

葡萄花色苷合成的影响因素研究进展

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摘要:花色苷是存在于葡萄果皮中的水溶性天然色素,属于类黄酮化合物。花色苷主要是由葡萄品种所决定的,但其生物合成还受到其他各种因素的调控,如气候(光照、温度和湿度)、土壤、植物激素、果实负载量等。本文对近年来影响葡萄花色苷合成的因素的研究进行综述,为改善葡萄果皮中花色苷的相关研究提供参考。

关键词:葡萄,花色苷,生物合成

Research on factors influencing the biosynthesis of anthocyanin in grape fruit

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Abstract: As a big family of flavonoid, anthocyanin was one kind of natural water-soluble pigment. Besides grape cultivars, the biosynthesis of anthocyanin was also modulated by climate, soil, plant hormones, load. Recent advances in researches on factors influencing the biosynthesis of anthocyanin were reviewed with the purposes to provide reference for study on improving anthocyanin in grape skin.

Key words: grape; anthocyanin; biosynthesis

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花色苷作为类黄酮家族最大的一个分支,影响着葡萄酒的色泽、口感及营养价值,且直接决定红葡萄酒和新葡萄酒的颜色^[1]。花色苷和单宁结合形成花色苷-单宁复合物,使得葡萄酒中的单宁软化,降低其苦涩味和粗糙感,使口感变得更加圆润。花色苷还能够清除自由基,其抗氧化性高于维生素C和维生素E^[2]。除了抗氧化性,花色苷的抗突变、抗癌、抗高血糖活性均很高^[3],赋予葡萄酒较高的营养价值。

花色苷存在于红葡萄最靠近表皮的3~4层细胞的液泡里^[4]。从转色期开始,伴随着糖分的积累,葡萄果皮中的花色苷也开始迅速积累^[5]。这一时期,花色苷主要以游离形式存在,随着葡萄的不断成熟,其含量也逐渐升高,并且单体之间开始聚合^[6]。花色苷的生物合成路径可以分为两个阶段:第一阶段为苯丙烷类代谢途径,即由苯丙氨酸转化为4-香豆酰CoA;第二阶段为类黄酮途径,由4-香豆酰CoA转化为各种黄酮类化合物^[7]。整个合成过程需要两类基因:编码酶的结构基因和编码转录因子的调节基因,图1简单展示了花色苷的合成路径及其过程中主要的结构

基因,*myb*相关基因是花色苷合成中的调节基因,其调节结构基因的时空表达,进而影响花色苷合成的强度。葡萄果皮中花色苷的差异是内外部因素共同调控的结果,本文将从五个方面简单介绍内外因对葡萄花色苷的调控。

1 葡萄品种

葡萄花色苷主要是由基因型决定的,品种不同,其果皮中花色苷的含量和分布也有所不同。这主要是由于不同葡萄品种花色苷合成基因的表达模式不同造成的。欧亚种葡萄是世界上种植最广泛的酿酒葡萄,与其他葡萄品种的区别在于其不含有花色苷双糖苷^[8]。Fraige Karina^[9]采用主成分分析的方法评估了品种之间的差异,证实了这一观点。欧亚种葡萄一般只含有花色苷单糖苷:矢车菊素糖苷、飞燕草素糖苷、矮牵牛素糖苷、芍药素糖苷和锦葵素糖苷及其酰化衍生物,以锦葵素糖苷及其衍生物为主,但是欧亚种黑皮诺中仅含有非酰化的花色苷^[10]。Arozarena Iñigo^[8]在连续两年测定了不同品质葡萄园的添帕尼尤、歌海娜和赤霞珠果皮中花色苷的组成后,发现赤

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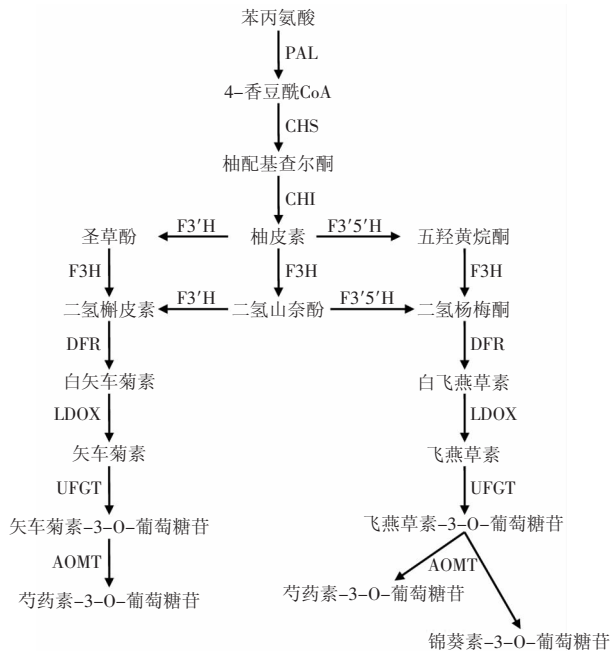


图1 花色苷的生物合成途径图

Fig.1 Simplified diagram of the anthocyanin biosynthetic pathway

注: PAL: 苯丙氨酸解氨酶 (phenylalanine ammonia-lyase); CHS: 查尔酮合成酶 (chalcone synthase); CHI: 查尔酮异构酶 (chalcone isomerase); F3H: 黄酮醇3-羟化酶 (flavanone-3-hydroxylase); F3'H: 类黄酮3'-羟化酶 (flavonoid 3'-hydroxylase); F3'5'H: 类黄酮3',5'-羟化酶 (flavonoid 3',5'-hydroxylase); DFR: 黄烷醇4-还原酶 (dihydroflavonol-4-reductase); LDOX: 无色花色素双加氧酶 (leucoanthocyanidin dioxygenase); UFGT: UDP-类黄酮-3-O-葡萄糖基转移酶 (UDP glucose:flavonoid-3-O-glucosyltransferase); AOMT: O-甲基转移酶 (anthocyanin O-methyltransferase)。

霞珠中乙酰化花色苷单体所占的比例明显高于添帕尼尤和歌海娜。Castellarin SD^[11]在分别测定了九个葡萄品种中编码酶的基因及四个转录因子的表达水平后,认为品种间基因的特定表达模式的差异是造成花色苷不同的主要原因。

同一葡萄品种在不同风土条件下生长,其果皮中的花色苷也有差异。这是因为花色苷的生物合成还受外部因素包括气候、土壤、植物激素和负荷等的调控。随着对花色苷代谢途径研究的不断深入,研究影响花色苷生物合成的外部因素,进而通过采取合适的栽培措施来改善葡萄的品质,以酿造高品质葡萄酒成为当前研究的热点。

2 气候因素

2.1 光照

在强紫外照射下,高等植物会启动防卫机制,通过酚类物质的积累选择性地吸收紫外线就是一种有效的方式^[12]。具体来说,由于转录因子控制花色苷生物合成中结构基因的时空表达^[13],而光照的刺激启动了光敏色素的信号传导途径,这些信号能进一步改变转录因子的表达水平,从而调控结构基因的表达^[14]。另外,光照还可以通过影响光合作用中糖、苯

丙氨酸等的合成来改变花色苷的积累^[15]。铺反光膜、摘叶处理是最常用的增大葡萄受光面积的方法。在转色前期进行摘叶处理能增大葡萄的受光面积,进而影响叶片中花色苷的合成。Wu Ben Hong^[16]研究了叶果比例对鲜食葡萄“京烟”花色苷的影响,证实适度减少留叶量有利于花色苷的积累,Jose TM^[17]从分子的角度探究了光照对欧亚种赤霞珠花色苷合成的影响,发现转色期移动基生叶以增大果穗受光面积能够提高结构基因 *Vvchs2*、*Vvldox*、*Vvomt* 和 *Vvufgt* 的表达水平,但是摘除80%基生叶处理抑制了这四种结构基因的表达。故不同葡萄品种的最适叶果比例有待探究。

不仅光照强度会影响花色苷的生物合成,光质也会对其产生影响,可见光主要诱导原花色素的合成并影响其组成,而紫外线主要诱导黄酮醇的生物合成^[18]。Kondo S^[19]研究发现夜间蓝光或红光照射均能提高 *Vvufgt*、*Vlmyba1-2* 和 *Vlmyba2* 的表达量,其中 *UFGT* 催化不稳定的花色素形成各种花色苷,调节基因 *myba1-2* 和 *myba2* 则调节 *ufgt* 的时空表达。值得注意的是,夜间蓝光照射比红光照射更能促进糖分和花色苷的积累。

2.2 温度

温度是影响葡萄花色苷积累的重要因素^[20]。一般认为,低温会促进花色苷的积累,而高温会抑制其合成^[21]。Mori K^[22]证实高温 (35 °C) 会加速赤霞珠葡萄果皮中除锦葵色素糖苷以外的其他单体的降解,但是花色苷合成基因以及类黄酮-3-O-葡萄糖基转移酶 *UFGT* 的活性并没有被显著抑制,这与 Takayoshi Yamane^[23]的研究结果不同,其发现20 °C下“*Aki Queen*”葡萄果皮花色苷合成中的7个结构基因以及调节基因 *Vvmyba1* 的表达量均高于30 °C下的表达量。两者的差异可能是由于实验所用的葡萄品种不同,故其对温度的响应结果不同。

此外,温度与其他环境因素之间的协同作用也逐渐被重视, Koshita Yoshiko^[24]认为低温和糖分积累协同作用促进了“*Aki Queen*”葡萄花色苷的合成,即当果实中糖分不足时,仅低温处理并不能促进花色苷的积累。Azuma Akifumi^[25]研究了温度和光照对“先锋”葡萄 (*V. ×labruscana*) 的影响,发现同时具备低温和光照条件才能诱导结构基因 *chs2* 和 *3h2* 的表达,另外,高温会显著降低 *dfr*、*ufgt* 和 *omt* 的表达水平,而三种编码转录因子的 *myb* 相关基因 *Vlmyba1-3*、*Vlmyba2* 和 *Vlmyba1-2* 对光照和温度的反应各不相同,其中温度显著影响 *Vlmyba1-3* 和 *Vlmyba2* 的表达水平,但对 *Vlmyba1-2* 的表达水平影响不大。

2.3 湿度

长期以来,限制水分供应是一种常用的提高葡萄多酚含量的农艺手段。水分胁迫提高葡萄果皮中花色苷的含量并不仅仅是通过限制果实的膨大,还通过提高类黄酮途径中结构基因的转录水平来促进花色苷的积累。Castellarin SD^[26]证实水分胁迫能够显著提高欧亚种美乐葡萄花色苷合成路径中的结构基因特别是 *ufgt*、*chs2*、*chs3* 和 *3h* 基因的转录水平。但

是, Bucchetti Barbara^[27]在测定了四年干旱胁迫下美乐果皮中的花色苷后, 认为减少水分是通过限制果实膨大来提高花色苷含量。赵权^[28]进一步证实干旱胁迫可显著提高山葡萄果皮总花色苷的含量以及单体二甲花翠素-3-葡萄糖苷的含量, 并使花色苷合成中的苯丙氨酸解氨酶PAL的活性有所提高。

3 土壤

土壤因素包含土壤类型、pH和营养元素等, 也会影响葡萄花色苷的生物合成。葡萄偏爱的土壤类型为砂砾土和沙壤土, 因为这两种土的通透性强, 吸热快、比热大, 使得昼夜温差大, 能够促进糖分和花色苷的积累^[29-30]。Koki Yokotsuka^[31]通过添加石灰岩和牡蛎壳粉的方式来改善葡萄园的弱酸性土壤环境, 发现这两种处理均能显著影响赤霞珠果皮中花色苷的组成。

适度的氮素胁迫有利于花色苷的生物合成, 而氮含量较高则会抑制花色苷的合成, 这与过量的氮元素加强了葡萄植株的营养生长, 降低了糖分积累有关, 更重要的是, 氮素供应影响了花色苷合成中相关基因的表达水平。Soubeyrand Eric^[32]从分子角度探究了氮元素对赤霞珠葡萄花色苷合成的影响, 氮素胁迫下, 一方面合成路径中结构基因*Vvdf*、*Vvldox*和*Vvaomi*及调节基因*Vvmyb1*的转录水平大大提高, 另一方面抑制花色苷合成的LBD蛋白质家族的转录因子*Vvldb39*的表达水平降低。Cheng Guo^[33]研究发现, 当土壤中水分和有机质的含量较少时, 赤霞珠的果粒重量减小但果皮重量增大, 使果皮中花色苷浓度增加, 另外, 酰化花色苷的比例也有所增加, 所酿造葡萄酒的颜色更加稳定。

4 植物激素

脱落酸(ABA)、乙烯等植物激素对花色苷的生物合成也发挥着重要的作用。脱落酸通过提高花色苷合成路径中基因的表达水平来促进花色苷的积累^[34], 马立娜^[35]和周莉^[36]的研究结果证实了这一观点, 并发现萘乙酸(NAA)处理会抑制花色苷的合成。另外, 还有研究表明脱落酸可以促进单糖的积累^[37], 而单糖正是花色素糖苷化必需的物质。乙烯则能够增强花色苷合成相关基因的转录, 使转录时期前移, 转录水平提高^[38]。从植物生理角度分析, 外源刺激物能够诱导植物积累特定的次级代谢产物来进行自身防御^[39]。外源使用苯并噻二唑(BTH)和茉莉酸甲酯(MeJ)可诱导葡萄中类黄酮特别是花色苷的积累^[40-41]。

5 负荷

葡萄叶片为葡萄植株的生长提供光合产物, 葡萄果粒则是容纳光合产物的载体。改变葡萄植株负荷即选择合适的留果量, 保证果实得到充足的糖分供应, 从而提高果皮中花色苷的含量。Fanzone Martín^[42]研究发现花后40 d疏穗50%促进了‘马贝克’葡萄果皮花色苷的生物合成, 提高了果皮中总花色苷的含量。张家荣^[43]对赤霞珠分别进行疏花、疏粒和疏穗三种处理, 发现疏粒处理能够加大葡萄的松散度, 改善果穗内部的光照条件, 这有利于降低葡萄果粒间的

温度, 从而有助于花色苷的积累。M Gil^[44]也证实了疏穗和疏粒处理均能提高西拉中花色苷的含量, 与对照相比, 两种处理下, 五种非酰化花色苷单体的比例并无明显差异, 但乙酰化和香豆酰化水平有差异。

6 结论与展望

花色苷作为葡萄和葡萄酒中主要的呈色物质, 对葡萄酒的品质具有重要影响, 因此, 葡萄花色苷合成的分子机理及其调控是当前的研究热点。未来外部因素对花色苷合成和分解代谢路径中的酶活性、结构基因和调节基因表达的影响仍需进一步探明, 另外, 还可以利用蛋白质组学对花色苷合成机理进行研究, 这将更有利于深化对葡萄花色苷代谢机理的认识, 为改善葡萄果皮中的花色苷提供理论依据, 从而有助于优质葡萄酒的研发。

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