

草莓果实花色苷合成途径相关结构基因 及 *FaMYB10* 表达模式差异分析

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摘要:【目的】分析不同颜色草莓果实中花色苷合成相关结构基因与 *FaMYB10* 的表达差异, 以及 *FaMYB10* 转录调控的功能验证。【方法】以栽培草莓‘红颜’‘小白’和‘白雪公主’为试材, 利用 RNA-seq 技术筛选花色苷合成差异表达基因并进行 qRT-PCR 验证, 利用同源重组法构建 35S:: *FaMYB10* 过表达载体, 通过离体果实瞬时转化验证其在果实花色苷合成调控中的作用。【结果】从 3 个品种不同时期果实转录组数据中筛选到 8 个参与果实花色苷合成的候选基因, 包括 *FaPAL*、*FaCHI*、*FaCHS*、*FaDFR*、*FaANS*、*FaUFGT*、*Fa3GT* 和 *FaGST*, 这 8 个候选基因在‘红颜’和‘小白’成熟过程中表达量逐渐升高, 且均显著高于‘白雪公主’, 基因表达模式与 3 个品种果实花色苷的合成积累模式相一致。qRT-PCR 结果表明, 果实发育成熟期 *FaMYB10* 在‘白雪公主’和‘小白’果实中的表达量均显著高于‘红颜’, 其表达模式与转录水平变化一致。‘小白’*FaMYB10-XB* 基因编码区序列存在 2 种类型, 其中 *FaMYB10-XB-2* 插入序列 GA; ‘白雪公主’*FaMYB10-SW* 编码区序列具有 ACTTATAC 插入。果实瞬时过表达结果证明 *FaMYB10* 可增加花色苷的合成和积累。【结论】不同表型果实各个时期, 果实总花色苷含量水平有显著差异; 8 个候选基因中, *FaCHI*、*FaDFR*、*FaANS* 和 *FaGST* 在果实发育成熟中, 白果类型的表达水平显著低于红果类型, 推测其可能是参与果实花色苷合成途径的关键结构基因; 然而, 白果类型 *FaMYB10* 的表达水平却显著高于红果类型; 果实瞬时转化证明 *FaMYB10* 是不同颜色果实花色苷合成的关键正调控因子, 但 *FaMYB10* 序列突变使其调控功能丧失, 可能是白果类型花色苷积累缺失的原因。

关键词: 草莓; 花色苷合成; 结构基因; 转录因子; 基因表达

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Expression analysis of *FaMYB10* transcription factor and structural genes related to anthocyanin biosynthesis in strawberry (*Fragaria × ananassa*) fruit

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Abstract: 【Objective】Fruit color is an important trait for strawberry fruit quality and commercial value. The color of fruits is determined by the content and components of anthocyanins. In plants, several enzyme genes and transcription factors are involved in anthocyanin biosynthesis, but the molecular mechanism underlying the variation in the color of strawberry fruits is still unclear. The study aimed to analysis the expression patterns of structural genes and *FaMYB10* in the fruits with different color of strawberry and to verify the function of the *FaMYB10* transcription regulator. It will provide the reference for the molecular mechanism of anthocyanin synthesis in strawberry fruits. 【Methods】Fruits from the three octoploid cultivated strawberry varieties (*Fragaria × ananassa* ‘Benihoppe’ with red skin and flesh; ‘Xiaobai’ with red skin and white flesh; ‘Snow White’ with white skin and flesh) were used as materials. Extraction of total anthocyanin was carried out by high performance liquid chromatography. The total RNA of fruit was extracted using an E.Z.N.A Plant RNA Kit. The first-strand cDNA was syn-

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thesized from the total RNA using a PrimerScript™ RT Reagent Kit with gDNA Eraser according to the manufacturer's instructions. Then, RNA-seq was used to analyze the transcriptomic change during three development and ripening stages of the three varieties. In addition, the expression levels of eight candidate genes were conducted by qRT-PCR. Homologous recombination technique was performed to insert the *FaMYB10* gene in the PBI121-GFP vector. After the constructed over-expression vector was transferred into *Agrobacterium* GV3101. *A. tumefaciens* strain GV3101 was resuspended in the *Agrobacterium* infiltration buffer ($10 \text{ mmol} \cdot \text{L}^{-1} \text{ MgCl}_2$, $10 \text{ mmol} \cdot \text{L}^{-1} \text{ MES}$, pH 5.6, $200 \mu\text{mol} \cdot \text{L}^{-1}$ acetosyringone) to a final OD_{600} of 0.6-0.8. The PBI121-*FaMYB10*-HY-GFP vector *Agrobacterium tumefaciens* was injected using a syringe with needle into the initial ripening fruits in vitro until the whole fruit was permeated. The fruit coloration was observed three or five days after post-injection.【Results】The total anthocyanin contents varied significantly with fruit developmental stages. In the ripening stage, the total anthocyanin contents of 'Benihoppe' was rapidly increased from $7.92 \text{ mg} \cdot \text{kg}^{-1}$ to $175.60 \text{ mg} \cdot \text{kg}^{-1}$, and the total anthocyanin contents of 'Xiaobai' was $15.2 \text{ mg} \cdot \text{kg}^{-1}$, which was only 8.66% of the 'Benihoppe'. However, the total anthocyanin contents were very low in 'Snow White'. The results of transcriptome analysis showed that the expression levels of genes related to the anthocyanins biosynthesis were significantly different between the red and the white strawberries. The expression of early biosynthetic genes including *FaPAL* (*gene22493*), *FaCHI* (*gene25025* and *gene25539*), and *FaCHS* (*gene3479*) and the late biosynthetic genes including *FaDFR* (*gene236*, *gene5738*, *gene7069* and *gene21883*), *FaANS* (*gene14037* and *gene15699*), *FaUFGT* (*gene21489* and *gene21620*), *Fa3GT* (*gene9227*, *gene16078*, *gene22086* and *gene22630*), *FaGST* (*gene2828*, *gene5140*, *gene9114* and *gene2665*) was significantly higher in the ripe fruits of 'Benihoppe' and 'Xiaobai' compared with the fruits of 'Snow White'. According to the results of qRT-PCR, the expression of *FaMYB10* significantly increased in the ripe fruits of 'Benihoppe', 'Xiaobai' and 'Snow White' compared with the fruits in initial developmental stage. Interestingly, in the full ripe stage of the fruits, the expression level of *FaMYB10* gene in the 'Snow White' and 'Xiaobai' was significantly higher than that of the red varieties 'Benihoppe', and its transcription level was not consistent with changes of the color phenotype of the fruits. Meanwhile, qRT-PCR analysis of eight candidate genes was consistent with the transcriptome results. By analysing the coding region sequences of the *FaMYB10* gene in the red and the white strawberry varieties showed that there were two sequence types of the *FaMYB10-XB-1* and the *FaMYB10-XB-2* was in 'Xiaobai' and the *FaMYB10-SW* in 'Snow White'. The coding sequences of *FaMYB10-XB-2* and *FaMYB10-SW* possessed GA and ACTTATAC base insertion respectively, which encoded truncated proteins. Meanwhile, the over-expression of *FaMYB10* in the strawberry induced coloration of the fruits compared with those of the control. It suggested that *FaMYB10* played a positive role in regulating anthocyanin biosynthesis.【Conclusion】There were significant differences in the total anthocyanin contents in different phenotypes, and anthocyanin content was closely related to the expression level of the genes related to the anthocyanin biosynthesis. Among the eight candidate genes, *FaCHI*, *FaDFR*, *FaANS* and *FaGST* showed significantly lower expression levels in the white fruit type than in the red fruit type during fruit ripening, suggesting that these genes might be vital structural genes in the anthocyanin biosynthesis. The expression level of *FaMYB10* in the white fruit type was significantly higher than that in the red fruit type. The transient transformation of fruit proved that *FaMYB10* was a key positive regulatory gene for the anthocyanin biosynthesis. *FaMYB10* gene sequence insertion mutation might result in the loss of intact *FaMYB10* protein function and then the loss of anthocyanin accumulation in the white octoploid strawberry.

Key words: *Fragaria × ananassa* Duch.; Anthocyanin biosynthesis; Structural gene; Transcription factor; Gene expression

色泽作为果实外观品质的重要组成部分,在一定程度上决定其商品价值。目前,草莓栽培中,果实色泽表型丰富,如白色、黄色、粉红色、红色及深红色等,这主要是由不同草莓基因型的果实中花色苷含量水平和组分不同所决定的^[1]。花色苷作为一类重要的天然类黄酮色素,广泛存在于植物叶、茎、花及果实等器官。植物中常见的花色苷为天竺葵色素、飞燕草色素、矢车菊色素、牵牛花色素、芍药色素和锦葵色素6种色素^[2-3]。草莓果实中已被鉴定出有25种花色苷组分,主要是天竺葵素和矢车菊素,而天竺葵素的含量远远高于矢车菊素,占总花色苷的70%以上^[4-5]。

目前,大多数结构基因和调控基因在草莓中得到广泛研究。Zhang等^[6]基于转录组分析结果,结构基因 *C4H*, *CHS*, *CHI*, *F3H*, *DFR* 和 *ANS* 在野生草莓 ‘Yellow Wonder’ 黄果中表达显著下调,这些基因的转录丰度与花色苷的积累呈正相关,推测可能是果实呈现黄色的原因。已有研究结果表明,发现 *CHS* 基因在草莓成熟期中高度表达,可能是参与类黄酮生物合成的关键酶^[7];在森林草莓 (*Fragaria vesca*) 和栽培草莓 (*F. × ananassa*) 中花青素羟基化模式不同主要体现 *F3'H* 和 *DFR1* 的表达模式上,而 *F3'H* 沉默后表现为白色或淡色表型^[8]; ‘阿尔比’ 草莓果实发育过程中, *DFR* 基因在幼果期和成熟期出现表达高峰^[9];野生草莓白果性状与 *ANS* 基因低量表达有关^[10]。此外,在草莓果实中,通过 *FaCHS*^[11]、*FaDFR*^[12]、*FaF3H*^[13]、*Fa4CL*^[14] 等基因沉默,表明它们主要

参与花色苷合成;前人已有大量的报道,认为 *Fv-MYB10*^[15]、*FaMYB10*^[16] 是花色苷合成的关键正调控转录因子, *FaMYB1*^[17]、*FaMYB5* 和 *FabHLH3Δ*^[18-19] 主要是草莓花色苷合成的关键负调控转录因子。

近年来,花色苷生物合成途径已基本被阐明,参与花色苷合成和调控的大部分基因在许多模式植物中得到分析和研究^[6,19-21],而对栽培草莓果实花色苷合成及其调控机制仍未完全阐明^[16,22],白果类型草莓花色苷积累缺失的原因仍需要进一步探究。笔者通过对不同颜色栽培草莓品种果实中8个参与花色苷生物合成途径相关基因的表达差异进行分析;同时,分析不同果实色泽类型 *FaMYB10* 基因序列和表达水平差异,探讨白果花色苷积累缺失的原因,并验证 *FaMYB10* 基因功能,以期为深入研究草莓果实花色苷合成的分子机制提供参考。

1 材料和方法

1.1 材料

‘红颜’(‘Benihoppe’,红皮红肉)、“小白”(‘Xiaobai’,红皮白肉)和‘白雪公主’(‘Snow White’,白皮白肉)草莓果实取自中国农业科学院郑州果树研究所草莓试验温室。根据果实发育时期分为6个时期:小绿果期(small green fruit, S1)、中绿果期(middle green fruit, S2)、大绿果期(large green fruit, S3)、白果期(white fruit, S4)、转色期(initial ripening fruit, S5)、成熟期(full ripe fruit, S6)(图1)。每个发育时



S1. 小绿果期;S2. 中绿果期;S3. 大绿果期;S4. 白果期;S5. 转色期;S6. 成熟期;S6-1. 成熟期纵切面。

S1. Small green fruit; S2. Middle green fruit; S3. Large green fruit; S4. White fruit; S5. Initial ripening fruit; S6. Full ripe fruit; S6-1. Vertical section of full ripe fruit.

图1 草莓果实的6个发育时期
Fig. 1 Six development stages of strawberry fruit

期采取 30 个大小一致、无病虫的果实(果皮果肉混合)进行混合,随机分为 3 组,每组 10 个果实。采样后迅速用液氮冷冻,保存于-80 ℃超低温冰箱备用。

1.2 方法

1.2.1 总花色苷含量测定 (1)样品预处理:选取草莓中绿果期(S2)、转色期(S5)、成熟期(S6)3 个时期的果实用液氮进行研磨,称取 5 g 样品于 50 mL 具塑料塞离心管中,加入提取液($V_{\text{无水乙醇}}:V_{\text{水}}:V_{\text{盐酸}}=2:1:1$)混匀,定容,摇匀 1 min,超声波 30 min。于沸水浴中水解 1 h,取出冷却后,用提取液再次定容。静置,取上清液,用 0.45 μm 水相滤膜过滤,待测。(2)总花色苷测定:采用 C18(4.6 mm × 250 mm)色谱柱(Dionex, American)进行花色苷测定,柱温 35 ℃,流动相 A:1%甲酸水溶液,B:1%甲酸乙腈溶液;梯度洗脱,流速为 0.8 mL · min⁻¹,进样量:20 μL;检测波长:530 nm。每个样品 3 次生物学重复。

1.2.2 RNA 提取、cDNA 文库构建及 RNA 测序 3 个品种分别选择 S2、S5、S6 等 3 个时期的果实,利用 E.Z.N.A Plant RNA Kit (Omega, USA)试剂盒提取果实总 RNA,反转录,构建 cDNA 文库,利用 Illumina 公司 HiSeq X Ten 平台 (Berry Genomics Corporation, Beijing)进行上机测序。

1.2.3 转录组数据分析 对于测序结果使用 Cuffnorm 软件^[23]进行读数测量,用 FRKM 分析基因的表达水平,采用 DEseq2 软件进行分析,筛选阈值为 log₂|FC|>1 和错误率(FDR) < 0.01, *p* 值 < 0.05^[24]。通过 BLAST 将 unigenes 进行比对。

1.2.4 qRT-PCR 表达模式分析 在生物信息学分析基础上筛选其中 8 个花色苷合成途径中的候选差异基因,并结合 KEGG 分析结果进行 GO 注释。利用 NCBI 的 Primer-BLAST 在线软件设计引物(表 1),以草莓 *Actin* 作为内参基因,使用 Light Cycler® 480

表 1 荧光定量 PCR 引物序列
Table 1 Primers used for gene expression analysis by qRT-PCR

基因 ID Gene ID	基因名称 Gene name	正向引物(5'-3') Forward primers	反向引物(5'-3') Reverse primers
gene25025	<i>CHI1</i>	AGGGGGATGGAGATACAGGG	CCGTCTTGCCCTTCCACTTA
gene25539	<i>CHI2</i>	GAAAGGAACCGAGCTTGCAG	GCACCCCATATTGAGACCCT
gene7069	<i>DFR1</i>	TCCACAGCGTCAAGTGAGTC	CTCCCTCTACAAACCGGCA
gene21883	<i>DFR2</i>	TGGCAATGGAAGAGGTGGTG	TGGTCACACAGACCAGGTTC
gene12712	<i>LAR</i>	TGATGGCACGGTTAAAGCCT	GTTCTGATGTCGTCCACCGT
gene14037	<i>ANS</i>	GAGTACGTGAGACCCGAAGAG	TTTCTCCCTCACCTTGATGTC
gene 2828	<i>GST</i>	GCAGGAGAGCAAAAGCAACC	GTTAGGACCACGCTCTGCAT
gene18250	<i>MYB10</i>	TGCCAGGAAGAAGCTGCCAAT	AGCTATTGTATTTCTCGGGGT
	<i>Actin</i>	CGAGGCTCAATCCAAAAGAG	TGGCCACATACATAGCAGGA

PCR 仪(Roche 公司)和 Light Cycler® SYBR Green Master MIX 试剂盒进行 qRT-PCR。反应体系为 15 μL: 7.2 μL 2 × Light Cycler 480 SYBR Green I Master mix (Roche), 0.4 μL 上游引物, 0.4 μL 下游引物, 5 μL ddH₂O, 2 μL cDNA; 反应程序为: 95 ℃, 5 min; 95 ℃, 10 s, 60 ℃, 20 s, 72 ℃, 20 s, 40 个循环; 反应结束后分析荧光变化曲线以及溶解曲线。用 2^{-ΔΔCt} 方法计算基因的相对表达量^[25]。每个试验样品均进行 3 次生物学重复。

1.2.5 *FaMYB10* 基因克隆 根据 GenBank 中栽培草莓 *FaMYB10* 基因序列(登录号 EU155162.1), 利用 Primer5.0 软件设计 CDS 全长特异性引物: MYB10- CDS- F:ATGGAGGGTTTCGGTGTGAG; MYB10- CDS- R: TCATACGTAGGAGATGTTGAC。

提取‘红颜’‘小白’和‘白雪公主’草莓果实成熟期总 RNA 并进行反转录成 cDNA,使用高保真酶 2×Phanta Max Master Mix (Dye Plus)进行 PCR 扩增,反应体系和反应程序参照说明书。PCR 产物使用 StarPrep 快速 DNA 胶回收试剂盒进行回收纯化,然后连接至 T 载体并转化至 DH5α,菌液 PCR 鉴定阳性单菌落并送至河南尚亚生物技术公司测序。提取测序正确的克隆质粒用于后续试验。

1.2.6 *FaMYB10* 表达载体构建和草莓果实瞬时转化 根据 *FaMYB10* 基因序列以及 PBI121-GFP 质粒多克隆位点的酶切位点设计带有 *Xba* I 和 *Bam* H I 的特异性引物: *FaMYB10*- *Xba* I - F: gagaacacgggggactctagaATGGAGGGTTTCGGTGTGAGA; *FaMYB10*- *Bam* H I - R: gcccttgctcaccatggatccTACG-

TAGGAGATGTTGACTAGATCATTG(下划线表示酶切位点)。用 *Xba* I 和 *Bam*H I 酶切质粒 pEASY-FaMYB10-HY-T 和 PBI121-GFP 载体,切胶回收 *FaMYB10-HY* 目的片段和线性化 PBI121-GFP 载体,使用无缝克隆试剂盒(ClonExpress® II)进行重组连接,并转化 DH5 α ,菌液 PCR 鉴定阳性克隆送至河南尚亚生物技术公司测序。测序正确的重组质粒命名为 PBI121-*FaMYB10-HY-GFP*。将重组质粒 PBI121-*FaMYB10-HY-GFP* 转入农杆菌 GV3101。参照 Zhao 等^[26]的方法进行草莓果实瞬时转化并进行略微改动。挑选、注射离体草莓果实(转色期)放入光照培养箱中,观察对照组及试验组表型变化。

1.2.7 数据统计与分析 采用 IBM SPSS Statistics (version 19.0)软件和 Excel2010 软件对试验数据进行分析;用 *t*-test 进行显著性分析。

2 结果与分析

2.1 果实不同发育时期总花色苷含量的变化

以‘红颜’‘小白’和‘白雪公主’草莓果实为试验材料,观察颜色的变化。其中,‘红颜’和‘小白’前3个时期(S1~S3)为绿色,S4为白果期,果实从转色期(S5)开始着色,成熟期(S6)果实表面全部为红色,‘红颜’颜色较‘小白’深。从果实纵切面看‘红颜’果肉为红色,而‘小白’果肉为白色;‘白雪公主’整个果实发育时期果皮的颜色从绿色变为白色(图1)。从果实颜色变化看,选取 S2、S5 和 S6 3 个具有代表性时期的果实测定总花色苷含量。

从表2中可以看出,‘红颜’和‘小白’总花色苷含量从 S2 到 S5 缓慢增加,S6 期达到峰值,‘红颜’总花色苷含量从 S5 期的 7.92 mg·kg⁻¹ 迅速升至 S6 期的

表2 不同发育时期果实中总花色苷含量

Table 2 Variations of anthocyanin contents of strawberry fruits at developmental stages

品种 Cultivar	w(总花色苷)Total anthocyanins content/(mg·kg ⁻¹)		
	中绿果 Middle green fruit(S2)	转色期 Initial ripening fruit(S5)	成熟期 Full ripening fruit(S6)
红颜 Benihoppe	0.75±0.07	7.95±0.92	175.60±2.26
小白 Xiaobai	0.30±0.00*	2.85±0.07*	15.20±2.26**
白雪公主 Snow White	0.10±0.00**	0.20±0.00**	0.70±0.00**

注:表中数据为平均值±标准误;以红颜果实为对照,不同果实在同一时期进行 *t* 检验,*和**分别表示在 $p < 0.05$ 和 $p < 0.01$ 差异显著。下同。

Note: All values are mean±SE, Benihoppe fruit is control, * and ** indicates significant difference at $p < 0.05$ and $p < 0.01$ by *t*-text. The same below.

175.60 mg·kg⁻¹,‘小白’S6 期总花色苷含量达到 15.20 mg·kg⁻¹,仅为‘红颜’的 8.66%;‘白雪公主’果实整个发育时期几乎没有检测到花色苷含量。‘红颜’‘小白’和‘白雪公主’果实3个时期总花色苷含量分别存在显著差异。因此,研究品种间花色苷合成途径相关基因的表达模式有利于揭示不同颜色草莓品种的花青素积累规律。

2.2 花色苷合成前期结构基因的表达分析

对‘红颜’‘小白’和‘白雪公主’果实发育阶段的3个主要时期进行 RNA-seq,分析花色苷合成途径相关结构基因的差异表达。花色苷合成途径相关结构基因由起始的苯丙氨酸经一系列酶催化最终合成花色苷,再经转运到细胞液泡贮存。苯丙氨酸解氨酶(PAL)将苯丙氨酸催化合成肉桂酸作为生物合成的起始步骤,由 *PAL*、*C4H*、*4CL*、*CHS* 和 *CHI* 合成类黄酮物质的前体,是花色苷合成途径的第一阶段(图2)。*FaPAL*(*gene22493*)在‘白雪公主’的整个发育阶段表达量都较低,而‘红颜’和‘小白’中,随着果实

发育阶段表达量呈上升趋势,S5 和 S6 时期表达量增加,‘红颜’表达量最高,分别是‘白雪公主’的 21 和 27 倍;但另一个 *FaPAL*(*gene24869*)基因的表达量在3个品种中差异不显著,说明 *FaPAL*(*gene22493*)可能是参与花色苷合成的重要基因。*Fa4CL*(*gene6051*、*gene16730*、*gene25800*、*gene27321* 和 *gene27480*)总体表达量 S2 期最高,S5 和 S6 时期表达量逐渐降低,该基因可能更多地参与类黄酮前体的合成过程。*CHI* 和 *CHS* 是合成花色苷的关键酶,其活性的升高或降低直接影响果实的着色,‘红颜’和‘小白’*FaCHI*(*gene25025*、*gene25539*)和 *FaCHS*(*gene3479*)的表达量在 S2 期比较低,S5 和 S6 期的表达量迅速上升,但‘小白’中的表达量显著低于‘红颜’,‘白雪公主’整个果实发育阶段这两个基因的表达水平均被抑制,这与果实总花色苷含量相一致。

2.3 花色苷合成后期结构基因的表达分析

F3H、*DFR*、*ANS*、*UFGT*、*3GT* 和转运花色苷的 *GST* 是主要负责花色苷合成和运输的关键酶基因(图2)。

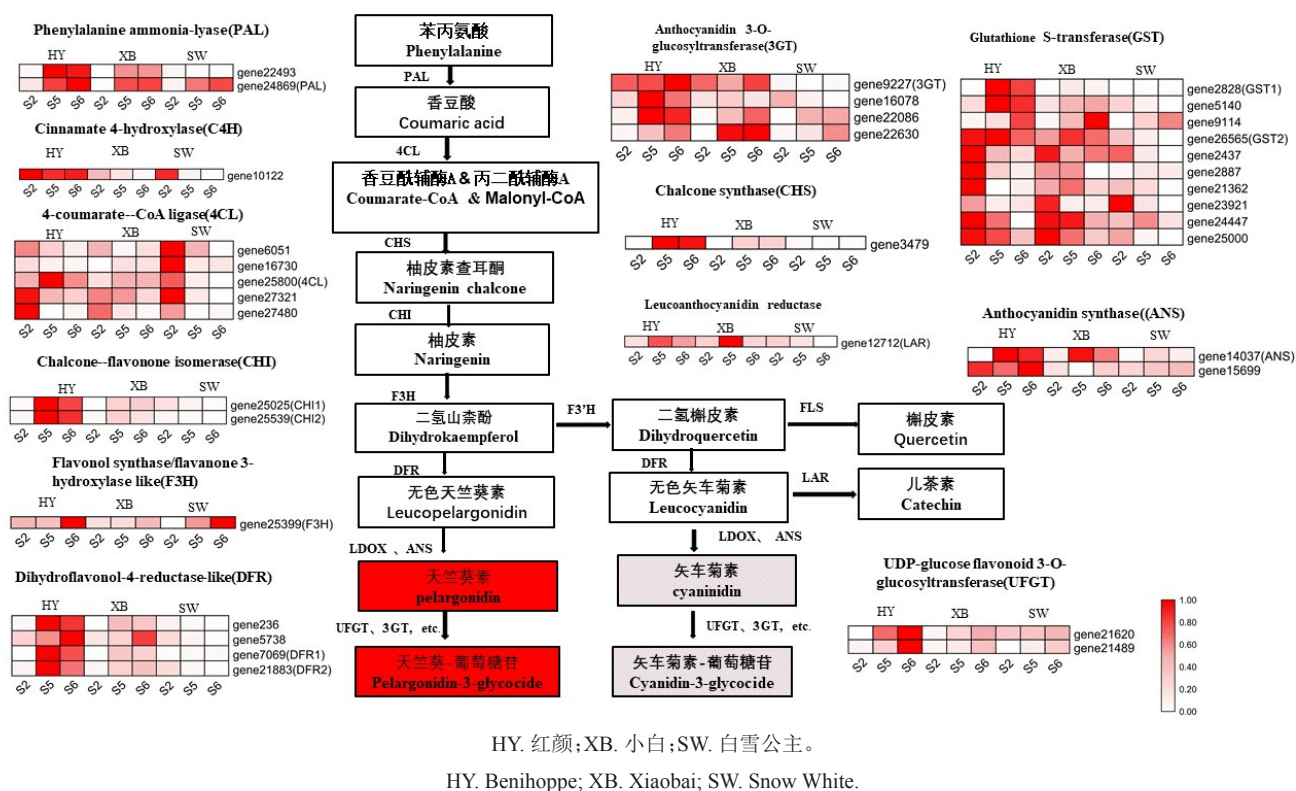


图2 草莓果实花色苷合成途径示意图及草莓果实中花色苷合成结构基因差异表达
 Fig. 2 Schematic of the anthocyanin biosynthetic pathway and differential expression of structural genes in three strawberry fruits

在果实发育的S5和S6期,‘红颜’和‘白雪公主’中FaF3H(gene25399)表达量高且二者之间差异不显著。FaDFR(gene236、gene5738、gene7069和gene21883)、FaANS(gene14037和gene15699)、FaUFGT(gene21489和gene21620)、Fa3GT(gene9227、gene16078、gene22086和gene22630)和FaGST(gene2828、gene5140、gene9114和gene2665)在3个品种中表达模式相似,S2期表达量低、S5和S6期达到峰值。在S5和/或S6期,这些基因在‘红颜’中表达量最高,‘小白’次之,‘白雪公主’一直维持在较低水平。另外,FaGST(gene2437、gene2887、gene21362、gene23921、gene24447和gene25000)在3个品种中前期表达量最高,果实发育后期表达量下降。花色苷合成途径后期相关结构基因的差异表达与总花色苷含量密切相关。

2.4 果实发育时期花色苷合成关键基因表达分析

对花色苷合成途径影响类黄酮前体物质的重要酶基因FaCHI、花色苷合成后期形成显色花青素的FaDFR和FaANS、负责花青素转运的FaGST及FaMYB10进行qRT-PCR分析(图3),结果表明,‘红

颜’‘小白’和‘白雪公主’中FaCHI1和FaDFR(FaDFR1和FaDFR2)表达量均在S1期出现一个峰值,随着‘红颜’和‘小白’果实着色加深,基因的表达量迅速增加,且在S5期‘小白’和‘白雪公主’表达量极显著低于‘红颜’。在‘红颜’和‘小白’中,随着果实的发育成熟,FaCHI2表达量逐渐上升,在S6期‘小白’和‘白雪公主’中的表达量极显著低于‘红颜’,而‘白雪公主’中FaCHI2表达量一直处于较低水平。在3个品种中,FaGST在果实的发育前期表达量很低,后期(S5和S6)迅速上升,但‘白雪公主’和‘小白’中的表达量极显著低于‘红颜’,此结果与FaGST后期参与花青素转运有关。同时,这些基因的表达变化趋势与转录组中的结果一致,证明了转录组数据分析结果的可靠性。

通过qRT-PCR分析草莓果实发育过程中FaMYB10基因表达水平变化(图3),结果表明,FaMYB10在3个品种中S1~S4期表达量极低,在S5至S6期表达量迅速上升。值得注意的是,在S5和S6时期,‘白雪公主’和‘小白’中FaMYB10表达量均极显著高于‘红颜’,其相对表达量分别是红颜的

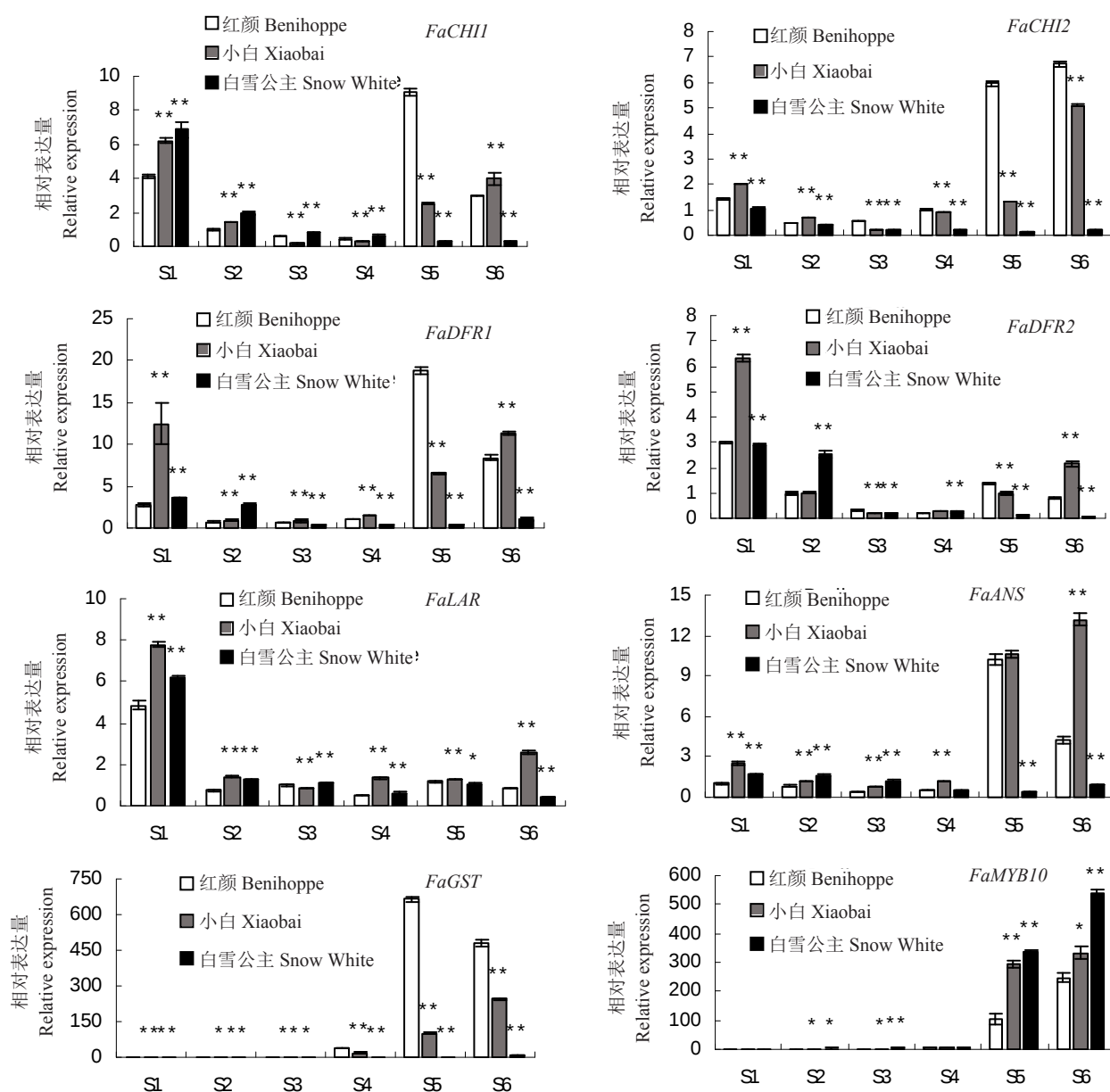


图3 果实发育过程中花色苷合成相关基因表达分析

Fig. 3 Relative expression of anthocyanin biosynthesis genes of three strawberry fruits

2.2倍和1.6倍,这一结果与果实花色苷含量呈负相关。

2.5 *FaMYB10*基因克隆及序列分析

qRT-PCR结果表明,*FaMYB10*在白果类型中的表达量显著高于红果类型。为进一步分析这种反常现象,分别从‘红颜’‘小白’和‘白雪公主’果实中克隆*FaMYB10*基因,进行CDS序列分析,结果发现(图4),与参考序列相比(登录号EU155162),‘红颜’*FaMYB10-HY*的CDS全长702 bp,编码233个氨基酸。‘小白’有2种序列类型,一种序列是*FaMYB10-XB-1*,与*FaMYB10-HY*序列一致,编码

233个氨基酸;另一种序列是*FaMYB10-XB-2*,在452~455区间有2个碱基GA的插入,仅编码154个氨基酸。‘白雪公主’*FaMYB10-SW*基因在491~500区间有8个连续碱基(ACTTATAC)的插入,编码179个氨基酸。*FaMYB10-XB-2*氨基酸序列由于2个碱基的插入,从而导致移码突变,蛋白翻译提前终止;*FaMYB10-SW*同样由于碱基插入而使蛋白翻译提前终止。推测氨基酸序列和结构的变化可能导致转录因子*FaMYB10*调控作用的丧失。

2.6 *FaMYB10*基因瞬时转化草莓果实表型分析

为了进一步研究基因功能,将过表达载体

FaMYB10-HY	ATGGAGGGTTTCGGTGTGAGAAAAGGTGCATGGACTAAAGAGGAAGATGAGCTTCTGAAACAGTTTCATCGAAATCCATGG	80
FaMYB10-XB-1	ATGGAGGGTTTCGGTGTGAGAAAAGGTGCATGGACTAAAGAGGAAGATGAGCTTCTGAAACAGTTTCATCGAAATCCATGG	80
FaMYB10-XB-2	ATGGAGGGTTTCGGTGTGAGAAAAGGTGCATGGACTAAAGAGGAAGATGAGCTTCTGAAACAGTTTCATCGAAATCCATGG	80
FaMYB10-SW	ATGGAGGGTTTCGGTGTGAGAAAAGGTGCATGGACTAAAGAGGAAGATGAGCTTCTGAAACAGTTTCATCGAAATCCATGG	80
FaMYB10 (EU155162)	ATGGAGGGTTTCGGTGTGAGAAAAGGTGCATGGACTAAAGAGGAAGATGAGCTTCTGAAACAGTTTCATCGAAATCCATGG	80
Consensus	atggagggtttcggtgtgagaaaaggtgcatggactaaagaggaagatgagcttctgaaacagtttcacgaaatccatgg	
FaMYB10-HY	AGAAGGCAAAATGGAAATCATGTTCTCTCAAATCAGGCTTAAACAGATGCAGGAAGAGCTGTAGACTGAGGTGGTGAATT	160
FaMYB10-XB-1	AGAAGGCAAAATGGAAATCATGTTCTCTCAAATCAGGCTTAAACAGATGCAGGAAGAGCTGTAGACTGAGGTGGTGAATT	160
FaMYB10-XB-2	AGAAGGCAAAATGGAAATCATGTTCTCTCAAATCAGGCTTAAACAGATGCAGGAAGAGCTGTAGACTGAGGTGGTGAATT	160
FaMYB10-SW	AGAAGGCAAAATGGAAATCATGTTCTCTCAAATCAGGCTTAAACAGATGCAGGAAGAGCTGTAGACTGAGGTGGTGAATT	160
FaMYB10 (EU155162)	AGAAGGCAAAATGGAAATCATGTTCTCTCAAATCAGGCTTAAACAGATGCAGGAAGAGCTGTAGACTGAGGTGGTGAATT	160
Consensus	agaaggcaaatggaaatcatgttctctcaaatacaggcttaaacagatgcaggaagagctgtagactgaggtggatgaatt	
FaMYB10-HY	ATTTGAAGCCGAATATCAAGAGAGGAGAGTTTGCAGAGGATGAAGTTGATTTGATCATCAGGCTTCATAAGCTTCTAGGA	240
FaMYB10-XB-1	ATTTGAAGCCGAATATCAAGAGAGGAGAGTTTGCAGAGGATGAAGTTGATTTGATCATCAGGCTTCATAAGCTTCTAGGA	240
FaMYB10-XB-2	ATTTGAAGCCGAATATCAAGAGAGGAGAGTTTGCAGAGGATGAAGTTGATTTGATCATCAGGCTTCATAAGCTTCTAGGA	240
FaMYB10-SW	ATTTGAAGCCGAATATCAAGAGAGGAGAGTTTGCAGAGGATGAAGTTGATTTGATCATCAGGCTTCATAAGCTTCTAGGA	240
FaMYB10 (EU155162)	ATTTGAAGCCGAATATCAAGAGAGGAGAGTTTGCAGAGGATGAAGTTGATTTGATCATCAGGCTTCATAAGCTTCTAGGA	240
Consensus	atttgaagccgaatatcaagagaggagagtttgcagaggatgaagttgatttgatcatcaggcttcataagcttctagga	
FaMYB10-HY	AACAGGTGGTCTTTAATTGCCGGAACGATTGCCAGGAAGAAGTCCCAATGATGTGAAGAACTATTGGAATACTTATCAAAG	320
FaMYB10-XB-1	AACAGGTGGTCTTTAATTGCCGGAACGATTGCCAGGAAGAAGTCCCAATGATGTGAAGAACTATTGGAATACTTATCAAAG	320
FaMYB10-XB-2	AACAGGTGGTCTTTAATTGCCGGAACGATTGCCAGGAAGAAGTCCCAATGATGTGAAGAACTATTGGAATACTTATCAAAG	320
FaMYB10-SW	AACAGGTGGTCTTTAATTGCCGGAACGATTGCCAGGAAGAAGTCCCAATGATGTGAAGAACTATTGGAATACTTATCAAAG	320
FaMYB10 (EU155162)	AACAGGTGGTCTTTAATTGCCGGAACGATTGCCAGGAAGAAGTCCCAATGATGTGAAGAACTATTGGAATACTTATCAAAG	320
Consensus	aacaggtggtcttttaattgccggaacgattgccaggaagaactgccaatgatgtgaagaactattggaatacttatacaaag	
FaMYB10-HY	GAAAAAGGATCAAAAGACGGCTTCATACGCAAGAAACTGAAAGTTAAACCCCGAGAAAAATACAATAGCTTACACAATTG	400
FaMYB10-XB-1	GAAAAAGGATCAAAAGACGGCTTCATACGCAAGAAACTGAAAGTTAAACCCCGAGAAAAATACAATAGCTTACACAATTG	400
FaMYB10-XB-2	GAAAAAGGATCAAAAGACGGCTTCATACGCAAGAAACTGAAAGTTAAACCCCGAGAAAAATACAATAGCTTACACAATTG	400
FaMYB10-SW	GAAAAAGGATCAAAAGACGGCTTCATACGCAAGAAACTGAAAGTTAAACCCCGAGAAAAATACAATAGCTTACACAATTG	400
FaMYB10 (EU155162)	GAAAAAGGATCAAAAGACGGCTTCATACGCAAGAAACTGAAAGTTAAACCCCGAGAAAAATACAATAGCTTACACAATTG	400
Consensus	gaaaaaggatcaaaagacggcttcatacgcaagaactgaaagttaaaccccgagaaaaatacaatagcttacacaattg	
FaMYB10-HY	TAAAGCCTCGACCACGAACCTTCATCAAAAGGTTCAATTTTACAGAGAGAGAA...CGCAAATATAGAGCATATCATTAG	478
FaMYB10-XB-1	TAAAGCCTCGACCACGAACCTTCATCAAAAGGTTCAATTTTACAGAGAGAGAA...CGCAAATATAGAGCATATCATTAG	478
FaMYB10-XB-2	TAAAGCCTCGACCACGAACCTTCATCAAAAGGTTCAATTTTACAGAGAGAGAA...CGCAAATATAGAGCATATCATTAG	480
FaMYB10-SW	TAAAGCCTCGACCACGAACCTTCATCAAAAGGTTCAATTTTACAGAGAGAGAA...CGCAAATATAGAGCATATCATTAG	478
FaMYB10 (EU155162)	TAAAGCCTCGACCACGAACCTTCATCAAAAGGTTCAATTTTACAGAGAGAGAA...CGCAAATATAGAGCATATCATTAG	478
Consensus	t a a g a c c t c g a c c a c g a a c c t t c a t c a a a a g g t t c a a t t t t a c a g a g a g a a . . . c g c a a a t a t a g a g c a t a a t c a t t c a g	
FaMYB10-HY	AAGTGAAGTTATAC...CAGTTCTTTACCAACAGAACCCACAGACTCTACAATTAGAAAATGTAAGTGAATTGGT	550
FaMYB10-XB-1	AAGTGAAGTTATAC...CAGTTCTTTACCAACAGAACCCACAGACTCTACAATTAGAAAATGTAAGTGAATTGGT	550
FaMYB10-XB-2	AAGTGAAGTTATAC...CAGTTCTTTACCAACAGAACCCACAGACTCTACAATTAGAAAATGTAAGTGAATTGGT	552
FaMYB10-SW	AAGTGAAGTTATAC...CAGTTCTTTACCAACAGAACCCACAGACTCTACAATTAGAAAATGTAAGTGAATTGGT	558
FaMYB10 (EU155162)	AAGTGAAGTTATAC...CAGTTCTTTACCAACAGAACCCACAGACTCTACAATTAGAAAATGTAAGTGAATTGGT	550
Consensus	aagtgagttatac...cagttctttaccaacagaaacccacagactctacaattagaaaatgtaactgattggt	
FaMYB10-HY	GGAAAGATTTCAGAAAGATAGTACAGAGAGCATTGATAGAACAATGTGTTCTGGTCTTGGTTTAGAGGATCATGACTTC	630
FaMYB10-XB-1	GGAAAGATTTCAGAAAGATAGTACAGAGAGCATTGATAGAACAATGTGTTCTGGTCTTGGTTTAGAGGATCATGACTTC	630
FaMYB10-XB-2	GGAAAGATTTCAGAAAGATAGTACAGAGAGCATTGATAGAACAATGTGTTCTGGTCTTGGTTTAGAGGATCATGACTTC	632
FaMYB10-SW	GGAAAGATTTCAGAAAGATAGTACAGAGAGCATTGATAGAACAATGTGTTCTGGTCTTGGTTTAGAGGATCATGACTTC	638
FaMYB10 (EU155162)	GGAAAGATTTCAGAAAGATAGTACAGAGAGCATTGATAGAACAATGTGTTCTGGTCTTGGTTTAGAGGATCATGACTTC	630
Consensus	g g a a a g a t t t c t c a g a a g a t a g t a c a g a g a g c a t t g a t a g a a c a a t g t g t t c g g t c t t g g t t t a g a g g a t c a t g a c t t c	
FaMYB10-HY	TTTCAAACTTTTGGGTTGAAGATATGCTACTATCGGCAAGCAATCATCTAGTCAACATCTCCTACGTATG	701
FaMYB10-XB-1	TTTCAAACTTTTGGGTTGAAGATATGCTACTATCGGCAAGCAATCATCTAGTCAACATCTCCTACGTATG	701
FaMYB10-XB-2	TTTCAAACTTTTGGGTTGAAGATATGCTACTATCGGCAAGCAATCATCTAGTCAACATCTCCTACGTATG	703
FaMYB10-SW	TTTCAAACTTTTGGGTTGAAGATATGCTACTATCGGCAAGCAATCATCTAGTCAACATCTCCTACGTATG	709
FaMYB10 (EU155162)	TTTCAAACTTTTGGGTTGAAGATATGCTACTATCGGCAAGCAATCATCTAGTCAACATCTCCTACGTATG	701
Consensus	ttcacaaacttttgggttgaagatatgtactatcggcaagcaatcatctagtcaacatctcctacgtatg	
FaMYB10-HY	1	MEGFGVRKGAWTKEEDELKQFIEIHGEGKWNHVLKSLNRCRKSCLRWVNYLKPNIKRGEFAEDEVLDLIRLHKLIG 80
FaMYB10-XB-1	1	MEGFGVRKGAWTKEEDELKQFIEIHGEGKWNHVLKSLNRCRKSCLRWVNYLKPNIKRGEFAEDEVLDLIRLHKLIG 80
FaMYB10-XB-2	1	MEGFGVRKGAWTKEEDELKQFIEIHGEGKWNHVLKSLNRCRKSCLRWVNYLKPNIKRGEFAEDEVLDLIRLHKLIG 80
FaMYB10-SW	1	MEGFGVRKGAWTKEEDELKQFIEIHGEGKWNHVLKSLNRCRKSCLRWVNYLKPNIKRGEFAEDEVLDLIRLHKLIG 80
FaMYB (ABX79947.1)	1	MEGFGVRKGAWTKEEDELKQFIEIHGEGKWNHVLKSLNRCRKSCLRWVNYLKPNIKRGEFAEDEVLDLIRLHKLIG 80
FaMYB10-HY	81	NRWSLIAGRLPGRTANDVKYWNQYQRKKDKQTASYAKKLKVKPRENTIAYTVRPRPRTFIKRFNFTERDANIEHNHSE 160
FaMYB10-XB-1	81	NRWSLIAGRLPGRTANDVKYWNQYQRKKDKQTASYAKKLKVKPRENTIAYTVRPRPRTFIKRFNFTERDANIEHNHSE 160
FaMYB10-XB-2	81	NRWSLIAGRLPGRTANDVKYWNQYQRKKDKQTASYAKKLKVKPRENTIAYTVRPRPRTFIKRFNFTERETQI----- 154
FaMYB10-SW	81	NRWSLIAGRLPGRTANDVKYWNQYQRKKDKQTASYAKKLKVKPRENTIAHTIVRPRPRTFIKRFNFTERYANIEHNHSE 160
FaMYB (ABX79947.1)	81	NRWSLIAGRLPGRTANDVKYWNQYQRKKDKQTASYAKKLKVKPRENTIAYTVRPRPRTFIKRFNFTERYANIEHNHSE 160
FaMYB10-HY	161	VSYSSTLPTPEPQTLQLENVTDWWKDFSEDSTESIDRTMCSGLGLEDDHFFTNFVWEDMVLASNDLVNISYV* 234
FaMYB10-XB-1	161	VSYSSTLPTPEPQTLQLENVTDWWKDFSEDSTESIDRTMCSGLGLEDDHFFTNFVWEDMVLASNDLVNISYV* 234
FaMYB10-XB-2	155	-----*----- 155
FaMYB10-SW	161	VSYSSTLPTPEPQTLQLENVTDWWKDFSEDSTESIDRTMCSGLGLEDDHFFTNFVWEDMVLASNDLVNISYV* 180
FaMYB (ABX79947.1)	161	VSYSSTLPTPEPQTLQLENVTDWWKDFSEDSTESIDRTMCSGLGLEDDHFFTNFVWEDMVLASNDLVNISYV* 234

红框表示序列插入;黑线表示 MYB-DNA-binding 结构域;*表示终止子。

The red box represents base sequence insertion; the black line represents the MYB-DNA-binding domain; *. Terminator.

图 4 *FaMYB10* 基因核苷酸和氨基酸序列分析

Fig. 4 Nucleotide and amino acid sequence analysis of *FaMYB10* gene

PBI121-FaMYB10-HY-GFP 转入 GV3101 农杆菌中, 分别注射‘红颜’‘小白’和‘白雪公主’转色期草莓果实中, 农杆菌浸染果实 3~5 d 后观察表型变化(图 5)。与阴性对照相比,‘红颜’红色果肉颜色变深,‘小白’和‘白雪公主’白色果肉颜色变红, 进一步证明 *FaMYB10*(*FaMYB10-HY*) 是参与花色苷合成的关键正调控因子, 而 *FaMYB10-XB-2* 和 *FaMYB10-SW* 序列突变导致功能丧失, 花色苷含量降低。

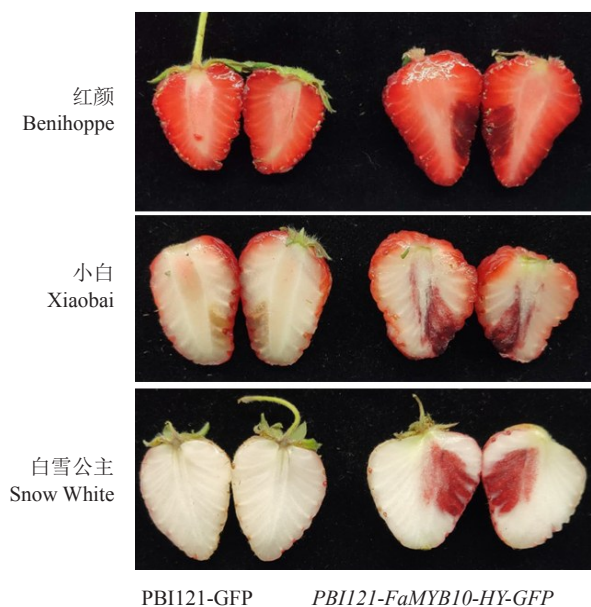


图 5 *PBI121-FaMYB10* 农杆菌侵染草莓果实表型变化
Fig. 5 The phenotype of strawberry fruit by *PBI121-FaMYB10-HY-GFP*

3 讨论

果实花色苷含量和组分是影响果实色泽品质的一个重要因素^[4], 花色苷是植物呈色的主要物质, 主要由一系列结构基因编码相关酶催化相关底物, 随后经过各种修饰被转运到液泡等部位储存^[27-28]。花色苷的积累与花色苷合成途径相关结构基因的表达水平密切相关。前人对花色苷的合成途径以及相关结构基因的分类和功能进行了大量研究, 目前, 梨^[29]、葡萄^[30]、石榴^[31]、草莓^[12]等植物中已分离并克隆了花色苷合成途径的结构基因。

花色苷合成途径中结构基因的表达水平与成熟果实着色程度有密切联系。苹果中, *CHS*、*F3H*、*DFR*、*ANS*、*UFGT* 等 5 个结构基因表达水平与花色苷积累呈正相关, 它们在黄色品种‘Orin’中的表达水平明显低于两个红色品种‘Jonathan’和‘Fuji’^[32]。‘Malay’白色苹果中无花色苷积累主要是由于合成花色苷关键基因 *UFGT* 不表达, 阻碍最终花色苷的

合成^[33]。本研究中,‘红颜’和‘小白’草莓果实中总花色苷含量在转色期和成熟期大量积累, 花色苷合成途径相关关键结构基因的表达模式与花色苷积累时期相一致。‘红颜’和‘小白’在整个果实发育成熟过程中, *FaCHI*、*FaDFR*、*FaANS* 和 *FaGST* 在 S5 和 S6 时期表达量迅速增加, 而‘白雪公主’中这些基因的表达量一直维持在较低水平, 与其白色果实表型性状相一致, 说明这些基因与草莓果实花色苷积累密切相关。其中, *FaCHI* 和 *FaDFR* 的表达呈现先降后升的模式, 分别在 S1 和 S6 时期出现两个高峰, 这与苹果^[34]和草莓^[9]的研究结果一致。Almeida 等^[20]认为 *DFR* 基因在果实发育过程中有两个高峰, 可能与草莓发育的过程中黄酮醇化合物的合成具有两个模式有关, 一个高峰可能对应黄烷-3-醇的生物合成, 另一个高峰对应花色苷和黄酮醇的生物合成。Lin 等^[12]通过 RNAi 沉默 *FaDFR* 后, 发现花青素糖苷下降了 91.3%, 而类黄酮生物合成的另一个分支产物槲皮素糖苷含量却增加了 73.1%, 说明绿色果实和成熟果实中 *FaDFR* 可能参与到其他不同生物合成途径中。

MYB 转录因子在植物花色苷积累中具有重要的调控作用。一般认为 *MYB10* 的表达水平与红色表型呈正相关, 在果实成熟中, *MYB10* 相对表达水平较高^[35]。甜樱桃中, 在果实成熟期‘Stalla’的 *Pa-MYB10* 表达水平显著高于‘Rainier’, 与花色苷积累相一致^[36]。森林草莓黄果和红果中, 果实发育前期 *FvMYB10* 表达量增加缓慢, 果实发育后期迅速增加^[37]。白果型森林草莓中, 过表达 *FvMYB10* 可使白果恢复红果表型^[15]。*MYB10* 基因序列变化导致调控功能改变,‘蜜脆’苹果果实上红绿条纹中 *MYB10* 转录本的差异积累是由 *MYB10* 启动子区域的差异甲基化导致的, 从而决定了花青素合成结构基因的转录和花色苷积累^[38]。红葡萄中, 果实颜色的差异主要由 *MYBA1* 基因中插入了反转录转座子导致的突变^[39]。Wang 等^[16]认为白果类型草莓是由于 *FaMYB10-2* 在编码该蛋白时 C 末端有 8 个碱基的插入, 从而导致翻译过程提前终止, 使其丧失功能导致果肉变白。本研究结果表明, *FaMYB10* 在白果、红果的转色期及成熟期表达量均较高, 但在‘白雪公主’中的相对表达量要显著高于‘红颜’和‘小白’, 红果类型和白果类型 *FaMYB10* 表达量与花色苷含量并不一致, 这些结果表明在 3 个不同果色表型中

*FaMYB10*调控花色苷合成发挥着不同的作用。这种差异的原因可能是MYB10序列结构存在差异。进一步分析*FaMYB10*基因序列结果显示MYB10在‘小白’品种中有2种序列类型,其中*FaMYB10-XB-2*有2个碱基“GA”的插入,从而使蛋白翻译过程提前终止,编码无功能的FaMYB10蛋白,推测可能是导致‘小白’果肉白色的因子,而*FaMYB10-XB-1*与*FaMYB10-HY*序列一致,可能参与果皮红色颜色的形成,这一推断还需进一步开展基因功能验证。而‘白雪公主’*FaMYB10-SW*在491-500区间有8个连续碱基“ACTTATAC”的插入,翻译蛋白过程也提前终止,这与Wang等^[16]报道的*FaMYB10-2*结果一致。‘红颜’‘小白’和‘白雪公主’果实中瞬时过表达*FaMYB10*,结果使白肉变红,表明FaMYB10仍是正调控花色苷合成的关键因子。

4 结 论

不同表型果实花青素积累存在显著差异,花色苷含量与花色苷合成的相关基因表达水平紧密相关;*FaCHI*、*FaDFR*、*FaANS*和*FaGST*在红色品种‘红颜’中表达最高,‘小白’次之,而‘白雪公主’表达量很低,推测可能是参与草莓果实花色苷合成途径的关键结构基因。*FaMYB10*在白果类型的表达水平显著高于红果类型,相对表达量与果实花色苷含量呈负相关。‘小白’*FaMYB10*基因编码区序列存在2种类型,其中*FaMYB10-XB-2*插入2个碱基GA,导致编码氨基酸序列和结构发生变化。结合果实瞬时过量表达验证,表明*FaMYB10*是不同颜色果实花色苷合成的关键正调控因子,但其序列变化引起功能丧失进而导致白果类型果实花色苷积累缺失。

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