

## 果实花青素生物合成分子机制研究进展

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**摘要:** 花青素是一种天然的水溶性植物色素, 与果实的品质性状密切相关, 有益于人体健康。花青素的积累是编码花青素生物合成途径的结构基因协同表达的结果, 而结构基因通常由MYB、bHLH和WD40这三类调节基因控制。现已从果实中分离了多种花青素合成的结构基因和调节基因。文章重点介绍了调节基因调控果实花青素生物合成的分子机制, 指出在MYB、bHLH和WD40互作的调控网络方面的研究还有很多空白。最新的研究揭示了果实成熟过程中生物内在因素和外界环境通过调节基因影响果实花青素生物合成。上述研究为在分子水平上更好的探索果实花青素的生物合成具有重要意义。

**关键词:** 果实; 花青素生物合成; 结构基因; 调节基因; 环境因子

## Recent Advances on the Molecular Mechanisms of Anthocyanin Synthesis in Fruits

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**Abstract:** Anthocyanins are important natural water-soluble, health-promoting pigments that have much effect on the quality of fruits. The anthocyanin biosynthetic pathway in fruits is clear. Anthocyanin accumulation depends on coordinated expression of structural genes encoding anthocyanin biosynthesis. Structural gene expression is regulated by regulatory genes, including MYB, basic helix-loop-helix (bHLH), and WD40 classes. The structural genes and the key regulatory genes controlling the pathway have been isolated in many fruits. Here we reviewed the recent advances in molecular mechanisms of regulatory genes in anthocyanin biosynthesis, including classes, concentration and functions of anthocyanins, transcriptional regulation of the anthocyanin biosynthesis, microRNAs regulation, as well as the internal and external influences on anthocyanin biosynthesis in fruits. Especially, we pointed many gaps remain in the regulatory network of MYB, bHLH and WD40 transcription factors. Furthermore, new discoveries have begun to reveal links between the internal influences (plant hormones, genetics, maturity, biological clock, etc.), external environmental influences (light, temperature, pH, nutrition, etc.) and the regulatory genes in anthocyanin biosynthesis during fruit ripening. These researches favor the anthocyanin biosynthesis of fruits at the molecular level from the point of view of interaction between the regulatory networks.

**Key words:** fruit; anthocyanin biosynthesis; structural gene; regulatory gene; environmental factors

色素在植物中广泛存在, 按其基本特性可分为脂溶性色素和水溶性色素。花青素(anthocyanin)是水溶性的植物色素, 是黄酮类代谢途径的分支产物。在自然条件下游离状态的花青素极少见, 主要以糖苷形式存在, 植物中常见的有天竺葵色素(pelargonidin)、矢车菊色素(cyanidin)、飞燕草色素(delphinidin)、芍药色素(peonidin)、牵牛色素(petunidin)及锦葵色素(malvidin) 6种(图1)。果色是果实品质的重要指标, 花青素是决定果色的主要色素, 也是果实成熟的标志。受到生物内在因

素和外界环境(农艺措施、光质和光强、温度、加工等)的共同影响, 不同果实中花青素种类和含量不同。就果实中花青素种类而言, 矢车菊色素是大多数果实的主要花青素, 如红皮苹果和红皮梨等; 飞燕草色素、锦葵色素、芍药色素、牵牛色

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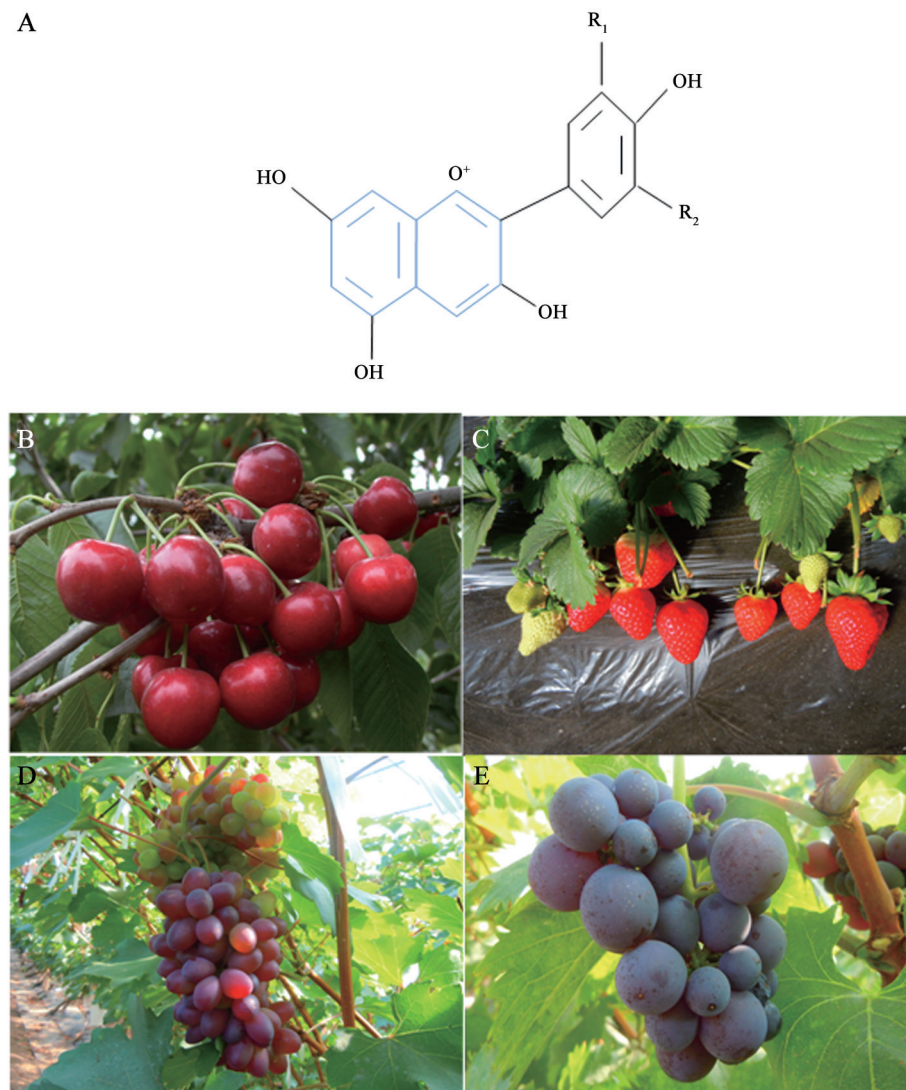


图1 花色素苷的化学结构及其含量丰富的果实

Fig.1 Chemical structures of the most common anthocyanidin aglycons and anthocyanin-rich fruits

参考Jaakola (2013)文献修改。A: 天竺葵色素 $R_1=R_2=H$ , 矢车菊色素 $R_1=OH$ 、 $R_2=H$ , 飞燕草色素 $R_1=R_2=OH$ , 芍药色素 $R_1=OCH_3$ 、 $R_2=H$ , 牵牛色素 $R_1=OCH_3$ 、 $R_2=OH$ , 锦葵色素 $R_1=R_2=OCH_3$ ; B: 樱桃; C: 草莓; D、E: 葡萄。

素主要存在于深蓝和紫色的果实中, 如蓝莓和紫葡萄等; 矢车菊色素和天竺葵色素主要存在于亮红色的果实中, 如草莓、樱桃等(Jaakola 2013)。就花青素含量而言, 果实花青素含量较高的果树有花楸果、石榴、红葡萄、越橘、覆盆子、蓝莓、樱桃等, 含量达到 $450 \text{ mg} \cdot (100 \text{ g})^{-1}$ 以上(Wu等2006; Kaspar等2013; Kayesh等2013)。在果实发育过程中, 其花青素含量随之发生了变化, 且不同品种之间有差异(Alcalde-Eon等2014)。花青素具有特殊的营养价值和保健功能, 能够帮助人体抵御某些癌症、心血管疾病以及一些与机体老化相关的疾

病(Yoshimoto等1999; Wang等2000; Kong等2003)。此外, 花青素有利于种子和花粉传播, 并且促进植物对低温、UV胁迫以及病菌攻击的适应(Hichri等2011)。因此, 果实花青素合成调控机理的相关研究是目前果树分子生物学研究的前沿课题, 对于果实花青素含量的提高、种质的创制等有重要科学意义(Hichri等2011; Petroni和Tonelli 2011; 葛翠莲等2012; 赵启明等2012; Jaakola 2013; 贾赵东等2014; 彭玉帅等2014; 刘晓芬等2013)。本文重点对果实花青素生物合成的调控基因及影响因素进行综述, 详述了果实发育过程中植物激素和环境因

素对调节基因的作用, 为进一步理解果实花青素生物合成以及分子改良提供借鉴。

### 1 花青素合成途径

植物花青素分布在特定的器官或组织中, 如植物的叶片、花、果实、种子等, 其含量受内在因子如生物钟和外界因子如光照、紫外光、温度和外源激素等影响(Procissi等1997; Harmer等2000; Piazza等2002)。在分子水平上, 影响花色苷代谢的基因分为结构基因和调节基因。结构基因就是直接编码花色苷生物合成酶类, 调节基因控制结构基因表达的强度和作用方式(包满珠1997)。花青素的代谢途径研究较为成熟, 如图2所示(Springob等2003; Hichri等2011; Petroni和Tonelli 2011)。苯丙氨酸是花色苷及其他类黄酮生物合成的直接前体, 首先由苯丙氨酸裂解酶(phenylalanine

ammonia-lyase, PAL)催化苯丙氨酸形成肉桂酸, 再经肉桂酸羟化酶(cinnamic acid 4-hydroxylase, C4H)和4-香豆酰-辅酶A连接酶(4-coumarate CoA ligase, 4CL)的催化形成香豆酰辅酶A, 香豆酰辅酶A和丙二酰辅酶A在查尔酮合成酶(chalcone synthase, CHS)催化下产生四羟基查尔酮, 然后在查尔酮异构酶(chalcone isomerase, CHI)、黄烷酮-3-羟化酶(flavanone 3-hydroxylase, F3H)的催化下产生二氢黄酮醇; 二氢黄酮醇在类黄酮-3'-羟化酶(flavonoid 3'-hydroxylase, F3'H)和类黄酮3',5'-羟化酶(flavonoid 3',5'-hydroxylase, F3'5'H)催化下生成双氢槲皮素和二氢杨梅黄酮, 它们在二羟黄酮醇-4-还原酶(dihydroflavonol-4-reductase, DFR)的催化下生成无色花青素, 经花色苷合成酶(anthocyanidin synthase, ANS)合成有色的花色苷, 最后在类黄酮-3-O-葡萄糖

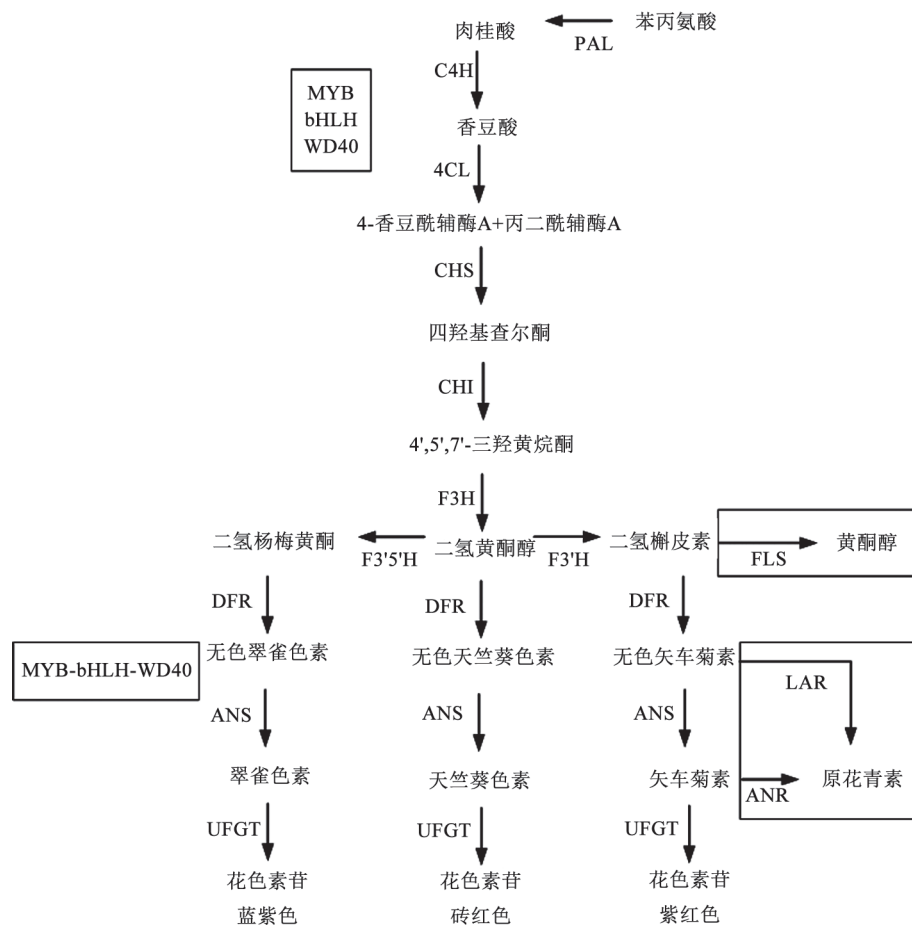


图2 花青素生物合成途径

Fig.2 Biosynthetic pathway of anthocyanin

参考Springob等(2003)、Hichri等(2011)、Petroni和Tonelli (2011)文献修改。

基转移酶(UDP glucose-flavonoid 3-O-glucosyl-transferase, UFGT)作用下将不稳定的花青素转变成蓝紫色、砖红色或紫红色的花色苷。

在果实发育过程中,随着果实颜色的加深,花青素的积累也随之增加。在果实花青素生物合成途径上游表达的关键酶基因包括*PAL*、*CHS*、*CHI*和*F3H*,这些酶基因同时也是黄酮醇类物质产生的上游基因,其表达与果实发育进程相关(Kumar和Ellis 2001; Muir等2001; Honda等2002; Lo Piero等2005)。在果实花青素生物合成途径下游表达的关键酶基因包括*DFR*、*ANS*和*UFGT*基因,一般情况下果实呈现出鲜艳的颜色时,发现这些酶基因高效表达(Kobayashi等2001; Honda等2002; Lo Piero等2005; 程建徽等2009; Wang等2010; Montefiori等2011; Han等2012; 王廿等2012)。但在荔枝果皮中*DFR*基因与其花青苷合成无密切关系(王惠聪等2004)。从分子角度而言,果实中花青素的积累是其生物合成的各类关键酶高效表达的结果,而这些关键酶的表达与调节基因的作用密切相关。

## 2 调节基因对果实花青素合成的作用机制

花青素合成结构基因的表达主要受特定调节因子的作用,主要的调节因子包括MYB转录因子、基本的螺旋-环-螺旋转录因子(bHLH)和色氨酸(W)和天冬氨酸(D) (WD40)重复蛋白(包满珠1997; Spelt等2000; Koes等2005; Chiu等2010; Hichri等2011; Schaart等2013)。MYB转录因子调控花青素合成的上游结构基因的表达,WD40/bHLH/MYB复合体调控花青素合成的下游结构基因的表达(Petroni和Tonelli 2011; 宫硇等2011)。在模式植物拟南芥中,MYB转录因子TT2、PAP1/PAP2、MYB75、MYB90、MYB113和MYB114, bHLH转录因子TT8、GL3和EGL3与WD40重复蛋白TTG1形成WD40/bHLH/MYB复合体,调控*DFR*、*ANS*、*UFGT*等下游基因的表达,进而影响花青素的生物合成(Gonzalez等2008)。最近,在多种果树上如葡萄和苹果等研究了转录因子MYB、bHLH、WD40调控花青素生物合成,这拓宽了我们对果实花青素合成分子调控机制的理解。更为重要的是,基因表达的调控是彼此联系,相互制约,构成复杂的基因表达调控网络。

### 2.1 MYB转录因子调控果实花青素合成

MYB转录因子含有保守的MYB结构域,每个

MYB结构域约由52个保守的氨基酸和间隔序列组成,这些氨基酸使MYB结构域折叠成螺旋-转角-螺旋(helix-turn-helix, HTH)结构(Dubos等2010)。根据MYB结构域的个数,MYB转录因子分为四类,单一MYB结构域(R1/2)蛋白、2个重复MYB结构域(R2R3)蛋白、3个重复MYB结构域(R1R2R3)蛋白和4个重复MYB结构域(4R)蛋白(Dubos等2010)。R1/2MYB蛋白的功能多样,*AtCPC*调控根毛发育;*HbSM1*在橡胶树中表达下调;*AtLHY*和*AtCCA1*控制昼夜节律;*MYBL2*参与类黄酮和油菜素内酯生物合成;1RMYB蛋白包括SANT/MYB结构域,参与染色质功能的调节(Wada等1997; Alabadi等2001; Grüne等2003; Dubos等2008; Ye等2012; Qin等2014)。R2R3MYB蛋白以N端含有由两个MYB结构域构成的DNA结合功能域为共同特征,在调控植物发育、细胞命运、代谢和对生物和非生物胁迫反应中发挥着重要作用(Dubos等2010)。R1R2R3蛋白调节细胞周期和细胞分裂(Ito 2005; Haga等2007)。植物4R蛋白所知甚少。

R2R3MYB通常含有一个[DE]Lx2[RK]x3Lx-6Lx3R基本结构域,参与调控花青素合成(Jin和Martin 1999; Zimmermann等2004; Allan等2008; Dubos等2010)。在模式植物拟南芥中,已鉴定125个R2R3MYB基因,其中13个与黄酮类代谢有关(Stracke等2001)。大多数参与果实花青素生物合成的MYB转录因子促进结构基因表达,从而导致果实中大量花青素积累。但是,少数MYB转录因子抑制结构基因表达,会导致花青素积累失常,表现在果实的果皮或果肉上花青素无积累或积累很少,如草莓*FaMYB1*和葡萄*VvMYB4* (Aharoni等2001; Matus等2008)。最近的研究发现,拟南芥中调控原花青素合成的MYB转录因子TT2和调控花青素合成的MYB转录因子PAP4 (*AtMYB114*),通过交换单个氨基酸, R2域Gly/Arg39连同R3结构域的4个氨基酸发生交换,实现原花青素合成向花青素合成转换,葡萄转录因子*VvMYBPA2*和*VvMYBA2*也发现了同样的规律,上述研究结果为我们提供了一种理解该问题的视角,即从进化上来说不同的黄酮类调节因子来自同一个祖先基因(Heppel等2013)。已在很多果树中鉴定了MYB转录因子,研究最为深入的是葡萄、苹果和草莓(表1)。

表1 果树中已经分离和鉴定参与调控花青素物合成途径的bHLH、MYB和WD40蛋白

Table 1 bHLH, MYB, and WD40 proteins involved in the regulation of the anthocyanin biosynthetic pathway identified in major fruit species

种类	植物	基因名称	GenBank登录号	参考文献
MYB	葡萄( <i>Vitis vinifera</i> )	<i>VvMYBA1</i>	AB097923	Kobayashi等2004 Walker等2007
		<i>VvMYBA2</i>	AB097924	Kobayashi等2004 Walker等2007
		<i>VvMYBA3</i>	AB097925	Kobayashi等2004
		<i>VvMYBA1a</i>	AB111100	Kobayashi等2004
		<i>VvMYBA1b</i>	AB111101	Kobayashi等2004
		<i>VvMYBA1c</i>	AB242302	Yakushiji等2006
		<i>VvMYBA1<sup>SUB</sup></i>	DQ345539	Lijavetzky等2006
		<i>VvMYBPA1</i>	AM259485	Bogs等2007
		<i>VvMYBPA2</i>	EU919682	Terrier等2009
		<i>VvMYB5a</i>	AY555190	Deluc等2006
		<i>VvMYB5b</i>	AAX51291	Deluc等2008
		<i>VvMYB4</i>	EF113078	Matus等2008
		<i>VlMYBA1-1</i>	AB073010	Kobayashi等2002
		<i>VlMYBA1-2</i>	AB073012	Kobayashi等2002
		<i>VlMYBA2</i>	AB073013	Kobayashi等2002
		<i>VlMYBB1-1</i>	AB073016	Kobayashi等2002
		<i>VlMYBB1-2</i>	AB073017	Kobayashi等2002
		<i>VlMYBC</i>	AB073014	Kobayashi等2002
		<i>VlMYBD</i>	AB073015	Kobayashi等2002
		<i>VlMYBA1-3</i>	AB427165	Azuma等2008
	<i>VvMYBA2r</i>	DQ886419	Walker等2007	
	<i>VvMYBA2w</i>	DQ886420	Walker等2007	
	苹果( <i>Malus domestica</i> )	<i>MdMYB1</i>	DQ886414	Takos等2006
		<i>MdMYBA</i>	AB279598	Ban等2007
		<i>MdMYB10</i>	EU153571	Espley等2007
		<i>MYB110a</i>	EB710109	Chagné等2013
	草莓( <i>Fragaria ananassa</i> )	<i>FaMYB1</i>	AAK84064	Aharoni等2001
		<i>FaMYB10</i>	EU155162	Lin-Wang等2010
	智利白草莓( <i>Fragaria chiloensis</i> )	<i>FcMYB1</i>	GQ867222	Salvaterra等2013
	野生草莓( <i>Fragaria vesca</i> )	<i>FvMYB10</i>	EU155163	Lin-Wang等2010
	树莓( <i>Rubus idaeus</i> )	<i>RiMYB10</i>	EU155165	Lin-Wang等2010
	梨( <i>Pyrus communis</i> )	<i>PcMYB10</i>	EU153575	Lin-Wang等2010
	沙子梨( <i>Pyrus pyrifolia</i> )	<i>PpyMYB10</i>	EU153576	Lin-Wang等2010
中国梨( <i>Pyrus×bretschneideri</i> )	<i>PbMYB10</i>	EU153577	Lin-Wang等2010	
西洋李( <i>Prunus insititia</i> )	<i>PiMYB10</i>	EU153579	Lin-Wang等2010	
欧洲李( <i>Prunus domestica</i> )	<i>PdmMYB10</i>	EU153580	Lin-Wang等2010	
红叶李( <i>Prunus cerasifera</i> )	<i>PcfMYB10</i>	EU153583	Lin-Wang等2010	
李子( <i>Prunus salicina</i> )	<i>PsMYB10</i>	EU155161	Lin-Wang等2010	
山杏( <i>Prunus armeniaca</i> )	<i>ParMYB10</i>	EU153578	Lin-Wang等2010	
杏( <i>Prunus dulcis</i> )	<i>PdMYB10</i>	EU155159	Lin-Wang等2010	
酸樱桃( <i>Prunus cerasus</i> )	<i>PcrMYB10</i>	EU153582	Lin-Wang等2010	
桃( <i>Prunus persica</i> )	<i>PprMYB10</i>	EU155160	Lin-Wang等2010	
枇杷( <i>Eriobotrya japonica</i> )	<i>EjMYB10</i>	EU153572	Lin-Wang等2010	
木瓜( <i>Cydonia oblonga</i> )	<i>CoMYB10</i>	EU153571	Lin-Wang等2010	
番茄( <i>Solanum lycopersicum</i> )	<i>ANT1</i>	AY348870	Lin-Wang等2010	
甘薯( <i>Ipomoea batatas</i> )	<i>IbMYB1</i>	AB258985	Lin-Wang等2010	

表1 (续)

种类	植物	基因名称	GenBank登录号	参考文献
bHLH	山竹( <i>Garcinia mangostana</i> )	<i>GmMYB10</i>	FJ197137	Palapol等2009
	梨( <i>Pyrus pyrifolia</i> )	<i>PyMYB10</i>	GU253310	Feng等2010; Qian等2014
	中国杨梅( <i>Myrica rubra</i> )	<i>MrMYB1</i>	GQ340767	Niu等2010
	甜橙( <i>Citrus sinensis</i> )	<i>Ruby</i>	JN402329	Butelli等2012
	甜樱桃( <i>Prunus avium</i> )	<i>PacMYBA</i>	KF974774	Shen等2014
	葡萄( <i>Vitis vinifera</i> )	<i>VvMYC1</i>	EU447172	Hichri等2010
		<i>VvMYCA1</i>	ABM92332	Matus等2010
	苹果( <i>Malus domestica</i> )	<i>MdbHLH3</i>	CN934367	Espley等2007
		<i>MdbHLH33</i>	DQ266451	
		草莓( <i>Fragaria ananassa</i> )	<i>FabHLH3</i>	JQ989284
		<i>FabHLH3A</i>	JQ989285	
		<i>FabHLH33</i>	JQ989286	
WD40	葡萄( <i>Vitis vinifera</i> )	<i>VvWDR1</i>	ABF66625	Matus等2010
		<i>VvWDR2</i>	ABF66626	
	苹果( <i>Malus domestica</i> )	<i>MdTTG1</i>	AF220203	Brueggemann等2010; An等2012
	石榴( <i>Punica granatum</i> )	<i>PgWD40</i>	AF485783	Ben-Simhon等2011

对于葡萄而言, *VvMYBA*转录因子家族基因多态性是栽培葡萄果实花青素含量变异的主要原因, 如红葡萄果实花青素含量高, 白葡萄果实中花青素含量低甚至没有(Fournier-Level等2010)。研究发现2个MYB基因(*VvMYBA1*和*VvMYBA2*)控制着红葡萄花青素合成的UFGT基因表达(Cutanda-Perez等2009)。而这些调节基因在白葡萄果实中处于失活状态, 研究发现这类基因发生了突变, *VvMYBA1*基因插入反转录转座子, *VvMYBA2*非保守编码区序列发生替换(Kobayashi等2004; Walker等2007)。大多数MYB转录因子功能特异性很高, 主要调控类黄酮途径的一个分枝。例如, 葡萄毛状根中*VvMYBA1*和*VvMYBA2*仅诱导与花青素生物合成和运输相关的基因表达(Cutanda-Perez等2009)。又如, *VvMYBPA1*和*VvMYBPA2*的表达激活原花青素途径中花青素还原酶基因(*ANR*)和无色花色素还原酶基因(*LAR*)的表达, 从而影响花青素生物合成(Bogs等2007; Terrier等2009)。同样, *VvMYB5a*基因能影响苯丙氨酸合成途径中结构基因的表达, 进而改变花青素、黄酮、单宁和木质素的代谢; *VvMYB5b*基因参与调节黄酮类化合物的合成, 能激活类黄酮合成途径中结构基因的启动子, 提高花青素和原花青素衍生物的含量(Deluc等2006, 2008)

对于苹果(*Malus domestica*)而言, MYB基因是栽培品种果实颜色差异的主要决定因素。不同的研究小组克隆到与苹果颜色密切相关的MYB基因主要是*MdMYB1*、*MdMYBA*和*MdMYB10*, 三者核苷酸具有98%的相似性, 其中*MdMYB1*和*MdMYBA*翻译相同的蛋白质, 而与*MdMYB10*有3个氨基酸的差异, *MdMYB1*、*MdMYBA*和*MdMYB10*可能是相同位点的等位基因(Ban等2007; Lin-Wang等2010)。Talos等(2006)从红色苹果‘Cripps’中分离了*MdMYB1*, 发现成熟果实中其转录水平与红色果皮花青素合成相关, 而且*MdMYB1*转录水平在红皮品种中比非红皮品种中高。Ban等(2007)从淡红色苹果‘Tsugaru’果皮中分离了*MdMYBA*基因, 并采用深红色苹果品种进行了表达分析, 结果发现*MdMYBA*基因表达调控具有很高的组织特异性和品种特异性, *MdMYBA*基因在深红色品种中比淡红色品种中表达高。Espley等(2007)从白肉品种‘Sciros’和红肉品种‘Red Field’中分别取果皮和果肉进行研究, 发现在果实发育过程中, *MdMYB10*表达与花青素含量强烈相关。有趣的是, 在苹果DNA水平上发现红肉果实的*MdMYB10*基因重排了23 bp的串联重复序列, 而在非红肉果实中, 这23 bp串联重复序列未重排(Espley等2009)。然而, *MdMYB10*基因重排了23 bp的串联重复序列不能

解释所有花青素相关表型。Chagné等(2013)采用遗传学和基因组学相结合的方法对红肉苹果栽培品种‘Sangrado’进行研究,确定了苹果MYB110a基因是一个古老加倍的基因,对红色果肉表型起关键作用;并证实红肉表型与MYB110a表达密切相关;将该基因转入烟草中,发现MYB110a上调可以促进烟草花青素生物合成;MYB110a染色体位置与苹果亚科中苹果进化中发生的全基因组加倍事件一致。

在草莓(*Fragaria* × *ananassa*)中,MYB转录因子调控果实花青素的机制不同于其他果树,MYB1抑制花青素的合成,而MYB10则促进花青素的积累,MYB10的相对转录水平在果实成熟组织中大量增加,这可能与草莓具有明显的两阶段类黄酮生物合成有关,未成熟果实中积累高水平的原花色素,而与颜色有关的花青素主要是在成熟的果实中产生(Schaart等2013; Fischer等2014)。草莓的FaMYB1抑制花青素的合成,可能与其C端具有一段保守序列有关,此段序列可能与激活子竞争结合靶基因,从而抑制成熟期果实中花青素合成(Aharoni等2001)。野生草莓(*F. vesca*)的FvMYB1在果实发育各阶段的相对表达量变化很小,这可能与果实颜色变化的特征有关,其果色变化较早,仅发生在表皮(Lin-Wang等2010)。从智利白果草莓(*F. chiloensis*)克隆的FcMYB1,是FaMYB1的同源基因,该基因在白果草莓表达量明显高于其在红果草莓‘Camarosa’中的表达量;采用RNA沉默的方法下调了白果草莓中FcMYB1的转录水平,实时定量PCR的结果表明ANS上调,而ANR和LAR则受到抑制,同时,果实中花青素的含量增加和几乎检测不到黄烷-3-醇含量,说明该转录因子在果实花青素和原花青素合成途径中起到平衡的作用(Salvatierra等2013)。从二倍体野生草莓克隆FvMYB10基因和从八倍体栽培草莓克隆FaMYB10基因,在两者果实成熟组织中,MYB10基因的相对转录水平平均大量增加,在八倍体栽培草莓中从蕾期到成熟期增加几乎是40 000倍;从花期到成熟期, FaMYB10的增加幅度比FvMYB1几乎多10倍以上,实验结果表明FaMYB10基因与草莓果实花青素的积累密切相关(Lin-Wang等2010)。

此外,相关研究在其他果实如梨(*Pyrus pyrifolia*)、樱桃(*Prunus avium*)、山竹(*Garcinia mangostana*)、杨梅(*Myrica rubra*)、甜橙(*Citrus sinensis*)等上也

有报道(Palapol等2009; Feng等2010; Niu等2010; Butelli等2012; Qian等2014; Shen等2014)。

上述研究表明, R2R3MYB转录因子在调节花青素生物合成中起关键作用,它们通过调节花青素合成路径中的结构基因的表达从而影响果实花青素的积累。

## 2.2 bHLH转录因子调控果实花青素合成

人们较多地关注MYB转录因子,而较为忽略bHLH转录因子。bHLH转录因子是调节果实花青素生物合成的另一类调节基因,能够独立地与DNA结合,也可以与MYB结合成二聚体再与DNA结合。人们对植物bHLH转录因子所知甚少,在拟南芥中已确定133个bHLH转录因子,其中至少113个是有表达的,但并没有确定哪些与花青素合成相关(Heim等2003)。目前,分离并鉴定的调控果实花青素合成途径的bHLH转录因子包括葡萄VvMYC1 (Hichri等2010; Matus等2010)、苹果Md-bHLH3和MdbHLH33 (Espley等2007; Xie等2012)和草莓FabHLH3 (Schaart等2013) (表1)。

当bHLH与MYB共表达时,可激活花青素生物合成的结构基因(Palapol等2009; Lin-Wang等2010; Niu等2010)。在模式植物拟南芥中, bHLH转录因子TT8、GL3、EGL3和MYB转录因子PAP1、PAP2、MYB113、MYB114相互作用调控花青素合成;在结构上两者互作的机制为MYB转录因子的一个特征域(D/E)Lx2(R/K)x3Lx6Lx3R与bHLH蛋白的N端特异性结合,该特征域位于R3重复区域,任何一个氨基酸的点突变都可以削弱与bHLH转录因子的相互作用,14个R2R3MYB蛋白和6个1RMYB蛋白都存在该特征域(Zimmermann等2004)。在葡萄中, bHLH转录因子(VvMYC1)与所有MYB转录因子(VvMYB5a/5b、VvMYBA1/A2和VvMYBPA1)互作,诱导和启动花青素或原花青素合成(Hichri等2010)。在苹果中, MYB转录因子(MdMYB10)高效诱导花青素生物合成依赖于两个bHLH蛋白(MdbHLH3和MdbHLH33)共表达(Espley等2007)。在转基因研究中,将果实专一性启动子驱动的MYB转录因子(*ros1*)和bHLH转录因子(*del*)基因转入番茄中,可激活花青素合成途径中的上游结构基因(PAL和CHI),从而获得富含花青素的紫色番茄(*Solanum lycopersicum* cv. ‘MicroTom’) (Butelli等2008)。转*ros1*和*del*基因草莓的根和叶的

颜色变成红紫色; 实时RT-PCR分析表明, 在转基因草莓中花青素合成的关键结构基因在转基因株系中的表达均上调, CHI上调幅度最大(金万梅和王华2014)。由此可见, MYB和bHLH转录因子彼此关联, 对花青素合成产生联合作用。

bHLH转录因子能调控葡萄花青素和原花青素的生物合成(Hichri等2010)。在拟南芥的角果和幼苗中, 以及圆叶牵牛的花和种皮中也发现bHLH转录因子有类似的功能(Nesi等2000; Park等2007)。此外, bHLH转录因子不但调节花青素的生物合成, 还在植物的生长发育方面发挥着作用, 比如参与表皮毛和根毛的形成等(Lloyd等1992; Heim等2003; Park等2007)。上述研究表明, bHLH转录因子可能参与多种生理过程, 重要的是在果实发育过程中, bHLH转录因子调节花青素的生物合成。

### 2.3 WD40重复蛋白调控果实花青素合成

调控花青素生物合成的另一类转录因子是WD40, 其一般含有4~16个串联重复的色氨酸(W)和天冬氨酸(D)构成的WD基元, 每个WD基元由总长约40个以色氨酸(W)和天冬氨酸(D)结尾的氨基酸序列组成。WD40蛋白可与多种蛋白质相互作用, 其作用可能是连接MYB与bHLH的中间介质, 三者形成复合物; WD40蛋白可能是一个对接平台, 可将该复合物从细胞质运往细胞核(Van Nocker和Ludwig 2003; Hichri等2011)。

到目前为止, 仅确定了少数几个参与花青素合成途径起调控作用的WD40蛋白。果实中确定的参与花青素合成途径调控的WD40蛋白更少, 仅在葡萄、苹果和石榴(*Punica granatum*)中确定了这种蛋白(表1)。对于葡萄而言, 其WD40蛋白(*VvWDR1*和*VvWDR2*)和bHLH蛋白(*VvMYC1*和*VvMYC1*)共表达与葡萄果实花青素积累密切相关, 与*VvMYB1*、*UFGT*和*ANR*表达也密切相关(Matus等2010)。石榴WD40蛋白(*PgWD40*)的表达水平与矢车菊色素含量呈正相关, *PgWD40*转录产物的稳态水平与结构基因*PgDFR*、*PgLDOX*转录产物强烈相关; *PgWD40*、*PgDFR*、*PgLDOX*表达与调节基因*PgAn1* (bHLH)和*PgAn2* (MYB)的表达相关(Ben-Simhon等2011)。与上述研究不同, 苹果WD40蛋白*MdTTG1*提高了果实中花青素的积累, 通过分析该基因在不同器官中的表达模式, 发现它们与苹果花青素的积累正相关; 酵母双杂交和

互补实验证明*MdTTG1*仅与bHLH (*MdbHLH3*和*MdbHLH33*)蛋白存在直接相互作用, 而无法与*MdMYB1*发生互作(Brueggemann等2010; An等2012)。这与拟南芥中三类调控因子之间的互作调控*DFR*和*BAN*基因的表达不同, 这种区别的内在机制有待进一步研究(Nesi等2000)。

此外, 与bHLH转录因子类似, WD40在调控花青素合成途径的同时, 也可以调控其他一些生理过程。例如, 拟南芥的WD40 (*TTG1*)还具有调节表皮毛分化的作用(Walker等1999)。

### 2.4 调节基因在花青素和原花青素合成积累调控网络中的作用

花青素合成积累是一个复杂的调控网络, 尤其是在调节基因方面。在模式植物拟南芥中已有报道(Nesi等2000; Zhang等2003; Gonzalez等2008; Petroni和Tonelli 2011)。首先, 所有的*TTG1*与bHLH和MYB转录因子互作调控次生代谢(花青素、原花青素和种皮粘液)和表皮细胞命运(表皮毛和根毛发生)。其次, MYB转录因子和bHLH转录因子功能有交叉。还有, 调控网络中存在互作和正负反馈, 如*MYBL2*抑制MYB和bHLH转录因子的表达; *TT8*正调控*MYBL2*的表达, 进而抵消*TT8*的正反馈机制。

调节基因在葡萄果实花青素合成积累的调控网络如图3所示。葡萄WD40蛋白和bHLH转录因子共表达与葡萄果实花青素积累密切相关(Matus等2010)。葡萄bHLH转录因子与MYB转录因子(*VvMYB5a/5b*、*VvMYB1/A2*和*VvMYBPA1*)互作, 诱导和启动花青素或原花青素合成(Hichri等2010)。葡萄MYB转录因子调控花青素合成方面的研究较为深入。首先, *VvMYB1*和*VvMYB2*决定葡萄果实的颜色, 而且, *VvMYB1*和*VvMYB2*两个基因位点是连锁的, 因此可以把这些基因看作是单个大的等位基因的一部分, 而这个大的等位基因可以认为是一个单倍型(haplotype) (Kobayashi等2004; Walker等2007; Azuma等2011)。*VvMYB1*和*VvMYB2*的等位基因*VvMYB1a*和*VvMYB2w*没有功能, *VvMYB1b*、*VvMYB1c*和*VvMYB2r*有功能(Kobayashi等2004; Walker等2007)。例如, 欧洲和美国葡萄杂交后代的单倍型包括HapA (具有*VvMYB2w*和*VvMYB1a*)、HapB (具有*VvMYB2w*和*VvMYB1b*)、HapC-N (具有*VvMYB2r*和



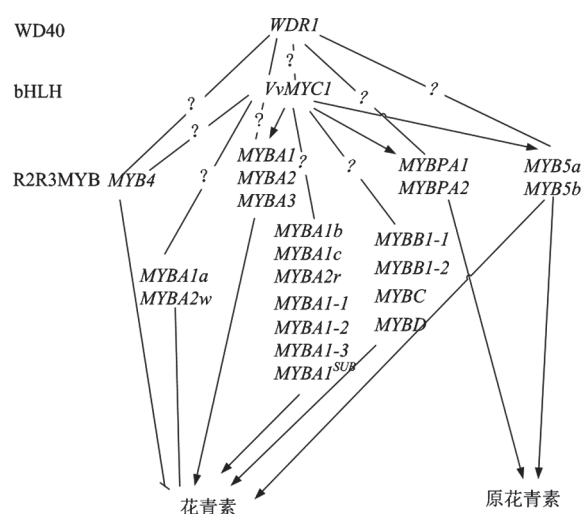


图3 葡萄果实花青素合成的基因调控网络  
Fig.3 Gene regulatory network of anthocyanin biosynthesis in grape

参考Kayesh等(2013)和Azuma等(2011)文献修改。

*VvMYBA1c*)、HapC-Rs (具有*VvMYBA2w*和*VvMYBA1c*)、HapE1 (具有*VIMYBA1-2*和*VIMYBA1-3*)、HapE2 (具有*VIMYBA2*和*VIMYBA1-3*); HapA没有功能,无法合成花青素;其他单倍型能够合成花青素;黑皮品种至少含有两个具有功能的单倍型,红皮品种仅含有一个具有功能的单倍型(Azuma等2008, 2011)。其次,欧洲葡萄东方品种群的4个品种中发现只存在*VvMYBA1<sup>SUB</sup>*,不存在其他的具有功能的*VvMYBA*基因(*VvMYBA1b*或*VvMYBA1c*)和*VIMYBA*基因(*VIMYBA1-3*或*VIMYBA1-2*) (Azuma等2008)。还有, *VvMYB5a/5b*基因调控花青素和原花青素生物合成途径中多个结构基因的表达(Deluc等2006, 2008), *VvMYBPA1*和*VvMYBPA2*基因调控原花青素生物合成(Bogs等2007; Terrier等2009)。

总之,在果实发育过程中,主要调节花青素生物合成的是MYB转录因子,受到研究者的多方关注。由于果实花青素生物合成分子调节机制很复杂,如果要全面准确地揭示其调控机制,也需要更多地关注bHLH和WD40转录因子。首先, bHLH转录因子能够与MYB结合成二聚体再与DNA结合,激活花青素生物合成途径中各类关键酶基因的表达;其次, WD40作用可能是连接MYB与bHLH的中间介质,三者形成复合物调节果实花青素的生物合成,关于这些方面仍然有许多空白,因此为了全面揭示花青素生物合成的分子调控机制,需要

更多关注MYB、bHLH和WD40作用的调控网络,只有这样,才能更好地阐述果实花青素生物合成的分子作用机制。

### 3 miRNAs对果实花青素生物合成的调控

微小RNAs (microRNAs, miRNAs)是基因表达的重要转录后调控因子,通过介导靶mRNAs的剪切或抑制其翻译来调控基因的表达,或者通过切割自己原始转录产物而合成反式作用小干扰RNAs (*trans-acting short interfering RNAs, ta-siRNA*),参与调控包括果实花青素生物合成在内的多个发育或生理过程(Bartel 2004; Floyd和Bowman 2004; Allen等2005; Solofoharivelo等2014)。

在模式植物拟南芥中发现, *AtTAS4-siR81(-)*调控*PAP1/MYB75*、*PAP2/MYB90*和*MYB113*的表达,进而影响花青素和黄酮的合成(Rajagopalan等2006)。缺磷导致拟南芥中*PAP1/MYB75*、*MYB90*和*MYB113*表达上调,激活花青素生物合成途径的结构基因;*PAP1/MYB75*上调,激活miR828和*AtTAS4*,诱导*AtTAS4-siR81(-)*上调,从而抑制*PAP1/MYB75*、*MYB90*和*MYB113*表达,这种自反馈调节能够在胁迫条件下使花青素的含量维持在合适的水平(Hsieh等2009)。与模式植物miRNAs研究相比,果树不仅生命周期长,而且基因组也很庞大,因此,miRNAs调控果实花青素生物合成的研究相对滞后。苹果*MdTAS4-siR81(-)*调控3个MYB和1个bHLH转录因子(*MdbHLH3*)的表达,影响花青素的积累;同时,miR828激活siRNA生物合成,调控7个与花青素合成相关的MYBs来加强这种反馈调节,以确保苹果果实发育过程中颜色的变化(Xia等2012)。在葡萄中也发现miRNAs对WD40的表达起调控作用,影响花青素的合成(Wang等2011, 2012)。由此可见,miRNAs激活,依赖MYB转录因子表达和siRNAs级联的基因调控网络,调节花青素相关的主要生理生化反应。因此,miRNAs调控对果实花青素生物合成,可提高果实中花青素的含量、改变果实的颜色。

### 4 生物内在因素和外界环境对果实花青素生物合成的影响

果实的颜色是生物内在因素和外界环境共同决定,多种信号刺激,转导,作用到转录因子,转录因子控制结构基因的表达,最终实现果实花青素含量的变化,其调控是复杂的调控网络(图4)。

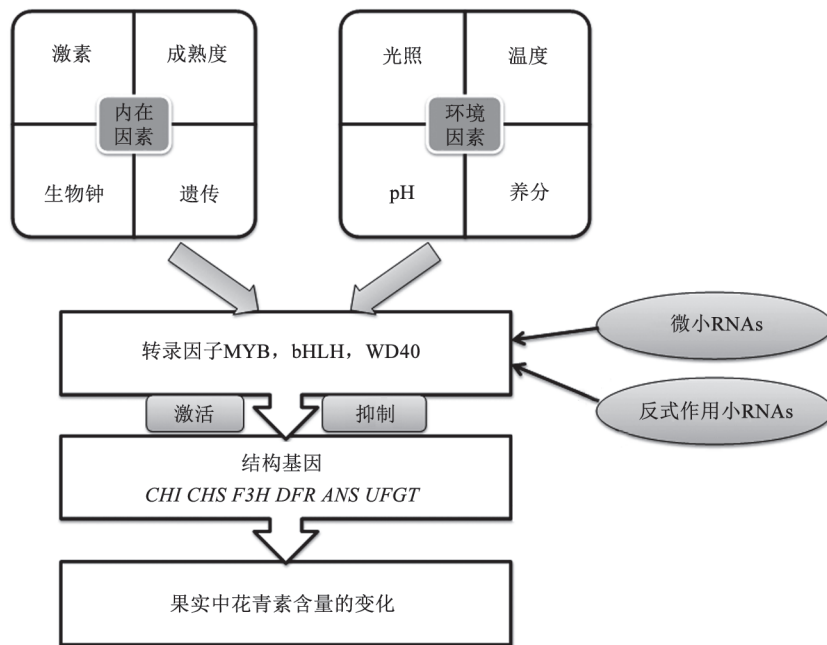


图4 果实花青素合成的调控网络

Fig.4 Regulatory network of anthocyanin biosynthesis in fruites

生物内在因素如植物激素等能够影响调节基因从而改变果实花青素的生物合成。ABA和乙烯是与果实成熟最相关的植物激素,对果实花青素的生物合成有重要影响。研究发现在拟南芥中,乙烯通过下调表达花青素合成正调控转录因子(*GL3*、*TT8*、*PAP1*),激活负调控因子*MYBL2*的表达,抑制糖或光诱导的花青素积累(Jeong等2010)。而外源乙烯激活葡萄花青素合成相关基因的表达(El-Kereamy等2003)。最近的研究表明,施加外源ABA导致葡萄果皮和樱桃中花青素迅速积累;进一步分析发现花青素合成途径中调节基因和结构基因的表达上调(Koyama等2010; Shen等2014)。另外,细胞分裂素能提高光和糖诱导的花色素生物合成,但是缺乏其在果实成熟相关的花青素生物合成中作用机制的研究(Das等2012)。

外界环境如温度、光照等也通过影响调节基因从而改变果实花青素的生物合成。在苹果中,低温诱导*MdbHLH3*蛋白磷酸化,增强其转录活性和与启动子结合能力,进而促进花青素合成(Xie等2012);高温迅速降低*MdMYB10*表达水平,进而减少果实着色(Lin-Wang等2011)。在收获前一周,把苹果‘Fortune’(红色品种)和‘Mutsu’(黄绿色品种)转动180度使其遮光面的果皮完全接受光照,与转

向的果实相比,没有转向的果实遮光面果皮*MdMYB10*和结构基因(*MdPAL*、*MdCHS*、*MdCHI*、*MdF3H*、*MdDFR1*、*MdLDOX*和*MdUFGT*)表达水平显著下调,花青素和黄酮醇含量也较低(Feng等2013)。在葡萄中,研究发现低温(15 °C)加光照处理条件下观察到葡萄果皮中积累了大量的花青素,而在高温(35 °C)或暗培养条件下观察到葡萄果皮中花青素的积累明显被抑制;定量RT-PCR分析表明,MYB转录因子(*VIMYBA1-3*、*VIMYBA1-2*和*VIMYBA2*)对温度和光照的响应差异很大,表明温度和光照通过影响类黄酮生物合成途径中的调节基因改变果皮中花青素的积累(Azuma等2012)。上述研究主要集中于光照和温度影响果实花青素的生物合成,低温和光照通过激活调节基因的转录活性和与启动子结合能力促进果实花青素积累,而高温和黑暗通过下调调节基因的转录活性抑制果实花青素积累。另外pH、养分等也影响果实花青素的生物合成(Fang等2013; Soubeyrand等2014)。其他的环境因子如水分、风速等,它们如何影响果实花青素生物合成及其分子调控机制有待进一步研究。

从调控网络的角度,揭示不同水平上果实花青素生物合成调控机制的研究非常少。Jaakola

(2013)认为花青素的调控网络是从环境因子到生物的发育调节,二者一起作用于调节基因,进一步控制结构基因,最终引起原花青素和花青素的积累。Carbone等(2009)以意大利2个地点的6个草莓栽培品种的果实为研究对象,研究了生物内在因素(如遗传)和外部影响因素(如环境因素)对黄酮类基因转录、酶的活性和代谢物的影响,结果表明,在草莓果实发育过程中,黄酮类化合物相关的基因表达和酶活的变化与基因型相关;基因型显著影响黄酮类代谢产物;黄烷-3-醇类、原花青素类的衍生物和花青素是含量最多的代谢产物;环境因素对黄酮醇含量和原花青素含量相关性状(表儿茶素/儿茶素比和聚合程度)影响明显。Åkerström等(2010)研究表明欧洲越橘的生长地点和亲本来源地影响其花青素的积累,亲本来自北纬或者北方的欧洲越橘其花青素含量较高;Uleberg等(2012)研究发现在不同的温度(12和18 °C)和光周期(12 h 自然光、24 h 自然光或24 h 自然光加红光)条件下,欧洲越橘北方型品种花青素含量比南方型品种高,上述两项研究说明遗传因素对于果实花青素的影响很强烈,而且环境因素也会影响果实的花青素积累。

## 5 讨论和展望

花青素是一种天然的水溶性植物色素,具有特殊的营养价值和保健功能,并且有利于植物传播种子和花粉、适应逆境。果实花青素合成分子调控机理的相关研究是目前果树分子生物学研究的前沿课题,在果树育种上有很高的应用价值,对于果实花青素含量的提高、种质的创制等具有重要的科学意义。近年来,转录因子MYB、bHLH和WD40对果实花青素生物合成的作用方面的研究已经取得了一定的进展。在今后的研究中,需要更多关注MYB、bHLH和WD40作用的调控网络,也要重视微小RNAs对调控基因表达的作用。实际上果实花青素生物合成调控是复杂的调控网络,生物内在因素和外界环境均可影响花青素的合成,从分子的角度看,基因表达的多种信号传递网络均可指向花青素合成的结构基因,其中的信号因子和信号传递的途径还有待于深入研究。

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