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植物花香代谢调节与基因工程研究进展

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摘要: 植物花香在吸引昆虫授粉、提高观赏价值和香精的商业价值方面具有重要的作用。随着分子生物学技术的发展,近年来植物花香基因被大量克隆,对花香化合物的合成与代谢的网络调控机制有了更深刻的认识,基因工程改良花香成为可能。对近年来植物花香的合成途径、花香的释放与基因调节、基因工程的研究进展进行了综述,并就存在的问题进行了分析,为花香的分子育种研究提供参考。

关键词: 花香化合物; 代谢调节; 生物合成; 基因工程; 研究进展
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Research Advances in Metabolic Regulation and Genetic Engineering of Floral Scent

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Abstract: Floral scent plays an important role in attracting insect pollinators and also enhances the economic value of ornamental plants and commercial value of perfumery. In recent years, with the development of molecular biological technology, especially many genes related to floral scent have been cloned, the regulation mechanism of biosynthesis and metabolism of floral scent compounds had be understood deeply, so that it was possible to change floral scents by genetic engineering technology. The advances in the biosynthesis path way, emission, and gene regulation of floral scent compounds were reviewed, and the strategies of changing floral scent by genetic engineering were discussed, and the molecular breeding of floral scent in the future was prospected.

Key words: Floral scent compound; Metabolic regulation; Biosynthesis; Genetic engineering; Review

植物花香化合物是植物花朵释放的次生代谢产物[1],它由许多低分子量、易挥发的化合物组成[2]。植物花香在香精香料工业、昆虫授粉和提高观赏作物商业价值等方面具有重要的作用。由于花香生物合成代谢的复杂性,以往的植物花香研究主要集中在花香成分鉴定方面[3-4]。自从密西根州立大学 Pichersky 等从仙女扇(*Clarkia breweri*)中克隆到第 1 个花香基因——芳樟醇合成酶基因(S-Linalool synthase, *LIS*)[5]后,金鱼草(*Antirrhinum majus*)、月季花(*Rosa chinensis*)等植物的花香基因[6-7]也相继被克隆,基因工程修饰花香的研究也取得一些进展[8-10]。利用基因工程技术改良花香、提高观赏植物的商业价值,植物花香化合物的生物合成调控成为研究热点。本文对近年来植物花香的合成途径、花香的释放与基因调节、基因工程的研究进展进行综述,为花香的分子育种研究

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提供参考。

1 植物花香化合物的代谢途径

目前已有990种(亚种)植物的花香成分被鉴定,包括萜烯类化合物、脂肪族化合物、苯基丙烷类/苯环型、含碳5支链化合物、含氮化合物、含硫化合物和其它环化物共7大类1719种,大多数花香化合物属于萜烯类化合物、脂肪族化合物和苯基丙烷/苯环型类化合物[4]。它们的合成处于基本代谢途径的末端,故称次生代谢途径[11-12]。大多数花香化合物的生物合成过程非常复杂,是由多个酶在不同组织中共同完成的[13]。

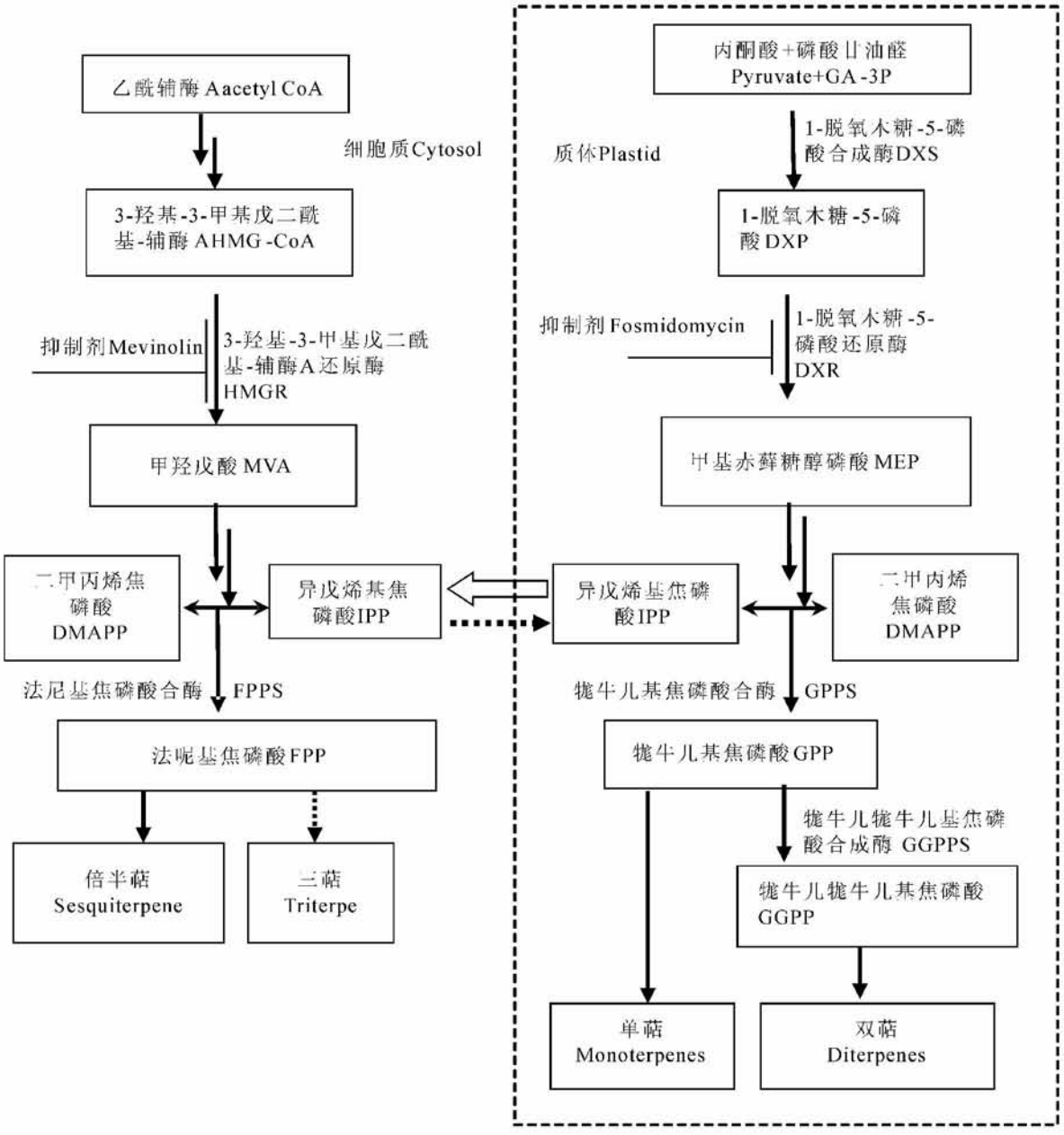

图1 萜烯类化合物代谢合成途径

Fig. 1 Synthesis pathways of terpenoid

HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme-A; HMGR: 3-hydroxy-3-methylglutaryl coenzyme A reductase 3-phosphate; DMAPP: Dimethylallyl pyrophosphate; DXP: 1-deoxy-D-xylulose-5-phosphate; GA-3P: D-glyceraldehyde-3-phosphate; IPP: Isopentenyl pyrophosphate; MVA: Mevalonate; MEP: Methyl-erythritol-phosphate; GPP: Geranyl diphosphate; FPP: Farnesyl diphosphate; GGPP: Geranylgeranyl diphosphate; GPPS: GPP synthase; DXS: 1-deoxy-D-xylulose-5-phosphate synthase; DXR: 1-deoxy-D-xylulose-5-phosphate reductoisomerase; FPS: FPP synthase; GGPPS: GGPP synthase.

1.1 萜烯类化合物合成途径

萜烯类化合物是植物天然花香化合物中最大的一类,在植物营养和花器官中普遍存在。萜烯类化合物合成途径包括来源于细胞溶质的甲羟戊酸途径(MVA)和来源质体的甲基赤藓糖醇磷酸途径(MEP)[14-15]。乙酰辅酶 A 是 MVA 途径的原初供体,经乙酰辅酶 A 酰基转移酶、3-羟基-3-甲基戊二酰基-辅酶 A 合成酶和 3-羟基-3-甲基戊二酰基-辅酶 A 还原酶(HMGR)等作用产生了异戊烯基焦磷酸(IPP)和二甲丙烯焦磷酸(DMAPP),其中 HMGR 为 MVA 途径的关键酶。丙酮酸和磷酸甘油醛是 MEP 途径的原初供体,在 1-脱氧木糖-5-磷酸合成酶、1-脱氧木糖-5-磷酸还原酶等酶作用下,也产生了 IPP 和 DMAPP,其中脱氧木酮糖磷酸盐还原异构酶(DXR)是 MEP 途径的限速酶[16]。IPP 与 DMAPP 缩合成单萜的前体物质牻牛儿基焦磷酸(GPP),最后经各种单萜合酶形成各种单萜类化合物。GPP 与 IPP 在法尼基焦磷酸合酶作用下,形成法呢基焦磷酸(FPP),即倍半萜的前体。FPP 与 IPP 在牻牛儿牻牛儿基焦磷酸合酶作用下形成牻牛儿牻牛儿基焦磷酸(GGPP),即双萜的前体。然后,单萜、倍半萜和双萜的前体物质在萜类合酶作用下形成三萜、四萜等萜类。这些萜类的前体再通过各种修饰酶如脱氢酶、还原酶、糖基转移酶和甲基转移酶等作用下形成各种萜烯[17]。经 MVA 途径产生的倍半萜和三萜类化合物的前体在细胞溶质中,经 MEP 途径产生单萜、双萜和四萜等化合物的前体在质体中[15,18-19]。尽管两个途径如何互作还不清楚,但最近的研究表明,它们之间可能通过 IPP 作为中间物质进行交流[20-22]。

1.2 脂肪族化合物合成途径

脂肪酸衍生物是植物花香化合物的第二大类,包括饱和与不饱和的短链醇类、醛类和酯类化合物。它以膜脂为源头,通过 C18 的脂肪酸降解亚麻酸和亚油酸的脂氧合酶途径(lipoxygenase pathway)形成。在脂氧合酶作用下,C18 的脂肪酸形成过氧化氢物,然后在脂氢过氧化物裂解酶作用下裂解形成 C12 和 C6 产物[23],这些 C6 产物包括(Z)-3-己烯醛和已烯醛[24],产物再经乙醇脱氢酶和酰基转移酶进一步转化成相应的醇类(Z-3-己烯醇或者已烯醇)或乙酸-3-乙烯酯[25]。脂肪酸在质体中合成,很多合成脂肪酸衍生物的基因已经被分离和鉴定,但这些基因是如何表达和调控的尚不明确[26]。

1.3 苯基丙烷类/苯环型化合物合成途径

苯基丙烷类的芳香族花香化合物起源于莽草酸途径(shikimic acid pathway),以苯丙氨酸为起始,经苯丙氨酸裂解酶(PAL)去氨基化产生肉桂酸,肉桂酸经羟基化酶和 O-甲基转移酶进一步修饰,形成木质素、防御性物质和色素等物质的中间体。部分含 9 个碳原子的苯丙类化合物的羧基经羟基化、酰基化和甲基化等分支途径产生了醛、醇、烷烃、烯烃、醚和酯类等挥发性物质,松柏醇是花香化合物代谢合成过程中的中间物质[27]。

苯环型化合物来源于苯丙类化合物,是将苯环侧链的 3 个碳原子中的 2 个缩化而成。通过同位素示踪实验和计算机辅助代谢通量分析揭示,矮牵牛(*Petunia hybrid*)有依赖辅酶 A 的 β-oxidative 途径和不依赖辅酶 A 的非 β-oxidative 途径,或两条途径结合[28]。在依赖辅酶 A 的 β-oxidative 途径中,肉桂酸通过辅酶 A 连接酶作用形成肉桂酰基辅酶 A、在烯酰辅酶 A 水合酶作用下形成 3-羟基-3-苯丙酰辅酶 A,然后,在脱氢酶作用下,将羟基转化成醛,形成 3-氧基-3-苯丙酰辅酶 A,最后通过克莱森(Claisen reaction)的逆反应生成苯甲酰辅酶 A。不依赖辅酶 A 的非 β-oxidative 途径以肉桂酸为底物,经过水合反应形成 3-羟基-3-苯丙酸,然后经醛醇反应的逆反应导致侧链降解形成苯甲醛。侧链碳原子的缩减也可以通过依赖辅酶 A 的非 β-oxidative 途径,但这种机制非常复杂,不同的植物,甚至同种植物根据生理条件不同有差异,苯环类化合物在合成途径中如何相互联络还需进一步研究[28]。

2 植物花香化合物释放与基因/酶的时空表达

不同植物释放的花香化合物在数量、种类和含量上存在差异。同种植物的花香化合物的时空差异可能发生在花器官的不同部位、同一植株的不同花朵,或者不同植株和居群之间[29]。植物释放花香化合物复杂的时空调节模式使植物在不同开花期所释放的花香化合物组分和含量发生变化[30],并能够为觅食者觉察[31-32]。

2.1 植物花香化合物释放空间模式与基因/酶的表达

一般来说,不同部位的花器官产生的花香化合物数量、种类和浓度不同[6,33-37]。大多数植物的花香来自花瓣[38],但雌蕊、雄蕊、花萼和蜜腺盘

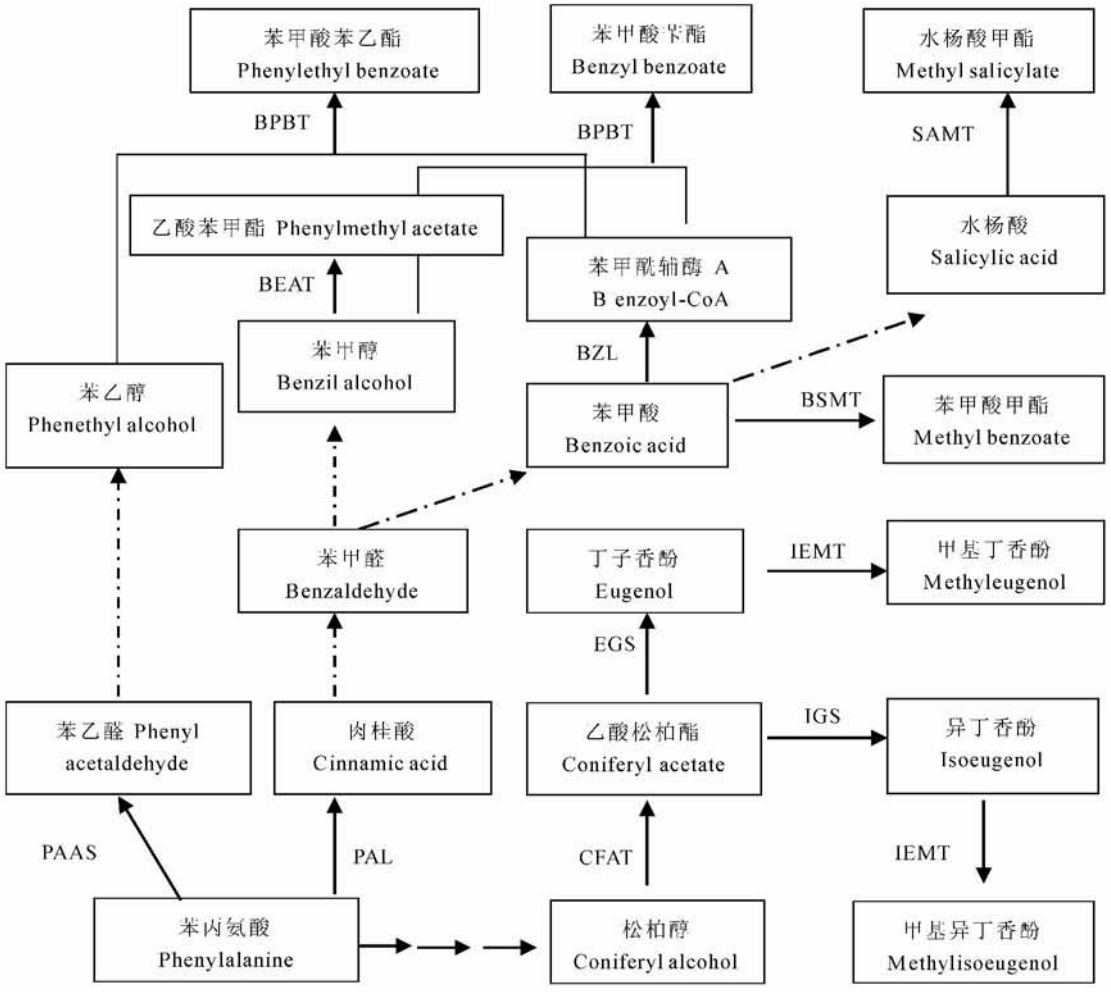

图2 苯环类和苯基丙酸类化合物的合成途径

Fig. 2 Synthesis pathways of benzenoids/phenylpropanoids

实线表示已证明途径,虚线表明是可能的途径 Solid lines indicate established biochemical reactions, and broken lines indicate possible steps; BEAT: 苯甲醇乙酰基转移酶 Benzylalcohol acetyltransferse; BPBT: 苯甲醇/苯基乙醇苯甲酰转移酶 Benzyl alcohol/phenylethanol benzoyltransferase; SAMT: 水杨酸羧基位甲基转移酶 Salicylic acid carboxyl methyltransferase; IEMT: (异)丁子香酚-O-甲基转移酶 (*iso*)-eugenol O-methyltransferase; BSMT: 苯甲酸/水杨酸羧基位甲基转移酶 Benzoic /Salicylic acid carboxyl methyl transferase; CFAT: 松柏醇酰基转移酶 Coniferyl alcohol acyltransferase; EGS: 丁子香酚合成酶 Eugenol synthase; IGS: 异甲基丁子香酚合成酶 (*iso*)-eugenol synthase; BZL: 苯甲酸辅酶A连接酶 Benzoate:CoA ligase; PAL: 苯丙氨酸裂解酶 Phenylalanine ammonia-lyase; PAAS: 苯乙醛合成酶 Phenylacetaldehyde synthase.

(nectary disks)等也能释放香气,如毛茛属植物 *Ranunculus acris* 的花瓣和雄蕊对花香具有同等的贡献[39],芸香科柑橘属植物 *Boronia megastigma* 的柱头能产生70%的精油[40]。植物花香化合物的释放还与性别有关,长豆角(*Ceratonia siliqua*)的雌花释放的花香化合物种类更丰富,但雄花释放的花香化合物含量更高[36]。花香化合物释放的部位受到生物合成中末端基因所在部位的影响,组织特异性表达分析结果表明,大多数基因在花瓣中的表达量最高,如仙女扇(异)丁子香酚-O-甲基转移酶基因(*IEMT*)[41]、苯甲醇乙酰基转移酶基因(*BEAT*)[42]、金鱼草的苯甲酸羧基位甲基转移酶基因(*BAMT*)[6]、橙花叔醇和芳樟醇合成酶基因(*AmNES/LIS*-1, *AmNES/LIS*-2)[43]、矮牵牛苯甲醇/苯基乙醇苯甲酰转移酶(BPBT)[28]、矮牵牛松柏醇酰基转移酶(PhCFAT)[27]、月季花间苯三酚氧位甲基转移酶基因(POMT)[44]等。柱头中表达量最高的是仙女扇的 *LIS* 基因[5]和 *BEBT* 基因[45],雄蕊表达量最高的有月季花氧位甲基转移酶基因(*RcOMT*1)[46]。此外,有些花香基因在营养器官也

有少量表达,如矮牵牛类胡萝卜素裂解双加氧酶基因(*PhCCD1*)[47]和仙女扇苯甲醇苯甲酰基转移酶基因(*BEBT*)[45]。

2.2 植物花香化合物释放的时间模式与基因/酶的表达

植物花香化合物的释放受到发育阶段的调节,一般随着开花进程花香化合物的种类、数量和含量逐渐升高,在授粉时达到高峰,此后显著下降,甚至花香化合物的种类也会发生改变[28,33,41-42,48-49]。植物花香的释放规律可能与吸引昆虫授粉及保护结实有关。开花进程中花香的释放模式受到三类蛋白酶的调控,一类是以仙女扇的 LIS、SAMT 和金鱼草的 BAMT 蛋白酶为代表,它们的酶活性水平随开花进程而增加,在花香强度达到最高之前的 12 ~ 24 h 的酶活性最高。花香停止释放后,该类蛋白酶的含量下降至最高峰的 40%~50%[6,41];第二类是以仙女扇 IEMT、BEAT、BEBT 和矮牵牛 BPBT 蛋白酶为代表,该类酶活性达到高峰后没有或仅轻微下降,可能这些酶还参与了花器官中其他的生物合成途径[28,42,45]。还有一类是以月季花的 OOMT 蛋白酶为代表,OOMT 的活性在开花前期没有显著增加,而是在花成熟时迅速达到高峰,随后立即下降至检测不到[48]。

此外,很多植物花香化合物的释放具有昼夜的节律性变化,这种可能与昆虫的活动规律有关。依靠蜜蜂授粉的金鱼草花朵释放的苯甲酸甲酯在白天有明显的高峰[6],而白三叶(*Trifolium repens*)花香化合物含量在上午7:00 至12:00 最高[50]。依靠蛾类授粉的矮牵牛释放的苯甲醛、苯甲酸甲酯和异丁子香酚等花香化合物在晚上最高[51-52]。然而,这种节律性与物种有很大的关系,同样依靠蛾类授粉的仙女扇,白天和晚上所释放的香气含量没有显著差异[33]。有些植物释放的花香化合物还受到光周期调控,如欧亚香花芥(*Hesperis matronalis*)释放的1,8-桉树脑在光照下释放最高,(E)-罗勒烯在光/暗转换时释放达到高峰,而乙酸苯甲酯在暗时达到最高[53]。除了光周期对植物花香释放有影响外,植物花香释放规律还受内源物质的调节,在 12 h/12 h 的光/暗交替下,大马士革玫瑰(*Rosa damascena* f. *semperflorens* 'Quatre Saisons')释放的花香化合物在光照 8 ~10 h 后出现释放高峰,之后无论在光或暗下,这种释放的节律性都将持续一段时间[54]。植物花香化合物释放节律的多样性表明植物中存在多种调控机制[53,55]。

花香化合物的释放节律还受末端的酶和前体底物水平的调节。如多花黑蔓藤(*Stephanotis floribunda*)释放的水杨酸甲酯受到末端基因 SAMT 或酶的直接调控,包括转录调节和转录后调节两种方式[56]。然而,很多花香化合物的释放节律与前体的底物水平变化一致,如矮牵牛和金鱼草的苯甲酸甲酯释放节律与 BSMT 和 BAMT 基因或酶活性水平并不同步,而是与直接前体物质(Precursor)苯甲酸的含量变化同步[57]。

3 植物花香基因工程

在过去的十几年中,许多植物的花香基因或酶被分离和鉴定,利用基因工程技术改良植物的花香成为人们关注的热点。通过外源基因的超表达[58-60]和内源基因的抑制表达[8,61]是花香基因工程最常用的两种方式。

表 1　植物花香代谢的基因工程

Table 1 Genes engineering in floral scents metabolism

基因 Gene	来源植物 Origin species	转化体 Transformated species	化合物 Compounds	文献 Reference
LIS	仙女扇 *Clarkia breweri*	番茄 *Lycopersicon esculentum*	(*S*)-芳樟醇/8-羟基芳樟醇(*S*)-Linalool, 8-Hydroxy linalool ↑	[65]
		矮牵牛 *Petunia hybrid*	芳樟醇配糖体 Linalool glycoside ↑	[8]
		香石竹 *D. caryophyllus*	(S)-芳樟醇/芳樟醇氧化物 (S)-Linalool, Linalool oxide ↑	[63]
BEAT	仙女扇 *C. breweri*	洋桔梗 *Eustoma*	—	[59]
GAS	菊苣 *Cichorium intybus*	拟南芥 *Arabidopsis thaliana*	大牻牛儿烯 A Germacrene A ↑	[17]
*TPS*10	玉米 *Zea mays*	拟南芥 *A. thaliana*	倍半萜烯 Sesquiterpene ↑	[66]
LIS/AmNES	草莓 *Fragaria ananassa*	拟南芥 *A. thaliana*	(*S*)-芳樟醇/配糖体芳樟醇/羟基芳樟醇 (S)-Linalool, Linalool glycoside, Hydroxy linalool ↑	[17]

续表(Continued)

基因 Gene	来源植物 Origin species	转化体 Transformated species	化合物 Compounds	文献 Reference
		拟南芥 *A. thaliana*	(3S)-(*E*)-橙花油(3S)-(*E*)-Neroli oil/(E)-DMNT ↑	[67]
		马铃薯 *Solanum tuberosum*	芳樟醇/配糖体芳樟醇/羟基芳樟醇 Linalool/Linalool glycoside/Hydroxy linalool ↑	[68]
TER PIN	柑橘 *Citrus limon*	烟草 *N. tabacum*	γ-萜品烯/柠檬烯/β-蒎烯 γ-Terpinene, Limonene, β-Pinene ↑	[10]
PS	广藿香 *Pogostemon cablin*	烟草 *N. tabacum*	薄荷醇/倍半萜烯 Menthol, Sesquiterpene ↑	[7]
LS	白苏 *Perilla frutescens*	烟草 *N. tabacum*	柠檬烯 Limonene ↑	[69]
		烟草 *N. tabacum*	柠檬烯 Limonene ↑	[69]
		烟草 *N. tabacum*	—	[69]
	留兰香 *Mentha spicata*	薄荷 *M. haplocaly*	—	[70]
		中国薄荷 *M. arvensis*	—	[71]
		辣薄荷 *M. piperita*	—	[71]
MS	辣薄荷 *M. piperita*	辣薄荷 *M. piperita*	薄荷呋喃/胡薄荷酮 Menthofuran, Menthone ↓; 薄荷醇 Menthol ↑	[72]
LH	辣薄荷 *M. piperita*	辣薄荷 *M. piperita*	柠檬烯 Limonene ↑, (异)薄荷酮/薄荷呋喃 (*iso*)-Menthone, Menthofuran ↓	[71]
		烟草 *N. tabacum*	(+)-反式-异薄荷二烯醇及其衍生物 (+)-*trans*-Isopiperitenol and derivatives ↑	[10]
BSMT	矮牵牛 *P. hybrid*	矮牵牛 *P. hybrid*	苯甲酸甲酯 Methyl benzoate ↓	[73]
PAAS	矮牵牛 *P. hybrid*	矮牵牛 *P. hybrid*	苯甲醛/苯乙醇 Benzaldehyde, Benzeneethanol ↓	[13]
BPBT	矮牵牛 *P. hybrid*	矮牵牛 *P. hybrid*	苯甲酸苄酯/苯甲酸苯乙酯 Benzyl benzoate, Phenylethl benzoate ↓; 苯甲醇/苯甲醛 Bentanol, Benzaldehyde ↑	[74]
CFAT	矮牵牛 *P. hybrid*	矮牵牛 *P. hybrid*	异丁子香酚 Isoeugenyl ↓	[75]
*ODO*1	矮牵牛 *P. hybrid*	矮牵牛 *P. hybrid*	苯环型化合物 Volaitle benzenoids ↓	[61]
PAR	草莓 *F. ananassa*	矮牵牛 *P. hybrid*	—	[76]
AAT	月季 *Rosa hybrid*	矮牵牛 *P. hybrid*	乙酸苯甲酯/乙酸苯乙酯 Benzyl acetate, Phenylethyl acetate ↑	[58]
	番茄 *L. esculentum*	矮牵牛 *P. hybrid*	2-苯基乙醇 2-Phenylethanol ↑; 苯乙醛 Phenylacetaldehyde ↓	[77]
*Pap*1	拟南芥 *A. thaliana*	矮牵牛 *P. hybrid*	苯环型/苯丙素类挥发物 Volaitle Phenylpropanoids/ Benzenoids ↑	[60]
CCD	番茄 *L. esculentum*	矮牵牛 *P. hybrid*	β-紫罗兰酮 β-Ionone ↓	[47]
		番茄 *L. esculentum*	已醇 Hexanol ↑	[78]
ADH		番茄 *L. esculentum*	β-紫罗兰酮/假紫罗酮/香叶基丙酮 β-Ionone, Pseudoionone, Geranyl acetone ↓	[47]
AADC	番茄 *L. esculentum*	番茄 *L. esculentum*	苯甲醛/苯基乙醇 Benzaldehyde, Phenylethanol ↑	[77]

LIS: 芳樟醇合成酶基因 Linalool synthase gene; *LIS/AmNES*: 芳樟醇/橙花油合成酶基因 Linalool/nerolidol synthase gene; *LS*: 柠檬烯合成酶基因 Limonene synthase gene; *TER*: γ-萜品烯合成酶基因 γ-Terpinene synthase gene; *PIN*: β-蒎烯合成酶基因 β-Pinene synthase gene; *PS*: 薄荷醇合成酶基因 Menthol synthase gene; *TPS*10: 萜烯合成酶基因 Terpenes synthase gene; *GAS*: 大牻牛儿烯 A 合成酶 Germacrene A synthase gene; *LH*: 柠檬烯-3-羟化酶基因 Limonene-3-hydroxylase gene; *MS*: 薄荷呋喃合成酶基因 Menthofuran synthase gene; *BSMT*: 苯甲酸/水杨酸羧基位甲基转移酶基因 Benzoic acid/salicylic acid carboxyl methyltransferase gene; *AADC*: 芳香族氨基酸脱羧酶基因 Aromatic L-amino acid decarboxylase gene; *AAT*: 醇酰基转移酶基因 Alcohol acetyltransferases gene; *ADH*: 乙醇脱氢酶基因 Alcohol dehydrogenase gene; *AOS*: 丙二烯氧化合酶基因 Allene oxide synthase gene; *BPBT*: 苯甲醇/苯基乙醇苯甲酰转移酶基因 Benzylalcohol/phenylethanol benzoyltransferase gene; *BSMT*: 苯甲酸/水杨酸羧基位甲基转移酶基因 Benzoic acid/salicylic acid carboxyl methyltransferase gene; *CCD*: 类胡萝卜素裂解双加氧酶基因 Carotenoid cleavage dioxygenase gene; *CFAT*: 松柏醇酰基转移酶基因 Coniferyl alcohol acetyltransferase gene; *HPL*: 氢过氧化物裂解酶基因 Hydroperoxide lyase gene; *ODO*1: 芳香族化合物转录因子 ODORANT1 transcription factor; *PAAS*: 苯乙醛合成酶基因 Phenylacetaldehyde synthase gene; *PAR*: 苯乙醛还原酶基因 2-Phenylacetaldehyde reductase gene; *LOX*: 脂肪氧合酶基因 Lipoxygenase gene; *Pap*1: 花青素产物转录因子 Anthocyanin pigment 1 transcription factor.

尽管目前在花香基因工程方面开展了大量工作,但仅有少量获得成功。通过转反义花色基因黄烷酮-3-羟化酶基因(*F3H*)能致使香石竹(*Dianthus caryophyllus*)的花色变浅,这是由于花青素合成受阻导致前体物质含量升高,促进了苯甲酸甲酯的生物合成,导致苯甲酸甲酯含量提高,最终表现为花色变浅[8],这种通过阻止竞争性途径的生物合成使前体物质含量升高,为花香基因工程的研究开辟了重要的途径。此外,Lücker 等通过转基因和两次杂交将柑橘(*Citrus limon*)的 3 个萜烯基因 *TER*、*LIM* 和 *PIN* 转到烟草中,转基因烟草释放的 β-蒎烯、柠檬烯、γ-萜品烯和单萜类产物增加[9-10]。

在植物花香基因工程中,绝大多数转基因植株的花香修饰没有成功,这可能与缺乏对代谢网络构成、酶的亚细胞定位、竞争途径、代谢渠道、通量控制环节和可能的反馈控制等方面的研究有关。花香修饰失败的原因有三个,其一是转基因植株缺少该酶适合的底物,Guterman 等将月季的乙酸香叶酯转移酶基因(*RhAAT*)导入矮牵牛中,转基因矮牵牛没有预期释放乙酸香叶酯,而喷施外源的香叶醇则导致大量乙酸香叶酯的产生[58]。Aranovich 等将 *BEAT* 基因导入洋桔梗(*Eustoma grandiflorum*),转基因植株没有产生预期的乙酸苯甲酯,在喷施外源底物苯甲醇时,转基因植株的花和叶片产生了大量乙酸苄酯,当喷施外源的醇化物如己醇、苯乙醇和异戊醇等,转基因植株产生的乙酸酯类化合物也显著增加[59],这些结果表明转基因植株体内相应底物的供应量决定了花香化合物的释放量。其二是有些合成的花香化合物以非挥发物形式存在,如导入 *LIS* 基因的矮牵牛花瓣和叶片都检测到 LIS 酶的高效表达,但大部分 S-芳樟醇被转化成不挥发的 S-芳樟酯-β-D-吡喃葡糖苷[62]。其三是转基因植株产生的花香化合物含量不高,或者被其他花香化合物掩盖。如转 *LIS* 基因的香石竹(*Dianthus caryophyllus* 'Eilat')产生的芳樟醇及顺式/反式芳樟醇含氧衍生物含量占挥发性物质的 10%,但转基因香石竹的芳樟醇还是不能为人所感知[63]。

4 展望

植物花香能够极大的提升观赏植物的商业价值,提高植物花香是观赏植物育种的长期目标。近年来,国内学者也开始探索用基因工程的方法改良植物花香的研究,如白姜花的倍半萜合成酶基因 *Hc-Sesqui*[79],蜡梅的法呢基焦磷酸合成酶基因 *CpFPPS*[80]等花香基因相继被克隆。通过超表达 HMGR 和 DXS 等关键酶使萜烯类化合物产量增加[81-83],表明基因工程改良植物花香具有广阔的应用前景。然而,目前该项研究工作还面临着巨大的挑战。一些花香化合物的关键前体物质的亚细胞定位还需要探索,如何精确控制花香释放的节律性[84]还有待于进一步研究。此外,由于大多数植物的花香化合物是受多个酶共同调控的复杂网络[13],试图改造某个酶或基因达到改良花香性状的可能性极低[64]。随着今后对植物花香代谢网络的构成、酶的亚细胞定位、竞争途径、代谢渠道、通量控制环节和可能的反馈控制等方面的深入研究,基因工程改良植物花香才能成为现实。

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