

· 专题论坛 ·

## 花青素转录因子调控机制及代谢工程研究进展

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**摘要** 花青素是广泛存在于植物中的一类重要的类黄酮化合物, 在植物生长发育和人类营养保健方面具有重要价值。花青素的生物合成途径已经解析得比较清楚, 但花青素的代谢调控网络还在不断完善。调控花青素生物合成的转录因子主要包括MYB、bHLH和WD40三大类, 这些转录因子通过激活或抑制CHS、ANS和DFR等花青素途径关键结构基因的表达水平, 进而决定花青素积累的部位与水平。该文结合国内外花青素生物合成与转录调控方面的研究进展, 简要介绍了花青素的生物合成途径, 归纳总结了模式植物中花青素代谢调控的分子机理, 尤其是MYB、bHLH和WD40三类主要转录因子的调控机理, 以及这些转录因子在观赏植物和水果等经济作物花青素代谢工程中的应用。该文将为系统阐明花青素的转录调控机制和利用代谢工程改良花青素的相关研究提供有益参考。

**关键词** 花青素, 转录因子, 转录调控, 代谢工程

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花青素(anthocyanins)与黄酮(flavones)、异黄酮(isoflavones)、黄酮醇(flavonols)和原花色素(proanthocyanidins)都属于类黄酮(flavonoids)化合物, 它们是广泛存在于植物中的一大类重要的次生代谢产物。花青素主要在植物的花、叶片和果实等器官中积累, 赋予植物丰富多彩的颜色, 使其具有重要的观赏价值和经济价值(Zhao and Tao, 2015)。花青素在植物生长和生存过程中以多种方式发挥作用(Winkel-Shirley, 2001; Dixon and Sumner, 2003; Zhu et al., 2017)。花青素具有保护植物免受生物侵害和吸引昆虫授粉的功能(Miller et al., 2011; Fan et al., 2016); 此外, 花青素可以响应生物与非生物胁迫(Shao et al., 2007)、清除氧自由基(Shih et al., 2007)以及保护植物免受高密度光照的伤害(Hughes et al., 2005)。近年来, 人们逐渐认识到花青素在抗癌、抗病和抗氧化方面的营养与保健功能(Zhu et al., 2011; Peiffer et al., 2016; Wei et al., 2018)。近期研究表明, 花青素可以降低血脂、减少胆固醇(Farrell et al., 2015)、提高青光眼视力(Shim et al., 2012)、治疗视网膜膜疾病(Tao et al., 2016)、修复记忆损伤(Jo et al., 2015)以及防治心血管疾病(Isaak et al., 2017)。花青

素不仅在植物生长过程中发挥重要作用, 而且对人体健康有益, 因此受到越来越多的关注, 成为植物次生代谢领域的研究热点。

目前, 随着分子生物学的发展, 花青素生物合成途径已经逐渐被阐明, 转录因子单独或协同调控花青素生物合成的分子机制也正在被不断完善。前人已经对花青素的生物合成和转运、环境因子对花青素合成的影响进行了详细的总结(胡可等, 2010; 祝志欣和鲁迎青, 2016)。本文着重综述调控花青素生物合成的主要转录因子的研究进展, 重点阐述MYB、bHLH和WD40三类转录因子调控花青素生物合成的分子机制以及它们在花青素代谢工程中的应用。

### 1 花青素的生物合成途径

花青素来源于类黄酮化合物的合成途径: 衍生于香豆酰辅酶A (4-coumaroyl CoA)和丙二酰辅酶A (malonyl CoA), 在查尔酮合酶(chalcone synthase, CHS)作用下合成查尔酮(chalcone)。查尔酮经查尔酮异构酶(chalcone isomerase, CHI)催化形成黄烷酮(flavonones)。黄烷酮再经过黄烷酮3-羟化酶(flavanone

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3-hydroxylase, F3H)催化形成二氢黄酮醇(dihydro flavonols)。在花青素特异的分支途径中,二氢黄酮醇还可以被黄酮羟化酶(flavonoid 3'-hydroxylase, F3'H和flavonoid 3',5'-hydroxylase, F3'5'H)催化,并在二氢黄酮醇还原酶(dihydroflavonol-4-reductase, DFR)作用下还原为无色花青素(leucoanthocyanidins),无色花青素在花青素合成酶(anthocyanidin synthase, ANS)的催化作用下形成花青素苷元(anthocyanidins),不稳定的花青素苷元经糖基转移酶(glycosyltransferase, UGTs)修饰形成稳定的花青素苷(anthocyanins) (Holton and Cornish, 1995; Zhang et al., 2014b)。常见的花青素苷元包括天竺葵素(pelargonidin)、飞燕草素(delphinidin)和矢车菊素(cyanidin) 3种基本花青素苷元,以及牵牛花素(petunidin)、芍药素(peonidin)和锦葵素(malvidin)等甲基化产物(Cabrita et al., 2000)。广义的花青素泛指各类花青素苷(Seeram and Nair, 2002)。

Pelletier等(1997)将类黄酮途径的基因分为早期生物合成基因(early biosynthetic genes, EBGs)和晚期生物合成基因(late biosynthetic genes, LBGs)两类;花青素生物合成途径中的EBGs即参与共同前体合成的基因,包括CHS、CHI、F3'H和F3H;LBGs指花青素生物合成途径中的下游基因,主要包括DFR、ANS和UGTs (图1)。

## 2 花青素合成的转录调控因子

MYB、bHLH和WDR是目前研究最多的3类调控花青素合成的主要转录因子家族,这3类转录因子的序列、表达特征和作用机制各不相同。它们广泛存在于模式植物、观赏植物和水果等经济作物中,具有物种特异性和多样性。本文重点对这3类转录因子在不同类型植物中的调控机制进行归纳总结。

### 2.1 MYB转录因子家族

MYB是植物中最大的转录因子家族,在植物生长发育(Song et al., 2011)、次生代谢调控(Borevitz et al., 2000)、植物激素信号转导(Abe et al., 2003)和胁迫应答(Zhang et al., 2012)等诸多方面发挥作用。MYB转录因子的N端含有1个保守的MYB结构域,根据MYB结构域的数量可将MYB转录因子分为4类:1R-MYB、

R2R3-MYB、3R-MYB和4R-MYB。其中,R2R3-MYB是数目最多的一类MYB转录因子(Dubos et al., 2010),也是调控类黄酮途径的重要转录因子,广泛参与调控花青素的生物合成。在拟南芥(*Arabidopsis thaliana*)、玉米(*Zea mays*)和矮牵牛(*Petunia hybrida*)等模式植物中均发现大量调控花青素生物合成的R2R3-MYB型转录因子。

#### 2.1.1 模式植物的MYB转录因子

第1个被发现调控花青素生物合成的R2R3-MYB转录因子是玉米中的C1 (Colorless-1),C1依赖光特异性地调控玉米糊粉层中CHS和DFR基因的表达(Cone et al., 1986; Paz-Ares et al., 1987; Pooma et al., 2002)。玉米中另一个不依赖光的基因PL (*Pl-Rhoades*)同样也通过影响DFR基因的表达而调控花和叶中花青素的积累(Cone et al., 1993a, 1993b; Pooma et al., 2002)。在模式植物拟南芥中调控花青素生物合成的MYB转录因子研究得最为清楚,其中PAP1 (Production of Anthocyanin Pigment 1)是调控花青素的关键转录因子。Borevitz等(2000)发现,激活PAP1的过量表达诱导花青素的大量积累,促使拟南芥叶片呈现深紫色(Borevitz et al., 2000)。过量表达PAP1影响CHS、CHI和ANS等基因的表达;其中与花青素合成相关的2个糖基转移酶基因UGT78D2和UGT75C1均被PAP1诱导表达,导致花青素中的矢车菊素含量显著增加(Tohge et al., 2005)。

拟南芥中花青素的积累还与PAP1是否受到生物或非生物因子诱导有关(Mitsunami et al., 2014; Nakabayashi et al., 2014; Onkokesung et al., 2014; Lee et al., 2016)。Maier等(2013)发现,光应答元件COP1/SPA (CONSTITUTIVELY PHOTOMORPHOGENIC1/SUPPRESSOR OF PHYA-105)能与PAP1和PAP2互作,在转录和转录后水平上影响花青素的合成。黑暗条件下,COP1/SPA降低PAP1和PAP2的表达,抑制花青素的合成。另一个光信号途径的元件HY5 (LONG HYPOCOTYL 5)通过结合PAP1启动子区域的G盒和ACE盒调控其表达(Shin et al., 2013)。同时,PAP1的表达也受到植物激素和蔗糖水平的影响。例如,生长素能够通过改变包括PAP1在内的调控基因的表达水平,进而影响pap1-D

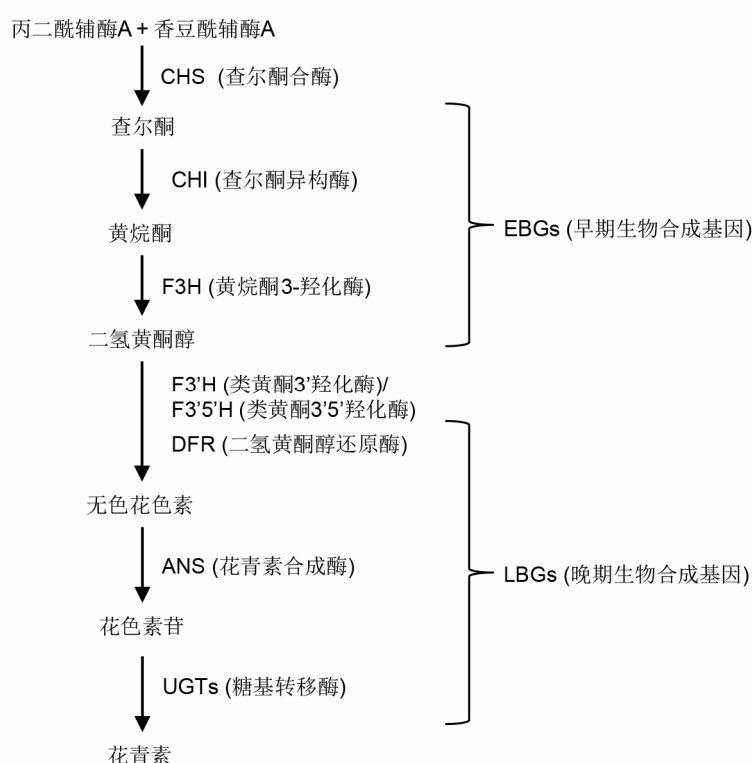


图1 花青素的生物合成途径

Figure 1 Simplified biosynthesis pathway of anthocyanins

突变体红色细胞中矢车菊素的含量(Liu et al., 2014)。在拟南芥苗期进行蔗糖诱导时发现*PAP1*基因的表达量增加20倍, 花青素大量积累(Broeckling et al., 2016)。

拟南芥中参与调控花青素合成的主要MYB转录因子还包括MYB113和MYB114, 这2个基因在拟南芥中过量表达所产生的表型与过量表达*PAP1*所产生的表型相似; 然而在bHLH的突变体中过量表达MYB113和MYB114时, 花青素含量降低, 说明MYB113和MYB114是bHLH依赖型的转录因子(Gonzalez et al., 2008)。由于*PAP1*不能在豆科模式植物蒺藜苜蓿(*Medicago truncatula*)中诱导花青素的积累, Peel等(2009)鉴定了与*PAP1*同源的*LAP1*(*Legume Anthocyanin Production 1*)基因, *LAP1*在紫花苜蓿(*M. sativa*)、蒺藜苜蓿和白三叶(*Trifolium repens*)中的过量表达均可大量积累矢车菊素类的花青素, 因此*LAP1*被认为是参与调控豆科植物花青素

生物合成的关键转录因子。通过*PAP1*和*LAP1*的对比研究, 发现不同植物中调控花青素合成的关键MYB转录因子具有一定的物种特异性。

在另一模式植物矮牵牛中, MYB型转录因子AN2(ANTHOCYANIN 2)和AN4都能够在转录水平影响*DFR*基因的表达。AN2在C端具有与玉米C1相似的结构域, 并且与C1功能相似, AN2调控矮牵牛花瓣中花青素的合成, 而AN4则调控花筒以及花药中花青素的合成(Quattrocchio et al., 1993, 1999)。除了AN2和AN4, 矮牵牛中调控花青素合成的MYB家族转录因子还包括DPL(DEEP PURPLE)和PHZ(PURPLE HAZE)。在矮牵牛中过量表达DPL和PHZ基因时, 植株整体呈现紫色, 飞燕草素、牵牛花素和锦葵素含量升高(Albert et al., 2011)。

在模式植物金鱼草(*Antirrhinum majus*)中, 调控花冠中花青素积累的*Rosea1*、*Rosea2*和*Venosa*基因均编码MYB转录因子, 但是它们具有种间特异性, 分

别调节不同的结构基因的表达水平: *Rosea1*能够提高*F3H*、*DFR*和*ANS*的表达水平, *Rosea2*仅调控*F3'H*的表达, *Vensoa*调控*CHI*、*F3H*、*F3'5'H*和*ANS*等多个结构基因的表达。这表明*Rosea1*、*Rosea2*和*Vensoa*等转录因子虽然存在一定的功能冗余, 但都各自调控相应的结构基因(Schwinn et al., 2006)。在烟草(*Nicotiana tabacum*)中特异表达*NtAN2*不仅能够激活*CHS*和*DFR*基因的表达, 还可以与bHLH型转录因子协同调控花青素的积累(Pattanaik et al., 2010)。上述模式植物中的研究表明, MYB家族转录因子在花青素合成途径中既可以独立调控*DFR*和(或)*ANS*的表达, 又可以作为MBW复合体的主要元件参与花青素的调控。

### 2.1.2 MYB转录因子调控果实中花青素

花青素赋予果实鲜艳的色彩, 其含量是许多果实如苹果(*Malus domestica*)、葡萄(*Vitis vinifera*)和草莓(*Fragaria ananassa*)的重要品质性状。目前, 对果实中花青素合成机理的研究也日益增多。其中, 葡萄作为一种富含花青素的果实, 其基因组测序已经完成, 对葡萄基因组转录因子的挖掘也鉴定了不少调控花青素的MYB型转录因子。巨峰葡萄(*V. labruscana*)的*MybA*基因能够促进与葡萄浆果花青素积累相关的糖基转移酶*UFGT*基因的表达, 在葡萄体细胞胚胎上诱导出现紫红色斑点(Kobayashi et al., 2002)。*VvMYB5a*和*VvMYB5b*则通过激活*ANS*的表达调控葡萄浆果成熟过程中花青素的积累(Deluc et al., 2008)。*VlmybA1-1*、*VlmybA1-2*、*VlmybA1-3*和*VlmybA2*以及其它转录因子均与葡萄浆果花青素的合成相关, 但其详细的分子机制目前尚不明确(Kobayashi et al., 2004; Geekiyanage et al., 2007; Azuma et al., 2008; Cutanda-Perez et al., 2009; Costantini et al., 2015; Sun et al., 2016)。

与葡萄相似, 苹果果皮颜色与花青素的积累密切相关, 多个MYB转录因子参与调控苹果果皮花青素的合成。在苹果红色果皮中表达量较高的*MdMYB1*基因在光照条件下表达量升高, 可诱导果皮积累花青素; *MdMYB1*还可以激活*DFR*和*UFGT*基因的表达(Takos et al., 2006)。与苹果红色果皮颜色相关的MYB转录因子还包括*MdMYBA*和*MdMYB3*, *MdMYBA*特异性地结合在*ANS*的启动子区调控花青素

合成, 其表达受到UV-B辐射和低温的诱导; 应用基因枪技术将*MdMYBA*基因转入不积累花青素的苹果子叶时, 子叶因花青素积累而形成紫红色的斑点(Ban et al., 2007)。过量表达*MdMYB3*基因能够转录激活*CHS*和*CHI*基因的表达(Vimolmangkang et al., 2013)。苹果的另一个转录因子*MdMYB10*与PAP1等MYB转录因子在保守结构域上高度相似, *MdMYB10*调控苹果果肉以及叶片中花青素的积累, 这一点不同于仅调控果皮花青素积累的转录因子(Espley et al., 2007)。过量表达*MdMYB10*的苹果愈伤组织中矢车菊素的含量升高, *DFR*基因的表达量也略有升高; 同时, *MdMYB10*还与*MdbHLH3*和*MdbHLH33*共表达促进花青素的生物合成(Espley et al., 2007)。在研究苹果红色果肉形成的过程中, Espley等(2009)发现, *MdMYB10*启动子区具有一段23 bp的串联重复序列, 这一特殊结构存在于各类红色果肉的苹果中, 而白色果肉苹果中则缺少这一序列; *MdMYB10*能够结合到这一重复区域, 从而促进花青素的积累, 说明*MdMYB10*启动子区的这段重复序列在苹果红色果肉形成过程中具有至关重要的作用, 这一发现是花青素机制研究领域的一个重要突破。*MdMYB10*的同源基因*MdMYB110*也能够调控苹果果肉中花青素的生物合成, 并且很可能通过参与形成MBW复合体激活*CHS*基因的表达(Chagné et al., 2013)。苹果*MdMYB9*和*MdMYB11*可以结合到*ANS*的启动子区, 而*MdbHLH3*能通过结合到*MdMYB9*和*MdMYB11*的启动子区与其共同调控苹果花青素的合成。同时, An等(2014)发现茉莉酸酯信号途径中的*MdJAZ* (Jasmonate ZIM-domain)蛋白能与*MdbHLH3*结合抑制其功能, 可见茉莉酸酯信号途径能够通过*MdbHLH3*转录因子影响花青素的合成调控。目前, 对苹果中调控花青素的转录因子的研究比较详尽, 研究结果对其它果实的相关研究具有一定的借鉴意义。

除了葡萄和苹果这2种研究相对较多的果实外, 山竹(*Garcinia mangostana*)、草莓、甜樱桃(*Prunus avium*)和梨(*Pyrus*)等常见水果中以及其它研究相对较少的果实中调控花青素的转录因子也陆续被报道(表1), 但对其分子机制的研究并不完善。在对这些MYB转录因子的研究中发现, 大多数MYB均与苹果*MdMYB10*同源, 功能也相似; 这些MYB型转录因子

表1 植物中调控花青素合成的MYB类转录因子

Table 1 MYB transcription factors (TFs) involved in the regulation of anthocyanin biosynthesis in plants

物种名称	拉丁名	基因名称	类型	参考文献
玉米	<i>Zea mays</i>	<i>C1</i>	激活	Cone et al., 1986
		<i>PL</i>	激活	Cone et al., 1993a
		<i>PL-BH</i>	激活	Cone et al., 1993b
拟南芥	<i>Arabidopsis thaliana</i>	<i>AtCPC</i>	抑制	Zhang et al., 2009
		<i>AtMYB60</i>	抑制	Park et al., 2008
		<i>AtMYBL2</i>	抑制	Matsui et al., 2008
		<i>AtMYB75/PAP1</i>	激活	Borevitz et al., 2000
		<i>AtMYB90/PAP2</i>	激活	Borevitz et al., 2000
		<i>AtMYB113</i>	激活	Gonzalez et al., 2008
		<i>AtMYB114</i>	激活	Gonzalez et al., 2008
		<i>AtMYB56</i>	激活	Jeong et al., 2018
		<i>LAP1</i>	激活	Peel et al., 2009
蒺藜苜蓿	<i>Medicago truncatula</i>			
矮牵牛	<i>Petunia hybrida</i>	<i>AN2</i>	激活	Quattrocchio et al., 1999
		<i>DPL</i>	激活	Albert et al., 2011
		<i>PHZ</i>	激活	Albert et al., 2011
		<i>MYBx</i>	抑制	Albert et al., 2014
		<i>MYB27</i>	抑制	Albert et al., 2014
		<i>ROSEA1</i>	激活	Schwinn et al., 2006
		<i>ROSEA2</i>	激活	Schwinn et al., 2006
金鱼草	<i>Antirrhinum majus</i>	<i>VENOSA</i>	激活	Schwinn et al., 2016
烟草	<i>Nicotiana tabacum</i>	<i>NtAN2</i>	激活	Pattanaik et al., 2010
苹果	<i>Malus domestica</i>	<i>MdMYB1</i>	激活	Takos et al., 2006
		<i>MdMYB3</i>	激活	Vimolmangkang et al., 2013
		<i>MdMYB6</i>	激活	Gao et al., 2011
		<i>MdMYB10</i>	激活	Espley et al., 2007
		<i>MdMYB110a</i>	激活	Chagné et al., 2013
		<i>MdMYB9</i>	激活	An et al., 2014
		<i>MdMYB11</i>	激活	An et al., 2014
		<i>MdMYBA</i>	激活	Ban et al., 2007
		<i>MdMYB16</i>	抑制	Wang et al., 2010
葡萄	<i>Vitis labruscana</i>	<i>VIMYBA1-1</i>	激活	Kobayashi et al., 2002
		<i>VIMYBA1-2</i>	激活	Cutanda-Perez et al., 2009
		<i>VIMYBA1-3</i>	激活	Azuma et al., 2008
		<i>VIMYBA2</i>	激活	Geekiyana et al., 2007
	<i>V. vinifera</i>	<i>VvMYB5a</i>	激活	Deluc et al., 2006
		<i>VvMYB5b</i>	激活	Deluc et al., 2008
		<i>VvMYBA1</i>	激活	Kobayashi et al., 2004
		<i>VvMYBA2</i>	激活	Kobayashi et al., 2004
		<i>VvMYBPA1</i>	激活	Passeri et al., 2017
		<i>VvMYBC2-L1</i>	抑制	Cavallini et al., 2015
		<i>VvMYBC2-L3</i>	抑制	Cavallini et al., 2015
西洋梨	<i>Pyrus communis</i>	<i>PcMYB10</i>	激活	Pierantoni et al., 2010
沙梨	<i>P. pyrifolia</i>	<i>PyMYB10</i>	激活	Feng et al., 2010
		<i>PyMYB114</i>	激活	Yao et al., 2017a

表1 (续) Table 1 (continued)

物种名称	拉丁名	基因名称	类型	参考文献
	<i>P. communis</i>	<i>PcMYB10</i>	激活	Wang et al., 2013
山竹	<i>Garcinia mangostana</i>	<i>GmMYB10</i>	激活	Palapol et al., 2009
甜樱桃	<i>Prunus avium</i>	<i>PacMYBA</i>	激活	Shen et al., 2014
		<i>PaMYB10</i>	激活	Starkevič et al., 2015
荔枝	<i>Litchi chinensis</i>	<i>LcMYB1</i>	激活	Lai et al., 2014
油桃	<i>Prunus persica</i>	<i>PpMYB10</i>	激活	Ravaglia et al., 2013
甜橙	<i>Citrus sinensis</i>	<i>CsRUBY</i>	激活	Butelli et al., 2012
杨梅	<i>Myrica rubra</i>	<i>MrMYB1</i>	激活	Niu et al., 2010
草莓	<i>Fragaria ananassa</i>	<i>FaMYB10</i>	激活	Medina-Puche et al., 2014
		<i>FaMYB1</i>	抑制	Aharoni et al., 2001
猕猴桃	<i>Actinidia chinensis</i>	<i>MYB110a</i>	激活	Fraser et al., 2013
亚洲杂交百合	<i>Lilium</i> spp.	<i>LhMYB6</i>	激活	Yamagishi et al., 2010
		<i>LhMYB12</i>	激活	Yamagishi et al., 2010
		<i>LhMYB12-Lat</i>	激活	Yamagishi et al., 2014
非洲菊	<i>Gerbera hybrida</i>	<i>GMYB10</i>	激活	Elomaa et al., 2003
菊花	<i>Chrysanthemum morifolium</i>	<i>CmMYB6</i>	激活	Liu et al., 2015
文心兰	<i>Oncidium gower</i>	<i>OgMYB1</i>	激活	Chiou et al., 2008
龙胆	<i>Gentian triflora</i>	<i>GtMYB3</i>	激活	Nakatsuka et al., 2008
紫苏	<i>Perilla frutescens</i>	<i>Myb-p1</i>	激活	Gong et al., 1999
番茄	<i>Lycopersicon esculentum</i>	<i>LeANT1</i>	激活	Mathews et al., 2003
红薯	<i>Ipomoea batatas</i>	<i>IbMYB1</i>	激活	Mano et al., 2007
马铃薯	<i>Solanum tuberosum</i>	<i>StAN1</i>	激活	Jung et al., 2009
		<i>StAN2</i>	激活	Jung et al., 2009
		<i>StMTF1</i>	激活	Rommens et al., 2008
结球甘蓝	<i>Brassica oleracea</i> var. <i>capitata</i>	<i>BoMYB2</i>	激活	Yuan et al., 2009
花椰菜	<i>B. oleracea</i> var. <i>botrytis</i>	<i>PURPLE</i>	激活	Chiu and Yeh, 2010
紫背天葵	<i>Gynura bicolor</i>	<i>GbMYB1</i>	激活	Shimizu et al., 2011
羽衣甘蓝	<i>B. oleracea</i> var. <i>acephala</i>	<i>BoPAP1</i>	激活	Zhang et al., 2012
淫羊藿	<i>Epimedium sagittatum</i>	<i>EsMYBA1</i>	激活	Huang et al., 2013a
		<i>EsMYB9</i>	激活	Huang et al., 2017
胡萝卜	<i>Raphanus sativus</i>	<i>RsMYB1</i>	激活	Lim et al., 2016
洋葱	<i>Allium cepa</i>	<i>MYB1</i>	激活	Schwinn et al., 2016
小麦	<i>Triticum aestivum</i>	<i>TaPL1</i>	激活	Shin et al., 2016
		<i>TaMYB3</i>	激活	Li et al., 2017
红掌	<i>Anthurium andraeanum</i>	<i>AaMYB2</i>	激活	Li et al., 2016a
大豆	<i>Glycine max</i>	<i>GmMYB-G20-1</i>	激活	Takahashi et al., 2011
梅	<i>Prunus mume</i>	<i>PmMYBa1</i>	激活	Zhang et al., 2017
葡萄风信子	<i>Muscari armeniacum</i>	<i>MaAN2</i>	激活	Chen et al., 2017
智利草莓	<i>Fragaria chiloensis</i>	<i>FcMYB1</i>	抑制	Salvatierra et al., 2013
芜菁	<i>B. rapa</i>	<i>BrMYB4</i>	抑制	Zhang et al., 2014a
银杏	<i>Ginkgo biloba</i>	<i>GbMYBF2</i>	抑制	Xu et al., 2014a
		<i>GbMYBFL</i>	激活	Zhang et al., 2018
杨树	<i>Populus trichocarpa</i>	<i>PtrRML1</i>	抑制	Hu et al., 2016

更多地与bHLH型转录因子互作调控*DFR*的表达, 但也可独立调控*ANS*和*UFGT*的表达。以上各种研究表明, *MYB10*及其同源基因在常见水果果实中普遍存在, *MYB10*在果实中调控花青素合成的机制可能是保守的。

### 2.1.3 观赏植物中的MYB转录因子

植物缤纷的颜色大多源于花青素的积累, 花青素的种类和含量决定了花瓣颜色, 而花瓣颜色是衡量鲜花品质的一个重要性状, 也是决定鲜花经济价值的重要因素。花色形成的分子机制一直是花青素研究领域的重点, 然而观赏植物中被报道的调控花青素合成的转录因子相对较少。非洲菊(*Gerbera hybrida*)中*GMYP10*的表达与其叶片和花中的花青素积累相关; *GMYP10*能与bHLH型转录因子*GMYP1*互作, 通过激活*DFR*调控花瓣中花青素的合成(Elomaa et al., 2003)。*GMYP10*在不同组织中诱导的花青素种类具有特异性, *GMYP10*促进愈伤组织和营养组织中矢车菊素的积累, 而在雄蕊中则促进天竺葵素的积累(Laitinen et al., 2008)。文心兰(*Oncidium goweri*)中的*OgMYB1*转录因子通过激活*CHI*和*DFR*促进红色花瓣中矢车菊素、飞燕草素、锦葵素和芍药素(Chiou and Yeh, 2008)的积累。龙胆(*Gentian triflora*)中的*GtMYB3*转录因子与*GtbHLH1*协同作用影响*F3'5'H*基因的表达, 进而促进花瓣中龙胆翠雀花素(gentioidephrin)的富集(Nakatsuka et al., 2008)。亚洲杂交百合(*Lilium spp.*)中的*LhMYB6*和*LhMYB12*与矮牵牛*AN2*同源, 能与*LhbHLH2*转录因子互作调控百合花中矢车菊素的生物合成, *LhMYB6*不仅影响百合花瓣上的花斑形成, 还受光诱导影响百合叶片花青素的积累; 而*LhMYB12*则调控百合花瓣和花药中花青素的合成(Yamagishi et al., 2010)。Yamagishi等(2014)还发现*LhMYB12*的等位基因*LhMYB12-Lat*也与百合花瓣上花青素局部积累形成的花斑相关。菊花(*Chrysanthemum morifolium*)中的*CmMYB6*主要调控紫色菊花发育时期花青素的合成, *CmMYB6*能够与bHLH型转录因子互作激活*DFR*的表达, 从而促进花青素的合成(Liu et al., 2015)。综上所述, 观赏植物中调控花青素生物合成的MYB型转录因子的调控具有组织特异性, 不同转录因子分别调控不同部位花青素的含量与种类。

### 2.1.4 其它植物中的MYB转录因子

除了水果和观赏植物之外, 蔬菜以及其它富含花青素的植物也是研究花青素调控机制的理想材料。目前, 已鉴定到影响花青素积累MYB转录因子的物种材料还包括各种蔬菜作物, 如马铃薯(*Solanum tuberosum*)、结球甘蓝(*Brassica oleracea*)、羽衣甘蓝(*B. oleracea*); 药用植物如淫羊藿(*Epimedium sagittatum*)和紫苏(*Perilla frutescens*); 作物如小麦(*Triticum aestivum*) (表1)。在这些转录因子基因中, 被证明通过影响*DFR*基因表达来调控花青素生物合成的MYB转录因子基因包括紫苏的*Myb-p1*和红掌(*Anthurium andraeanum*)的*AaMYB2*; 被证明通过与bHLH家族转录因子互作来促进花青素积累的包括结球甘蓝的*BoMYB2*、花椰菜的*Pr*、紫背天葵(*Gynura bicolor*)的*GbMYB1*和淫羊藿的*EsMYBA1*; 其它植物中的MYB转录因子基因仅被发现参与调控花青素的积累, 但都缺乏具体的分子机制研究。

## 2.2 bHLH转录因子家族

碱性螺旋-环-螺旋(basic helix-loop-helix, bHLH)蛋白在动植物中高度保守, 具有保守的氨基酸序列(Morgenstern and Atchley, 1999)。bHLH转录因子被发现参与植物生长发育(Song et al., 2013)、信号转导(Zhang et al., 2009)和次生代谢(Zhang et al., 2003)等诸多生物学过程(Toledo-Ortiz et al., 2003)。

### 2.2.1 模式植物中的bHLH转录因子

bHLH转录因子是调控花青素合成的另一大类转录因子家族(表2)。玉米中调控花青素合成的主要bHLH转录因子包括*R1*、*B1*、*Lc* (Leaf color)和*Sn*, 是最早发现的一批调控花青素合成的bHLH家族转录因子(Chandler et al., 1989; Ludwig et al., 1989; Consonni et al., 1992)。其中, *Lc*能够通过激活*CHS*和*DFR*的表达, 互补矮牵牛*an2/an11*双突变体中花青素缺失的表型(Quattrocchio et al., 1993)。金鱼草中的*DELILA*基因能够调控*DFR*的表达, 促进花青素的积累(Almeida et al., 1989)。

拟南芥中调控花青素合成的bHLH家族转录因子*TT8* (TRANSPARENT TESTA 8)、*GL3* (GLABRA3)和*EGL3* (ENHANCER OF GLABRA 3)均与玉米的*R*转录因子同源。*TT8*作为调控花青素的重要bHLH型

表2 植物中调控花青素合成的主要bHLH转录因子

Table 2 bHLH transcription factors (TFs) involved in the regulation of anthocyanin biosynthesis in plants

物种名称	拉丁名	基因名称	类型	参考文献
玉米	<i>Zea mays</i>	<i>R1</i>	激活	Chandler et al., 1989
		<i>Lc</i>	激活	Ludwig et al., 1989
		<i>B1</i>	激活	Chandler et al., 1989
		<i>Sn</i>	激活	Tonelli et al., 1991
金鱼草	<i>Antirrhinum majus</i>	<i>DELILA</i>	激活	Martin et al., 1991
矮牵牛	<i>Petunia hybrida</i>	<i>JAF13</i>	激活	Spelt et al., 2000
		<i>AN1</i>	激活	Spelt et al., 2000
拟南芥	<i>Arabidopsis thaliana</i>	<i>GL3</i>	激活	Payne et al., 2000
		<i>EGL3</i>	激活	Zhang et al., 2003
		<i>MYC146</i>	激活	Ramsay et al., 2003
		<i>TT8</i>	激活	Nesi et al., 2000
蒺藜苜蓿	<i>Medicago truncatula</i>	<i>MtTT8</i>	激活	Li et al., 2016b
苹果	<i>Malus domestica</i>	<i>MdbHLH3</i>	激活	Xie et al., 2012
		<i>MdbHLH33</i>	激活	Espley et al., 2007
		<i>MdMYC2</i>	激活	An et al., 2016
烟草	<i>Nicotiana tabacum</i>	<i>NtAn1a</i>	激活	Bai et al., 2011
		<i>NtAn1b</i>	激活	Bai et al., 2011
菊花	<i>Chrysanthemum morifolium</i>	<i>CmbHLH</i>	激活	Xiang et al., 2015
非洲菊	<i>Gerbera hybrida</i>	<i>GMYC1</i>	激活	Elomaa et al., 2003
荔枝	<i>Litchi chinensis</i>	<i>LcbHLH1</i>	激活	Lai et al., 2016
		<i>LcbHLH3</i>	激活	Lai et al., 2016
龙胆	<i>Gentian triflora</i>	<i>GtbHLH1</i>	激活	Nakatsuka et al., 2008
杨梅	<i>Myrica rubra</i>	<i>MrbHLH1</i>	激活	Liu et al., 2013
花椰菜	<i>Brassica oleracea</i> var. <i>botrytis</i>	<i>BobHLH1</i>	激活	Chiu and Li, 2012
结球甘蓝	<i>B. oleracea</i> var. <i>capitata</i>	<i>BoTT8</i>	激活	Yuan et al., 2009
紫背天葵	<i>Gynura bicolor</i>	<i>GbMYC1</i>	激活	Shimizu et al., 2011
杂交百合	<i>Lilium</i> spp.	<i>LhbHLH1</i>	激活	Nakatsuka et al., 2009
番茄	<i>Lycopersicon esculentum</i>	<i>AH</i>	激活	Qiu et al., 2016
大丽花	<i>Dahlia variabilis</i>	<i>DvIVS</i>	抑制	Ohno et al., 2011
圆叶牵牛	<i>Ipomoea purpurea</i>	<i>bHLH2</i>	激活	Park et al., 2007
甜橙	<i>Citrus sinensis</i>	<i>CsMYC2</i>	激活	Cultrone et al., 2010
紫苏	<i>Gynura bicolor</i>	<i>Myc-rp</i>	激活	Gong et al., 1999
葡萄	<i>Vitis vinifera</i>	<i>VvbHLH1</i>	激活	Xu et al., 2014b
		<i>VvMYC1</i>	激活	Hichri et al., 2010
		<i>VvMYCA1</i>	激活	Hichri et al., 2010
		<i>Myc-F3G1</i>	激活	Yamazaki et al., 2003
甜樱桃	<i>Prunus avium</i>	<i>PabHLH3</i>	激活	Starkevič et al., 2015
梨	<i>Pyrus pyrifolia</i>	<i>PybHLH3</i>	激活	Yao et al., 2017a
		<i>LhbHLH2</i>	激活	Nakatsuka et al., 2009
马铃薯	<i>Solanum tuberosum</i>	<i>StbHLH1</i>	激活	Payyavula et al., 2013
		<i>StbHLH2</i>	激活	Payyavula et al., 2013
		<i>AH</i>	激活	Qiu et al., 2016



转录因子, 主要调控幼苗和荚果中*DFR*的表达, 参与形成MBW复合体(Baudry et al., 2004)。Zhang等(2003)发现*gl3/egl3*双突变体缺少花青素, 而且*DFR*的表达量远低于野生型。分别过量表达*GL3*和*EGL3*都能够回复*ttg1*突变体中花青素缺失的表型, 说明*GL3*和*EGL3*参与花青素的生物合成(Payne et al., 2000; Zhang et al., 2003)。EGL3还被证明能够与PAP1共表达促进花青素的积累, 可见EGL3在调控花青素合成的功能方面强于GL3 (Zhang et al., 2003)。Gonzalez等(2008)发现, 过量表达*GL3*还能够上调*F3'H*、*DFR*、*ANS*、*TT8*和*PAP2*的表达水平。*GL3*还参与氮胁迫环境条件下花青素的积累: 在缺氮条件下, *GL3*功能失活的植株中不合成花青素, 而且*DFR*的表达量远低于野生型(Feyissa et al., 2009)。模式植物矮牵牛中调控花青素合成的bHLH类转录因子主要包括JAF13和AN1, JAF13能够与AN2互作激活*DFR*的表达, 促进花青素合成(Quattrocchio et al., 1998)。AN1不仅能够与AN2结合激活*DFR*的表达, 还可以直接激活*DFR*的表达(Spelt et al., 2000)。AN1与AN2和AN4之间的协同互作具有组织特异性, AN2在矮牵牛叶片中的表达可以诱导AN1表达, 而在花药中AN1可以诱导AN4表达(Spelt et al., 2000)。

近年来, 其它模式植物中有关bHLH转录因子调控花青素合成的研究逐渐增多。MtTT8是蕤藜苜蓿中与拟南芥TT8同源的bHLH型转录因子。*mttt8*突变体表现为花青素缺失表型, 在突变体中过量表达*MtTT8*其毛状根中花青素的含量增加; 同时, 在拟南芥*tt8*突变体中表达*MtTT8*也能产生花青素, 恢复突变体花青素缺失的表型。*MtTT8*能与*MtLAP1*和*MtWD40-1*转录因子互作形成MBW复合体, 进而共同调控花青素的合成(Li et al., 2016b)。另一个模式植物烟草中的NtAn1a和NtAn1b转录因子能够在烟草花中促进花青素的积累, NtAn1a与烟草NtAn2互作激活*DFR*和*CHS*的表达, 从而调控花青素的合成(Bai et al., 2011)。模式植物中的这些研究表明, 绝大部分bHLH型转录因子需要与MYB互作, 独立行使功能的较少。虽然花青素生物合成途径包含*CHS*、*CHI*、*F3H*、*DFR*和*ANS*等多个基因, 但MYB和bHLH转录因子主要调控*DFR*和*ANS*基因的表达(尤其是*DFR*), 表明*DFR*在花青素合成途径中具有关键作用。

## 2.2.2 非模式植物中的bHLH转录因子

非模式植物中bHLH型转录因子调控花青素的机制与模式植物相同, 主要包括调控*DFR*基因的表达以及与MYB转录因子互作两个方面。苹果MdbHLH3和MdbHLH33共同与MdMYB10转录因子互作, 促进果实颜色变红(Espley et al., 2007)。受低温诱导的MdbHLH3转录因子还能够结合到*DFR*的启动子区, 与MdMYB1共同促进花青素的积累(Xie et al., 2012)。MdMYB1还可以通过参与茉莉酸途径正调控花青素的合成(An et al., 2016)。荔枝(*Litchi chinensis*) LcbHLH1和LcbHLH3转录因子同样能与LcMYB1共同作用, 促进花青素积累(Lai et al., 2016)。非洲菊GMYC1特异地在花冠和心皮部位调控*DFR*活性, 与AN2和GMYB10互作促进*DFR*的表达(Elomaa et al., 2003)。菊花CmbHLH转录因子能够激活*DFR*的表达, 在与CmMYB6转录因子共表达时促进花青素的合成(Xiang et al., 2015)。能够与MYB共同作用调控花青素积累的bHLH类转录因子还包括龙胆的*GtbHLH1* (Nakatsuka et al., 2008)、杨梅(*Myrica rubra*)的*MrbHLH1* (Liu et al., 2013)、花椰菜(*Brassica oleracea* var. *botrytis*)的*BobHLH1* (Chiu et al., 2010)、结球甘蓝(*B. oleracea* var. *capitata*)的*BoTT8* (Yuan et al., 2009)和紫背天葵中的*GbMYC1* (Shimizu et al., 2011)。

bHLH类转录因子基因也陆续在其它植物中被鉴定(表2), 但目前还未在其它植物中发现与其互作的MYB转录因子。目前, 对这些植物中bHLH的功能研究还不够深入, 它们在不同植物花青素调控网络中的上下游关系还不十分明确。

## 2.3 WD40重复蛋白家族

WD40重复蛋白(WD40 repeat proteins, WDR)主要存在于真核生物中, 具有保守而特异的二肽重复基序, 每个重复大概有40多个氨基酸残基; 重复的WD40基序在蛋白质互作时作为支架起固定作用(Mishra et al., 2012)。WDR参与植物非生物胁迫、生长发育和类黄酮的合成(Walker et al., 1999; Huang et al., 2008; Miller et al., 2016)。

矮牵牛的AN11 (ANTHOCYANIN11)是第1个被发现调控花青素生物合成的WDR蛋白, 其相应的编码基因是通过转座子标签法被鉴定的。AN11作用于

AN2上游,可能通过参与1个信号转导串联体系激活AN2的表达,从而调控矮牵牛花青素的合成(de Vetten et al., 1997)。拟南芥的TTG1 (TRANS-PARENT TESTA GLABRA 1)与矮牵牛AN11转录因子同源,其能够调控一系列生长发育过程,包括表皮毛的生长、种子黏液质的形成和种子内含物的积累等;TTG1通过影响*DFR*的表达调控花青素的合成(Walker et al., 1999);酵母双杂交实验证明,TTG1可以与bHLH型转录因子GL3互作(Payne et al., 2000)。Gonzalez等(2008)进一步证实bHLH依赖型(bHLH-dependent) MYB转录因子和TTG1依赖型(TTG1-dependent) bHLH转录因子能够调控*DFR*及*ANS*的表达,即PAP1和GL3/EGL3互作, GL3和TTG1互作调控花青素的生物合成;从而提出了MYB/bHLH/TTG1转录复合体调控花青素合成的机制。Shi和Xie (2011)在研究*pap1-D*突变体时发现,在富含花青素的红色细胞中, *PAP1*、*TT8*、*GL3*和*TTG1*基因的表达量高于野生型,同时*DFR*和*ANS*的表达量也升高;他们认为在拟南芥的红色细胞中存在1个TTG1-GL3/TT8-PAP1复合体调控花青素的合成。随后,Zhou等(2012)发现在*pap1-D*突变体的红色细胞中, *PAP1*-GL3/TT8-TTG1复合体的表达受氮的影响,氮水平可以调控花青素的合成。MYB-bHLH-WD40三元复合体的调控机制在除花青素之外的类黄酮分支途径中也广泛存在,因此Albert等(2014)将调控花青素生物合成的MYB-bHLH-WDR三元复合体命名为MBW复合体。

拟南芥中WDR类TTG1参与MBW复合体调控类黄酮化合物合成的机制研究得较为详细,其它物种中WDR蛋白调控的功能研究相对较少。目前已有报道的调控花青素合成的WDR均与AN11和/或TTG1同源,例如,玉米中的PAC1 (PALE ALEURONE COLOR 1)和MP1基因(Carey et al., 2004),紫苏中的PFWD也被证明通过与MYC互作进而调控花青素的积累(Sompornpailin et al., 2002)。作者所在课题组通过正向遗传筛选的方法在蒺藜苜蓿中鉴定了1个WDR类的*MtWD40-1*基因。*MtWD40-1*不仅可以互补拟南芥*ttg1*突变体,而且可以回复蒺藜苜蓿*Tnt1*突变体的表型,过量表达*MtWD40-1*导致蒺藜苜蓿毛状根中花青素含量回复,证明*MtWD40-1*参与调控苜蓿花青素的生物合成;但与TTG1不同的是, *MtWD40-1*虽

然也参与种子原花青素和黏液质的积累,但它并不影响表皮毛和根毛的形成,这是*MtWD40-1*与TTG1功能显著不同之处(Pang et al., 2009)。其它植物中WDR调控花青素生物合成的研究相对较少,仅限于对该类转录因子的挖掘(表3),它们与TTG1或其它功能明确的WDR类蛋白在结构与功能方面的异同还有待进一步研究。

## 2.4 其它类型转录因子

### 2.4.1 bZIP类转录因子

目前,对花青素途径转录因子的研究主要集中在MYB、bHLH和WDR三大类转录因子家族,对其它类型的转录因子家族研究相对较少。近年来, bZIP (basic leucine zipper)家族的转录因子也被证明参与调控花青素的生物合成。bZIP是植物中一大类转录因子家族,由1个亮氨酸拉链二聚体和1个DNA结合结构域构成,参与调控诸多生物学过程(Banerjee and Roychoudhury, 2017),如植物生长发育(Gibbalová et al., 2017)、环境胁迫应答(Wang et al., 2017)和光信号转导(Ang and Deng, 1994)。拟南芥中影响植物光形态建成的bZIP转录因子HY5是第1个被报道参与花青素合成调控途径的bZIP型转录因子。Lee等(2007)在研究HY5转录因子结合位点时,发现HY5能够调控*F3H*和*CHS*基因的表达;随后的研究还证明, HY5和PIF3 (Phytochrome Interacting Factor 3)共同作用,直接结合在*C4H*、*F3'H*和*DFR*启动子的特异区域(Shin et al., 2007)。此外, HY5还可以结合到*PAP1*的启动子上正调控花青素的合成(Shin et al., 2013)。HY5还可以结合到*MYBL2*的启动子上,通过组氨酸修饰抑制*MYBL2*的表达,从而促进花青素合成(Nguyen et al., 2015)。*miR858*是HY5潜在的靶基因,它可以与HY5共同作用参与调控花青素的合成(Wang et al., 2016)。An等(2017)通过同源克隆的方法获得了苹果*MdHY5*基因, *MdHY5*基因能够结合到*MdMYB10*的启动子区,诱导苹果花青素的积累;且*MdHY5*不仅与拟南芥HY5具有相同的功能,都受光和脱落酸诱导,而且还参与氮信号途径。bZIP型HY5转录因子与MYB、bHLH和WDR三类转录因子的区别在于HY5主要通过结合到保守启动子区行使功能,通过调控参与花青素生物合成的转录因子促进花青素的积累,而其它3类转录因子主要通过调控*DFR*和

**表3** 植物中调控花青素合成的WD40转录因子**Table 3** WD40 transcription factors (TFs) involved in the regulation of anthocyanin biosynthesis in plants

物种名称	拉丁名	基因名称	类型	参考文献
矮牵牛	<i>Petunia hybrida</i>	AN11	激活	De Vetten et al., 1997
拟南芥	<i>Arabidopsis thaliana</i>	TTG1	激活	Walker et al., 1999
玉米	<i>Zea mays</i>	PAC1	激活	Carey et al., 2004
		MP1	激活	Carey et al., 2004
蒺藜苜蓿	<i>Medicago truncatula</i>	WD40-1	激活	Pang et al., 2009
苹果	<i>Malus domestica</i>	MdTTG1	激活	Brueggemann et al., 2010
石榴	<i>Punica granatum</i>	PgWD40	激活	Ben-Simhon et al., 2011
红薯	<i>Ipomoea batatas</i>	IbWD40	激活	Dong et al., 2014
葡萄	<i>Vitis vinifera</i>	VvWDR1/2	激活	Matus et al., 2010
马铃薯	<i>Solanum tuberosum</i>	StWD40	激活	Payyavula et al., 2013
		StAN11	激活	Li et al., 2014
杨梅	<i>Myrica rubra</i>	MrWD40-1	激活	Liu et al., 2013
紫苏	<i>Gynura bicolor</i>	PFWD	激活	Sompornpailin et al., 2002
甜樱桃	<i>Prunus avium</i>	PaWD40	激活	Starkevič et al., 2015
圆叶牵牛	<i>Ipomoea purpurea</i>	WDR1	激活	Morita et al., 2006
花椰菜	<i>Brassica oleracea</i> var. <i>botrytis</i>	BoWD40	激活	Chiu and Li, 2012
苦荞麦	<i>Fagopyrum tataricum</i>	FtWD40	激活	Yao et al., 2017b

ANS基因的表达参与花青素的合成。bZIP型转录因子是否参与调控光、氮素和脱落酸等非生物因子介导的花青素代谢调控网络还有待进一步研究。

#### 2.4.2 负转录因子

花青素生物合成正调控因子的分子调控机制一直是花青素领域研究的热点。随着研究的深入,更多的负调控因子也被逐渐鉴定,它们的作用机制也正在被阐明。模式植物拟南芥中调控花青素合成的负转录因子主要包括MYBL2、MYB2、SPL9 (SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9)和CPC (CAPRICE)。mybl2和myb2突变体中的花青素含量增加,F3H、DFR和ANS的表达量升高(Dubos et al., 2008; Jun et al., 2015)。MYBL2转录因子具有独特的TLLLFR抑制结构域,通过与TT8结合抑制DFR和TT8的表达(Matsui et al., 2008)。在拟南芥中过量表达CPC时,花青素含量降低(Zhang et al., 2009; Zhu et al., 2009)。研究表明,CPC在氮胁迫下的花青素合成过程中具有反馈抑制作用(Nemie-Feyissa et al., 2014)。而SPL9转录因子通过破坏MBW复合体的稳定性抑制DFR的表达,从而抑制花青素积累(Gou et al., 2011)。矮牵牛中MYB27通过与AN1互作抑制AN1

的表达,同时MYB27的表达在高光下受到抑制;相反,MYBx作为MYB27的竞争性抑制因子,却在高光条件下表达(Spelt et al., 2000; Albert et al., 2011)。Albert等(2014)的研究表明,MYB27还与JAF13和AN11互作,通过结合bHLH型转录因子破坏MBW复合体的形成,在花青素代谢网络中行使负调控功能。

除了模式植物,非模式植物中调控花青素途径的负转录因子也逐渐被报道,特别是富含花青素的经济作物。葡萄中的VvMYBC2-L1和VvMYBC2-L3在矮牵牛中表达时能够抑制花瓣中花青素的积累,两者均可以与AN1结合,影响MBW复合体的稳定性;MYBA1在VvMYBC2-L1抑制花青素合成过程中起到平衡作用(Cavallini et al., 2015)。在葡萄皮中特异表达VvMYB4-like能够下调DFR和ANS的表达;在烟草中异源表达VvMYB4-like时,花青素的积累受到抑制(Pérez-Díaz et al., 2016)。苹果MdMYB6作为一个负调控转录因子,过表达时能够抑制包括DFR和CHS在内的多个结构基因的表达,从而减少花青素的合成(Gao et al., 2011)。智利草莓(*Fragaria chiloensis*)中的FcMYB1能够抑制苹果表面花青素的积累,当其表达量降低时,ANS的表达量升高(Salvatierra et al., 2013)。芜菁(*Brassica rapa*) BrMYB4基因的表达受

UV-B诱导,它可以结合到*C4H*的启动子区使其抑制花青素的合成(Zhang et al., 2014a)。杨树(*Populus trichocarpa*) *PtrRML1* (R3 MYB-LIKE1)在拟南芥中过量表达时能够抑制*GL3*的表达,从而抑制花青素的积累(Hu et al., 2016)。

综上所述,目前花青素生物合成的负调控因子主要是MYB转录因子,这类转录因子的分子调控机制可分为3类:一是通过抑制*DFR*等结构基因的表达行使功能;二是与其它正调控转录因子结合抑制正调控因子的表达,从而减少花青素积累;三是通过结合参与MBW复合体形成的转录因子,破坏MBW复合体的稳定性,进而行使抑制功能。

### 3 花青素代谢工程

#### 3.1 通过关键结构基因进行代谢工程改良

花色改良一直是花青素代谢工程的研究热点之一,由于传统以杂交和诱变为主的观赏植物育种技术很难培育出富含飞燕草素的蓝色花朵,而通过代谢工程的方法修饰花青素结构能够使花朵呈现纯正的蓝色,更加贴合消费者的需求,因此代谢工程为花色改良育种提供了新思路。其中,通过改变关键结构基因的表达水平,进而改变单个花青素产物是花色改良的一个重要策略。矮牵牛是一种常见的观赏植物,也是研究花青素代谢调控的经典模式植物。Meyer等(1987)将玉米来源的*DFR*基因导入矮牵牛*RL01*突变体,使含有矢车菊素和飞燕草素的浅粉色突变体花朵变为含有天竺葵素的砖红色花朵。Fukusaki等(2004)通过RNA干扰技术(RNA interference)抑制蝴蝶草(*Torenia hybrida*) *CHS*基因的表达,使富含锦葵色素和芍药色素的蓝色蝴蝶草变为花青素缺失的白色蝴蝶草。Boase等(2010)将飞燕草素合成途径的关键酶基因*F3'5'H*反向转入仙客来(*Cyclamen persicum*)中,抑制了*F3'5'H*基因的表达,使花青素总量降低80%,飞燕草素含量降低,矢车菊素比例相对升高,使花朵颜色由深粉色变浅。在康乃馨(*Dianthus caryophyllus*)、玫瑰(*Rosa rugosa*)和菊花中过量表达*F3'5'H*基因可以使花朵合成飞燕草素,从而呈现蓝紫色(Katsumoto et al., 2007; Brugliera et al., 2013)。Noda等(2017)将风铃草(*Campanula medium*)来源的*Cam-F3'5'H*基因和蝶豆(*Clitoria ternatea*)来源的*CtA3'*

*5'GT*基因转入菊花,使其因飞燕草素积累而显现出纯正的蓝色,从而改良了菊花颜色。

另外,利用微生物生产花青素作为化妆品和食品添加剂也是代谢工程的一个发展方向。Yan等(2005)将苹果来源的*F3H*和*ANS*、红掌来源的*DFR*以及矮牵牛来源的*3-GT*基因转入大肠杆菌,第1次在大肠杆菌中产生了含量较低的天竺葵素和矢车菊素,在微生物中重建了花青素的合成途径。该实验证明,在充分阐明花青素代谢途径的基础上,在微生物中目标导向性地重建花青素的合成途径切实可行。

#### 3.2 通过调控关键基因进行代谢工程改良

异源过量表达转录因子基因是花青素代谢工程的另一种重要策略。来源于紫苏的*Myc-rp* (Gong et al., 1999)、葡萄的*VIMYB2* (Geekiyana et al., 2007)、苹果的*MdMYB1* (Talos et al., 2006)、*MdMYB110a* (Chagné et al., 2013)、*MdMYB3* (Vimolmangkang et al., 2013)、*MdbHLH3* (Xie et al., 2017)和拟南芥的*PAP1* (Gatica-Arias et al., 2012; Qiu et al., 2014)在烟草中异源表达时均能促进花青素的大量积累。山竹*GmMYB10*在烟草中与*AtbHLH*共表达时可以激活*DFR*的表达,促进花青素的合成(Palapol et al., 2009)。荔枝*LcMYB1*也能够与*LcbHLH1*和*LcbHLH3*在烟草中共同作用,从而促进烟草叶片中花青素的积累(Lai et al., 2016)。在模式植物烟草和拟南芥中,探索外源花青素合成调控基因功能的一系列实验不断取得成功,为代谢工程在作物中的蓬勃发展提供了具有借鉴意义的理论和实践基础。

Butelli等(2008)将金鱼草的*DELILA*和*ROSEA1*基因转入番茄(*Solanum lycopersicum* cv. 'Micro-Tom'),使转基因番茄的果皮和果肉因大量富集酰基化花青素而呈现出深紫色,转基因番茄的花青素含量达2.83 mg·g<sup>-1</sup>,远高于富含花青素的蓝莓等水果;将这些高花青素含量的转基因番茄粉添加到患癌症小鼠的饮食中,发现小鼠的寿命延长,该结果证明了花青素具有抗癌的特性,引起了广泛关注。在本课题组的一项研究中,将*PAP1*和*Lc*共表达转入稀有的野生药用植物天山雪莲(*Saussurea involucreta*),转基因株系的愈伤组织和嫩枝中均出现至少4类矢车菊素的衍生物;同时,花青素合成途径中多数结构基因的表达受到诱导,其中*CHS*基因表达量变化最为显著

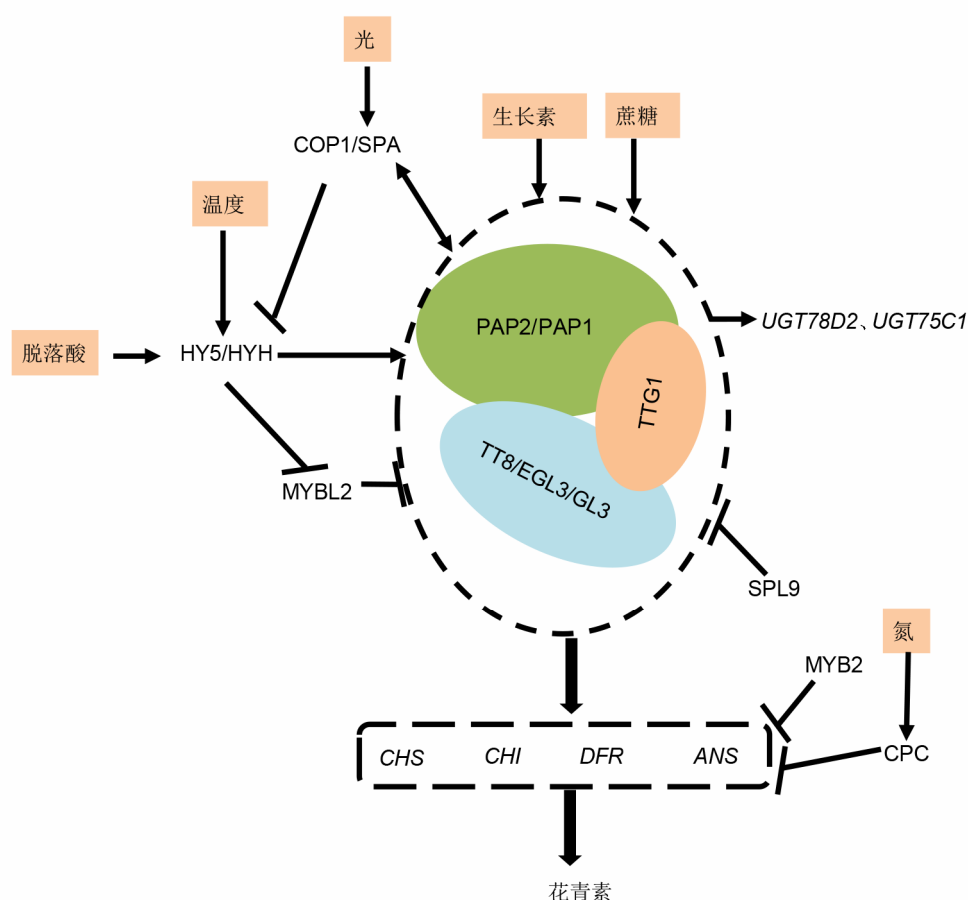


图2 拟南芥MBW转录复合体参与花青素的代谢调控网络

拟南芥PAP1/PAP2、TT8/EGL3/GL3和TTG1组成的MBW复合体主要激活*CHS*、*CHI*、*DFR*和*ANS*的表达进而调控花青素的积累。MBW复合体的功能不仅受环境因子的影响,还受负调控因子的调控。外源施加生长素和蔗糖直接影响花青素的积累。光信号直接作用于COP1/SPA受体,温度和脱落酸通过诱导HY5/HYH基因的表达,进而间接作用于MBW复合体中的MYB型转录因子。负调控因子MYBL2、SPL9、MYB2和CPC则通过负调控MBW复合体或者结构基因的表达,进而抑制花青素的积累。

Figure 2 Summary of MBW transcription complex in the regulation of anthocyanins in *Arabidopsis*

The MBW complex composes of PAP1/PAP2, TT8/EGL3/GL3 and TTG1, which mainly regulates anthocyanin accumulation in *Arabidopsis thaliana* by the regulation of the expression levels of key structural genes, including *CHS*, *CHI*, *DFR* and *ANS*. The function of the MBW complex is not only affected by environmental factors, but also by negative regulators. The application of auxin and sucrose can directly affect the accumulation of anthocyanins. The light signal directly acts on COP1/SPA receptor, and temperature and ABA induce the expression of *HY5/HYH* gene, which in turn affect the MYB transcription factor in the MBW complex. The negative regulatory factors such as MYBL2, SPL9, MYB2 and CPC, negatively regulate the expression levels of MBW complex or structural genes, and then reduce the accumulation of anthocyanins.

(Qiu et al., 2013)。He等(2017)将*PAP1*基因转入烟草,在转基因烟草中检测到1种具有药用价值的花青素Cyanidin 3-O-rutinoside,这种花青素的含量占总花青素含量的98%,这种转基因烟草可以作为工业上提取大量具有药用功能的花青素的原料。Zhu等

(2017)将来源于彩叶草(*Solenostemon scutellarioides*)的*CHS*、*CHI*、*F3H*、*F3'H*、*DFR*、*ANS*以及来源于玉米的转录因子基因*Lc*和*P1*构建入同一个载体中,在水稻胚乳特异表达启动子的驱动下转化水稻,培育出了富含矢车菊素和芍药素的“紫晶米”(Zhu

et al., 2017; 朱丽和钱前, 2017)。这一研究使在主要粮食作物中产生花青素成为可能, 同时富含花青素的转基因水稻的成功培育极大地促进了花青素代谢工程研究的发展。

## 4 展望

花青素作为类黄酮化合物代谢途径的1种重要分支产物, 在生物和非生物胁迫应答、人体保健、抗氧化以及抗病等方面具有重要的研究和应用价值。随着花青素代谢机制的逐渐阐明特别是调控花青素途径转录因子的分离和功能鉴定, 对转录因子的研究已经为花青素开发利用提供了目标基因和理论基础。调控花青素的3类主要转录因子MYB、bHLH和WDR形成1个复杂的调控网络; 其中, WDR是形成MBW复合体的保守元件, 目前已被鉴定的WDR蛋白均参与MBW复合体的形成, 但水果和观赏植物等非模式植物中的WDR及其功能还有待进一步研究。bHLH型转录因子主要通过与MYB转录因子互作的方式调控DFR和ANS的表达, 它们在MBW复合体中主要作为与MYB和WDR互作的功能元件。在MBW复合体中, 研究最充分的MYB转录因子在各种植物中广泛存在, 有的MYB转录因子可以独立调控以DFR为主的结构基因, 有的MYB转录因子则能够参与形成MBW复合体。MYB型转录因子在叶片、果实和花朵等不同组织器官中的表达具有一定的组织特异性, 尤其花青素在果实果皮与果肉中, 以及不同颜色观赏植物花瓣中的积累均受到不同MYB转录因子的调控。果实中花青素的积累通常还会受到光照、温度和植物激素等非生物因子的影响, 通过激活MYB和bHLH转录因子参与非生物因子介导的花青素合成调控; 然而, 目前除了HY5调控光照影响花青素积累的分子机制研究取得了一定进展之外, 更多的互作因子还有待发现, 光诱导调控花青素积累的信号通路还有待进一步明确(图2)。

在花青素的转录调控网络中, 还有一些关键问题有待阐明。例如, 各种非生物因子是与单个转录因子作用影响花青素合成, 还是与多个转录因子协同作用影响花青素合成? 既影响结构基因又影响调控基因的负转录因子间是否存在一定的联系? 导入结构基因或编码MYB/bHLH型转录因子的基因可以明显提高花青素的含量, 也是花青素代谢工程中常见的策

略, 过量表达WDR蛋白或者抑制负调控基因是否也能在花青素代谢工程中发挥作用, 导致花青素含量提高? 总而言之, 解决这些问题将有助于阐明花青素的合成及其调控模式, 有助于更好地采用代谢工程的方法调控植物花青素途径, 改良植物品质, 提高有益的花青素成分和含量, 创造有价值的产品, 进而造福于人类。

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## Recent Advances in the Regulation Mechanism of Transcription Factors and Metabolic Engineering of Anthocyanins

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**Abstract** Anthocyanins are among the most important flavonoid compounds widely present in plants. Anthocyanins play significant roles in plant growth and development as well as human nutrition and health care. The anthocyanin biosynthetic pathway has been widely documented, and the anthocyanin metabolic regulation network is being constantly improved. The transcription factors that regulate anthocyanin biosynthesis mainly include three classes: MYB, bHLH and WD40 proteins. The proteins regulate the accumulation, location and levels of anthocyanins by activating or suppressing the expression of key structural genes, including *CHS*, *ANS* and *DFR*. This review briefly introduces the anthocyanin biosynthetic pathway and summarizes the molecular mechanism of transcriptional regulation based on recent progress. It mainly focuses on the molecular mechanism of MYB, bHLH and WD40 transcription factors in the regulation of anthocyanins in model plants. In addition, it summarizes the use of these transcription factors in anthocyanin metabolic engineering in ornamental plants and fruit crops. This review will provide valuable references for the in-depth investigation of transcriptional regulation and improving anthocyanins by metabolic engineering.

**Key words** anthocyanins, transcription factor, transcriptional regulation, metabolic engineering

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