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转色初期喷施BTH对‘红地球’葡萄着色和果实品质的影响

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摘 要:【目的】研究植物诱抗剂BTH对葡萄果实花色素苷生物合成、着色和品质的影响。【方法】以‘红地球’葡萄植株为试材,在葡萄果实转色初期对果穗喷施30~120 mg·L^{-1}的BTH溶液,定期采样测定葡萄果实的形态、色度、花色素苷和糖酸累积等品质特征的变化。【结果】BTH处理‘红地球’葡萄果实的颜色指数CIRG和总花色素苷含量均得到显著提高,处理效果依次为60 mg·L^{-1} > 120 mg·L^{-1} > 30 mg·L^{-1};参与花色素苷生物合成的*PAL*、*CHS1*、*CHS2*、*CHI1*、*CHI2*、*F3'H*、*F3H1*、*F3H2*、*DFR*、*LDOX*、*UFGT-2*和*VvmybyA1*等基因和转录因子的相对表达量显著提高,表明BTH诱导葡萄花色素苷含量的增加与花色素苷生物合成途径相关基因的上调表达有关;*F3'5'H*基因显著下调表达,且花色素苷单体主要以*F3'H*支路产物矢车菊素-3-*O*-葡萄糖苷(Cy)和芍药素-3-*O*-葡萄糖苷(Pn)为主,*F3'5'H*支路产物锦葵素-3-*O*-葡萄糖苷(Mv)、矮牵牛素-3-*O*-葡萄糖苷(Pt)和飞燕草素-3-*O*-葡萄糖苷(Dp)含量极少,据此推测BTH处理‘红地球’葡萄可能主要通过*F3'H*支路合成花色素苷。BTH处理使葡萄果实中可溶性固形物含量显著提高,可滴定酸含量显著下降,固酸比(SSC/TA)显著提高;60 mg·L^{-1} BTH处理还对提高葡萄果实的可溶性总糖含量有显著效果;除120 mg·L^{-1} BTH处理导致葡萄果实横径变小外,其他浓度处理对葡萄果实形态均无显著影响。【结论】‘红地球’葡萄转色初期喷施一定浓度的BTH溶液可显著促进避雨栽培条件下葡萄的着色和转熟,并提高果实品质,且以60 mg·L^{-1} BTH处理效果最好。

关键词:‘红地球’葡萄;BTH;转色期;着色;花色素苷;果实品质

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Effects of BTH application at veraison on berry coloration and quality of ‘Red Globe’ berries

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Abstract:【Objective】The growing season of table grapes in southern China happens in rainy season with serious rain-transmitted diseases, which cause serious losses. Rain-shelter cultivation is an effective method to prevent rain-transmitted diseases in the production of table grapes and has been widely applied in southern China, although rain shelter reduces light penetration and leads to significant delay in the accumulation of sugar and pigments. ‘Red Globe’ (*Vitis vinifera* L.) is one of the important table grapes cultivated under rain-shelter in southern China but often has the problem of poor coloration due to weak light and high temperature. Plant elicitors are regarded as effective tools in inducing the biosynthesis of second-

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ary metabolites such as flavonoids in plants. In this work, BTH (benzo (1,2,3) thiadiazole-7- carbothioic acid S-methyl ester, Bion®), one of the synthetic elicitors, was sprayed on 'Red globe' grape clusters, and the color parameters (L^*, a^*, b^*), the anthocyanin content, the expression of genes involved in anthocyanin biosynthesis as well as qualities in 'Red globe' grape were analyzed to evaluate the effects of BTH on table grapes.【Methods】The experiment was carried out in a 4-year-old 'Red Globe' vineyard under rain-shelter cultivation in Xundian county, Kunming City from July 10th to August 9th of 2014. Four treatments were studied (the control and three BTH doses) in a complete randomized block design. There were three blocks with a total of 12 plots, each of which had five grapevines. Ten grape clusters were kept in each grapevine with 70-80 berries in every cluster. Grape clusters were evenly sprayed with water or three concentrations of BTH solutions (30 mg·L^{-1}, 60 mg·L^{-1} and 120 mg·L^{-1}) at 10:00 in the morning at the early stage of veraison. 4 and 7 days later, the grape clusters were sprayed again avoiding spraying the chemical onto the leaves. On the 10th day after the first treatment, ten grape berries were randomly sampled from the upper, middle and lower parts of grape clusters in each of the 12 plots, and berry sampling was carried out every five days till harvest (30th day). And berry length, diameter, shape index (length/diameter), weight and CIE parameters were measured. Color indexes for red grapes (CIRG) included the values of L^*, a^* and b^*. Soluble solids (SSC), titratable acid (TA) and total soluble sugar contents were analyzed after grape flesh was homogenated. Grape skins were peeled from berries and grinded in liquid nitrogen and anthocyanin content was determined with photospectrometry method. Individual anthocyanins were detected with high-performance liquid chromatography (HPLC) method.【Results】The CIRG and total anthocyanin content of 'Red Globe' berries treated with BTH in the early stage of veraison were all significantly increased compared to control berries. The results showed that 60 mg·L^{-1} BTH treatment had the highest values of both CIRG and total anthocyanin content, followed by 120 mg·L^{-1}, and then 30 mg·L^{-1}. Meanwhile, the expression of genes involved in anthocyanins biosynthesis such as *PAL*, *CHS1*, *CHS2*, *CHI1*, *CHI2*, *F3'H*, *F3H1*, *F3H2*, *DFR*, *LDOX*, *UFGT-2* and *VvmybyA1* were significantly up-regulated while *F3'5'H* gene at harvest was significantly down-regulated by preharvest treatment of 60 mg·L^{-1} BTH. The down-regulation of *F3'5'H* gene might block the flux to the delphinidin branch pathway, while the cyanidin synthesis pathway was enhanced because of the significant up-regulation of *F3'H* gene. The individual anthocyanins in 'Red Globe' grape skins detected by HPLC mainly were derivatives from the cyanidin pathway such as peonidin-3-*O*-glucoside (Pn) and cyanidin-3-*O*-glucoside (Cy); derivatives from the delphinidin branch such as malvidin-3-*O*-glucoside (Mv), petunidin-3-*O*-glucoside (Pt) and delphinidin-3-*O*-glucoside (Dp) accounted for only a very small percentage of the total anthocyanins. Thus, we suggested that BTH might stimulate anthocyanin biosynthesis mainly through *F3'H* branch. BTH treatments significantly increased soluble solid content and decreased titratable acid content, which resulted in significant increase in SSC/TA. BTH treatments did not affect berry diameter, length, shape index and weight except that 120 mg·L^{-1} BTH treatment significantly decreased berry diameter at the 30th day.【Conclusion】These results suggested that BTH, as an effective elicitor in activating plant systemic acquired resistance (SAR), is effective in enhancing red color by up-regulating genes and transcription factors involved in anthocyanin biosynthesis, accelerating ripeness and improving quality of 'Red Globe' berries under rain-shelter cultivation. Among all the treatments, 60 mg·L^{-1} BTH treatment was the most effective one.

Key words: 'Red Globe' grape; BTH; Veraison; Coloration; Anthocyanin; Fruit quality

‘红地球’葡萄(*Vitis vinifera* ‘Red globe’)果肉味甜硬脆、色泽鲜艳、耐贮运、经济效益高,近年来随着避雨栽培技术的推广和应用,在我国南方地区得到快速发展[1]。避雨栽培能够阻隔雨水,降低叶幕区域的湿度,阻断多种病害的传播与流行,使葡萄病害显著降低[2]。但避雨薄膜的使用阻挡了光的透过性,降低了光照强度[3]和光合有效辐射,延缓了糖分的累积、葡萄的着色和成熟[2,4-5],不利于葡萄及早上市。着色水平是影响红色葡萄商品性状的重要因子之一,而着色的深浅则主要由果皮中花色素苷的累积量所决定[6]。

苯并(1, 2, 3)噻二唑-7-硫代羧酸甲酯(benzo (1, 2, 3) thiadiazole-7-carbothioic acid S-methyl ester, BTH)是植物诱抗剂中的一类,因能诱导植物次生代谢物质(如酚类、黄酮类物质和植保素等)的累积从而激发对病原菌的防御机制,在诱导作物抗性方面的研究广泛[7-9],且对葡萄灰霉病、霜霉病和黄金病等病害的抗性诱导效果较好[10-12]。由于BTH的毒理学风险远低于传统农药[13-14],有研究将其用于诱导水果特定次生代谢物质的合成,如BTH可促进草莓、酿酒葡萄果实中花色素苷的累积[13-14],并提高葡萄酒的颜色深度及香气物质含量[14-15]。此外,还有研究关注了水果采前或采后BTH处理对果实贮藏期间的抗病性及品质的影响,如甜瓜、沙糖橘、杏等[16-18]。

BTH能够诱导葡萄植株产生系统性获得抗性(SAR)从而提高对病原菌的抵抗力,且作用持久期长的特征已得到证实,其应用可以减少常规农药的使用次数和使用量,从而达到减量增效、提高果品安全性的目标。但BTH是否兼具改善‘红地球’葡萄果实的着色潜力及其作用机制、对果实品质的影响及适合的施用浓度等尚不清楚。笔者旨在研究葡萄转色初期采用不同浓度BTH溶液喷施‘红地球’葡萄果穗,并观测葡萄果实在转熟过程中的形态、着色及与品质相关指标的变化,研究BTH对‘红地球’葡萄着色、花色素苷合成及糖酸累积的影响,探索利用植物诱抗剂BTH改善葡萄着色和品质的可能性。

1 材料和方法

1.1 材料

本试验于2014年4月至9月在云南省昆明市寻甸县云南农业大学大河桥农科教实践教学基地内进行,葡萄园土壤为黏土,年均气温为14.9 ℃,年均降雨量为1 022.4 mm,年平均日照时数为2 061.6 h。供试材料为4 a生‘红地球’葡萄,采用避雨栽培方式,架式为高宽垂V形水平架,东西行向,株行距为1.0 m×2.8 m,采用规范化管理。处理所用的药剂为苯并(1,2,3)噻二唑-7-硫代羧酸甲酯(BTH),商品名为Bion®(50% WG)(先正达)。

试验设质量浓度为30、60和120 mg·L^{-1}的BTH水溶液处理组以及蒸馏水对照组(CK)。采用随机区组设计,选择长势一致、葡萄果实进入转色初期(2014年7月10日果面着色约20%)的‘红地球’葡萄植株,共设3个区组,每2株为1小区,每株保留10串果穗(每串留70~80粒果实)。处理组和对照组均添加含量为0.1%的吐温80以增加展着效果,混匀后用高压喷雾器喷施葡萄果穗,喷至果穗溶液覆盖均匀且滴水为止。为保证喷施效果,于首次喷施后的第3天和第6天各再喷施1次。自第10天起,每5 d采1次样,直至第30天。采样时每个小区从果穗的上、中、下部位随机摘取10粒葡萄果实,标记好后放入冰盒内迅速带回实验室。将果实冲洗并吸干水分后迅速测量果粒的纵径、横径、单果质量及色度等指标;将果实在冰浴上小心剥下果皮,在液氮下研磨成粉,贮于-80 ℃超低温冰箱中备用;果肉用于物性分析、可溶性固形物、可滴定酸和可溶性糖的提取和测定。

1.2 葡萄果实形态和品质指标的测定

葡萄果实的纵径、横径指标采用数显游标卡尺测定;单果质量采用电子天平测定;可溶性固形物含量(SSC)采用手持数显糖度计(日本Atago PAL-3型)测定;可滴定酸含量采用瑞士万通一体式电位滴定仪(916 Ti-Touch)氢氧化钠滴定法测定[19]。

葡萄果肉可溶性糖的提取参照Wang等[11]的方法并有改动。准确称取2 g葡萄果肉匀浆于15 mL离心管中,加入10 mL 95%的乙醇溶液,在40 ℃条件下超声波震荡提取10 min,之后在8 000 r·min^{-1}、4 ℃条件下离心10 min,分离上清液后再重复提取2次,合并上清液并于40 ℃下减压蒸馏浓缩,分次用去离子水溶解残留物并定容至10 mL,用0.45 μm滤膜过滤后待测;可溶性总糖含量的测定采用苯酚-硫酸法[20]。

1.3 葡萄果实色度和花色素苷含量的测定

将手持式色差计(Konika-Minolta CR 410)光源

设定为D65，观察角为10°，将探头放置在葡萄果实的赤道部位，测得CIE颜色指标L^*（亮度）、a^*（红/绿颜色分量）、b^*（蓝/黄颜色分量）值，每粒果实分别测定赤道3个不同点的L^*、a^*、b^*值，测定结果取平均值。利用a^*和b^*值可以计算出色度（C^*）值，$C^*=\sqrt{a^{*2}+b^{*2}}$；并计算出色调角（h）值，h = arctangent b^*/a^*，进而计算红色葡萄果实颜色指数*CIRG*（Color Index of Red Grape），$CIRG=(180-h)/(L^*+C^*)$，该指数评价果实外观色泽的标准为：$CIRG<2$为黄绿，$2\leq CIRG<4$为粉红，$4\leq CIRG<5$为红色，$5\leq CIRG<6$为深红，$CIRG\geq 6$为蓝黑[21]。

果皮总花色素苷的提取参照张家荣[22]的方法，总花色素苷含量的测定采用紫外-可见光分光光度法测定，参照Amerine等[20]的方法。HPLC检测样品的制备参照张家荣[22]的方法。葡萄果皮花色苷单体含量的测定参照赵悦等[23]的方法。

1.4　葡萄果皮花色素苷生物合成主要酶基因表达的RT-PCR分析

1.4.1　引物设计　内参基因选用*KyActin1*（Accession No. AB073011.1），参考文献[24]；基因*CHS2*、*CHS3*、*CHI1*、*CHI2*、*F3H1*、*LDOX*和*VvmybyA1*的引物参考文献[25]；*F3H2*的引物参考文献[24]；*F3'H*、*F3'5'H*、*DFR*引物参考文献[26]；基因*PAL*、*CHS1*和*UFGT*的引物采用Primer 3（v.0.4.0）设计，详见表1，引物由上海桑尼生物技术有限公司合成。

表1　用于RT-PCR的特异引物序列设计

Table 1 Specific primers for real-time PCR

基因名称 Gene name	登录号 Accession No.	正向引物序列（5'-3'） Forward primer（5'-3'）	反向引物序列（5'-3'） Reverse primer（5'-3'）
KyActin1	AB073011.1	GATTCTGGTGATGGTGTGAGT	GACAATTTCCCGTTCAGCAGT[24]
PAL	JN858957	GGTGGAGAGTTTCTGCGTGA	CCGACAACTCCACACTCACA
CHS1	AB015872	AAGGCCATCAAGGAATGGGG	AGCAGCCCTGTTGGTACATC
CHS2	AB066275	GAAGATGGGAATGGCTGCTG	AAGGCACAGGGACACAAAAG[25]
CHS3	AB066274	TCGGCTGAGGAAGGGCTGAA	GGCAAGTAAAGTGGAAACAG[25]
CHI1	X75963	CAGGCAACTCCATTCTTTTC	TTCTCTATGACTGCATTCCC[25]
CHI2	VitiB655	TCCAGATCAAGTTCACAGCA	GAAACAAGAGCCTCAAAGAA[25]
F3H1	X75965	CCAATCATAGCAGACTGTCC	TCAGAGGATACACGGTTGCC[25]
F3H2	VitiA130	CTGTGGTGAACTCCGACTGC	CAAATGTTATGGGCTCCTCC[24]
F3'H	AB113261	GCCTCCGTTGCTGCTCAGTT	GAGAAGAGGTGGACGGAGCAAATC[26]
F3'5'H	AB213606	AAACCGCTCAGACCAAAACC	ACTAAGCCACAGGAAACTAA[26]
DFR	X75964	GAAACCTGTAGATGGCAGGA	GGCCAAATCAAACTACCAGA[26]
LDOX	X75966	AGGGAAGGGAAAACAAGTAG	ACTCTTTGGGGATTGACTGG[25]
UFGT	AF000372	TAGCACATGAGGCAGTTGGG	CCTCCCATTGAGCCTTTGGT
VvmybyA1	AB097923	TAGTCACCACTTCAAAAAGG	GAATGTGTTTGGGGTTTATC[25]

1.4.2　RNA的提取与cDNA合成　葡萄果皮总RNA的提取采用Tiangen公司的植物总RNA提取试剂盒（RNA plant plus Reagent），按照说明步骤操作，采用琼脂糖凝胶电泳检测总RNA的质量。cDNA第1链的合成参考TaKaRa Super RT Kit说明书，将检测合格并定量的总RNA逆转录成cDNA。

向置于冰浴中的试管中加入如下反应混合物，模板RNA：总RNA，2 μg；引物：Oligo$(dT)_{18}$（50 μmol·L^{-1}），1 μL；5×Reaction Buffer，4 μL；RNase Inhibitor（40 U·μL^{-1}），1 μL；dNTP Mix（10 mmol·L^{-1}），1 μL；AMV RT（200 U·μL^{-1}），1 μL；加水至20 μL轻轻混匀后，反应混合物在50 ℃反应60 min；在70 ℃加热10 min结束反应，置冰上进行后续试验或冷冻保存。

1.4.3　荧光定量PCR反应　制备DNA模板：针对每1个需要测量的基因和内参基因，选择1个确定表达该基因的cDNA模板进行PCR反应：2×SYBR real-time PCR premixture，10 μL；10 μmol·L^{-1}的PCR特异引物F，0.4 μL；10 μmol·L^{-1}的PCR特异引物R，0.4 μL；cDNA，1 μL；加水至总体积为20 μL。

Real-time PCR反应：将按反应体系配置的PCR反应溶液置于Realtime PCR仪上进行PCR反应，反应程序如下：95 ℃预热4 min，95 ℃变性15 s，57 ℃退火15 s，72 ℃延伸25 s，35个循环。每个体系反应完成后得到1个Ct值，目的基因相对于内参基因Kyactin1的转录表达量参照Schmittgen等[27]的方法。

1.5　精密仪器和药品规格

日本Konika-Minolta CR 410型手持式色差计；日本Atago PAL-3型手持数显糖度计；瑞士万通一体式电位滴定仪（916 Ti-Touch）；Thermo Nanodrop

2 000超微量光分光度计；德国Sigma 3-16 KL型高速冷冻离心机；Shimadzu UV-1 780紫外-可见分光光度计；Shimadzu高压液相色谱；葡萄糖、果糖、蔗糖均为色谱级标样；矢车菊素-3-*O*-葡萄糖苷、芍药素-3-*O*-葡萄糖苷、矮牵牛素-3-*O*-葡萄糖苷、飞燕草素-3-*O*-葡萄糖苷和锦葵素-3-*O*-葡萄糖苷标样均购自于Sigma-Aldrich公司（中国）。

1.6 统计分析

采用Excel 2010对原始数据进行标准化处理和作图，分别采用SPSS 19数据处理软件的单因素ANOVA和一般线性模型（GLM）多变量（multivariate）进行方差分析，处理间的多重比较采用Duncan's测试，显著性水平设定为α=0.05。

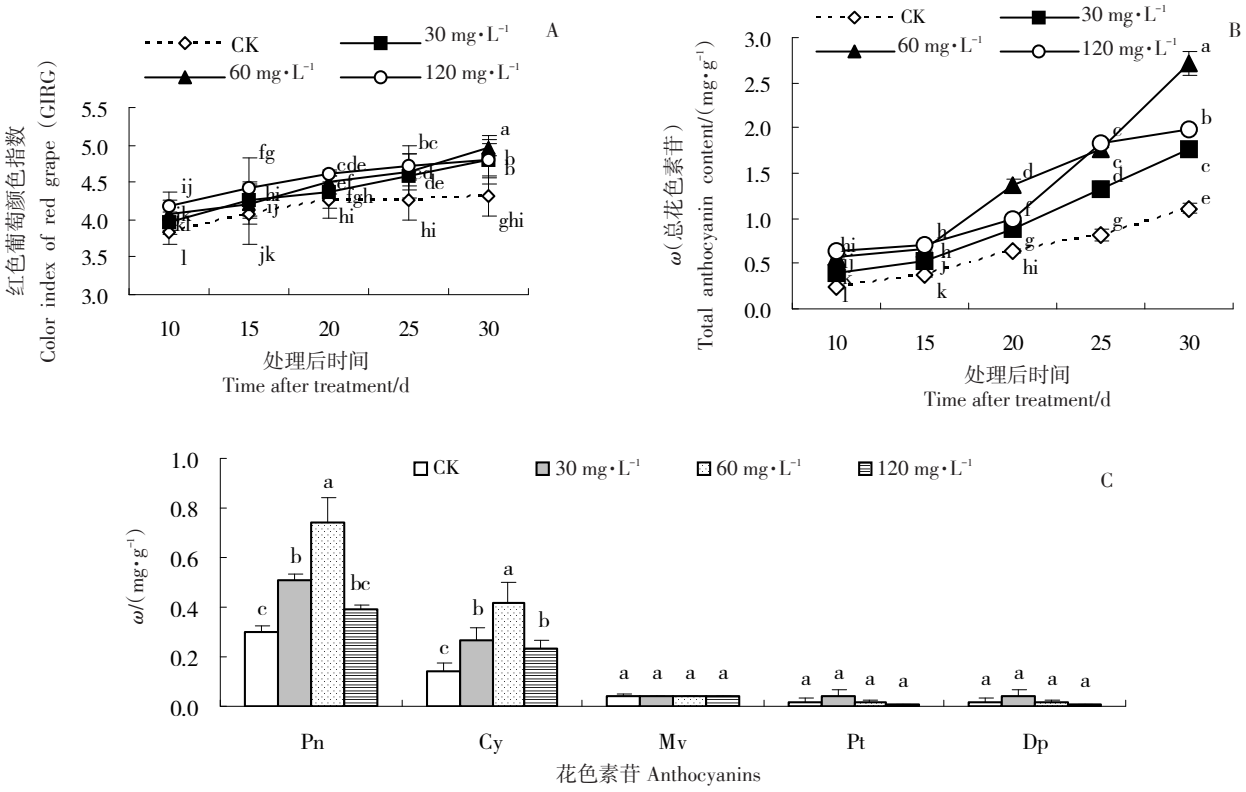
2 结果与分析

2.1 BTH处理对葡萄果实形态指标的影响

BTH处理后30 d，葡萄果实的纵径、果形指数和单果质量等形态指标均未受显著影响，但120 mg·L^{-1} BTH处理使果实横径降低1.74%，且差异显著，其他浓度处理对果实横径大小均无显著影响。说明中、低浓度BTH处理不影响葡萄果实的形态指标。

2.2 BTH处理对葡萄果皮颜色指数（CIRG）和花色素苷含量变化的影响

'红地球'葡萄果皮的CIRG和总花色素苷含量在BTH处理后30 d内均呈逐渐增大的趋势，BTH处理增大了葡萄果皮的CIRG及总花色素苷含量，且随时间推移与对照的差异均逐渐增大（图1-A、B）。处理后30 d，葡萄果皮CIRG和总花色素苷质量浓度均显著高于对照，按由大到小排序依次为60 mg·L^{-1} > 120 mg·L^{-1}>30 mg·L^{-1}；对照葡萄果皮CIRG偏小，颜色偏向于红色，BTH处理CIRG偏大，颜色偏向于深红色，与葡萄果皮的实际颜色相符（图2）；总花色素苷含量分别是对照的2.45、1.80和1.60倍，且差异显著。花色素苷单体以Cy和Pn为主，Mv、Pt和Dp含量极低，BTH处理主要增加了葡萄果皮中Cy和Pn



Pn、Cy、Mv、Pt 和 Dp 分别代表芍药素-3-*O*-葡萄糖苷、矢车菊素-3-*O*-葡萄糖苷、锦葵素-3-*O*-葡萄糖苷、矮牵牛素-3-*O*-葡萄糖苷和飞燕草素-3-*O*-葡萄糖苷。不同小写字母表示在 $P<0.05$ 上差异显著。下同。

Pn, Cy, Mv, Pt and Dp separately stand for peonidin-3-*O*-glucoside, cyanidin-3-*O*-glucoside, malvidin-3-*O*-glucoside, petunidin-3-*O*-glucoside and delphinidin-3-*O*-glucoside. Different small letters indicate significant difference at $P<0.05$. The same below.

图1 BTH处理对葡萄果皮CIRG、总花色素苷和花色素苷单体含量的影响

Fig. 1 Effects of BTH treatment on CIRG, contents of total anthocyanins and individual anthocyanins in grape skins

2014年7月20日、7月30日和8月9日分别为BTH首次处理后10、20和30 d。

2014-07-20, 2014-07-30, 2014-08-09 were the 10, 20 and 30 d after first BTH treatment, respectively.

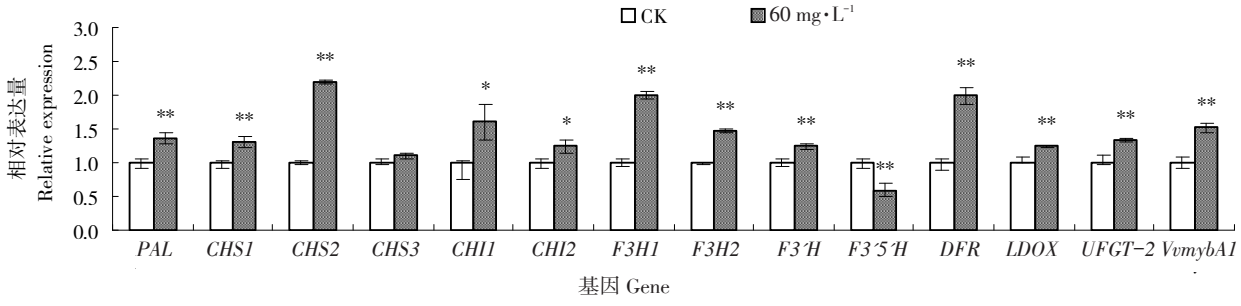
图2　BTH处理后葡萄果穗和果粒着色变化的过程

Fig. 2　Changes in coloration of grape clusters and berries after BTH treatments

的含量，30 mg·L^{-1}、60 mg·L^{-1}和120 mg·L^{-1} BTH处理Cy含量分别高于对照91.67%、200%和64.29%，且差异显著；Pn含量除120 mg·L^{-1} BTH处理外均显著高于对照，3者含量分别高于对照67.82%、145.30%和28.95%；Cy和Pn含量均以60 mg·L^{-1} BTH处理最高，且与其他处理差异显著（图1-C）。可见，BTH处理改善葡萄着色效果显著，且主要通过促进Cy和Pn的合成而增加总花色素苷的含量。

2.3　BTH处理对葡萄果实花色素苷生物合成主要基因表达的影响

图3显示了BTH处理后30 d时60 mg·L^{-1} BTH处理与对照葡萄果皮中花色素合成途径中主要基因



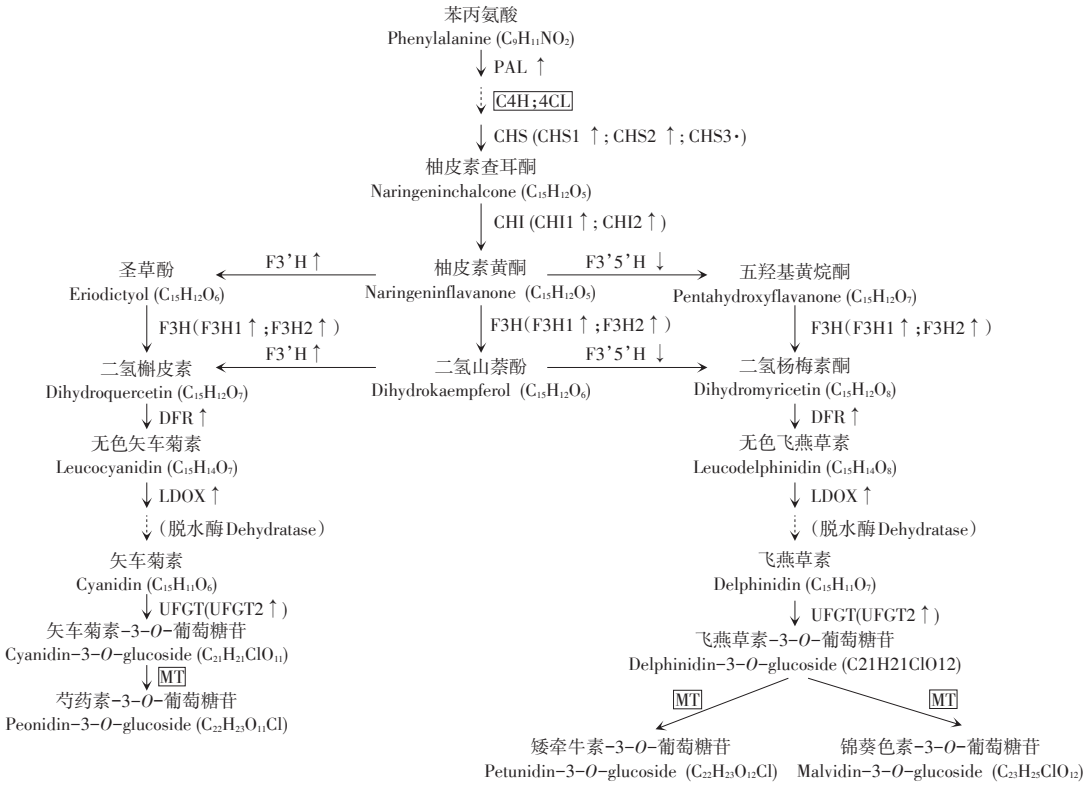
PAL. 苯丙氨酸解氨酶；CHS1、CHS2和CHS3. 查尔酮合成酶；CHI1和CHI2. 查尔酮异构酶；F3H1和F3H2. 黄烷酮-3-羟化酶；F3’H. 类黄酮-3’-羟化酶；F3’5’H. 类黄酮-3’5’-羟化酶；DFR. 二氢黄酮醇-4-还原酶；LDOX. 无色花青素双加氧酶；UFGT-2. 类黄酮3-*O*-糖基转移酶；VvmybA1. 转录因子。*表示在$P<0.05$差异显著，**表示在$P<0.01$差异极显著。

PAL. Phenylalanine ammonia-lyase; CHS1, CHS2 and CHS3. Chalcone synthase 1, 2 and 3, respectively; CHI1 and CHI2. Chalcone isomerase 1 and 2, respectively; F3H1 and F3H2. Flavanone-3-hydroxylase 1 and 2, respectively; F3’H. Flavonoid-3’-hydroxylase; F3’5’H. Flavonoid-3’, 5’-hydroxylase; DFR, Dihydroflavonol reductase; LDOX. Leucoanthocyanidin dioxygenase; UFGT-2. UDP-glucose flavonoid 3-*O*-glucosyltransferase; VvmybA1.Transcription factor MYB90. * indicates significant difference at $P<0.05$ and ** at $P<0.01$.

图3　葡萄成熟时果皮花色素苷生物合成途径中主要基因的RT-PCR表达分析

Fig. 3 Relative expression of the main genes involved in anthocyanin biosynthetic pathway

和转录因子的相对表达量，图4显示了BTH处理对该合成途径主要基因和转录因子表达的调节规律。结果显示BTH处理*PAL*、*CHS1*、*CHS2*、*CHI1*、*CHI2*、*F3'H*、*F3H1*、*F3H2*、*DFR*、*LDOX*、*UFGT-2*和*VvmybA1*等基因和转录因子的相对表达量均显著或极显著上调；而*F3'5'H*基因的相对表达量则极显著下调，说明BTH处理有促进花色素生物合成途径大部分基因上调表达的作用。



↑表示基因上调差异表达；↓表示基因下调差异表达；·表示无差异表达；加框基因未作检测。

↑ means that gene is up-regulated expression and ↓ means down-regulated expression; · means no difference expression; framed genes were not detected.

图4 花色素苷生物合成途径基因表达变化[28]

Fig. 4 Expression of anthocyanin biosynthesis pathway genes[28]

2.4 BTH处理对葡萄果实糖、酸累积变化的影响

BTH处理后30 d内，葡萄果实的可溶性固形物和固酸比均呈逐渐增大的趋势，而可滴定酸含量呈逐渐降低的趋势，BTH处理显著促进了固酸比和可滴定酸的趋势变化，并对可溶性固形物含量的增加也具有一定效果（图5-A~C）；此外，BTH处理在短期内（处理后第10天）具有增加可溶性总糖含量的效果，但仅60 mg·L^{-1} BTH处理能够持续增加可溶性总糖含量并保持显著差异（图5-D）。综合来看，BTH处理对葡萄有促早熟和改善果实品质的作用。

3 讨 论

着色水平是影响红色鲜食葡萄市场价格的重要因素。研究表明红色葡萄颜色指数（CIRG）可作为评价葡萄果实着色水平的重要指标，其值越大表示葡萄颜色越深[20]。本试验在葡萄转色初期采用BTH处理葡萄果穗，通过对处理后30 d内葡萄果实色度的定期观测，发现BTH处理有促进葡萄果皮CIRG提高和颜色加深的效果，这与孙晓文等[29]采用茉莉酸甲酯处理'圣诞玫瑰'葡萄后的果皮颜色和CIRG的变化趋势一致。

葡萄果实的花色素苷在转色后开始迅速积累，并于果实成熟时达到最高含量[30]。本研究在葡萄转色初期采用BTH处理后，葡萄果皮中的总花色素苷含量在30 d内均呈持续增加的趋势，BTH处理可显著增加葡萄果皮中花色素苷的含量，且随时间延长与对照的差异逐渐增大，这与Marcello等[14]在酿酒葡萄'美乐'（'Merlot'）转色末期应用BTH提高了葡萄果皮中花色素苷含量的报道一致，但BTH促进酿酒

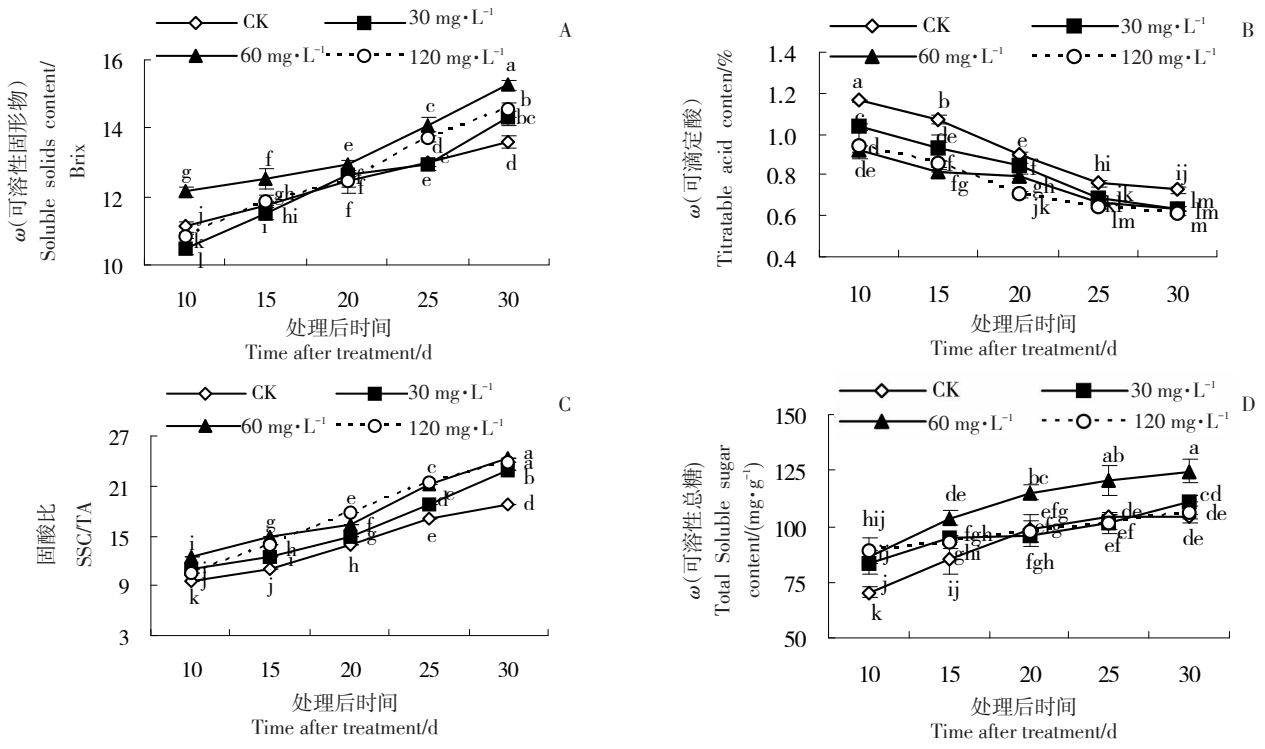


图5　BTH处理后葡萄果实中可溶性固形物含量、可滴定酸含量、固酸比及可溶性总糖含量的变化规律

Fig. 5 Changes in contents of soluble solids, titratable acid, SSC/TA and soluble sugar content in grape berries of different BTH treatments

葡萄花色素苷合成的机制未知。葡萄果皮中花色素苷的种类和含量决定了葡萄果皮的颜色和深度[31]，葡萄果皮中的花色素苷主要有Pn、Cy、Mv、Pt和Dp等糖苷以及酰基化和对香豆酰基化的糖苷衍生物等，因品种不同花色素的种类差异很大[32]。程建徽等[33]报道，‘红地球’葡萄果皮主要含有Pn和Cy，2者占总花色素苷的70%以上，本试验也发现了相似的结果，HPLC检测显示‘红地球’葡萄果皮中的花色素主要为Pn和Cy，Mv、Pt和Dp含量极低，以Pn含量居多，Cy含量次之，BTH处理后葡萄果皮中Cy含量均显著增加，Pn含量除120 mg·L⁻¹ BTH处理外均显著增加，因此Pn和Cy含量的增加是葡萄果皮中总花色素苷含量增加的主要原因。BTH处理使‘红地球’葡萄花色素苷含量显著增加，可能与花色素苷生物合成途径中*PAL*、*CHS1*、*CHS2*、*CHI1*、*CHI2*、*F3'H*、*F3H1*、*F3H2*、*DFR*、*LDOX*、*UFGT-2*和*VvmybyA1*等基因和转录因子的显著上调表达密切相关，这一结果与ABA诱导‘京优’葡萄花色素苷合成中相关基因表达的结果相似[34]，但BTH对花色素苷合成的诱导是否与葡萄内源ABA含量的变化有关，目前未见报道，有待于后续研究。BTH处理使*F3'H*基因显著上调表达，*F3'5'H*基因显著下调表达，*F3'H*支路产物Pn和Cy合成增加，但*F3'5'H*支路合成的Mv、Pt和Dp含量均较低，且与对照并无显著差异，说明‘红地球’葡萄可能主要通过*F3'H*支路合成花色素苷，且BTH处理促进了*F3'H*支路花色素苷的合成。

葡萄果实在发育过程中可溶性固形物和糖的累积及酸的转化是果实转熟的重要特征，3者的含量是决定葡萄内在品质的重要指标[35]。果实中的糖不仅是果实香气和口感品质的重要来源，而且还具有重要的信号分子功能，能够调节涉及防御响应和代谢进程的一系列基因的转录水平，进而影响果实的成熟和次生代谢产物的合成[36]。本试验‘红地球’葡萄在BTH喷施果穗处理后，果实的SSC含量显著增加，TA含量显著降低，固酸比显著提升，对葡萄果实具有明显的促熟效果，这与ABA和水杨酸(SA)在葡萄上的应用效果类似[30,35]。

4　结　论

在‘红地球’葡萄转色初期，果穗喷施BTH溶液可显著提高果皮花色素苷含量，改善着色程度。这一效应与其诱导葡萄花色素苷生物合成途径主要基

因的上调表达有关。此外，BTH处理还提高了葡萄果实可溶性固形物含量和固酸比，降低了可滴定酸含量，改善了果实品质，处理浓度以60 mg·L^{-1}最好。

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