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# 紫玉兰‘红元宝’*M13GT1* 基因的克隆及表达分析

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**摘要:** UDP-类黄酮 3-O-葡萄糖基转移酶(3GT)是花青素生物合成途径的重要催化酶之一。为研究其在紫玉兰花青素苷合成途径中的作用,该文以紫玉兰品种‘红元宝’(*Magnolia liliflora* ‘Hongyuanbao’)为材料,根据转录组测序获得的 3GT 序列设计引物,利用 RT-PCR 技术克隆花青素苷生物合成途径中的结构基因 *M13GT1*,并对其进行生物信息学和表达模式分析。结果表明:(1)*M13GT1* 基因的 cDNA 序列长度为 1 863 bp,其中最长开放阅读框(ORF)为 1 374 bp,编码一条 457 aa 的肽链,相对分子质量为 49.37 kDa,理论等电点(pI)为 6.04。(2)氨基酸序列比对显示其具备典型的植物次生产物糖基转移酶信号序列(PSPG box)。(3)系统发育分析结果表明,*M13GT1* 蛋白与小苍兰、矮牵牛、番薯等物种的 3GT 蛋白聚在一支。(4)qRT-PCR 结果显示 *M13GT1* 基因的表达具有时空特异性,在花中的表达量最高,在嫩叶和老叶中有少量表达,而在根和茎中几乎不表达;随着花的发育,*M13GT1* 基因的表达量呈现先降低后升高的趋势,并在盛花期达到最高。上述结果表明,*M13GT1* 可能参与类黄酮 3-O 的糖基化修饰,本研究结果将为木兰属植物花色育种研究奠定基础。

**关键词:** 紫玉兰, 花色, 糖基转移酶, 基因克隆, 表达分析

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## Cloning and expression analysis of *M13GT1* in *Magnolia liliflora* ‘Hongyuanbao’

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**Abstract:** UDP-flavonoid 3-O-glucosyltransferase (3GT) is one of the important catalytic enzymes in the anthocyanin biosynthesis pathway. To study the function of 3GT in anthocyanin biosynthesis of *Magnolia liliflora*, *M. liliflora*

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‘Hongyuanbao’ was employed as materials. Primers were designed based on the 3GT sequence obtained from the transcriptome database of *M. liliflora* ‘Hongyuanbao’, and the structural gene *Ml3GT1* in anthocyanin biosynthesis pathway was cloned by RT-PCR (reverse transcription-PCR), and its bioinformatics and expression pattern were analyzed. The results were as follows: (1) The cDNA sequence length of *Ml3GT1* was 1 863 bp, and the open reading frame was 1 374 bp, encoding 457 amino acid residues. The relative molecular weight of *Ml3GT1* was 49.37 kDa, and its isoelectric point was 6.04. (2) The deduced amino acid sequence of *Ml3GT1* contains a conserved plant secondary product glycosyltransferase signature sequence (PSPG box). (3) Results of the phylogenetic analysis showed that *Ml3GT1* protein was closely relative to 3GT proteins from *Freesia hybrida*, *Petunia × hybrida*, and *Ipomoea batatas*. (4) Results of fluorescence quantitative PCR (qRT-PCR) revealed that *Ml3GT1* has spatio-temporal specificity, with the highest expression level in flowers, the lower expression level in young leaves and old leaves, and little expression in roots and stems; With the development of flowers, the expression level of *Ml3GT1* gene decreased first, then increased, and showed the highest expression level at the fully-flowering period. These results suggest that *Ml3GT1* may be involved in flavonoid 3-O-glycosylation. This study will lay a foundation for the flower and color breeding of *Magnolia* plants.

**Key words:** *Magnolia liliflora*, flower color, glycosyltransferase, gene cloning, expression analysis

花色是观赏植物重要的品质性状之一,花青素苷 (anthocyanin) 是由花青素苷元 (anthocyanidin) 和糖组成的糖苷,是影响花色的一类重要色素物质 (戴思兰和洪艳, 2016)。目前,关于花青素的生物合成途径的研究已经比较深入。图 1 显示花青素生物合成通路上的 3 条主链产生矢车菊素 (cyanidin)、天竺葵素 (pelargonidin) 以及飞燕草素 (delphinidin), 矢车菊素甲基化生成芍药色素 (peonidin), 矮牵牛色素 (petunidin) 和锦葵色素 (malvidin) 则由飞燕草素不同程度的甲基化而来,共同构成了自然界中 6 种主要花色素 (朱丽娟等, 2012)。对花青素苷元进行糖基化修饰,可形成稳定的花青素苷并对呈色具有重要作用 (招雪晴等, 2017)。

在植物花色形成过程中,UDP-类黄酮 3-O-葡萄糖基转移酶 (UDP-flavonoid 3-O-glucosyltransferase, 3GT) 通常作用于花青素苷合成途径的最后一步,能够催化 UDP-葡萄糖中的糖分子转移到花色素的 C3 羟基位点上,将花青素转化生成该通路上第一个稳定的花青素苷 (Sui et al., 2011)。花青素在自然条件下性质不稳定,易通过糖苷键与糖形成花青素苷 (Nakatsuka et al., 2008),构成花青素苷的单糖主要有葡萄糖、鼠李糖、半乳糖、木糖和阿拉伯糖 (庄维兵等, 2018)。从化学的角度来看,糖的结合增加了花青素苷的稳定性和水溶性,花青素最常在 C3 位发生 O-糖基化,其次是 C5 位的糖基化,糖基化使花青素很容易从细胞质的生产部位转移到液泡 (Nakatsuka et al., 2008),从而使颜色稍微向红色转变 (Tanaka et al., 2008)。由此可见,糖基化对花色

的形成起到关键作用。

第一个被发现可以催化花青素 C3 羟基糖基化的酶是玉米 (*Zea mays*) Bronze-1 (X13500),由于花青素的积累较少, Bronze 突变体呈现出苍白的色粒 (Dooner & Nelson, 1977; Larson & Coe, 1977)。迄今为止, 3GT 基因已经在矮牵牛 (*Petunia hybrida*) (Jonsson et al., 1984)、三花龙胆 (*Gentiana triflora*) (Tanaka et al., 1996)、荷兰鸢尾 (*Iris hollandica*) (Yoshihara et al., 2005)、小苍兰 (*Freesia hybrida*) (Sui et al., 2011)、滇牡丹 (*Paeonia delavayi*) (王毅等, 2017)、葡萄风信子 (*Muscari armeniacum*) (杜灵娟等, 2017) 等观赏植物中克隆得到。在大多数植物中, 3GT 的表达常常与花青素的积累正相关,如 *Fh3GT* 和 *MdUFGT*, 然而在有的植物中,如 *Ze3GT*, 其表达并不呈现正相关的趋势 (Ban et al., 2009; Sui et al., 2011; Hu et al., 2016; Qian et al., 2021)。 *PeUFGT3* 在蝴蝶兰 (*Phalaenopsis equestris*) 红色形成中起着重要作用,抑制 *PeUFGT3* 的表达导致花青素含量的显著下降 (Chen et al., 2011)。舒庆艳等 (2018) 利用 VIGS 技术沉默紫斑牡丹 (*Paeonia suffruticosa*) *PsUF3GT* 基因, 3G 型糖苷和 3G5G 型糖苷都有不同程度的降低。由此可见, 3GT 基因在植物花色形成中具有重要作用。

紫玉兰 ‘红元宝’ (*Magnolia liliflora* ‘Hongyuanbao’) 为木兰科 (Magnoliaceae) 木兰属 (*Magnolia*) 的多年生落叶灌木,是紫玉兰 (*Magnolia liliflora*) 的栽培品种,该品种花色深于紫玉兰。课题

组前期研究显示,紫玉兰和紫玉兰‘红元宝’花被片中均含有 4 种在 C3 位芸香糖苷修饰的花青素苷,即矢车菊素 3-O-芸香糖苷-5-O-葡萄糖苷(Cy3Ru5G)、矢车菊素 3-O-芸香糖苷(Cy3Ru)、芍药素 3-O-芸香糖苷-5-O-葡萄糖苷(Pn3Ru5G)、芍药素 3-O-芸香糖苷(Pn3Ru)(Wang et al., 2019)。花青素的 C3 位芸香糖苷化修饰分两步完成,首先在 3GT 作用下 C3 位发生 O-葡萄糖基化,随后鼠李糖转移酶(3-O-rhamnosyltransferase, 3RT)才可以转移 O-鼠李糖苷与 O-葡萄糖苷相连形成芸香糖苷基团(Yamazaki et al., 2002)。由此推测紫玉兰‘红元宝’花色形成过程中,3GT 基因对 4 种花青素苷的合成具有重要作用。为进一步揭示其功能,本研究基于转录组数据,筛选出 3GT 基因,并对其克隆和表达分析,研究结果为木兰属植物花色形成机理研究提供重要依据。

## 1 材料与方 法

### 1.1 试验材料

紫玉兰‘红元宝’十年生实生苗种植于浙江农林大学平山试验基地(119° 42' 54.67" E、30°15'50.09" N),选择生长健壮且无病虫害的植株,于 2019 年 4 月采取花蕾期(bud period)(S1)、露色期(dew color period)(S2)、初花期(initial flowering period)(S3)、半花期(half-flowering period)(S4)、盛花期(fully-flowering period)(S5)5 个时期的最外轮花瓣(图 2),锡纸包裹,液氮速冻固样。由于紫玉兰‘红元宝’为花叶同放,同时采集根、茎、老叶、嫩叶等组织部位的样品,保存于-80 °C 冰箱,用于 RNA 提取和荧光定量分析。

### 1.2 总 RNA 的提取、检测与 cDNA 的合成

使用诺禾致源 UltraClean Polysaccharide and phenol Plant RNA Purification Kit (DNA free) RNA 提取试剂盒(NHUC002S),提取紫玉兰‘红元宝’S1~S5 时期花瓣和根、茎、老叶、嫩叶等组织部位的总 RNA。使用 PrimeScript™ RT Master Mix (Perfect Real Time)(TaKaRa code: RR036A)进行反转录反应。反转录后得到的 cDNA 保存在-20 °C 冰箱备用。

### 1.3 *MI3GT1* 基因克隆

利用本课题组前期构建的紫玉兰‘红元宝’花瓣转录组数据库(结果未发表),以 anthocyanidin/flavonoid 3-O-glucosyltransferase 为检索词,对注释到

Nr(non-redundant protein database)数据库的基因注释进行筛选,对得到的序列 CL3388.Contig1 进一步通过 NCBI 的 BLAST 功能(<https://blast.ncbi.nlm.nih.gov/Blast.cgi>)进行 blast 比对。在开放阅读框(open reading frame, ORF)框的两侧使用 Prime Prime 5.0 软件设计引物(表 1),引物由杭州有康生物技术有限公司合成。将紫玉兰‘红元宝’花发育的 S1~S5 共 5 个时期的 cDNA 等量混匀,稀释 10 倍作为模板,进行 RT-PCR 扩增。20 μL PCR 反应体系如下:上下游引物(10 μmol · L<sup>-1</sup>)各 1 μL, cDNA 模板 1 μL, Premix Taq 10 μL, ddH<sub>2</sub>O 7 μL。反应程序:95 °C 预变性 5 min 后运行 35 个循环(95 °C 变性 30 s, 59.6 °C 30 s, 72 °C 2 min); 72 °C 10 min, 10 °C 5 min。PCR 反应结束后,通过 1%(w/v)琼脂糖凝胶电泳分离目的片段。切胶后,使用 TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver. 4.0 (TaKaRa code: No.9762)试剂盒按照使用说明书进行胶回收。回收后,取适量的回收产物连接至 pMD™18-T Vector (TaKaRa code: No.6011)。连接产物转化大肠杆菌 *Escherichia coil* DH5α Competent Cells (TaKaRa code: No. 9057),蓝白斑筛选后挑单克隆进行菌落 PCR 鉴定,条带正确的送至杭州有康生物技术有限公司进行 DNA 测序。

表 1 紫玉兰‘红元宝’*MI3GT1* 基因克隆及实时荧光定量引物

Table 1 Primers for cloning and real-time quantitative PCR of *MI3GT1* in *Magnolia liliflora* ‘Hongyuanbao’

引物名称 Primer name	序列(5'-3') Sequence (5'-3')	用途 Function
<i>MI3GT1</i> -F	TACCCCAAACCCCATCCCACC	扩增 ORF
<i>MI3GT1</i> -R	CTTCTCCGCTTCTTGCCCAT	To amplify ORF
<i>MI3GT1</i> -qPCR-F	GACCGCTTCGCCACAAA	实时荧光定量
<i>MI3GT1</i> -qPCR-R	CAATCCCTCCCTCGCTTCT	Real-time quantitative PCR
<i>MbTEF</i> -F	AGGTTGAGAATGGTGAGACTGT	扩增内参基因
<i>MbTEF</i> -R	TCACGCACGGAATCATTACATT	To amplify reference gene

### 1.4 *MI3GT1* 基因的生物信息学分析

使用 SnapGene 4.1.8 软件去除载体序列,获得目的基因序列;使用 DANMAN 7.0 软件将核苷酸序列翻译成蛋白质序列;利用 NCBI 提供的 ORF finder (<https://www.ncbi.nlm.nih.gov/orffinder/>)分



析序列的编码区;利用 CDD 蛋白保守结构域数据库 (<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>) 分析蛋白的保守域;利用 ProtParam (<https://web.expasy.org/protparam/>) 在线软件对紫玉兰‘红元宝’M13GT1 蛋白序列的分子量、等电点、不稳定系数、脂肪指数和亲疏水性等理化性质进行预测;利用 protscale 在线网站分析该蛋白质的亲/疏水性 (<https://web.expasy.org/protscale/>);利用 SOPMA 在线软件 ([https://npsa-prabi.ibcp.fr/cgi-bin/secpred\\_sopma.pl](https://npsa-prabi.ibcp.fr/cgi-bin/secpred_sopma.pl)) 预测 M13GT1 蛋白序列的二级结构;利用 SWISS-MODEL 在线网站 ([swissmodel.expasy.org](http://swissmodel.expasy.org)) 预测 M13GT1 蛋白的三级结构;使用 DNAMAN 7.0 软件进行多序列比对,并使用 MEGA 6.0 软件构建系统发育进化树,系统发育进化树的构建在序列比对后,采用邻接法建树 (neighbor-joining, NJ), bootstrap 重复 1 000 次获得分支可信度。

### 1.5 M13GT1 在花开放的不同时期与组织的表达分析

使用 Light Cycler 480 II (Roche) 实时定量 PCR 仪进行基因表达相对定量分析。反应体系:模板 2  $\mu\text{L}$ , 上下游引物各 0.8  $\mu\text{L}$ , 荧光染料 BCG Qper Master Mix (2 $\times$ ) 10  $\mu\text{L}$  和 6.4  $\mu\text{L}$  ddH<sub>2</sub>O。通过两步法进行 qRT-PCR, 扩增程序为 95  $^{\circ}\text{C}$  预变性 30 s 后运行 40 个循环 (95  $^{\circ}\text{C}$  5 s, 60  $^{\circ}\text{C}$  30 s), 然后再运行 (95  $^{\circ}\text{C}$  5 s, 60  $^{\circ}\text{C}$  1 min, 95  $^{\circ}\text{C}$  15 s)。对反转录得到的 cDNA 分别进行 5 倍数梯度稀释, 以 *MbTEF* 为内参基因 (王宁杭等, 2019), 每个样品设置 3 个生物学重复。采用  $2^{-\Delta\Delta Ct}$  计算目的基因的相对表达量。使用 SigmaPlot 14.0 软件进行数据分析和绘图。

## 2 结果与分析

### 2.1 M13GT1 基因克隆及序列分析

紫玉兰‘红元宝’的 RT-PCR 结果显示, 扩增产物长 1 863 bp (图 3), 将测序得到的序列通与转录组序列 CL3388.Contig1 进行比对, 核苷酸序列相似性为 99.84%。利用 NCBI 的 ORF finder 分析发现其最长开放阅读框 1 374 bp, 编码 457 个氨基酸, 推导的氨基酸序列与转录组序列相似性为 100%。将该基因命名为 *M13GT1*, 在 GenBank 登录号为 MW454862。

通过 NCBI 的 CDD 蛋白保守结构域数据库, 对紫玉兰‘红元宝’M13GT1 蛋白保守区进行预测, 结

果表明, M13GT1 具备尿嘧啶二磷酸-糖基转移酶结构域 (PLN02670), UDP-葡萄糖醛基/葡萄糖基转移酶保守域 (UDPGT), 表明 M13GT1 属于糖基转移酶超家族, 具有 GT1\_Gtf-like 结构域, 表明其属于植物界中最大的家族 1 糖基转移酶 (GT1s)。按照蛋白质的三维折叠模式, 糖基转移酶可以大致划分为 GT-A、GT-B 和 GT-C 三大类 (Coutinho et al., 2003), 预测结果显示, M13GT1 为 GT-B 类的糖基转移酶。

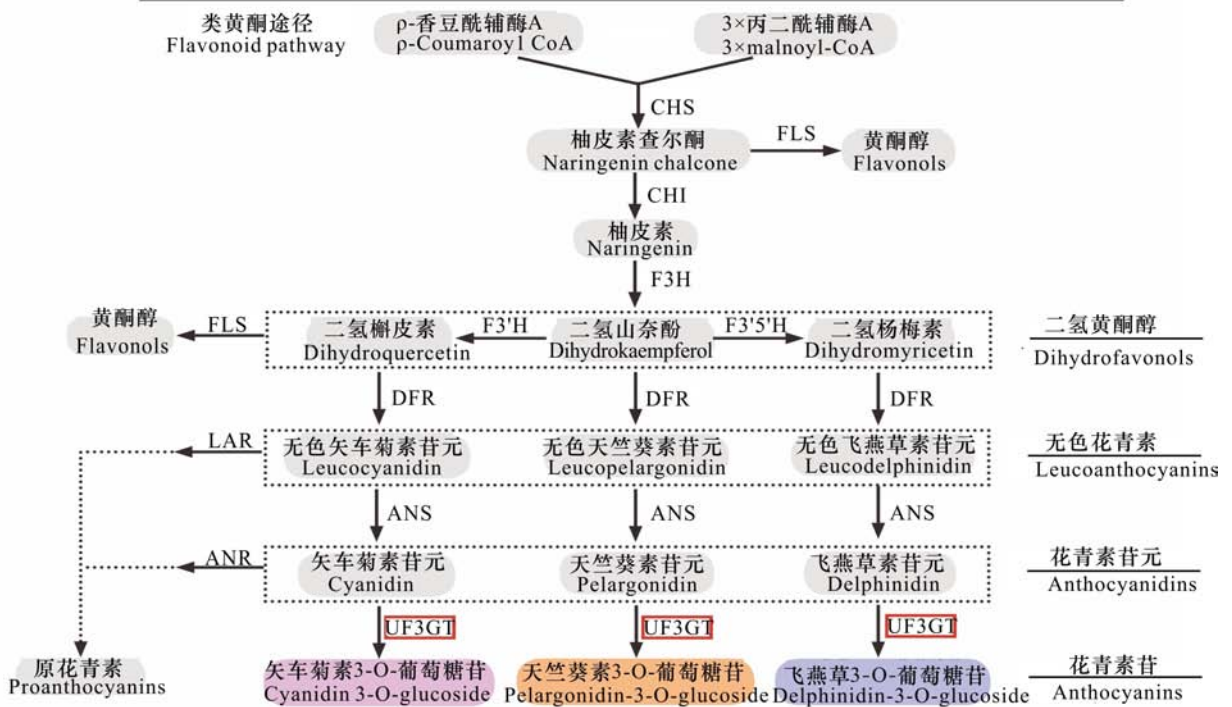
利用 NCBI 的在线 Blastp 功能, 将 M13GT1 的氨基酸序列与其他植物的氨基酸序列进行在线比对, 结果表明, M13GT1 与沉水樟 (*Cinnamomum micranthum*) 3GT 的相似性最高, 达 60.35%, 与荷花 (*Nelumbo nucifera*)、杨梅 (*Morella rubra*)、海枣 (*Phoenix dactylifera*)、美洲葡萄 (*Vitis labrusca*)、蓖麻 (*Ricinus communis*) 等物种 3GT 的相似性较高, 为 49.00%~54.27%。

使用 DNAMAN 7.0 软件将 M13GT1 推导的氨基酸序列与其他物种已发表的 3GT 氨基酸序列进行多序列比对。图 4 结果显示, M13GT1 与拟南芥、苹果、玉米、矮牵牛、葡萄、三花龙胆 3GT 蛋白的相似性分别为 46.20%、44.20%、41.60%、38.40%、47.20%、41.80%, 且 M13GT1 与其他 3GT 蛋白类似, 其 C 端具备典型的由 44 个氨基酸组成的保守序列, 即植物次生产物糖基转移酶信号序列 (PSPG box), 表明 M13GT1 具备植物糖基转移酶特征, 可能参与次生代谢产物的糖基化修饰。M13GT1 的 PSPG 基序为 WAPQTMVLGHVALGAFVTHCGWNSVMESITAGVPMICRPFFGDQ, 已有的研究表明, 糖供体特异性部分由 PSPG 盒中的最后氨基酸残基确定, 当为 Q 时使用葡萄糖作为糖供体, 当为 H 时则使用半乳糖 (Kubo et al., 2004), M13GT1 的 PSPG 基序最后一个残基为 Q, 表明 M13GT1 可能属于 UDP-类黄酮糖基转移酶, 使用 UDP-葡萄糖作为糖供体。

### 2.2 M13GT1 基因的生物信息学分析

通过 ProtParam 在线软件分析 M13GT1 氨基酸的理化性质, 结果表明: M13GT1 编码 457 个氨基酸, 分子式为 C<sub>2216</sub>H<sub>3457</sub>N<sub>601</sub>O<sub>638</sub>S<sub>20</sub>, 相对分子质量为 49.37 kDa, 理论等电点 (pI) 为 6.04, 小于 7, 说明其为酸性蛋白; 其中带负电荷氨基酸残基 (Asp + Glu) 数为 50, 带正电荷氨基酸残基 (Arg + Lys) 数为 45, 丙氨酸含量最多, 占 10.9%, 酪氨酸含量最少, 占 0.9%; 该蛋白不稳定系数为 40.71,

一般苯丙素途径  
General phenylpropanoid pathway



**CHS.** 查尔酮合成酶; **FLS.** 黄酮醇合成酶; **CHI.** 查尔酮异构酶; **F3H.** 黄烷酮 3-羟基化酶; **F3'H.** 类黄酮-3'-羟基化酶; **F3'5'H.** 类黄酮-3',5'-羟基化酶; **DFR.** 二氢黄酮醇还原酶; **ANS.** 花青素合成酶; **UF3GT.** UDP-类黄酮 3-O-葡萄糖基转移酶; **LAR.** 无色花青素还原酶; **ANR.** 花青素还原酶。

**CHS.** Chalcone synthase; **FLS.** Flavonol synthase; **CHI.** Chalcone isomerase; **F3H.** Flavanone 3-hydroxylase; **F3'H.** Flavonoid-3'-hydroxylase; **F3'5'H.** Flavonoid-3',5'-hydroxylase; **DFR.** Dihydroflavonol reductase; **ANS.** Anthocyanin synthase; **UF3GT.** UDP-flavonoid 3-O-glucosyltransferase; **LAR.** Leucoanthocyanin reductase; **ANR.** Anthocyanin reductase.

图 1 花青素生物合成途径

Fig. 1 The biosynthetic pathway of anthocyanin(Petroni & Tonelli, 2011; Liu et al., 2018)

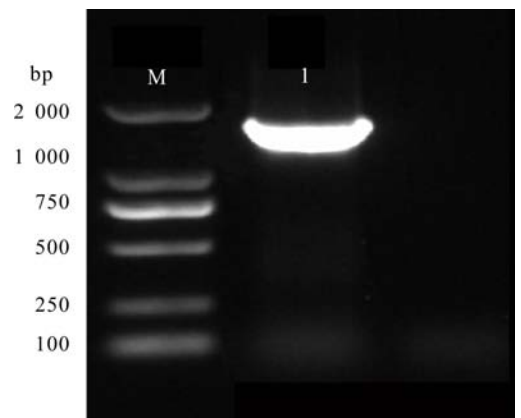


图 2 不同开花阶段的紫玉兰‘红元宝’

Fig. 2 Flowering stages of *Magnolia liliflora* ‘Hongyuanbao’

不稳定系数大于 40,表明其为不稳定蛋白;总平均亲水性的负值表示亲水性,正值表示疏水性,且正值越大表示疏水性越强,*MI3GT1* 总平均亲水性为 0.090,说明该蛋白是疏水性蛋白。

通过 SOPMA 在线网站对紫玉兰‘红元宝’3GT1 蛋白的二级结构预测(图 5),结构表明 *MI3GT1* 蛋



M. DL2000 DNA Marker; 1. *MI3GT1*。

图 3 紫玉兰‘红元宝’*MI3GT1* 基因克隆

Fig. 3 Gene cloning of *MI3GT1* from *Magnolia liliflora* ‘Hongyuanbao’

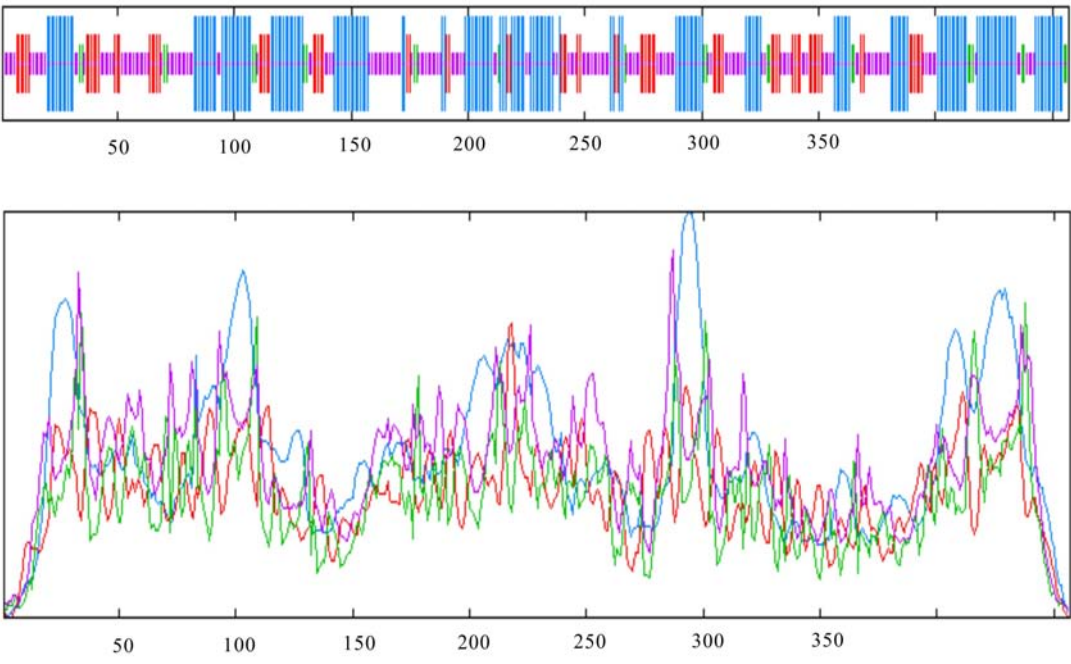


拟南芥 <i>Arabidopsis thaliana</i> (NP197207.1)	.....MTKPSIPTRDSEAVLAFPPGTHAPELLITVRRRLASASP...STVFSFNTACSSNLSLFGSDE...ACRPNANRVYTIADGVPEG	80
苹果 <i>Malus domestica</i> (AF117267.1)	MAAPLPIEI EFSSTNGPPLALAYNREAVVAVFPPTSHASALLETVRRRLATALP...NTLFSFPTSRKSSLSFNSNI...TNAPPNRVYTVADGVPEG	95
玉米 <i>Zea mays</i> (AY167672.1)	.....MAPADGESSPPPEAVVAVFPPTSHASALLETVRRRLATALP...NTLFSFPTSRKSSLSFNSNI...TNAPPNRVYTVADGVPEG	86
矮牵牛 <i>Petunia × hybrida</i> (AB027454.1)	.....MTTSCLEALLAEPFSSHAPELLITVRRRLASASP...STVFSFNTACSSNLSLFGSDE...ACRPNANRVYTIADGVPEG	73
葡萄 <i>Vitis vinifera</i> (BAB41024.1)	.....NSQTTTNPVAVLAFPPGTHAPELLITVRRRLASASP...STVFSFNTACSSNLSLFGSDE...ACRPNANRVYTIADGVPEG	76
三花龙胆 <i>Gentiana triflora</i> (D85186.1)	.....MSPVSAVLAEPFSSHAPELLITVRRRLASASP...STVFSFNTACSSNLSLFGSDE...ACRPNANRVYTIADGVPEG	74
M13GT1	.....MFAKPPPEAVLAFPPGTHAPELLITVRRRLASASP...STVFSFNTACSSNLSLFGSDE...ACRPNANRVYTIADGVPEG	76
拟南芥 <i>Arabidopsis thaliana</i> (NP197207.1)	YVFGKPCEDI ELENAPENIRSLDASVAIDCK...CISGLIEMDFVFGVHLADELGVVTFVI...SGLKSLISVYVHLLIETI...CGITGRENLLI	172
苹果 <i>Malus domestica</i> (AF117267.1)	EESVVPVRCGLFVEMAEACGVKAWLEAARAAAGGARVTCVQDF...VWPAADAASAGAPVWVIV...VAVSALLAEIRLALREIVG...DQAAANRVEPL	183
玉米 <i>Zea mays</i> (AY167672.1)	NCKNPVGLAEIKLEI...CATPINFKVKVAEEETGV...KFSGLFSDAFVFSKLAEKI...NVPVIAFV...AAASCSLSVLYGDFIRS...NDETSL	161
矮牵牛 <i>Petunia × hybrida</i> (AB027454.1)	YVAGRPCEAILELCAAPENFRREI...AKAEIVETGV...EVLKEMDFVFGVHLADELGVVTFVI...SGLKSLISVYVHLLIETI...CGITGRENLLI	192
葡萄 <i>Vitis vinifera</i> (BAB41024.1)	YVAGRPCEAILELCAAPENFRREI...AKAEIVETGV...EVLKEMDFVFGVHLADELGVVTFVI...SGLKSLISVYVHLLIETI...CGITGRENLLI	173
三花龙胆 <i>Gentiana triflora</i> (D85186.1)	YVFGKPCEDI ELENAPENIRSLDASVAIDCK...CISGLIEMDFVFGVHLADELGVVTFVI...SGLKSLISVYVHLLIETI...CGITGRENLLI	171
M13GT1	YVPLGKPCEDI ELENAPENIRSLDASVAIDCK...CISGLIEMDFVFGVHLADELGVVTFVI...SGLKSLISVYVHLLIETI...CGITGRENLLI	174
拟南芥 <i>Arabidopsis thaliana</i> (NP197207.1)	GV...ISGAE...KIRVKE...PFGVFCNLSVFSKALHQL...GLR...LP...AVAVF...NSPELE...LPTLN...RSRFRK...Y...N...F...L...GLL...S...T...L...C...L...V...C...E...P...H...G...C	267
苹果 <i>Malus domestica</i> (AF117267.1)	VEKNVY...G...L...N...RI...K...D...A...G...V...I...F...C...N...L...S...V...F...S...K...A...L...H...Q...L...R...L...P...R...A...V...A...V...F...N...S...P...E...L...E...L...P...T...L...N...R...S...R...F...R...K...Y...N...F...L...G...L...L...S...T...L...C...L...V...C...E...P...H...G...C	284
玉米 <i>Zea mays</i> (AY167672.1)	I...S...H...P...L...A...S...Y...R...V...R...D...P...E...G...V...S...G...D...F...Y...Y...I...L...L...H...N...H...K...C...C...P...R...S...A...A...V...A...L...N...P...C...L...P...P...V...T...A...A...L...A...E...I...L...P...N...C...P...P...G...P...H...L...L...A...E...I...D...A...T...A...A...P...P...H...G...C	278
矮牵牛 <i>Petunia × hybrida</i> (AB027454.1)	N...I...P...F...S...T...L...K...I...S...E...N...P...P...V...A...E...N...L...P...P...M...Y...N...L...H...H...K...A...A...V...V...I...N...S...P...E...L...E...L...P...T...L...N...R...S...R...F...R...K...Y...N...F...L...G...L...L...S...T...L...C...L...V...C...E...P...H...G...C	253
葡萄 <i>Vitis vinifera</i> (BAB41024.1)	N...I...P...F...S...T...L...K...I...S...E...N...P...P...V...A...E...N...L...P...P...M...Y...N...L...H...H...K...A...A...V...V...I...N...S...P...E...L...E...L...P...T...L...N...R...S...R...F...R...K...Y...N...F...L...G...L...L...S...T...L...C...L...V...C...E...P...H...G...C	251
三花龙胆 <i>Gentiana triflora</i> (D85186.1)	LF...N...I...P...G...L...S...K...V...R...F...D...C...E...G...I...F...V...C...N...L...S...F...S...K...A...L...H...Q...L...R...L...P...R...A...V...A...V...F...N...S...P...E...L...E...L...P...T...L...N...R...S...R...F...R...K...Y...N...F...L...G...L...L...S...T...L...C...L...V...C...E...P...H...G...C	269
M13GT1	LF...N...I...P...G...L...S...K...V...R...F...D...C...E...G...I...F...V...C...N...L...S...F...S...K...A...L...H...Q...L...R...L...P...R...A...V...A...V...F...N...S...P...E...L...E...L...P...T...L...N...R...S...R...F...R...K...Y...N...F...L...G...L...L...S...T...L...C...L...V...C...E...P...H...G...C	262
拟南芥 <i>Arabidopsis thaliana</i> (NP197207.1)	LAWKEKRS...G...S...V...M...I...S...F...G...I...M...T...P...P...C...E...L...A...I...A...E...G...E...S...K...V...P...F...V...S...K...E...K...S...L...V...L...P...K...G...L...D...R...T...R...E...C...G...I...V...P...V...A...P...Q...V...E...L...D...K...E...A...T...C...F...V...H...G...V...S...V...L	363
苹果 <i>Malus domestica</i> (AF117267.1)	LSWDEKQCAP...S...M...V...I...S...F...G...I...M...T...P...P...C...E...L...A...I...A...E...G...E...S...K...V...P...F...V...S...K...E...K...S...L...V...L...P...K...G...L...D...R...T...R...E...C...G...I...V...P...V...A...P...Q...V...E...L...D...K...E...A...T...C...F...V...H...G...V...S...V...L	383
玉米 <i>Zea mays</i> (AY167672.1)	LAWGRQP...G...A...V...S...F...G...I...M...T...P...P...C...E...L...A...I...A...E...G...E...S...K...V...P...F...V...S...K...E...K...S...L...V...L...P...K...G...L...D...R...T...R...E...C...G...I...V...P...V...A...P...Q...V...E...L...D...K...E...A...T...C...F...V...H...G...V...S...V...L	376
矮牵牛 <i>Petunia × hybrida</i> (AB027454.1)	LWIKERK...E...S...M...V...S...F...G...I...M...T...P...P...C...E...L...A...I...A...E...G...E...S...K...V...P...F...V...S...K...E...K...S...L...V...L...P...K...G...L...D...R...T...R...E...C...G...I...V...P...V...A...P...Q...V...E...L...D...K...E...A...T...C...F...V...H...G...V...S...V...L	349
葡萄 <i>Vitis vinifera</i> (BAB41024.1)	LWIKERK...E...S...M...V...S...F...G...I...M...T...P...P...C...E...L...A...I...A...E...G...E...S...K...V...P...F...V...S...K...E...K...S...L...V...L...P...K...G...L...D...R...T...R...E...C...G...I...V...P...V...A...P...Q...V...E...L...D...K...E...A...T...C...F...V...H...G...V...S...V...L	357
三花龙胆 <i>Gentiana triflora</i> (D85186.1)	LWIKERK...E...S...M...V...S...F...G...I...M...T...P...P...C...E...L...A...I...A...E...G...E...S...K...V...P...F...V...S...K...E...K...S...L...V...L...P...K...G...L...D...R...T...R...E...C...G...I...V...P...V...A...P...Q...V...E...L...D...K...E...A...T...C...F...V...H...G...V...S...V...L	355
M13GT1	LWIKERK...E...S...M...V...S...F...G...I...M...T...P...P...C...E...L...A...I...A...E...G...E...S...K...V...P...F...V...S...K...E...K...S...L...V...L...P...K...G...L...D...R...T...R...E...C...G...I...V...P...V...A...P...Q...V...E...L...D...K...E...A...T...C...F...V...H...G...V...S...V...L	360
拟南芥 <i>Arabidopsis thaliana</i> (NP197207.1)	ESVSGGVPM...Q...R...P...F...D...Q...R...I...N...G...R...A...V...E...V...E...I...G...T...I...I...N...G...V...E...K...D...F...E...K...L...E...V...L...V...C...E...L...G...K...K...K...N...A...K...K...E...L...A...Y...E...A...S...S...N...R...S...E...N...R...C...L...L...A...V...N...I...I...S...P...S...G...N...Y...Q	460
苹果 <i>Malus domestica</i> (AF117267.1)	ETIAGRVPM...Q...R...P...F...D...Q...R...I...N...G...R...A...V...E...V...E...I...G...T...I...I...N...G...V...E...K...D...F...E...K...L...E...V...L...V...C...E...L...G...K...K...K...N...A...K...K...E...L...A...Y...E...A...S...S...N...R...S...E...N...R...C...L...L...A...V...N...I...I...S...P...S...G...N...Y...Q	482
玉米 <i>Zea mays</i> (AY167672.1)	EGVSSGVPA...Q...R...P...F...D...Q...R...I...N...G...R...A...V...E...V...E...I...G...T...I...I...N...G...V...E...K...D...F...E...K...L...E...V...L...V...C...E...L...G...K...K...K...N...A...K...K...E...L...A...Y...E...A...S...S...N...R...S...E...N...R...C...L...L...A...V...N...I...I...S...P...S...G...N...Y...Q	471
矮牵牛 <i>Petunia × hybrida</i> (AB027454.1)	EGISCGVPM...Q...R...P...F...D...Q...R...I...N...G...R...A...V...E...V...E...I...G...T...I...I...N...G...V...E...K...D...F...E...K...L...E...V...L...V...C...E...L...G...K...K...K...N...A...K...K...E...L...A...Y...E...A...S...S...N...R...S...E...N...R...C...L...L...A...V...N...I...I...S...P...S...G...N...Y...Q	448
葡萄 <i>Vitis vinifera</i> (BAB41024.1)	ESVAGGVPM...Q...R...P...F...D...Q...R...I...N...G...R...A...V...E...V...E...I...G...T...I...I...N...G...V...E...K...D...F...E...K...L...E...V...L...V...C...E...L...G...K...K...K...N...A...K...K...E...L...A...Y...E...A...S...S...N...R...S...E...N...R...C...L...L...A...V...N...I...I...S...P...S...G...N...Y...Q	456
三花龙胆 <i>Gentiana triflora</i> (D85186.1)	ESI FCRVPM...Q...R...P...F...D...Q...R...I...N...G...R...A...V...E...V...E...I...G...T...I...I...N...G...V...E...K...D...F...E...K...L...E...V...L...V...C...E...L...G...K...K...K...N...A...K...K...E...L...A...Y...E...A...S...S...N...R...S...E...N...R...C...L...L...A...V...N...I...I...S...P...S...G...N...Y...Q	453
M13GT1	ESI TAGVPM...Q...R...P...F...D...Q...R...I...N...G...R...A...V...E...V...E...I...G...T...I...I...N...G...V...E...K...D...F...E...K...L...E...V...L...V...C...E...L...G...K...K...K...N...A...K...K...E...L...A...Y...E...A...S...S...N...R...S...E...N...R...C...L...L...A...V...N...I...I...S...P...S...G...N...Y...Q	457

黑色阴影和其他阴影框分别表示相同和相似的氨基酸,下划线表示 PSPC 保守基序。  
 Black shaded and other shaded boxes show identical and similar amino acids, and the underline indicates the PSPC conservative motif.

图 4 推导的 M13GT1 氨基酸序列与其他物种 3GT 氨基酸序列比对

Fig. 4 Multiple alignment of deduced M13GT1 amino acid sequences and 3GT amino acid sequences from other plants



蓝线.  $\alpha$ -螺旋; 紫线. 无规则卷曲; 红线. 延伸链; 绿线.  $\beta$ -转角。  
 Blue line.  $\alpha$ -helix; Purple line. Random coil; Red line. Extended strand; Green line.  $\beta$ -turn.

图 5 M13GT1 蛋白二级结构预测结果

Fig. 5 Results of M13GT1 protein secondary structure prediction

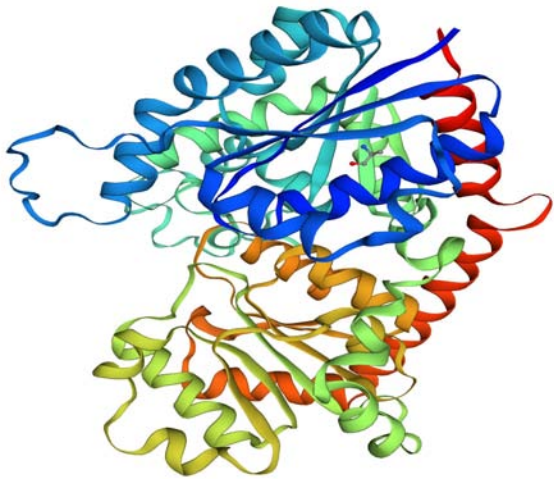


图 6 *MI3GT1* 蛋白三级结构预测结果

Fig. 6 Results of *MI3GT1* protein tertiary structure prediction

白中  $\alpha$ -螺旋(39.39%)和无规则卷曲(38.29%)含量最多,而延伸链和  $\beta$ -转角含量分别为 16.85%和 5.47%。将 *MI3GT1* 序列提交到 SWISS-MODEL 在线网站预测编码蛋白质的三维结构,3D 预测结果显示(图 6),QMEAN 分值在  $-4 \sim 0$  之间,越接近于 0,表明待测蛋白与模板蛋白的匹配度越好,可信度越高,*MI3GT1* 的 QMEAN 分值为  $-0.88$ ,表明 *MI3GT1* 与模板蛋白 UDP-葡萄糖类黄酮 3-O 糖基转移酶(2c1x.1.A)匹配度较好,相似性较高,达 52.67%,推测其可能为类黄酮 3-O 糖基转移酶;GMQE 可信度评分为 0.83,介于  $0 \sim 1$  之间,且较接近 1,说明以上述蛋白模板构建的模型质量较好;*MI3GT1* 具备 GT-B 家族典型的 Rossmann 折叠结构域,表明其为 GT-B 家族成员,这也与 CDD 的预测结果一致。

### 2.3 *MI3GT1* 氨基酸序列系统发育分析

将紫玉兰‘红元宝’*MI3GT1* 与其他植物中已报道的参与类黄酮途径的糖基转移酶共同构建系统发育进化树,图 7 结果显示,GTs 分为 5GTs、7GTs、3GTs 进化分支,*MI3GT1* 与小苍兰 *Fh3GT1* 亲缘关系较近聚为一支,属于 3GTs 进化大支,该分支还有葡萄 *Vv3GT*、草莓 *Fa3GT*、矮牵牛 *Ph3GT*、番薯 *Ib3GT*,且与其他类黄酮糖基转移酶 5GTs、7GTs 的亲缘关系较远,表明其可能与 *Fh3GT1* 功能类似,属于 3GT 糖基转移酶家族成员,参与类黄酮 3-O 的糖基化。

### 2.4 *MI3GT1* 基因在花开放的不同时期与不同组织中的表达分析

以紫玉兰‘红元宝’花开放不同时期(S1~S5)以及老叶、嫩叶、根、茎等组织部位的 cDNA 为模板,探究 *MI3GT1* 基因在紫玉兰‘红元宝’中的表达模式(图 8)。结果表明,随着花的开放进程,*MI3GT1* 基因的表达量呈现先降低后升高的趋势,其表达量在盛花期(S5)达到峰值。各组织部位的荧光定量结果显示,*MI3GT1* 基因具有组织特异性,在花中的表达量最高,在老叶和嫩叶中有少量表达,而在根和茎中几乎不表达,且其在花朵开放的各个时期中的表达量均高于其他组织部位。

## 3 讨论与结论

本文克隆得到紫玉兰‘红元宝’*MI3GT1* 基因,生物信息分析发现 *MI3GT1* 序列具有植物次生产物糖基转移酶信号序列——PSPG box,推测其可能参与次生代谢产物的糖基化修饰。GTs 根据其催化位点的不同,可分为 3-O-糖基转移酶(3GTs)、5-O-糖基转移酶(5GTs)、7-O-糖基转移酶(7GTs)(王应丽等,2014)。系统发育树聚类结果显示 *MI3GT1* 和小苍兰 *Fh3GT1* 亲缘关系较近,聚为一支,进一步表明其可能和 *Fh3GT1* 功能类似,具有参与类黄酮 3-O 的糖基化修饰的功能(Sun et al., 2016)。

有研究表明,3GT 具有花器官组织特异表达模式(王毅等,2017)。滇牡丹(王毅等,2017)和葡萄风信子(杜灵娟等,2017)中 3GT 基因在花青素苷大量积累的组织中高表达,而在少量积累和无花青素苷的组织中,3GT 表达量极低甚至不表达。与前人研究结果一致,在本研究中,*MI3GT1* 基因在花中大量表达,而在其他营养器官组织中几乎不表达或者表达量很低,表明 *MI3GT1* 可能参与花青素苷的生物合成。

3GT 参与小苍兰和葡萄风信子花瓣中花青素苷的生物合成,其表达量往往与花瓣中花青素苷的积累呈正相关(Sui et al., 2011;梁沛雯等,2019)。但是 *MI3GT1* 基因表达模式与小苍兰和葡萄风信子不同,其表达量随着花的开放呈现先下降后上升的趋势,这可能与不同植物花瓣中花青素苷的糖苷类型不同有关。葡萄风信子花瓣中花青素苷糖苷类型均为葡萄糖苷(梁沛雯等,2019),因此葡萄风信子花青素苷的积累与 3GT 基因表达

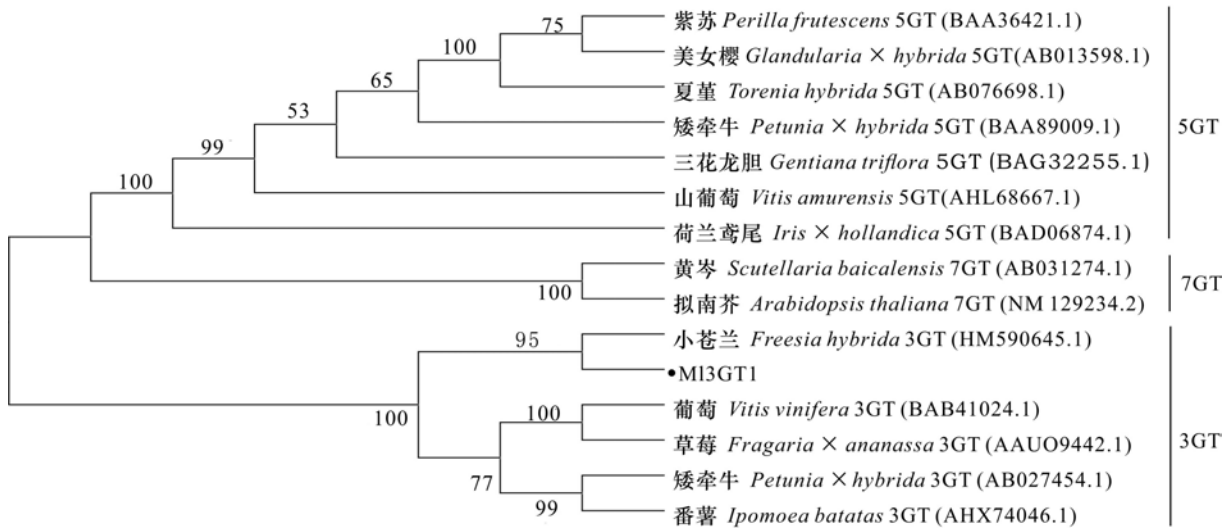


图 7 M13GT1 蛋白与其他植物的 GT 蛋白的系统进化树

Fig. 7 Molecular phylogenetic tree of M13GT1 protein and GT proteins from other plants

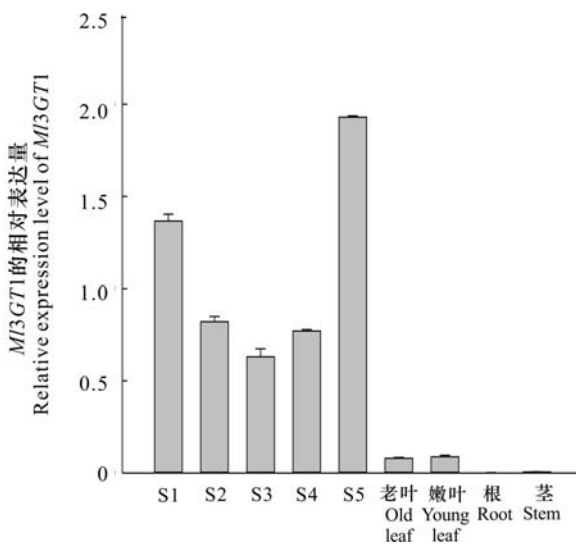


图 8 M13GT1 在紫玉兰‘红元宝’花开放的不同时期以及组织部位的表达分析

Fig. 8 Expression analysis of M13GT1 in different flowering periods and tissue parts of *Magnolia liliflora* ‘Hongyuanbao’

量紧密相关。在紫玉兰‘红元宝’花瓣中,4 种花青素苷 C3 位均发生芸香糖基化修饰,其中 Pn3Ru 含量最高(约占总花青素苷含量的 71%)(Wang et al., 2019)。由于花青素的 C3 位芸香糖苷化修饰依次由 3GT 和 3RT 催化完成(Yamazaki et al., 2002),紫玉兰‘红元宝’芸香糖苷型花青素苷的合

成不仅受 3GT 基因的调控,同时还与 3RT 基因的表达有关。由此表明 M13GT1 参与调控紫玉兰‘红元宝’的花青素苷的生物合成,但是该基因不是紫玉兰‘红元宝’花青素苷生物合成的限速酶基因。

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