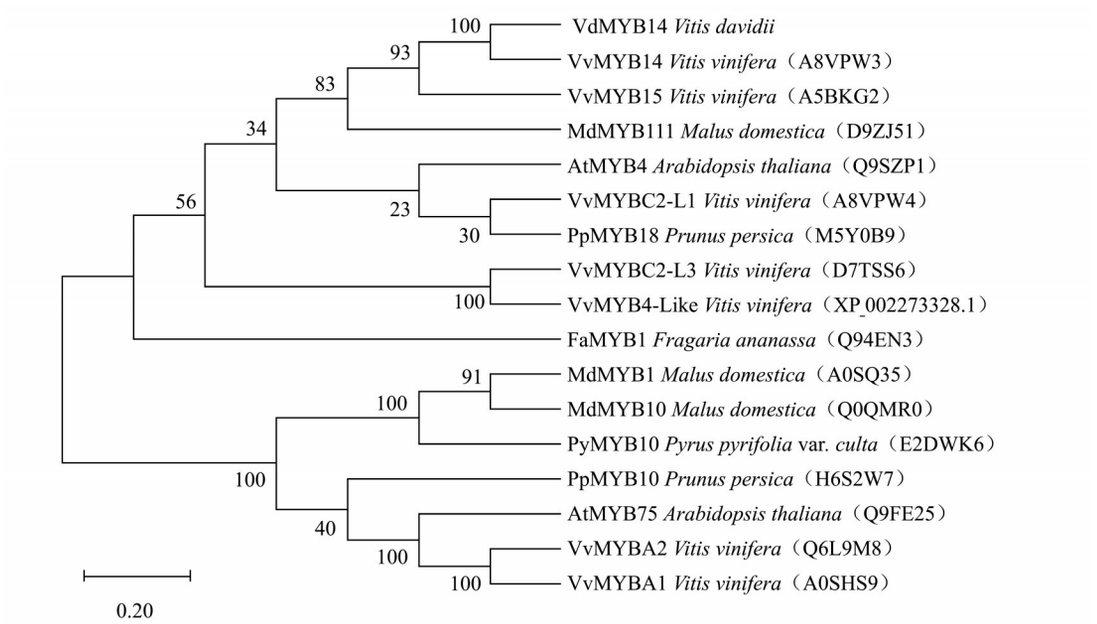


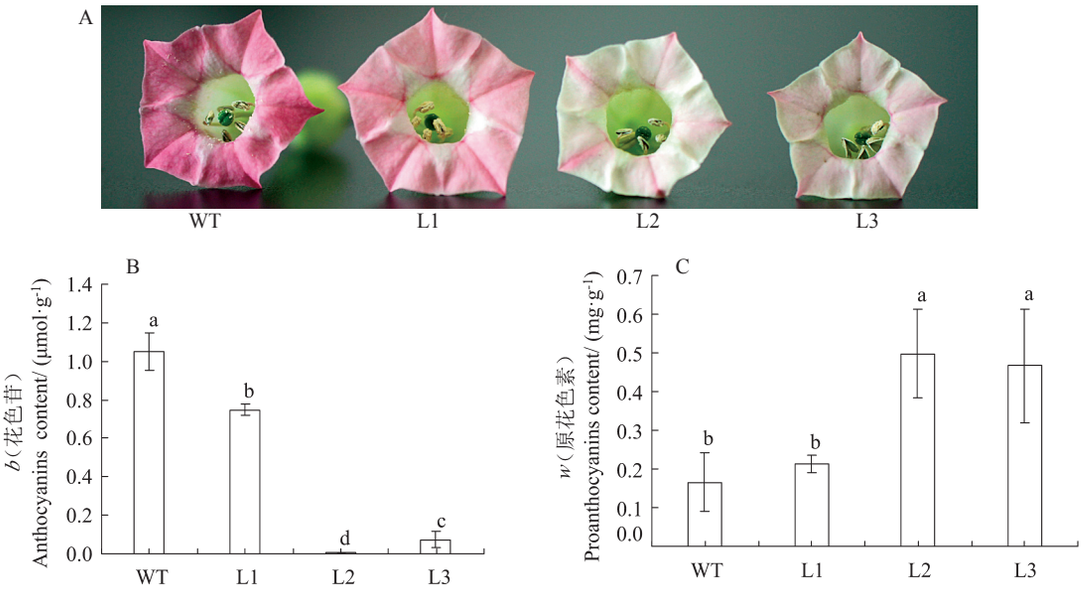
图4 VdMYB14与其他植物MYB转录因子的系统发生关系

Fig. 4 Phylogenetic relationship of VdMYB14 with other plant MYB transcription factors

成中具有相似的调控功能。

2.4 VdMYB14基因影响烟草花中花色苷和原花色素的积累

鉴定野生型和不同转基因植株盛花期的烟草花瓣颜色。野生型烟草花瓣盛开时为红色，转基因烟草在生长发育过程中，花瓣的着色程度明显低于野生型，到盛花期时花色与野生型差异明显，甚至出现近白色花瓣，并且不同转基因植株之间的花色深浅也存在差异（图 5-A）。检测野生型和转基因烟草花中花色苷和原花色素含量发现，转基因植株花瓣花色苷含量明显低于野生型烟草（图 5-B），但是原花色素的含量高于野生型烟草（图 5-C）。



WT. 野生型；L1、L2、L3. 转基因植株的三个株系；A. 烟草花瓣表型；B. 总花色苷含量变化；C. 原花色素含量变化。不同小写字母表示在 $p < 0.05$ 水平上差异显著。下同。

WT. Wild type; L1, L2, L3. Three lines of transgenic plants; A. Tobacco petal phenotype; B. Change of Total anthocyanin content; C. Change of proanthocyanins content. Different small letters indicate significant differences at the 0.05 level. The same below.

图5　*VdMYB14*转基因烟草花瓣表型及其花色苷和原花色素含量

Fig. 5　Floral phenotypes, anthocyanin content and proanthocyanidin content of transgenic tobacco plants expressing *VdMYB14*

为了进一步阐明 *VdMYB14* 基因对烟草花中花色苷和原花色素合成的调控机制，利用 qRT-PCR 分析了类黄酮生物合成途径中部分结构基因的表达水平（图 6）。与野生型相比，过表达 VdMYB14 烟草花中多个类黄酮途径中结构基因的表达量出现显著差异。其中，上游结构基因 *NtCHI*、*NtDFR* 的表达量显著升高，而 *NtCHS*、*NtF3H* 和 *NtANS* 的表达水平未发生显著变化。过表达 *VdMYB14* 烟草花中，原花色素合成关键基因 *NtLAR* 和 *NtANR* 的表达量显著上调，而花色苷合成关键基因 *NtUFGT* 的表达量显著下调，表明 *VdMYB14* 转录因子能够促进原花色素的合成，抑制花色苷的积累。

3　讨　论

果实颜色对葡萄的商品价值具有重要影响，葡萄果实颜色主要由果皮中花色苷含量和组分决定[1]。MYB 转录因子在调控花色苷生物合成中发挥重要作用[12]。葡萄中，VvMYBA1 和 VvMYBA2 转录因子为葡萄果实花色苷合成的重要调控因子[30-31]。苹果中 MdMYB9、MdMYB1/MdMYB10、MdMYB11 等多个 MYB 转录因子参与花色苷的合成调控[32-33]。近些年，在葡萄中报道了多个对葡萄果实花色苷具有负调控作用的 MYB 转录因子。葡萄 VvMYB4-like 转录因子能够抑制烟草花中 *NtANS*、*NtDFR*、*NtUFGT* 等花色苷合成途径中结构基因的表达，从而抑制花色苷的积累，影响烟草花瓣的正常着色[13]。*VvMYBC2-L3* 在矮牵牛中异源表达后，能够与 bHLH 家族中的花色苷合成调控因子 PhAN1 蛋白结合，抑制 PhAN1 的转录调控作用，从而使花色苷合成关键基因 *CHS*、*DFR*、*UFGT* 的表达量出现下调，进而导致花中花色苷和原花色素的含量下降[22]。系统发育分析表明，VdMYB14 蛋白与苹果 MdMYB111 具有较高同源性，而 *MdMYB111* 基因能够抑制苹果愈伤组织花色苷的积累[34]，从而推测 *VdMYB14* 基因可能也参与花色苷合成的负调控过程。

此外，有研究表明，欧亚种葡萄 *VvMYB14* 与 *VvMYB15* 参与调控白藜芦醇的合成[35]。苯丙氨酸在 PAL、C4H 和 4CL 等一系列酶催化作用下完成苯丙烷途径，合成了 4-香豆酰-CoA。4-香豆酰-CoA 经 CHS 催化便进入类黄酮合成途径，而在 STS 的催化作用下则进入白藜芦醇合成途径[9, 36]。本研究主要关注花色苷合成的调控，因此只检测了类黄酮途径中结构基因的表达水平，但不排除刺葡萄

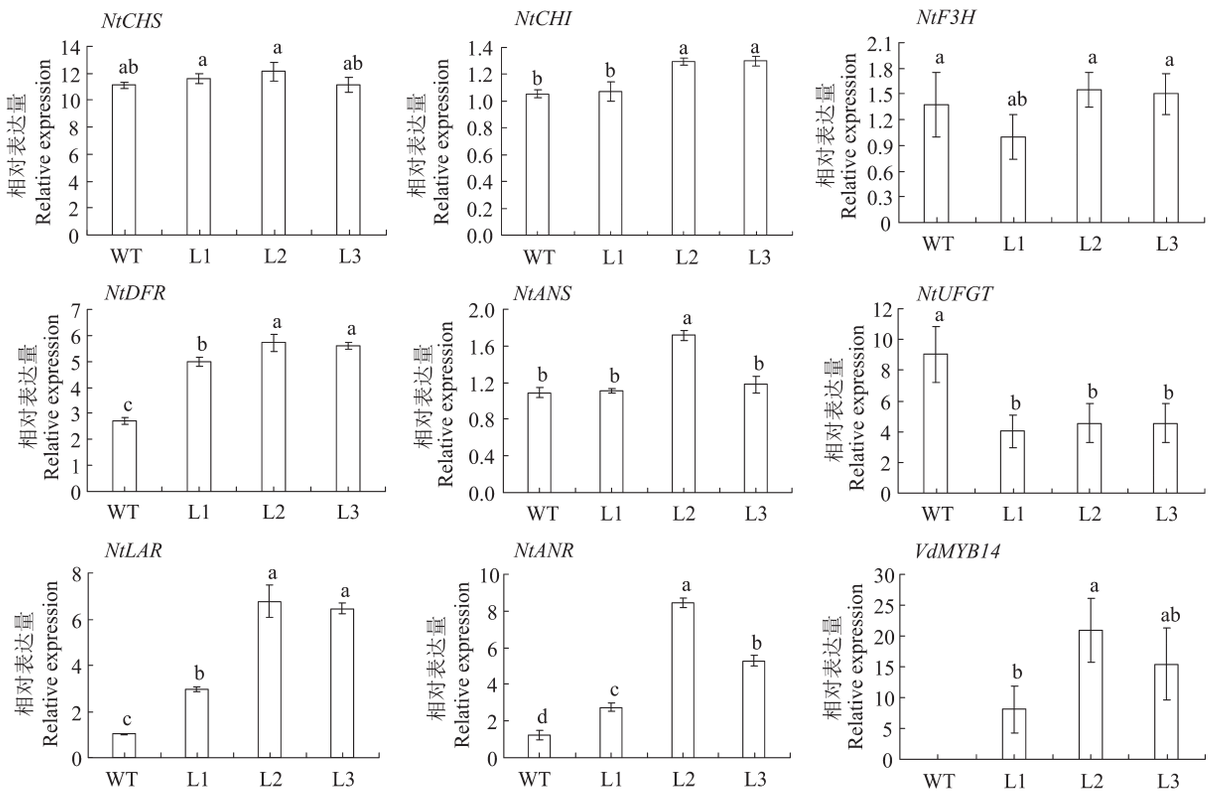


图6 定量分析野生型和转基因烟草花中类黄酮合成相关调控基因的转录水平

Fig. 6 Quantitative analyses of transcript levels of flavonoid-related biosynthetic genes in petals of wild-type and transgenic tobacco expressing *VdMYB14*

VdMYB14 基因也参与调控白藜芦醇的生物合成。实际上，一个 MYB 转录因子参与多个代谢途径的现象也早有报道。如苹果 *MdMYB10* 转录因子，不仅是果皮中花色苷合成的主要调节因子[37]，而且在苹果果皮中过表达 *MdMYB10* 基因后发现，*MdPAL*、*MdDFR*、*MdANS*、*MdFLS*、*MdANR* 等基因的表达水平显著升高，从而促进了香豆酸、绿原酸、花色苷、表儿茶素的积累[15]，表明 MYB 转录因子在植物中可以调控多个靶基因，参与多种物质的代谢过程[38]。刺葡萄 *VdMYB14* 基因与欧亚种葡萄 *VvMYB14* 基因同源性高达 98.4%，表明 *VdMYB14* 基因可能也参与葡萄白藜芦醇的合成调控，具体调控作用有待进一步研究。

本研究中，过表达 *VdMYB14* 基因能够促进原花色素合成途径特异性基因 *NtANR* 和 *NtLAR* 的表达，抑制花色苷合成关键基因 *UFGT* 的表达，导致中间产物无色花色素和花色素多用于原花色素的合成，使原花色素的含量升高，花色苷含量降低。有关此类调控方式，在葡萄上也已见报道。葡萄 *VvMYBPA1* 在烟草中过表达，能够促进转基因烟草花中原花色素合成，同时抑制花中花色苷积累[39]。有研究发现，其他植物中 MYB 基因也表现出与 *VdMYB14* 类似的调控方式。彩叶草 *SsMYB3* 通过调控类黄酮途径下游结构基因 *DFR*、*UFGT* 的表达，促进花色苷和原花色素合成过程中的共同中间产物向原花色素途径通量转移，从而抑制了花色苷的合成，但对该合成途径上游结构基因的表达水平没有明显影响[40]。在本研究中，*VdMYB14* 在葡萄果实发育的早期表达量较高，随着果实进入转色期，其表达水平逐渐减低，这说明 *VdMYB14* 在果实发育早期可能抑制了花色苷的积累，至于 *VdMYB14* 具体的调控机制仍需开展进一步研究进行阐释。

4 结 论

本研究探究了葡萄 *VdMYB14* 基因在调控花色苷和原花色素合成中的作用。结果表明，VdMYB14 转录因子能够促进原花色素合成关键结构基因的表达量，抑制花色苷合成关键结构基因的表达，导致类黄酮途径的中间产物多流向于原花色素途径，从而促进了原花色素的积累，抑制了花色苷的生物

合成。本研究对进一步探究葡萄果实类黄酮合成调控机制具有重要意义。

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