臭氧影响植物花青素与类胡萝卜素代谢的研究进展

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摘要: 臭氧是光化学污染的主要成分, 其对全球生态环境尤其是对植物产生重要影响。花青素和类胡萝卜素是植物呈色密切相关的色素, 臭氧影响植物花青素和类胡萝卜素的代谢已受到多方关注, 本文从植物花青素和类胡萝卜素组分与功能、合成途径, 植物吸收臭氧途径、特征, 到臭氧影响花青素和类胡萝卜素合成调控的分子机制, 系统地阐述植物在臭氧信号诱导下花青素和类胡萝卜素代谢途径中的调控作用, 为深入揭示臭氧对植物生理功能的影响提供科学依据。

关键词: 臭氧; 花青素; 类胡萝卜素; 信号途径; 分子机制

近地面空气中氮氧化物(NOx)和挥发性有机物(VOCs)等前体物在一定的环境条件下形成的臭氧(O_3)属于二次污染物, 对植物生长和人类健康造成极大的伤害(Fuhrer等1997; Karnosky等2007; Büker等2015)。近几十年来, 随着城市化和工业化进程的加快, 全球近地层臭氧浓度已升高到35~40 $nL \cdot L^{-1}$, 且正以每年0.5%~2.5%的速率增长, 预计到2100年将超过70 $nL \cdot L^{-1}$ (Sitch等2007)。我国近地层臭氧浓度增加较快, 2005到2010年间上升约7%, 平均浓度变化范围23.5~33.7 $nL \cdot L^{-1}$ (Feng等2015; Verstraeten等2015)。从2012年7月4日到8月30日监测臭氧浓度的变化, 北京山区的臭氧浓度平均为105.39 $nL \cdot L^{-1}$, 公园中为68.49 $nL \cdot L^{-1}$, 发现18种植物出现了较为明显的臭氧伤害症状(张红星等2014)。臭氧胁迫通过气孔限制和非气孔限制, 影响植物叶片对光能的利用, 导致其光合速率降低, 改变同化产物的分配(Karnosky等2007; 许宏等2007; 列淦文等2014)。臭氧污染显著降低植物开花的数量、生物量, 甚至导致提前开花(Hayes等2012; Leisner和Ainsworth 2012)。

植物色素在植物生命活动的许多方面起着重要作用, 尤其是花青素和类胡萝卜素。花青素和类胡萝卜素是植物体内重要的次生代谢产物, 与植物多种组织器官的呈色有关, 具有抵御低温、干旱、真菌感染, 防御紫外线伤害、虫害, 吸引授粉者, 利于种子传播等功能, 对植物的生长繁殖及对环境的适应有重要意义(Koes等2005; 刘晓芬等2013; Hichri等2011; Jaakola 2013; 王华等2015)。

有关大气臭氧浓度升高对花青素、类胡萝卜素等次生代谢产物的影响, 目前研究主要集中于代谢产物含量和组分的变化; 臭氧胁迫下花青素、类胡萝卜素等代谢产物合成或者降解途径结构基因及其转录因子的分析研究等。

1 植物花青素与类胡萝卜素的组分、功能与合成途径

1.1 植物花青素和类胡萝卜素的组分与功能

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, 有利于种子和花粉传播。对蔷薇亚属200种植物花瓣花青苷的调查发现了11种花青素, 并以矢车菊素3,5-葡萄糖苷为主(Mikanagi等2000)。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, 不同果实或者花中花青素种类和含量不同(Petroni和Tonelli 2011; Jaakola 2013; 王华等2015)。

类胡萝卜素是一类重要的脂溶性天线色素, 主要分布在光合反应中心复合体的光合膜上, 在光合作用中发挥着重要的作用。它也是一类重要的次生代谢物, 积累到一定浓度后会在植物叶片、花瓣和果实中呈现出黄色、橙色, 有利于吸引传粉者和散播种子(Demmig-Adams等1996; Dall'Osto等2007)。类胡萝卜素在不同组织器官中或在不同发育阶段累积不同。如在番薯(*Ipomoea batatas*)开放的花朵中, 类胡萝卜素主要组分是β-隐黄质、玉米黄质和β-胡萝卜素, 而花瓣发育的早期阶段和叶片中, 类胡萝卜素主要组分是黄体素、紫黄质和β-胡萝卜素(Yamamizo等2010)。又如在玫瑰(*Rosa rugosa*)果

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实中, 检测到21种类胡萝卜素, 其中11种叶黄素和10种胡萝卜素(Zhong等2016)。

1.2 植物花青素和类胡萝卜素合成途径

植物花青素合成途径较为成熟, 主要有3条, 分别形成蓝紫色的飞燕草素、砖红色的天竺葵素和紫红色的矢车菊素(图1) (Hichri等2011; Jaakola 2013; 王华等2015)。苯基丙氨酸是花青素生物合成的最初底物, 苯基丙氨酸在苯丙氨酸解氨酶(phenylalanine ammonia-lyase, PAL)催化下形成肉桂酸, 经肉桂酸-4-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, 乙酸在乙酰辅酶A羟化酶的作用下生成丙二酰辅酶A, 4-香豆酰辅酶A与丙二酰辅酶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)催化下生成二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, 经花色素合成酶(anthocyanidin-synthase, ANS)合成有色的花青素, 最后在类黄酮-3-*O*-葡糖基转移酶(UDP glucose-flavonoid 3-*O*-glcosyl-trans-ferase, UFGT)作用下将花青素转变成蓝紫色、砖红色或紫红色的花色素苷。花青素、原花青素、黄酮醇合成途径之间有交叉。二氢黄酮醇在黄酮醇合成酶(flavonol synthase, FLS)催化下生成黄酮醇。原花青素由黄烷-3-醇或表-黄烷-3-醇聚合而成。黄烷-3-醇是由无色花青素经过无色花青素还原酶(leucoanthocyanidin reductase, LAR)催化得到, 而表-黄烷-3-醇是由花青素经过花青素还原酶(anthocyanidin-reductase, ANR)催化得到。

类胡萝卜素的生物合成途径是类异戊二烯合成体系中的一个分支, 由一系列不同的阶段组成(图2) (Tanaka和Ohmiya 2008; Cazzonelli和Pogson 2010; 高慧君等2015; Arango等2016; Dani等2016; Zhang等2016; 朱运钦等2016)。其前体物质是异戊二烯焦磷酸(isopentenyl pyrophosphate, IPP), IPP通过质体中的2-C-甲基-D-赤藓醇-4-磷酸(2-C-methyl-D-erythritol 4-phosphate, MEP)途径合成, 是重要的

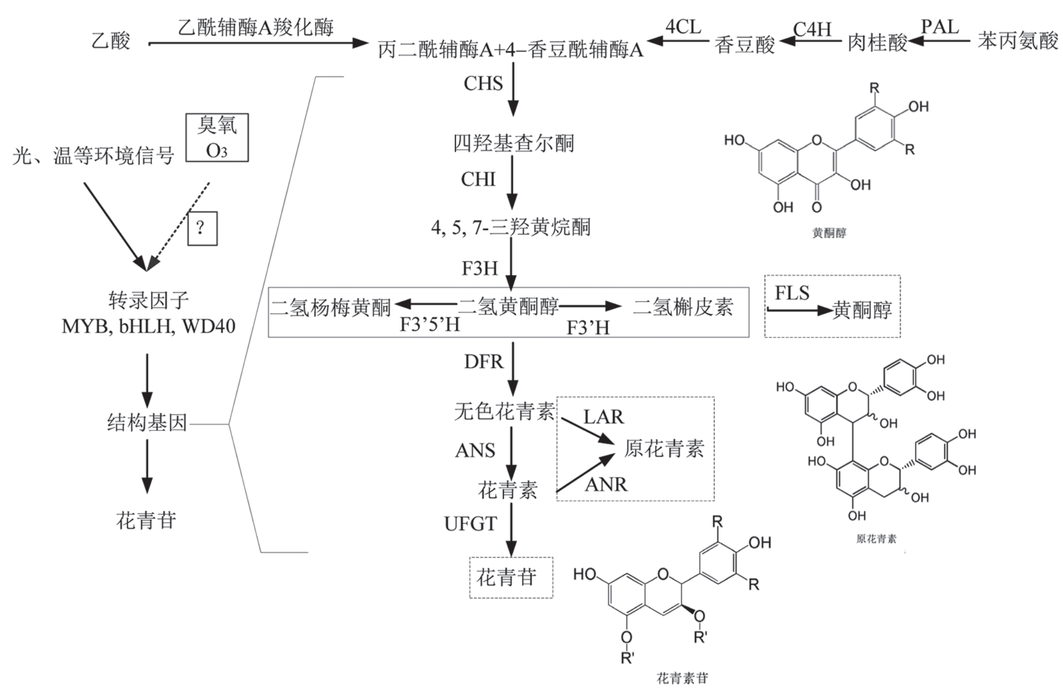

图1 花青素合成途径

Fig.1 The biosynthesis pathway of anthocyanin

参考Hichri等(2011)和Jaakola (2013)并略有修改。

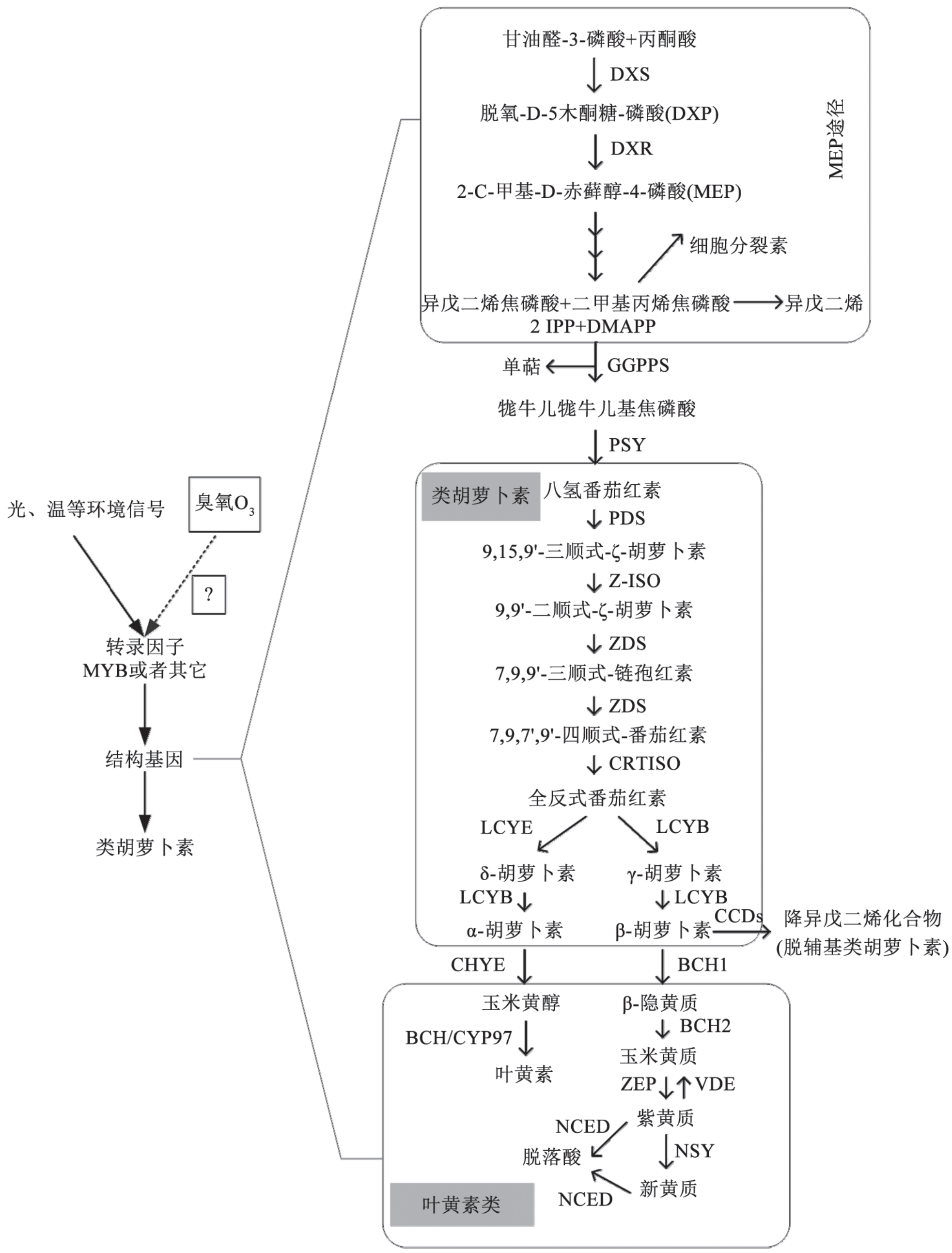

图2 类胡萝卜素合成途径

Fig.2 The biosynthesis pathway of carotenoid

参考Tanaka和Ohmiya (2008)、Cazzonelli和Pogson (2010)、高慧君等(2015)、Arango等(2016)、Dani等(2016)、Zhang等(2016)和朱运钦等(2016)并略有修改。

萜类物质合成前体。在IPP异构酶(isopentenyl pyrophosphate isomerase, IPI)和牻牛儿牻牛儿基焦磷酸合酶(geranylgeranyl di-phosphate synthetase, GGPPS)的作用下, 二甲基丙烯焦磷酸(dimethylallyl diPhosPhate, DMAPP)与3个IPP缩合生成牻牛儿牻牛儿基焦磷酸(geranylgeranylpyrophosphate, GGPP)。两分子GGPP在八氢番茄红素合成酶(phytoene synthase, PSY)作用下形成无色的类胡萝卜素—八氢番茄红素。八氢番茄红素通过八氢番茄红素脱氢酶(phytoene desaturase, PDS)、ζ-胡萝卜素异构酶(ζ-carotene isomerase, Z-ISO)、ζ-胡萝卜素脱氢酶(zeta-carotenedesaturase, ZDS)和胡萝卜素异构酶

(carotenoid isomerase, CRTISO)的共同作用下生成粉红色的全反式番茄红素。番茄红素环化是类胡萝卜素进一步合成的分支点: 一条途径是在番茄红素β-环化酶(Lycopene bate cyclase, LCYB)的作用下产生β-胡萝卜素; 另一条途径是在番茄红素β-环化酶(LCYB)和番茄红素ε-环化酶(lycopeneε-cyclase, LCYE)的共同作用下合成α-胡萝卜素。α-胡萝卜素在β-胡萝卜素羟化酶(beta-carotene hydroxylase, BCH)作用下合成叶黄素。β-胡萝卜素经过BCH羟基化反应生成β-隐黄质, 经羟基化生成玉米黄质, 再经玉米黄质环氧酶(zeaxanthin epoxidase, ZEP)催化生成花药黄质, 进而生成紫黄质; 在新黄质合成酶(NSY)的催化作用下生成新黄质, 是脱落酸(abscisic acid, ABA)的合成前体。

2 植物吸收臭氧途径和特征

气孔是臭氧进入叶片最主要的途径, 与植物臭氧伤害的形成密切相关(Nowak和Dwyer 2007; 高峰等2017)。环境中臭氧浓度一定时, 气孔导度是植物吸收臭氧的关键限制因子, 气孔导度的变化受到自身的生物学特性和环境因子的影响, 如冠层位置、树龄、温度、光强、土壤含水量等(Jarvis 1976; Wieser等2000)。目前, 现有的大部分研究主要集中在林木和农作物上, 如加那利松(*Pinus canariensis*)、挪威云杉(*Picea abies*)、瑞士五叶松(*Cembran pine*)、欧洲山毛榉(*Fagus sylvatica*)、欧洲落叶松(*Larix decidua*)、冬小麦(*Triticum aestivum*)、大豆(*Glycine max*)、西兰花(*Brassica oleracea* var. *italica*)、白菜(*Brassica pekinensis*)等吸收臭氧的影响机制(Morgan等2003; Wieser等2003, 2006; Nunn等2007; Köstner等2008; Braun等2010; Feng等2011; Rozpądek等2015)。

植物吸收臭氧研究的重点主要集中在植物吸收臭氧速率与特点等方面。植物吸收臭氧速率差别较大。如: 美国14个城市森林冠层吸收臭氧速率变化范围为1.82~2.10 $g·m^{-2}$ (Nowak和Dwyer 2007); 北京市森林冠层吸收臭氧速率约为5.50 $g·m^{-2}$ (Yang等2005)。功能型树种(常绿树种与落叶树种、阔叶树种与针叶树种)是影响城市林木吸收臭氧速率的重要原因。如: 罗马市不同功能型的树种吸收臭氧速率差异大, 如常绿阔叶林冬青栎(*Quercus ilex*)和欧洲栓皮栎(*Q. suber*)、落叶阔叶林苦栎(*Q. cerris*)、针叶树意大利伞松(*Pinus pinea*)在2003和2004年吸收臭氧速率分别是0.8和0.4~0.5 $g·m^{-2}$、0.7和0.6 $g·m^{-2}$、0.2~0.8和0.4~0.8 $g·m^{-2}$ (Manes等2011); 奥地利帕彻科菲尔山常绿树种吸收臭氧速率显著高于落叶树种(Wieser等2003)。植物吸收臭氧特点对群落吸收臭氧功能影响显著, 围绕日变化、季节变化、年度变化特点等方面开展了大量研究。这方面研究的主要观点有: (1)夜间欧洲白桦(*Betula pendula*)林木能够通过气孔途径吸收臭氧, 引起较大伤害, 因而建议评价臭氧引起夜间林木伤害的指标应考虑夜间吸收臭氧量(Matyssek等1995)。(2)林木吸收臭氧季节变化差异性较大, 如: 北京市典型绿化树种吸收臭氧速率夏季较高(Wang等2012)。(3)林木吸收臭氧年度变化较大, 如2003~2004年罗马市不同功能型的树种吸收臭氧能够互补, 在不同的气候条件下维持稳定的群落吸收臭氧功能(Manes等2011)。这些研究结果对于环境空气质量的改善和植物的管理起到了极大的促进作用。

3 臭氧对植物花青素、类胡萝卜素代谢的影响

臭氧对花青素和类胡萝卜素代谢的影响是比较复杂的, 目前此类研究多集中在臭氧胁迫下植物的花青素或者类胡萝卜素含量的变化方面。臭氧可刺激类苯丙烷代谢, 增加了苯丙酸类合成途径的生物合成活性, 引起花青素相关化合物的积累。如: 327 $nL·L^{-1}$臭氧浓度处理彩萼石楠(*Calluna vulgaris*) 24 w, 花青素含量显著增加(Foot等1996); 高臭氧浓度处理会增加火炬松(*Pinus taeda*)原花青素含量(Booker等1996); 急性臭氧处理会增加巴西乡土树种原花青素含量(Moura等2014); 70 $nL·L^{-1}$臭氧浓度处理西兰花(*Brassica oleracea* var. *italica*)和白菜(*Brassica pekinensis*) 3 d, 成熟后的花青素含量增加(Rozpądek等2015); 浓度为200 $nL·L^{-1}$的臭氧处理蜜蜂花(*Melissa officinalis*) 3 h, 酚类物质代谢途径相关酶(莽草酸脱氢酶、苯丙氨酸解氨酶和肉桂醇脱氢酶)活性增加2倍多, 花青素含量增加2倍(Tonelli等2015)。花青素具有水溶性, 胁迫后叶片花青素含量上升以降低细胞渗透势, 进而促进其对环境的适应能力(Chalker-Scott 2002)。

臭氧通过植物气孔进入叶片内部, 直接影响原生质膜上的类脂或蛋白质, 造成类胡萝卜素合成被抑制。如: 2倍外界臭氧浓度处理4 w大的2个

莴苣(*Lactuca sativa*)品种‘Valladolid’和‘Morella’幼苗60 d, 叶片叶绿素和类胡萝卜素含量显著降低(Calatayud和Barreno 2004); 在南极半岛, 2种苔藓植物(*Cephaloziella varians*和*Sanionia uncinata*)新黄质合成增加, 紫黄质、叶黄素、玉米黄质和β-胡萝卜素的浓度明显是受到了臭氧的影响(Newsham等2002); 120 nL·L^{-1}臭氧熏蒸耐臭氧和臭氧敏感的杨树(*Populus deltoides*×*P. trichocarpa*), 发现臭氧耐受性与很多因素相关(Ryan等2009)。

最近的研究注意到了在臭氧处理下植物叶片和果实的花青素和类胡萝卜素有变化。1.34×10^{3} nL·L^{-1}臭氧浓度处理1年生元宝枫(*Acer truncatum*) 1个生长季, 在植物光合叶片中早生叶的花青苷和类黄酮相对含量显著升高了34.1%和7.3%, 而类胡萝卜素却下降了9.6% (李丽等2016)。171.6×10^{3} nL·L^{-1}臭氧浓度处理辣椒(*Capsicum annuum*) 62 d, 在辣椒皮中类胡萝卜素含量增加了52.8%, 总酚化合物含量增加17% (Bortolin等2016)。

4 臭氧影响花青素和类胡萝卜素合成的分子机制

4.1 臭氧影响花青素合成的分子机制

在臭氧作用的花青素合成调控网络中, 臭氧胁迫作为最初的信号, 其主要是通过气孔进入植物叶片细胞间空隙, 经反应形成一系列的活性氧自由基(ROS), ROS累积能激活多个信号, 如应激激素等(Vainonen和Kangasjärvi 2015; 高峰等2017)。以JA、SA和ABA等作为信号分子, 调控各类转录因子如*MYB*的表达水平, 转录因子单独或与其他蛋白互作形成复合体, 通过与其调控的下游结构基因启动子区的顺式作用元件结合而直接调控结构基因的表达, 影响植物花青素合成(图1) (包满珠1997; Spelt等2000; Koes等2005; Hichri等2011; Petroni和Tonelli 2011; 葛翠莲等2012; Jaakola 2013; 刘晓芬等2013; Vainonen和Kangasjärvi 2015; Zhao和Tao 2015)。

在矮牵牛(*Petunia hybrida*)中, 转录复合体主要调控*DFR*和*CHS*这两个关键结构基因的表达, 进而促进花瓣或花药花青素积累(Quattrocchio等1993)。在金鱼草(*Antirrhinum majus*)中, MYBs转录因子Rosea和Venosa分别调控不同的结构基因, 促进花青素在花瓣不同部位积累(Schwinn等2006)。在月季中, *RhMYB10*和结构基因*UFGT* (*RhUF3GT1*、*RhUF3GT2*和*RhUF3GT3*)对于花青素合成起重要的调节作用(Lin-Wang等2010; Fukuchi-Mizutani等2011)。

长期臭氧胁迫可诱导银杏(*Ginkgo biloba*)幼苗叶片活性氧积累, 诱发信号通路, 进一步影响色素的形成和积累(高姗姗2016)。Puckette等(2008)采用300 nL·L^{-1}臭氧熏蒸臭氧敏感苜蓿(*Medicago sativa*)品种‘Jemalong’和耐臭氧的品种‘JE154’ 6 h, 苜蓿叶片的信号转导、激素合成路径、黄酮合成等相关基因表达均上调。

由此可见, 在臭氧胁迫下, 臭氧可作为环境刺激信号、通过信号转导, 作用到转录因子, 转录因子调控结构基因的表达, 最终实现植物花青素含量变化。

4.2 臭氧影响类胡萝卜素合成的分子机制

有关臭氧胁迫影响类胡萝卜素合成的分子机制的研究较少, 主要集中在植物中类胡萝卜素合成的基因调控机制上。类胡萝卜素生物合成路径中的一系列结构基因表达水平与类胡萝卜素的合成积累密切相关, 可直接影响类胡萝卜素的组分及含量。已有报道, 在胡萝卜中有功能注释的36个类胡萝卜素合成相关基因中八氢番茄红素合成酶(PSY)是类胡萝卜素合成途径首要限速酶(欧承刚等2017)。*PSY*基因的序列差异是调控类胡萝卜素合成的一种机制, 主要表现为可变剪切及SNP位点的变化(王慧等2014)。也有学者采用基因编辑技术CRISPR/Cas9, 对苹果*PSY*基因进行编辑, 观察到白化表型的植株(Nishitani等2016)。类胡萝卜素裂解双加氧酶(carotenoid cleavage dioxygenases, CCDs)基因作为酶降解途径的关键基因, 也是影响类胡萝卜素含量的重要因子之一(Schulz等2016)。Ureshino等(2016)对黄色落叶杜鹃(*Rhododendron japonicum*)和白花常绿杜鹃(*R. kiusianum*×*R. indicum*)开花过程中类胡萝卜素进行研究, 结果表明随着花开放, *PSY*和*PDS*基因表达在黄色落叶杜鹃花瓣比白花常绿杜鹃和杂交后代中显著增加, *CCD4*基因表达在白花常绿杜鹃和杂交后代中比黄色落叶杜鹃花瓣高。橙红色桂花(*Osmanthus fragrans*)品种‘Yanhong Gui’花瓣呈色的主要物质是α-胡萝卜素和β-胡萝卜素, 通过转录组数据的分析, 发现10个基因(*OfCRTISO1*、*OfDXS2*、*OfGGPS2*、*OfGGPS4*、 *OfLCYE1*、*OfPDS1*、*OfPSY1*、*OfZDS1*、*OfZISO1*和*OfZ-ISO2*)与类胡萝卜素合成正相关(Zhang等2016)。

类胡萝卜素很大程度上是由结构基因的转录调控决定, 但是转录因子的相关研究很少。Sagawa等(2016)确定了*RCP1*转录因子对类胡萝卜素积累有正调控作用, *RCP1*基因失去功能引起类萝卜素合成途径中结构基因的下调和类胡萝卜素含量的降低, *RCP1*基因过表达类胡萝卜素含量回升。另外, *RCP1*基因与花青素合成相关的转录因子R2R3-MYB亚类6和7明显不同(Dubos等2010), 由此可知, R2R3-MYB类基因不但调节花青素生物合成, 也调节类胡萝卜素生物合成(图2), 在未来的研究中需关注这类MYBs转录因子。

5 总结和展望

随着城市化和工业化进程的加快, 在今后相当长的时间里, 全球近地层臭氧浓度呈现不断增加的趋势。臭氧不但影响全球的自然环境, 而且影响植物的生理生态, 也包括影响植物花青素和类胡萝卜素的代谢。臭氧通过气孔进入植物细胞, 影响植物叶片的光能利用。植物在自然适应过程中, 形成一套机制来缓解臭氧胁迫伤害。臭氧可影响植物花青素、类胡萝卜素等代谢产物。在臭氧胁迫下, 臭氧可作为环境刺激信号、通过信号转导, 作用到转录因子, 转录因子进而激活一系列花青素、类胡萝卜素等合成路径的结构基因的表达, 最终实现植物花青素、类胡萝卜素等物质积累, 这一机制有助于理解臭氧胁迫下花青素、类胡萝卜素合成与相关环境信号之间, 以及合成途径相关结构基因与调控基因之间的互相联系。

生物内在因素和外界环境均可影响植物花青素和类胡萝卜素的合成, 从分子的角度看, 在环境信号如臭氧等诱导下植物花青素和类胡萝卜素合成途径的酶基因表达是关键, 但是作为信号分子的臭氧, 其受体是什么, 信号如何传递, 在基因水平上如何起作用, 此类研究报道很少, 也就是说应答臭氧胁迫的内在分子机制的研究并不完全清楚, 尚需进行大量和深入研究。

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Effects of atmospheric ozone on anthocyanin and carotenoid of plants

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Abstract: Ozone, which is the main component of photochemical oxidants, is a potential risk factor in global environment. Anthocyanins and carotenoids represent the major pigments in plants. Studies have been raised about effects of atmospheric ozone on anthocyanin and carotenoid of plants. This review provided an overview of anthocyanin and carotenoid components, functional roles, biosynthetic pathways, and ozone uptake by various plants. It also presented the molecular mechanisms underlying the anthocyanin and carotenoid biosysthesis responsing to elevated ozone. Our review revealed the regulation role of related genes in anthocyanins and carotenoids biosysthesis when exposed to elevated ozone, providing a theoretical basis for understanding the role of ozone in plant physiology.

Key words: ozone; anthocyanins ; carotenoids; signaling pathways; molecular mechanisms

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