果 树 学 报 2022，39(8)：1358-1367
Journal of Fruit Science

DOI:10.13925/j.cnki.gsxb.20210512

转录因子FabHLH148参与草莓果实的颜色发育

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摘 要：【目的】探究碱性螺旋-环-螺旋（basic helix-loop-helix，bHLH）转录因子在草莓果实成熟过程中的功能。**【方法】**基于转录组测序和基因组数据库，克隆*FabHLH148*基因；分析其理化性质、保守结构域、预测编码的蛋白质结构、系统进化树、亚细胞定位等；采用RT-qPCR检测其在草莓中的时空表达水平，并构建过表达（over expression，OE）和RNA干扰（RNA interference，RNAi）载体，利用农杆菌介导瞬时侵染草莓果实，观察记录表型，检测*FabHLH148*表达水平，并测定花色素苷含量。**【结果】***FabHLH148*基因CDS区全长687 bp，编码228个氨基酸，预测蛋白分子质量24.89 ku，理论等电点（pI）为11.65；该基因属于bHLH超家族，因其与二倍体草莓*FvbHLH148*序列相似度最高，所以将其命名为*FabHLH148*；亚细胞定位显示，FabHLH148主要定位在细胞核。RT-qPCR结果表明，*FabHLH148*在草莓不同组织部位均有表达，在果实中有较高表达并随果实成熟表达量显著升高。过表达*FabHLH148*能够促进果实着色以及花色素苷的积累，通过RNAi调低*FabHLH148*的表达则作用结果相反，且*FabHLH148*在OE组果实中表达水平显著高于对照，RNAi组果实中表达水平显著低于对照（$p<0.01$）。**【结论】**转录因子*FabHLH148*属于bHLH超家族，可促进草莓果实着色。

关键词：草莓；*FabHLH148*；转录因子；表达模式；花色素苷

中图分类号：S668.4 **文献标志码：**A **文章编号：**1009-9980(2022)08-1358-10

Transcription factor FabHLH148 is involved in the color development of strawberry fruit

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Abstract:【Objective】Strawberry is the most widely cultivated small berry fruit in the world due to its unique flavor as well as high nutritional and economic value. Meanwhile, it is a prominent problem to improve the quality of strawberry in cultivation. Coloring is an important evaluation index of fruit quality. Anthocyanin is the main component affecting the color of strawberry fruit. The basic helix-loop-helix (bHLH) transcription factor family as the second largest transcription factor families in plants has been proved to be involved in many processes of plant growth, development, morphogenesis and stress response. Some members of bHLH transcription factors play crucial regulatory roles in plant anthocyanin synthesis. To analyze the function of bHLH transcription factors during strawberry fruit coloring, the *FabHLH148* gene was cloned and we used physiological and molecular biology methods to reveal the function of FabHLH148.【Methods】Firstly, according to the reported transcriptome data of strawberry fruits in five development periods (SG, LG, Wt, IR and PR), a gene increasing rapidly with fruit ripening was screened. As it contained a bHLH superfamily conserved domain, it had the highest sequence similarity with diploid strawberry *FvbHLH148* (GenBank accession: XM_004295010.2), and the gene was named *FabHLH148*. The total RNA from strawberry cultivar Bebihoppe fruit was extracted using the plant total RNA extraction kit (Huayueyang, China) according to the manufacturer's protocol. First-strand cDNA was synthesized and the reverse transcription was carried out using Hifair® Ⅲ 1st Strand

收稿日期：2021-10-25 **接受日期：**2022-03-30

基金项目：国家自然科学基金项目（32102362，32030100）

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cDNA Synthesis SuperMix (YEASEN, China) and then, the full-length CDS sequence of the *FabHLH148* gene was obtained by PCR. Secondly, some bioinformatics techniques were used in this research. ExPASy website was referred to analyze the molecular weight, isoelectric point and liposoluble index of encoding protein. The conserved domains of FabHLH148 were predicted using NCBI Conserved Domains. The secondary and tertiary structure of FabHLH148 was analyzed by DNA star software and the online software SWISS-MODEL separately. MEGA 5.1 software was used to construct phylogenetic tree of *FabHLH148* homologous proteins. Thirdly, we used RT-qPCR to detect the expression level of *FabHLH148* in strawberry including various organs and developmental stages. Its subcellular localization was observed by Agrobacterium mediated transient transformation of tobacco mesophyll cells. Finally, the full-length CDS sequence of *FabHLH148* was constructed into pSuper1300 vector by homologous recombination to obtain over-expression vector; the 32−383 bp CDS sequence of *FabHLH148* was constructed into pK7GWIWG(Ⅱ)RR by Gateway method to obtain RNAi vector. These vectors were transformed into *Agrobacterium*. Based on this, the strawberry fruits in the de-green period were infected by the *Agrobacterium*-mediated transient infection, and the phenotypes were photographed and recorded at 0, 3, 5 and 7 days after injection. The achenes were removed 7 days after injection, and only the injection parts were frozen in liquid nitrogen and stored at −80 ℃. Then, we detected the expression level of *FabHLH148* and anthocyanin content in different transgenic strawberry fruits. 【Results】Bioinformatics analysis showed that the whole open reading frame (ORF) of *FabHLH148* was 687 bp encoding 255 amino acids. The results of amino acid physicochemical properties analysis showed that putative FabHLH148 protein was $C_{1080}H_{1809}N_{353}O_{312}S_5$, with a molecular weight of 24.89 ku and a theoretical isoelectric point (pI) of 11.56, so it was an alkaline protein. The protein contained 10 negatively charged amino acid residues (Asp + Glu), 42 positively charged amino acid residues (Arg + Lys), with an instability coefficient of 54.49 and average hydrophilicity of −0.636, indicating that the protein was an unstable hydrophobic protein. Conserved domain analysis showed that FabHLH148 contained a typical bHLH superfamily conserved domain. Therefore, FabHLH148 protein was clustered into the bHLH superfamily and shared high identity with amino acid sequence of FvbHLH148 (99.56%). Transient expression in *Nicotiana benthamiana* showed the green fluorescent signal of Super1300: FabHLH148-GFP was found in the nucleus and cytoplasm of epidermal cells in leaves. Analyses of qRT-PCR showed that *FabHLH148* was expressed in different organs of strawberry, with the highest relative expression in full red fruit and lowest in achene. It was highly expressed in fruit and reached its peak at full red fruit, which suggested that it played a crucial role in strawberry fruit ripening. Based on *Agrobacterium* transient infection of strawberry fruit and RT-qPCR, the expression level of *FabHLH148* in the overexpression group was significantly higher than that in the control group while the expression level in RNAi group was significantly lower than the control group. The fruit coloration of overexpression group was faster and the anthocyanin content was higher than that from the control group, while the effect was opposite when the expression of *FabHLH148* was reduced by RNA interference.【Conclusion】A homolog of *FvbHLH148* denoted as *FabHLH148* was cloned in cultivated strawberry. *FabHLH148* encoded a classic bHLH transcription factor and was located in nucleus and cytoplasm. *FabHLH148* was expressed much higher in fruit than in the rest of organs and up-regulated significantly during fruit ripening. Up-regulated or down-regulated expression of *FabHLH148* led to a significant induction or reduction of anthocyanin in transient transgenic strawberry fruits. These results suggested that *FabHLH148* could play an important role in strawberry fruit coloring.

Key words: Strawberry; *FabHLH148*; Transcription factor; Expression pattern; Anthocyanins

草莓(*Fragaria*×*ananassa* Duch.)属于多年生草本果树,具有独特的风味和极高的营养、经济价值,其栽培面积和产量位于世界小浆果首位[1]。改善草莓果实品质是目前草莓生产面临的突出问题。草莓果实品质形成受植物激素、矿质营养和生态环境等因素的调节[2-4]。花色素苷含量在植物色泽形成过程中发挥重要作用,是果蔬气味和颜色的重要组成部分,因此是评价果实品质的重要指标[5-6]。

花色素苷参与植物的发育和防御,能够影响种子品质、植物产品的涩味,提高植物产品的农艺、工业和营养价值[7]。此外,花色素苷还具有清除自由基、抗氧化和抑菌等食品保健功能,对人类健康也具有重要意义[8]。花色素苷属于类黄酮化合物[7],许多植物中的合成途径已经明确[9],催化酶及其编码基因已经得到广泛鉴定,包括查尔酮合成酶(CHS)、查尔酮异构酶(CHI)、黄烷酮-3-羟化酶(F3H)、二氢类黄酮还原酶(DFR)、花色素苷合成酶(ANS)、类黄酮3,5-糖基转移酶(UFGT)[10-11]。光和温度等环境因素通过影响花色素苷合成相关基因的表达起作用,目前已有报道*FvMYB10*、*FaRIF*和*FvTCP9*等转录因子参与调控草莓果实花色素苷的生物合成[12-14]。

研究表明,植物碱性螺旋-环-螺旋(basic helix-loop-helix,bHLH)家族的部分成员在植物花色素苷合成过程也发挥着重要的调控作用[15]。bHLH转录因子作为植物第二大类转录因子,不仅在植物生长发育和生理代谢途径中具有重要作用[16],还参与调节类黄酮与花青素的合成,植物形态建成以及胁迫响应等过程[17]。bHLH家族成员包含两个保守结构域:碱性区域和螺旋-环-螺旋(helix-loop-helix,HLH)区域[18],既可以作为转录激活子又可以作为转录抑制子起作用。目前已经在草莓[19]、杜梨(*Pyrus betulaefolia*)[20]、苹果(*Malus domestica*)[21]、葡萄(*Vitis vinifera*)[22]和黑果枸杞(*Lycium ruthenicum*)[23]等植物中鉴定出bHLH转录因子。本研究在转录组分析的基础上,发现了一个bHLH超家族转录因子*FabHLH148*,并对*FabHLH148*进行了克隆、生物信息学分析、表达模式检测以及功能的初步分析,为提高草莓果实品质提供理论基础。

1 材料和方法

1.1 试验材料与试剂

植物材料是北京农学院温室种植的红颜草莓。栽培条件为温度17~26 ℃、相对湿度为60%~80%、光照14 h/黑暗10 h。根据已有研究将草莓果实分为7个生长阶段,分别是小绿(small green,SG)、大绿(large green,LG)、褪绿(de-green,DG)、白果(white,Wt)、始红(initial red,IR)、片红(partial red,PR)和全红(full red,FR),分别在开花后9、13、16、19、21、22和25 d后拍照。所用烟草为北京农学院科技综合楼植物培养室种植的本氏烟草。

RNA提取试剂盒购自北京华越洋公司,反转录试剂盒购自YEASEN公司,凝胶回收试剂盒和质粒提取试剂盒购自Axygen公司,核酸内切酶购自NEB公司,无缝克隆以及Phanta高保真酶购自诺唯赞公司,植物花色素苷提取试剂盒购自索莱宝公司,所用感受态购自上海唯地生物有限公司,所用引物由上海生工生物有限公司合成,测序由睿博兴科公司完成。

1.2 *FabHLH148*基因生物信息学分析

草莓*FabHLH148*全长CDS序列分析采用NCBI Blast程序比对;利用NCBI Conserved Domains进行蛋白质的保守结构域预测;利用在线软件ExPASy分析蛋白理化性质;利用DNA star软件进行蛋白质二级结构分析;利用在线软件SWISS-MODEL进行蛋白质三级结构预测。利用MEGA 5.1构建系统进化树。

1.3 草莓总RNA提取与cDNA第1条连的合成

草莓总RNA的提取参照北京华越洋公司的植物总RNA提取试剂盒说明书进行,利用ND5000超微量分光光度计测定及调整RNA浓度,反转录实验参照YEASEN公司Hifair® Ⅲ1st Strand cDNA Synthesis SuperMix for qPCR(gDNA digester plus)试剂盒说明书进行。

1.4 *FabHLH148*基因CDS全长的克隆

使用SnapGene软件,根据NCBI中*FvbHLH148*(GeneBank登录号:XM_004295010.2)基因的CDS区设计基因克隆引物(表1)。以红颜草莓全红果实的cDNA为模板进行PCR扩增,PCR体系参照诺唯赞公司2×Phanta®Mix Master Mix说明书,程序为:95 ℃,3 min;95 ℃,15 s;65 ℃,15 s;72 ℃,1 min;循环34次;72 ℃,5 min。PCR产物经琼脂糖凝胶检测正确后,使用Axygen凝胶回收试剂盒进行纯化回收。纯化后的目的基因片段连接pEASY®-Blunt Simple克隆载体,转化Trans1 T1感受态细胞,进行

表1 本研究中所用引物

Table 1 The specific primers of this study

引物名称 Primer name	引物序列（5’-3’） Primer sequence（5’-3’）
FabHLH148-F	ATGGCGTCAACAACTCTGATCTCCAATC
FabHLH148-R	ACTCGACGGCGGCGGCGAAGA
FabHLH148-Sal Ⅰ-F	CTGCAGGGGCCCGGGGTCGACATGGCGTCAACAACTCTGATCTCCAATC
FabHLH148-Spe Ⅰ-R	CATGGTACCGGATCCACTAGTACTCGACGGCGGCGGCGAAGA
attB *FabHLH148*-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTAGCCACCTCCTCCGATCAT
attB *FabHLH148*-R	GGGGACCACTTTGTACAAGAAAGCTGGGTCTGCTGCCGGAACTTGATCTT
FabHLH148-RT-F	AACAACTCTGATCTCCAATCCCG
FabHLH148-RT-R	ATTTGGAGGAGTAGAGATGCTGC
FaACTIN-RT-F	TGCATATATCAAGCAACTTTACACTGA
FaACTIN-RT-R	ATAGCTGAGATGGATCTTCCTGT

阳性克隆筛选并测序。

1.5 *FabHLH148*过表达载体构建

采用同源重组的方法，设计同源重组的引物（表1）。以测序成功的pEASY®-Blunt Simple-*FabHLH148*质粒为模板，克隆带有pSuper1300:GFP过表达载体同源臂的基因片段，并将pSuper1300:GFP载体用*Sal* Ⅰ-HF、*Spe* Ⅰ-HF内切酶酶切，经琼脂糖凝胶回收线性化的载体片段。通过诺唯赞ClonExpress® Ⅱ One Step Cloning Kit进行连接，转化DH5α大肠杆菌感受态细胞，筛选阳性克隆进行测序。

1.6 *FabHLH148* RNAi载体构建

通过Gateway的方法构建*FabHLH148* RNAi载体，设计入门载体引物（表1）。克隆*FabHLH148*基因32~383 bp为目的序列，通过琼脂糖凝胶电泳验证PCR结果，胶回收目的片段经BP反应构入pDONR221载体，反应体系参照赛默飞Gateway® BP Clonase™ Ⅱ Enzyme Mix说明书，反应程序为25 ℃过夜，反应结束后加入1 μL Proteinase K，37 ℃反应10 min。将BP反应产物转化大肠杆菌Trans1-T1感受态细胞，筛选阳性克隆并进行测序。提取入门载体pDONR221-$FabHLH148_{352}$质粒通过LR反应构入pK7GWIWG（Ⅱ）RR，得到RNAi载体，反应体系参照赛默飞Gateway® LR Clonase™ Ⅱ Enzyme Mix说明书进行。筛选阳性克隆，提取*FabHLH148* RNAi载体质粒并转化GV3101农杆菌感受态细胞。

1.7 亚细胞定位

将Super1300:FabHLH148-GFP质粒转化GV3101农杆菌感受态。筛选扩繁阳性克隆，制备农杆菌侵染液，侵染5~6周长势良好的烟草叶片。48 h后剪取注射部位的烟草叶片，避光浸泡在稀释好的DNA荧光染料DAPI染液（1 μg·mL^{-1}，现配现用）中30~60 min，用激光共聚焦显微镜观察FabHLH148亚细胞定位。试验3次重复。

1.8 *FabHLH148*瞬时侵染草莓果实

扩繁阳性*FabHLH148* OE、转化空载体的农杆菌（CK）和RNAi农杆菌，制备农杆菌侵染液，挑取长势相近的褪绿时期果实每种农杆菌各注射10个，注射后做好标记并在注射0、3、5和7 d时拍照记录表型，注射7 d后取样，去掉瘦果只切取注射部分液氮速冻后保存在-80 ℃冰箱。试验3次重复。

1.9 草莓果实花色素苷含量测定

取-80℃保存的*FabHLH148* OE、CK以及RNAi组果实，液氮研磨成粉末后称取0.1 g，用于花色素苷的提取，试验步骤以及花色素苷含量的测定参照索莱宝植物花色苷含量检测试剂盒（索莱宝，北京，中国）说明书进行。试验3次重复。

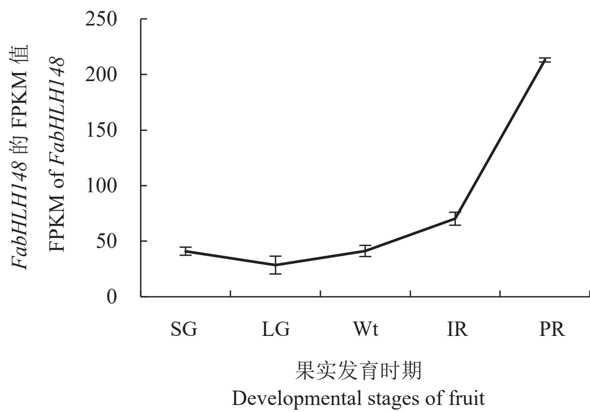
1.10 *FabHLH148*荧光定量分析

RT-qPCR检测基因相对表达量，反应体系参照Trans-Start® Top Green qPCR Super Mix试剂盒（全式金，中国）说明书，PCR反应体系为10 μL，反应程序为94 ℃，30 s；94 ℃，5 s；60 ℃，15 s；72 ℃，10 s；40个循环。草莓*FabHLH148*基因和内参基因的荧光定量的引物见表1。

2 结果与分析

2.1 *FabHLH148*生物信息学分析

根据实验室前期获得的八倍体草莓果实转录组测序结果[24]，筛选得到一个从始红（IR）到片红（PR）时期表达显著升高的基因（图1），该基因与二倍体



SG. 小绿果时期；LG. 大绿果时期；Wt. 白果时期；IR. 始红果时期；PR. 片红果时期。下同。

SG. Small green fruit; LG. Large green fruit; Wt. White fruit; IR. Initial red fruit; PR. Partial red fruit. The same below.

图 1 *FabHLH148* 基因在草莓果实不同发育时期的 FPKM 值

Fig. 1 FPKM of *FabHLH148* in different development periods of strawberry fruit

草莓 *FvbHLH148*（GenBank 登录号：XM_004295010.2）相似度最高，因此将其命名为 *FabHLH148*。*FabHLH148* 基因 CDS 全长 684 bp，共编码 228 个氨基酸。对氨基酸序列分析，发现其在第 153~202 位氨基酸处包含一个保守的 bHLH 结构域（图2），表明其属于 bHLH 超家族。氨基酸理化性质分析结果表明：*FabHLH148* 预测编码的蛋白质分子式为 $C_{1080}H_{1809}N_{353}O_{312}S_5$，分子质量为 24.89 ku，理论等电点（pI）为 11.56，为碱性蛋白，含有负电荷氨基酸残基（Asp+Glu）共 10 个，正电荷氨基酸残基（Arg+Lys）共 42 个，不稳定系数为 54.49，亲水性平均值为-0.636，由此推断该蛋白为不稳定的疏水性蛋白。FabHLH148 二级结构预测结果（图3）显示，其含有的 *α*-螺旋、*β*-折叠、无规则卷曲、柔性区域等基本上分布均匀，其三级结构预测如图 4 所示。从 NCBI 数据库获得不同种植物的 FabHLH148 同源蛋白序列进行比对，构建系统进化树（图5），其中二倍体草莓（*Fragaria vesca* subsp. *vesca*）FvbHLH148 蛋白（GenBank 登录号：XP_004295058.1）与八倍体草莓 FabHLH148 的相似度最高，达 99.56%，其次是与月季（*Rosa chinensis*）RcbHLH147 蛋白（GenBank 登录号：XP_024197449.1），序列相似度达 79.25%。

图 2 FabHLH148 的结构域分析

Fig. 2 Analysis of conserved domain of FabHLH148

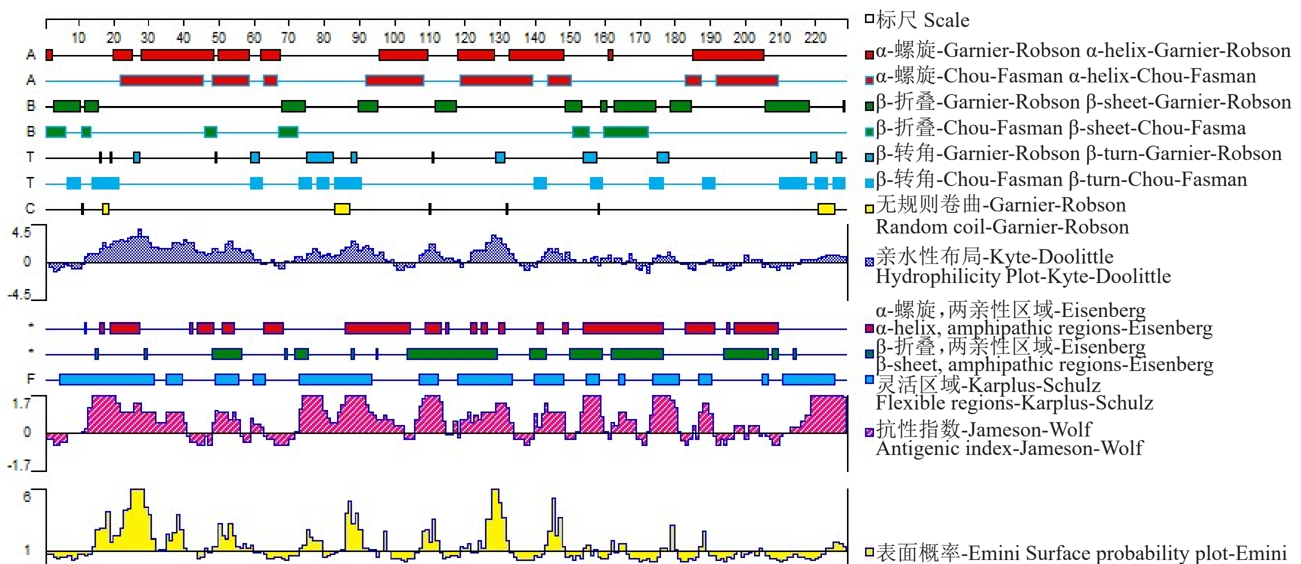
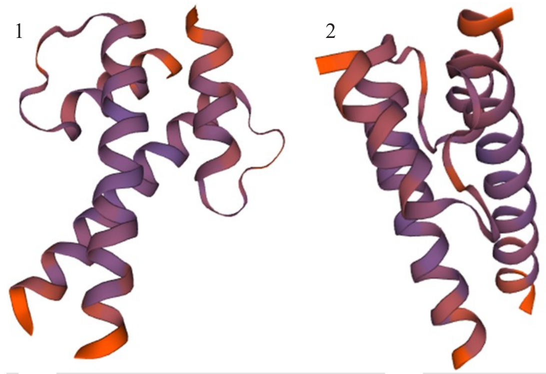


图 3 FabHLH148 蛋白质二级结构分析

Fig. 3 Analysis of protein secondary structure of FabHLH148



1~2. FabHLH148 蛋白质三级结构不同视图。

1-2. Different views of the tertiary structure of FabHLH148 protein.

图4　SWISS-MODEL 预测 FabHLH148 蛋白质三级结构

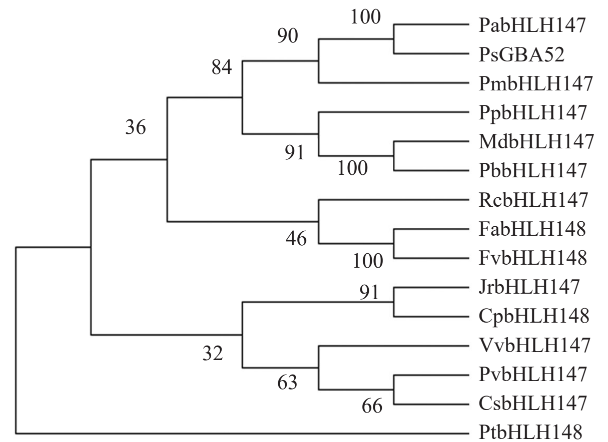
Fig. 4　The tertiary structure of FabHLH148 protein predicted with SWISS－MODEL

2.2　FabHLH148亚细胞定位

为观察FabHLH148的亚细胞定位，进行了农杆菌介导的烟草叶肉细胞瞬时转化实验。实验结果表明FabHLH148-GFP蛋白在细胞核和细胞质中有定位，且在细胞核中与DNA荧光染料DAPI共定位（图6）。细胞核定位为FabHLH148行使转录因子功能提供了空间基础。

2.3　*FabHLH148*的表达模式

为检测*FabHLH148*在草莓中的表达模式，通过RT-qPCR检测其在草莓不同组织部位的表达（图7-A），发现其在草莓不同组织部位均有表达，在全红果实中的相对表达量最高，在瘦果中的相对表达量最低。其次，还检测了*FabHLH148*在草莓果实不同发育时期的表达水平，结果显示该基因从褪绿到片红时期的表达量缓慢升高，片红到全红时期表达量迅速升高（图7-B），这为*FabHLH148*在草莓果实成熟中发挥功能提供了基础。



Pa. 樱桃；Ps. 山杏；Pm. 梅花；Pp. 桃；Md. 苹果；Pb. 杜梨；Rc. 月季；Fa. 草莓；Fv. 森林草莓；Jr. 核桃；Cp. 番木瓜；Vv. 葡萄；Pv. 阿月浑子；Cs. 黄瓜；Pt. 毛果杨。

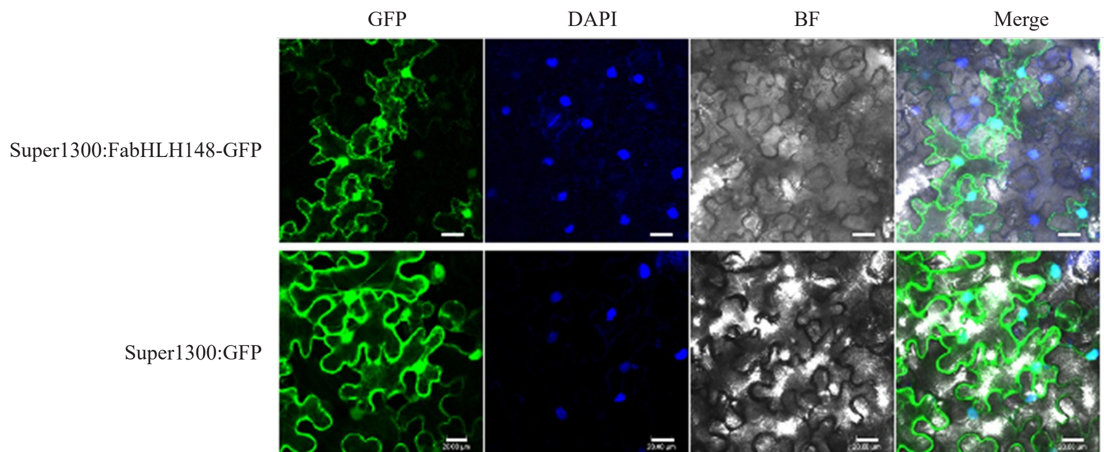
Pa. *Prunus avium*; Ps. *Prunus sibirica*; Pm. *Prunus mume*; Pp. *Prunus persica*; Md. *Malus domestica*; Pb. *Pyrus bretschneideri*; Rc. *Rosa chinensis*; Fa. *Fragaria × ananassa*; Fv. *Fragaria vesca* subsp. vesca; Jr. *Juglans regia*; Cp. *Carica papaya*; Vv. *Vitis vinifera*; Pv. *Pistacia vera*; Cs. *Cucumis sativus*; Pt. *Populus trichocarpa.*

图5　FabHLH148 同源蛋白的系统进化树分析

Fig. 5　Phylogenetic tree analysis of FabHLH148 homologous proteins

2.4　*FabHLH148*促进草莓果实着色

为进一步明确*FabHLH148*在草莓果实成熟中的功能，分别构建*FabHLH148* OE和RNAi载体，转化农杆菌，通过农杆菌介导瞬时侵染草莓果实，记录表型（图8-A），检测*FabHLH148*表达水平（图8-B），

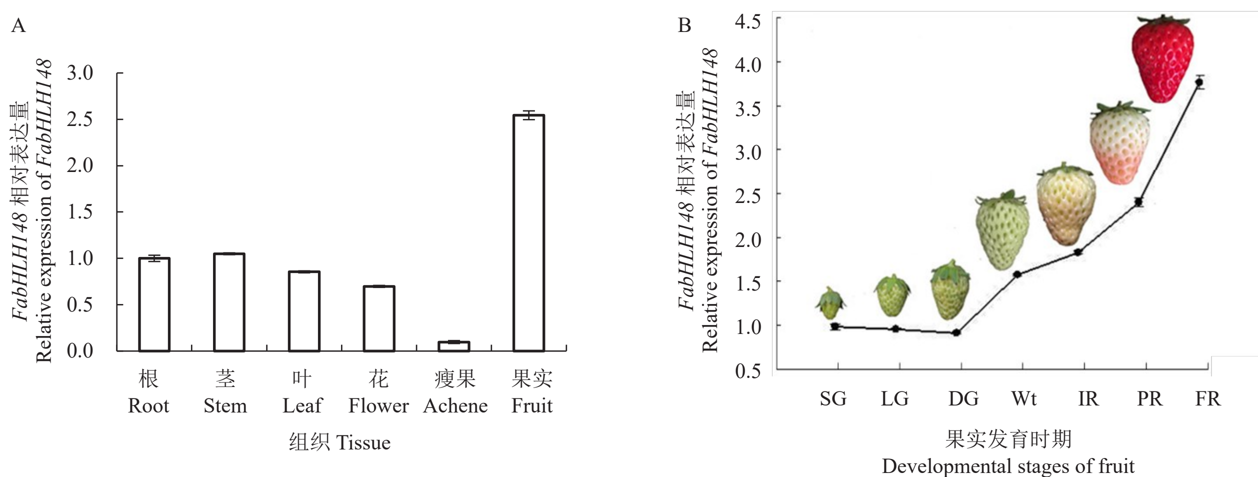


GFP. 绿色荧光蛋白通道；DAPI. DNA 荧光染料通道；BF. 明场；Merge. 合并图片。Bars= 20 μm。

GFP. Signal (green); DAPI. Signal (blue); BF. Bright field; Merge. Overlay signals. Bars: 20 μm.

图6　FabHLH148 亚细胞定位

Fig. 6　Subcellular localization of FabHLH148

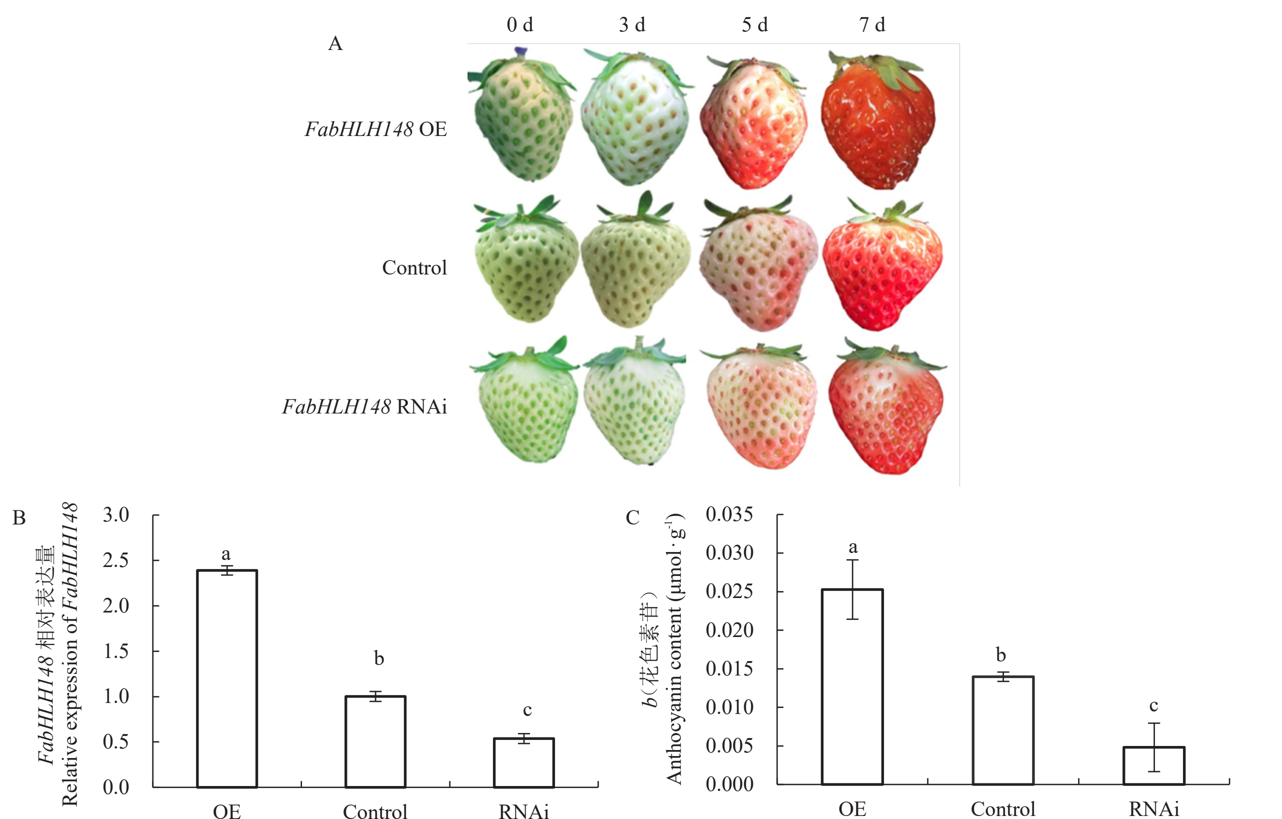


A. *FabHLH148* 在草莓不同器官的相对表达量；B. *FabHLH148* 在草莓果实不同发育时期的相对表达量。

A. Relative expression level of *FabHLH148* in different organs of strawberry; B. Relative expression level of *FabHLH148* in different developmental stages of strawberry fruit.

图 7 *FabHLH148* 在草莓不同组织部位(A)及发育时期(B)的相对表达量

Fig. 7 Relative expression level of *FabHLH148* in different tissues and developmental stages of strawberry



A. 包含 *FabHLH148* OE、CK 和 *FabHLH148* RNAi 农杆菌注射草莓进行表型观察。B. RT-qPCR 分析 *FabHLH148* OE、CK 和 *FabHLH148* RNAi 组果实中 *FabHLH148* 基因的相对表达量，采用算法为 $2^{-\triangle\triangle ct}$。C. *FabHLH148* OE、CK 和 *FabHLH148* RNAi 组草莓果实的花色素苷含量。不同小写字母表示在 $p<0.05$ 差异显著。

A. RT-qPCR was used to analyze the relative expression levels of *FabHLH148* in *FabHLH148* OE, CK and *FabHLH148* RNAi group of strawberry fruits, and the algorithm was$2^{-\triangle\triangle ct}$. B. *FabHLH148* OE, CK and *FabHLH148* RNAi Agrobacterium were injected into de-green strawberry fruit for phenotypic observation. C. Anthocyanin content of *FabHLH148* OE, CK and *FabHLH148* RNAi group of strawberry fruit. Different small letters indicate significant difference at $p<0.05$.

图 8 FabHLH148 正调控草莓果实成熟

Fig. 8 FabHLH148 positively regulated strawberry fruit ripening

并测定不同转基因果实的花色素苷含量（图8-C）。结果表明，*FabHLH148*过量表达能够促进草莓果实着色以及花色素苷的积累；通过RNAi降低*FabHLH148*表达则抑制草莓果实着色以及花色素苷的积累。以上结果初步说明*FabHLH148*在草莓果实着色过程中起重要作用。

3 讨　论

已有研究表明bHLH转录因子参与花色素苷调控。Cui等[25]研究表明*PyMYB10*、*PybHLH*和*PyWD40*转录因子形成三元复合体MBW（MYB-bHLH-WD40）来调控云南红梨中花色素苷的合成与积累。来自bHLH超家族Ⅲ亚家族的*TT8*（Transparent testa 8）、*GL3*（Glabra 3）和*EGL3*（Enhancer of Glabra 3）能够与*TTG1*（Transparent testaglabra1，WD40蛋白家族）和*MYB*（myeloblastosis protein，MYB蛋白家族）形成MBW复合物，调控拟南芥和番茄中花色素苷的合成[24-28]。在苹果中发现两种bHLH转录因子MdbHLH3[29]和MdbHLH33[30]能够调控花色素苷的积累：Xie等[31]从苹果中分离得到*MdbHLH3*，其通过N端的两个区域（1~23和186~288位氨基酸）与*MdMYB1*互作，激活下游花色素苷合成相关基因*MdDFR*和*MdUFGT*的表达，从而促进低温条件下苹果花色素苷的积累；而*MdbHLH33*通过与*MdMYB16*互作形成异源二聚体来减弱*MdMYB16*对花色素苷的抑制作用[32]，从而促进花色素苷的积累。*NtbHLH1*能够作为调节因子与*NtMYB6*互作从而促进中国水仙中花色素苷的积累[33]。以上研究表明bHLH家族转录因子主要与MYB超家族互作来调控植物花色素苷的合成与积累。范祺祺[34]通过对彩色马铃薯块茎花色素苷合成代谢的转录组学研究，筛选出193个注释在bHLH类转录因子中的差异基因。王沛捷等[35]从紫色马铃薯bHLH转录因子家族中鉴定出5个参与花色素苷合成的基因。吴楠[36]在前期卵叶牡丹叶片转录组数据的基础上，筛选出与叶片花色苷合成相关的bHLH转录因子*PqbHLH1*，并获得异源表达*PqbHLH1*的拟南芥株系，与野生型拟南芥相比，其主根颜色呈红褐色，积累有少量的花色素苷，此外，过表达株系中*AtCHS*、*AtCHI*、*AtF3H*、*AtUFGT*、*AtF3' H*和*AtFLS*等基因的表达水平较野生型均显著增加。Zhao等[37]从红颜草莓以及其突变体小白、白雪公主中筛选出来自不同组织和果实不同发育时期的113个同源bHLH基因，RT-qPCR结果显示，7个选定的*FabHLH*基因（*FabHLH17*、*FabHLH25*、*FabHLH27*、*FabHLH29*、*FabHLH40*、*FabHLH80*和*FabHLH98*）响应果实花色素苷生物合成和激素信号传导；蛋白质互作预测揭示了4种bHLHs（*FabHLH25*、*FabHLH29*、*FabHLH80*和*FabHLH98*）参与果实花色素苷生物合成和激素信号转导。以上研究表明bHLH转录因子家族在植物花色素苷合成与积累过程中具有重要的调控作用。但关于bHLH转录因子家族转录因子*FabHLH148*少有研究。

笔者在本试验中成功从红颜草莓克隆出*FabHLH148*基因，蛋白质保守结构域分析发现FabHLH148具有一个bHLH超家族结构域。其预测编码的氨基酸序列同二倍体森林草莓（*Fragaria vesca* subsp. *vesca*）、月季（*Rose chinensis*）具有较高的同源性，尤其是蔷薇科植物同源性较高，其编码的蛋白功能可能类似。亚细胞定位分析显示*FabHLH148*主要在细胞核中表达。通过RT-qPCR检测*FabHLH148*的时空表达，其在草莓的不同组织部位均有表达，在果实中较高表达，且在全红时期表达量达到峰值，暗示其在草莓果实着色过程中起重要作用。瞬时侵染草莓果实验结果表明过表达*FabHLH148*能够促进草莓果实着色以及花色素苷的积累，通过RNAi降低*FabHLH148*表达水平则结果相反。以上结果表明*FabHLH148*参与草莓果实颜色发育。探究草莓中*FabHLH148*转录因子的表达模式及功能，为bHLH超家族在草莓生长发育以及形态建成等相关研究奠定基础，为提高草莓果实品质相关研究提供参考。

4 结　论

从八倍体红颜草莓中克隆转录因子*FabHLH148*，并提供一系列证据表明*FabHLH148*能够促进红颜草莓果实成熟过程中花色素苷的积累，其调控的分子机制有待进一步研究。

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