花香的生物合成、调控及基因工程研究进展

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摘要　　花香能提高观赏植物的审美特性, 并且在植物的繁衍中起着重要作用。近年来, 随着分子生物学的发展, 花香分子水平的研究呈现加速发展的趋势, 已成为当前的一大研究热点。该文主要论述了花香的生物合成途径及关键酶基因、分子水平的调控和共调控探索、花香基因工程策略, 以期为花香性状改良提供参考。

关键词　　花香; 生物合成; 分子调控; 共调控; 基因工程

植物的花香是一系列低分子量挥发性物质的混合物。一般认为, 花香能够吸引传粉昆虫和抵制草食性动物[1-2]。它不但对很多植物的生殖具有重要作用, 而且在植物染病后, 可以作为免疫信号激起防卫反应, 从而确保植物的产量和质量[3]。此外, 从植物花香挥发物中提取的香精香料也是很多轻工业和化妆品的重要原料, 具有重要的应用价值[4]。

与花色、花型等其它花朵性状相比, 花香的研究相对滞后。直到近几年, 花香的研究才逐渐深入, 花香的物质成分及其生物合成途径逐渐明确[5-6], 生物合成途径中一些关键酶基因相继被研究和应用[7]。现实中, 一些观赏价值很高的花卉, 如蝴蝶兰(*Phalaenopsis amabilis*)、文心兰(*Oncidium luridum*)等, 虽然色泽鲜艳, 但缺少香味。如何利用基因工程技术调控甚至改造植物花香挥发物, 从而提高花香质量和增强植物防御能力[8-11], 已成为当前植物花香研究的热点。

1 花香的生物合成途径、关键酶及关键酶基因

目前, 已知的花香挥发物大约有2 000多种[12], 分为3大类, 即萜烯类化合物(terpenoids)、苯丙酸类化合物/苯环型化合物(benzenoids/phenylpropanoids)和脂肪酸衍生物(aliphatic compounds)[13]。花香挥发物被认为是次级代谢物的一部分, 它们只在特定植物种类中产生或者只控制植株的特定形态功能, 而初级代谢产物则分布广泛。但是, 初级代谢途径和次级代谢途径不能完全分开。大多情况下, 次级代谢是初级代谢的一个末端分支。目前, 主要研究的是萜烯类化合物和苯丙酸类化合物/苯环型化合物。

1.1 萜烯类化合物

在萜烯类化合物中, 最常见的是单萜和倍半萜, 均由若干个异戊二烯(C5)单元组成。这些C5单元的生物合成由细胞质中的甲瓦龙酸(mevalonic acid, MVA)途径和质体中的甲基赤藓糖醇(methylerythri-tol)途径组成。一分子C5化合物异戊烯二磷酸(iso-pentenyl diphosphates, IPP)和一分子C5化合物二甲基烯丙基焦磷酸酯(dimethylallydiphosphate, DMAPP)在GPP合酶催化下生成具有C10骨架的牻牛儿基焦磷酸酯(geranyl pyrophosphate, GPP)[5], 作为合成单萜的前体[14]。两分子的IPP和一分子的DMAPP在FPP合酶催化下, 合成具有C15骨架的法呢基焦磷酸酯(farnesyl pyrophosphate, FPP), 作为合成倍半萜的前体[15]。大部分单萜类和倍半萜类化合物只需前体的一步反应就能合成, 这为导入外源基因、生成新的芳香化合物提供了可能(图1A)。

1.2 苯丙酸类化合物/苯环型化合物

苯丙酸类化合物是以苯丙氨酸(phenylalanine)为起点, 经过一系列复杂的分支途径合成, 合成产物大多不具有挥发性, 只有在C9位上酰基化或甲基化, 才具有芳香。苯环型化合物则依赖于苯丙酸类

途径中的一个分支途径——肉桂酸(cinnamic acid)途径, 具有挥发性[16]。苯环型化合物和一些苯丙氨酸类化合物合成的第一步是在苯丙氨酸解氨酶(pheammonialyase, PAL)的催化下, 苯丙氨酸生成反式肉桂酸。然后, 反式肉桂酸通过依赖乙酰辅酶A的β氧化(CoA-dependent β-oxidative)途径和不依赖于乙酰辅酶A的非β氧化途径(CoA-independent non-

β-oxidative)减去两个C原子, 形成苯环型化合物[6]。苯丙酸类化合物/苯环型化合物的合成途径一般需要几步反应才能完成, 要生成新的芳香化合物, 单靠导入某个关键酶基因很难达到目的(图1B)。

1.3 脂肪酸衍生物

脂肪酸衍生物包括小分子的醇类、醛类和脂类。合成途径主要通过多烯脂肪酸类氧化(oxidized
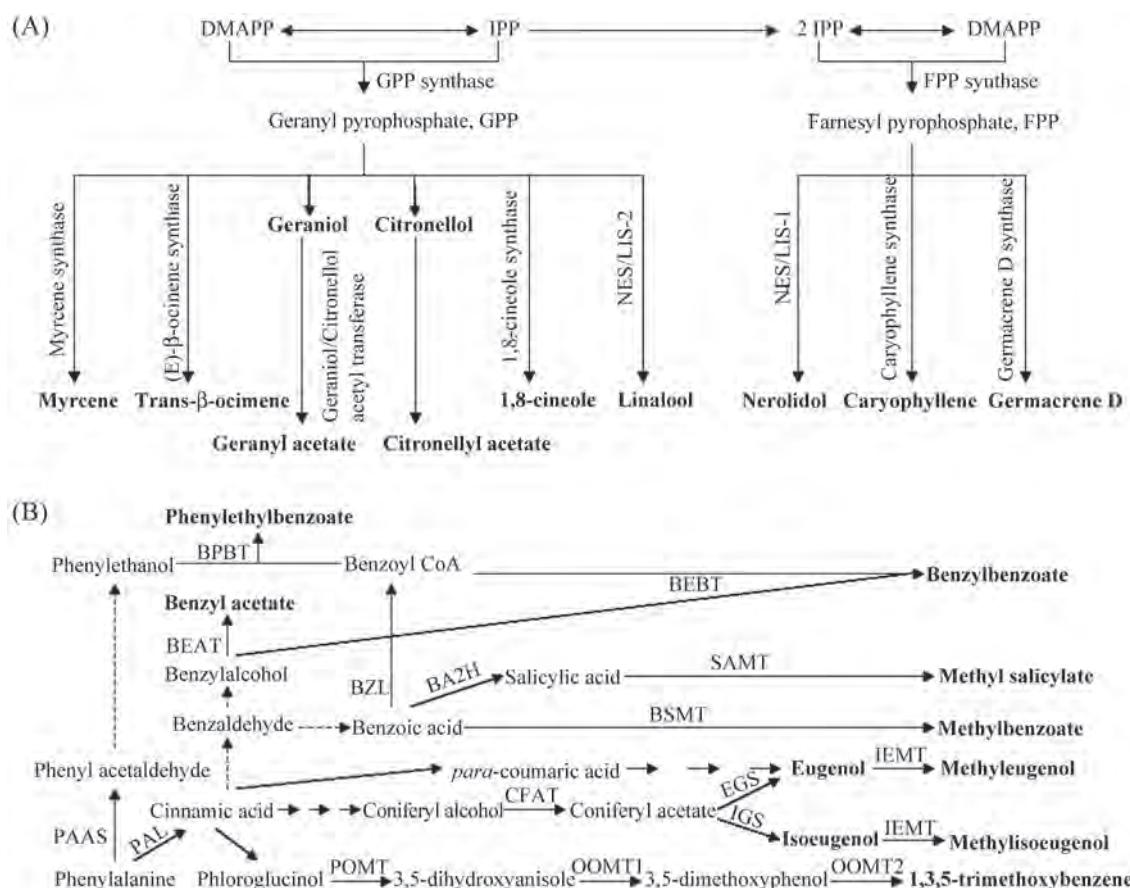
A: 单萜烯和倍半萜烯合成途径; B: 苯丙酸类/苯环型化合物合成途径。BA2H: 苯甲酸2 -羟化酶; BEAT: 苯甲醇乙酰基转移酶; BEBT: 苯甲醇甲酰基转移酶; BPBT: 苯甲醇/苯乙醇苯甲酰基转移酶; BSMT: 苯甲酸/水杨酸羧位甲基转移酶; BZL: 苯甲酸:辅酶A连接酶; CFAT: 松柏醇酰基转移酶; EGS: 丁子香酚合成酶; IEMT: 丁子香酚甲基转移酶; IGS: 异丁子香酚合成酶; NES/LIS: 橙花叔醇/芳樟醇合成酶; OOMT: 苔黑粉氧位甲基转移酶; PAAS: 苯乙醛合成酶; PAL: 苯丙氨酸解氨酶; POMT: 间苯三酚氧位甲基转移酶; SAMT: 水杨酸羧位甲基转移酶。实线表示已确定的反应, 虚线表示可能的反应; 文字加粗部分表示最终的花香挥发物。

A: the biosynthesis of monoterpenes and sesquiterpenes; B: the biosynthesis of phenylpropanoids/benzenoids. BA2H: benzoic acid 2-hydroxylase; BEAT: acetyl CoA:benzylalcohol acetyltransferase; BEBT: benzoyl-CoA:benzyl alcohol benzoyl transferase; BPBT: benzoyl-CoA:benzyl alcohol/phenylethanol benzoyl transferase; BSMT: S-adenosyl-L-methionine:benzoic acid/salicylic acid carboxyl methyltransferase; BZL: benzoate:CoA ligase; CFAT: coniferyl alcohol acyltransferase; EGS: eugenol synthase; IEMT: S-adenosyl-L-methionine:(iso)eugenol O-methyltransferase; IGS: isoeugenol synthase; NES/LIS: nerolidol/linalool synthases; OOMT: orcinol O-methyltransferases; PAAS: phenylacetaldehyde synthase; PAL: phenylalanine ammonialyase; POMT: phloroglucinol O-methyltransferase; SAMT: S-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase. Solid lines indicate established biochemical reactions, and broken lines indicate possible steps for which enzymes have not yet been characterized. The floral scent volatiles are shown in bold.

图1 花香挥发物的生物合成途径

Fig.1 Pathways that lead to floral scent volatiles

polyenoic fatty acids)和随后的脂肪氧合酶途径(the lipoxygenase pathway)[17]。近年来，脂肪氧合酶途径中的一些基因被分离和鉴定[18]。但是，到目前为止尚没有基因被克隆的报道。

1.4 花香生物合成过程的关键酶及基因

在花香生物合成途径的关键酶中，芳樟醇合酶(linalool synthase, LIS)是第一个被分离纯化的酶[19-20]。LIS能催化GPP合成芳樟醇，没有其他副产物，被认为是单一产物酶。LIS单一产物酶的特性在矮牵牛(*Petunia hybrida*)和康乃馨(*Dianthus caryophyllus*)中得到了进一步证实[21-22]。单一产物酶的研究为新合成某一特定的花香挥发物提供了可能。与LIS相反，桉叶素合酶(cineol synthase, CIN)为多产物酶。在烟草(*Nicotiana suaveolens*)中，CIN催化GPP合成的主产物为桉叶素，与其伴随产生的还有七种副产物，即反式β罗勒、α蒎烯、β蒎烯、月桂烯、柠檬烯(limonene)、桉烯(sabinene)和α松油烯(α-terpineole)[23]。多产物酶的发现为同时释放几种花香挥发物提供了思路。除LIS和CIN之外，花香生物合成中的很多关键酶被分离纯化[6,24-25]，这些关键酶的基因也相继被克隆和研究(表1)。

2 花香生物合成的调控

2.1 分子水平的调控

花香挥发物在植物特定组织中合成，其合成部位即为芳香物释放部位。花瓣是产生花香最主要

表1 花香生物合成过程中的关键酶基因
Table 1 Floral scent-synthesizing genes

关键酶基因 Genes	参考文献 References
Clarkia breweri linalool synthase, *CbLIS*	[20]
Arabidopsis thaliana linalool synthase, *AtLIS*	[61]
Antirrhinum majus ocimene synthase, *AmOCS*	[24]
A. majus myrcene synthase, *AmMYR*	
A. majus nerolidol/linalool synthases-1, *AmNES/LIS-1*	[36]
A. majus nerolidol/linalool synthases-2, *AmNES/LIS-2*	
Nicotiana suaveolens cineol synthase, *NsCIN*	[23]
Rosa hybrida geraniol/citronellol acetyl transferase	[28]
A. thaliana caryophyllene synthase	[61]
R. hybrida germacrene D synthase, *RhGDS*	[62]
C. breweri S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferas, *CbSAMT*	[63]
Stephanotis floribunda S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferas, *SfSAMT*	[34]
A. majus S-adenosyl-L-methionine:benzoic acid carboxyl methyltransferase, *AmBAMT*	[64]
Petunia hybrida S-adenosyl-L-methionine:benzoic acid/salicylic acid carboxyl methyltransferase, *PhBSMT*	[37,65]
A. thaliana S-adenosyl-L-methionine:benzoic acid/salicylic acid carboxyl methyltransferase, *AtBSMT*	[66]
N. suaveolens S-adenosyl-L-methionine:benzoic acid/salicylic acid carboxyl methyltransferase, *NsBSMT*	[33]
C. breweri acetyl-CoA:benzylalcohol acetyltransferase, *CbBEAT*	[67]
C. breweri benzoyl-CoA:benzylalcohol benzoyltransferase, *CbBEBT*	[64]
P. hybrida benzoyl-CoA:benzyl alcohol/phenylethanol benzoyl transferase, *PhBPBT*	[6]
P. hybrida coniferyl alcohol acetyltransferase, *PhCFAT*	[47]
Petunia axillaris parodii coniferyl alcohol acetyltransferase, *PapCFAT*	[68]
Rosa Chinensis phloroglucinol O-methyltransferase, *RcPOMT*	[30]
R. hybrida orcinol O-methyltransferases-1, *RhOOMT-1*	[29]
R. hybrida orcinol O-methyltransferases-2, *RhOOMT-2*	
C. breweri S-adenosyl-L-methionine:(iso)eugenol O-methyltransferase, *CbIEMT*	[32]
Ocimum basilicum eugenol synthase1, *ObEGS1*	[69]
P. hybrida isoeugenol synthase1, *PhIGS1*	
C. breweri eugenol/isoeugeno synthase1,CbEGS1、*CbIGS1*	[70]
P. axillaris parodii eugenol/isoeugeno synthase1, *PapEGS*、*PapIGS*	[68]
Rosa×damascena Mill phenylacetaldehyde reductase, *rose-PAR*	[71]

的组织, 一些基因只在花瓣中特异表达, 如非洲茉莉(Stephanotis floribunda)中的SfSAMT和金鱼草(Antirrhinum majus)中的AmBAMT[26-27]。还有一些基因, 虽然在其它组织中也有表达, 但在花瓣中表达水平最高, 如仙女扇(Clarkia breweri)中的CbLIS、CbIEMT、CbBEAT、CbSAMT, 月季(Rosa hybrid)中的RhPOMT、RhOOMT、RhAAT、RhGDS[19,28-32]。在细胞水平上, 花香相关基因在花器官的表皮细胞中表达, 以便其表达产物快速挥发到空气中去[32-34]。Rohrbec等[35]利用细胞免疫定位方法检测到花香挥发物的相关酶大多集中在表皮细胞和膜上。在亚细胞水平上, 花香基因表达和挥发物合成部位的研究较少。Nagegowda等[36]采用功能基因组学方法, 研究金鱼草的两个具有95%同源性的橙花叔醇和芳樟醇合成酶基因(AmNES/LIS-1 & AmNES/LIS-2), 结果表明, AmNES/LIS-1在细胞质中表达产生橙花叔醇(Nerolidol), AmNES/LIS-2在质体中表达产生芳樟醇(Linalool)。

花香挥发物的生物合成还存在发育调节(开花期香味达到最高峰)和昼夜周期调节, 这主要受相关基因的转录水平和翻译后水平调控[37-40]。白天释放挥发性物质的金鱼草(A. majus)和夜间释放挥发物质的非洲茉莉(S. floribunda)、烟草(N. suaveolens)的主要挥发性物质均为苯甲酸甲酯。研究表明, 苯甲酸甲酯的释放节律与三种甲基转移酶基因(AmBAMT、SfSAMT和NsBSMT) mRNA的水平波动一致[27,34,40]。在刚开花时, NsBSMT的转录水平达到最高[27]。在开花后的第四天, AmBAMT的转录水平达到最高, 为苯甲酸甲酯的释放最高期[26]。甲基转移酶的蛋白质水平与苯甲酸甲酯释放节律没有显著相关性[39], 但是蛋白质的甲基化会随着释放节律的波动而波动[33,40]。

花香挥发物的表达还受到酶底物量和相对浓度的调节[5]。当底物缺乏时, 即使导入关键酶基因, 也不能合成相应挥发物[41]。Aranovich等[41]将从仙女扇中克隆的BEAT基因导入洋桔梗(Eustoma grandiflorum), 虽然在转基因植株中检测到BEAT基因的表达和相应酶的活动, 却没有相应挥发物的产生, 而在加入外源底物苯甲醇的情况下, 转化植株的花和叶中都有乙酸苄酯的产生。在月季花瓣中, 乙醇乙酰基转移酶(alcohol acetyltransferase, RhAAT)的表达表现出昼夜节律, 白天合成乙酸香叶酯(geranyl acetate), 夜晚不合成, 好像被生物钟控制, 但当底物香

叶醇(geraniol)短缺时, 即使连续光照, 其产物也不再合成[42]。还有一种情况, 当某一类酶, 如甲基转移酶(methyltransferase)或乙酰基转移酶(acyltransferases)等, 可以催化多种底物时, 各种底物的浓度往往能决定最终产物的种类和代谢物的流向, 从而最终决定花香是否产生以及花香的类型[6,37]。例如: 水杨酸羧基位甲基转移酶(S-adenosyl-L-methionine: salicylic acid carboxyl methyltransferas, SAMT)虽然在体外对水杨酸有很高的催化作用, 但在非洲茉莉(S. floribund)中, SAMT是以甲基化苯甲酸为底物, 因为在非洲茉莉的花组织中水杨酸底物量不充足[27,33]。

2.2 共调控探索

2.2.1 花香与花色共调控 花香与花色共调控的一个可能机制是通过对上游途径的调控, 改变代谢流, 使得下游调控产生叠加作用[43]。例如, 莽草酸途径是苯环型化合物和花青素合成的上游途径, 通过对莽草酸途径的调控, 可以达到花香花色共调控的目的[44]。MYB (V-myb avian myeloblastosis viral oncogene homolog)转录因子是植物中最重要的转录因子之一, 能够调控多种代谢途径, 在莽草酸生物合成途径中起重要作用[45]。Ben Zvi等[44]将拟南芥MYB转录因子pap1 (production of anthocyanin pigment 1)导入矮牵牛中, 使pap1过表达。结果表明, 转基因植株在夜间产生的挥发性苯环型化合物含量增加了10倍, 花色也增深。与此同时, 苯环型化合物和花青素的合成前体——苯丙酸的利用率增加, 由原来的30%增加到57%。这说明pap1的过表达对苯丙酸的调控产生叠加作用, 并且pap1在植物生长前期即花青素合成以前就已表达。Verdonk等[38]利用RNAi技术沉默矮牵牛的MYB转录因子ODORANT1 (Odo1), Odo1属于MYB转录因子R2R3类型, 结果导致苯环型花香挥发物苯甲酸苄酯(benzyl benzoate)、乙酸苄酯(benzyl acetate)和异丁子香酚(isoeugenol)的合成受阻, 而花青素途径不受影响, 花色仍然保持原有的紫色没有改变。这可能是由于花青素在开花前期已合成, 而Odo1在花成熟后才开始表达, 使得Odo1对花青素合成没有影响。

花香花色共调控的另一个可能机制是花香与花色的生物合成途径存在竞争关系, 且来自共同的代谢途径, 通过抑制某一途径改变代谢流使得另一途径过表达[46]。苯甲酸和花青素均来自苯丙酸类代谢途径, 而类黄酮3-羟化酶(flavonoid 3-hydroxylase,

F3H)是形成花青素的一个关键酶。Zuker等[47]将反义F3H序列转入康乃馨, 大大减弱了转基因植株中F3H基因的表达和类黄酮3-羟化酶的活性, 封锁了花青素合成途径。结果显示, 花色由原来的橘红色变淡或消失, 香味挥发物苯甲酸的释放量却大大增加, 并且这种香味的改变能被嗅觉所感知。

2.2.2　花香与植物激素共调控
目前, 花香与植物激素共调控的研究较少, 植物激素信号传导的负调控以及两化合物之间存在并联途径是可能的调控机理。在矮牵牛(P. hybrida)花上外施乙烯, 能使七种芳香挥发物的释放量减少, 其中包括苯甲酸甲酯。施用10小时后, 花的导管、气孔等部位PhBSMT1、PhBSMT2、PhCFAT的mRNA水平降低[48-49]。鉴于传粉后, 花的不同部位产生乙烯[50]。由此推测, 花通过乙烯信号的传导产生授粉后调控, 其中包括花香生物合成与释放的负调控[48]。Orlova等[51]利用RNAi技术沉默了矮牵牛的BPBT基因, 导致转基因植株挥发物中缺少苯甲酸苯乙酯(phenylethylbenzoate)和苯甲酸苄酯(benzylbenzoate)。有趣的是, 在BPBT表达受到抑制的同时, 转基因植株的外部形态也发生了改变, 花叶增大、茎增粗、节间增长、植物激素量增加。推测苯丙酸合成途径与植物激素调控间存在一定的联系, 苯丙酸生物合成与植物激素转录增强可能存在并联途径, 使得BPBT基因的沉默改变了代谢流途径, 导致植物激素量增加。

共调控为植物研究者改良花的性状提供了新的思路和技术策略, 但在分子水平上, 花香生物合成的调控、信号机理及共调控机理尚需进一步明确[44]。值得我们思考的是, 在共调控的竞争途径调控中, 代谢流重新分配虽不会对植物产生有害的影响, 但代谢流从一种途径转向另一途径减少了植物基本化合物的合成, 会降低植物的生存能力和健康状况[52]; 在花香挥发物合成改变的同时, 植物其它性状的改变, 如花色增深、植物激素量增加, 是否是人们所期望的, 是否会对植物的生长发育有负面影响, 需要做进一步的研究和验证。

3　植物花香基因工程的策略
3.1　引入外源基因
通过引入外源的花香生物合成相关基因, 产生新的芳香挥发物。引入外源基因, 目前的方法主要有三个: (1) 引入单个外源基因。将从仙女扇中克隆

的LIS基因导入矮牵牛[21]和康乃馨[22]中, 结果表明, 有芳樟醇的产生。但是人的嗅觉感觉不到芳香的改变, 因为矮牵牛中挥发性的芳樟醇大多被转化为非挥发性的芳樟醇的配糖[21], 康乃馨中产生的挥发性芳樟醇的量没有达到人类嗅觉的阈值或芳樟醇香味被其它芳香物覆盖[22]; (2) 同时引入多个外源基因。Lücker等[53]将3个柠檬单萜合成酶基因导入烟草中, 这些酶以GPP为底物, 在35S启动子的调控下, 合成β-蒎烯、柠檬烯、γ-萜品烯(γ-terpinene)等目的芳香挥发物, 并且这些香味可以被人的嗅觉感知; (3) 依次引入多个外源基因, 产生多步反应。Lücker等[54]将留兰香(Mentha spicata)中克隆到的柠檬烯-3-羟化酶(limonene-3-hydroxylase)的cDNA导入上述已转入3个单萜合成酶基因的烟草中。经过两步酶调控反应, 新合成的柠檬烯在柠檬烯-3-羟化酶催化下, 形成了反式异薄荷烯醇(trans-isopiperitenol)目的挥发物和一些非目的挥发物。

引入外源基因在植物花香基因工程的研究中有了一些成功的应用, 但也存在着一些问题: 一是转化植株中会产生不期望的代谢途径, 释放非目的挥发物[53-54]; 二是转化植株的芳香挥发物可能会被修饰成非挥发性的物质[53]; 三是转基因植株即便产生了挥发性物质, 也可能不被人类的嗅觉所感知[22]。通过引入外源基因改良花香性状需要进一步完善。

3.2　调控内源基因
植物内源的花香生物合成相关基因表达增强或减弱, 使原有花香挥发物的量增加或减少。花香挥发物释放量增加的方法主要是阻断花香生物合成的竞争代谢途径, 改变代谢流的流向, 从而增加目的产物的合成[47]; 花香挥发物释放量减少的方法主要是通过基因沉默, 阻断一些原有花香挥发物的产生[48,55]。目前, 调控内源基因应用的主要方法有: (1) 反义抑制。Zuker等[47]将反义F3H序列转入康乃馨, 封锁了花青素合成途径, 使花色橘红色变浅, 花香成分苯甲酸的释放量大大增加; (2) 病毒介导的基因沉默(VIGS)。Spitzer等[55]以花青素途径中的矮牵牛查耳酮合酶(chalcone synthase, CHS)基因为报告基因, 利用VIGS技术分别沉默矮牵牛的BSMT基因、PAAS基因和ODORANT1基因, 使BSMT基因表达产物水杨酸甲酯(methylsalicylate)释放量减少10倍, 苯甲酸甲酯(methylbenzoate)的释放量减少7倍, PAAS基因表达产物苯乙醛(phenylacetaldehyde)和ODOR-

*ANTI*基因表达产物苯丙酸类化合物的释放量也都显著降低; (3) RNAi技术。Underwood等[48]利用RNA干涉技术沉默了矮牵牛中*BSMT*基因, 导致转基因植株缺少主要的花香产物苯甲酸甲酯。这种改变能轻易地被人类的嗅觉捕捉。RNAi技术在矮牵牛的*PAAS*、*CFAT*基因上同样得到了成功的应用[49,56]。

调控内源基因表达能够比较有效地控制植物花香挥发物的释放, 这一点已经被证明。但是, 植物体中的挥发物质具有防御外来侵害和吸引昆虫传粉的作用, 通过调控内源基因使挥发物丧失, 这是否会引起植物抵抗力的降低, 或者是否会引起授粉率的降低, 从而影响植物自身的生长和繁衍, 需要进一步探讨[57]。

3.3 转录因子的调控

转录因子能够激活或抑制某些基因的转录, 从而控制不同合成途径的多步反应。(1) 引入外源转录因子。Ben Zvi等[44]通过导入外源转录因子MYB-*pap1*, 使矮牵牛花香与花色生物合成的下游调控产生叠加作用, 达到改变挥发物释放量和花色的目的; (2) 抑制内源转录因子。Spitzer-Rimon等[58]利用VIGS技术抑制矮牵牛转录因子MYB-*EOBII* (emission of benzenoids II), 使整个苯丙酸途径合成受阻, 导致花香挥发物如丁子香酚、苯甲酸苄酯等释放量显著降低。转录因子调控花香的研究虽然刚刚起步, 却为花香的基因工程提供了一个新的策略。

4 展望

近年来, 花香受到越来越多植物研究者的关注。在延长鲜花开放时间及采后花的寿命选育过程中, 大量香味基因型正在逐步消失[59]。这为植物的花香研究提出了新的问题, 同时也为花香研究开辟了新的领域。随着基因工程技术的发展和成熟, 将基因工程与花香研究结合起来, 成为破解当前花香研究困境的一条捷径。目前, 植物花香物质代谢途径的研究正逐步深入, 代谢途径中关键酶基因相继被克隆和分析, 这些成果为植物花香基因工程的发展提供了坚实的理论基础。分子水平调控和共调控的进一步探索, 为花香的基因工程提供了新的思路。Zuker[47]、Ben Zvi[44]、Lücker[53-54]等学者的研究初步证明了这些思路的可行性。花香转录因子策略的尝试, 为同时改变多种花香挥发物提供了可能, Verdonk[38]、Ben Zvi[44]和Spitzer-Rimon[58]等的试验显示

了转录因子策略的应用价值。随着研究的进一步深入, 相信花香基因工程领域将会取得更多的成果。

但是, 我们也要认识到, 植物花香基因工程的发展还受到一些瓶颈的制约。比如, 酶亚细胞的定位、花香生物合成的竞争途径、各代谢途径之间的内在联系和可能的反馈调控等尚未明确。这在一定程度上制约了花香基因工程的进展, 使得花香的性状改良有时不能取得预期的结果[60], 产生不期望的非目的产物[21-22]。在今后的工作中, 我们还需要采用传统方法和高通量方法阐明更多的香味物质合成途径及与其他途径的内在联系, 进一步研究酶亚细胞定位和分子水平的调控机理, 为花香基因工程提供更加完善的理论基础。

参考文献 (References)

1   Knudsen ET, Tollsten L. Trends in floral scent chemistry in pollination syndromes: Floral scent composition in mothpollinated taxa. Bot J Linn Soc 1993; 113(3): 263-84.

2   Paré PW, Tumlinson JH. *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. Plant Physiol 1997; 114(4): 1161-7.

3   Shulaev V, Silverman P, Raskin I. Airborne signaling by methyl salicylate in plant pathogen resistance. Nature 1997; 385(6618): 718-21.

4   冯立国, 周 力, 陶 俊, 孟祥申. 慧兰花香成分研究. 安徽农业科学 2009; 37(35): 17465-6.

5   Tholl D, Kish CM, Orlova I, Sherman D, Gershenzon J, Pichersky E, *et al*. Formation of monoterpenes in *Antirrhinum majus* and *Clarkia breweri* flowers involves heterodimeric geranyl diphosphate synthases. Plant Cell 2004; 16(4): 977-92.

6   Boatright J, Negre F, Chen X, Kish CM, Wood B, Peel G, *et al*. Understanding *in vivo* benzenoid metabolism in petunia petal tissue. Plant Physiol 2004; 135(4): 1993-2011.

7   Dudareva N, Pichersky E. Metabolic engineering of floral scent of ornamentals. J Crop Improvement 2006; 18(1/2): 325-46.

8   Lücker J, Verhoeven HA, van der Plas LHW, Bouwmeester HJ. In Biology of Floral Scent. Florida: CRC Press, 2010: 321-37.

9   Pichersky E, Dudareva N. Scent engineering: Toward the goal of controlling how flowers smell. ELSEVIER 2007; 25(3): 105-10.

10 Degenhardt J, Gershenzon J, Baldwin IT, Kessler A. Attracting friends to feast on foes: Engineering terpene emission to make crop plants more attractive to herbivore enemies. Curr Opin Biotechnol 2003; 14(2): 169-76.

11 Aharoni A, Jongsma MA, Bouwmeester HJ. Volatile science? Metabolic engineering of terpenoids in plants. Trends Plant Sci 2005; 10(12): 594-602.

12 Dunkel M, Schmidt U, Struck S, Berger L, Gruening B, Hossbach J, *et al*. Super Scent-a database of flavours and scents.

Nucleic Acid Res 2009; 37: D291-4.

13   Croteau R, Kutchan TM, Lewis NG. Natural products: Secondary metabolites/biochemistry and molecularbiology of plants. Michigan Academician: ASPP 2000; 1250-318.

14   Chang TH, Hsieh FL, Ko TP, Teng KH, Liang PH, Wang AH. Structure of a heterotetrameric geranyl pyrophosphate synthase from Mint (*Mentha piperita*) reveals intersubunit regulation. Plant Cell 2010; 22(2): 454-67.

15   Xiang L, Zhao K, Chen L. Molecular cloning and expression of *Chimonanthus praecox* farnesyl pyrophosphate synthase gene and its possible involvement in the biosynthesis of floral volatile sesquiterpenoids. Plant Physiol Bioch 2010; 48(10/11): 845-50.

16   Knudsen JT, Tollsten L. Trends in floral scent chemistry in pollination syndromes-floral scent composition in moth-pollinated taxa. Bot J Linn Soc 1993; 113(3): 263-84.

17   Feussner I, Wasternack C. Lipoxygenase catalyzed oxygenation of lipids. Fett-Lipid 1998; 100(4/5): 146-52.

18   Feussner I, andWasternack C. The lipoxygenase pathway. Annu Rev Plant Biol 2002; 53: 275-97.

19   Pichersky E, Lewinsohn E, Croteau R. Purification and characterization of S-linalool synthase, an enzyme involved in the production of floral scent in *Clarkia breweri*. Arch Biochem Biophys 1995; 316(2): 803-7.

20   Dudareva N, Cseke L, Blanc VM, Pichersky E. Evolution of floral scent in Clarkia: Novel patterns of S-linalool synthase gene expression in the *C. breweri* flower. Plant Cell 1996; 8(7): 1137-48.

21   Lücker J, Bouwmeester HJ, Schwab W, Blaas J, van der Plas LH, Verhoeven HA. Expression of Clarkia S-linalool synthase in transgenic petunia plants results in the accumulation of S-linalyl-beta-D-glucopyranosid. Plant J 2001; 27(4): 315-24.

22   Lavy M, Zuker A, Lewinsohn E, Larkov O, Ravid U, Vainstein A, *et al*. Linalool and linalool oxide production in transgenic carnation flowers expressing the *Clarkia breweri* linalool synthase Gene. Mol Breeding 2002; 9(2): 103-11.

23   Roeder S, Hartmann AM, Effmert U, Piechulla B. Regulation of simultaneous synthesis of floral scent terpenoids by the 1,8-cineole synthase of *Nicotiana suaveolens*. Plant Mol Biol 2007; 65(1/2): 107-24.

24   Dudareva N, Martin D, Kish CM, Kolosova N, Gorenstein N, Fäldt J, *et al*. (*E*)-*β*-Ocimene and myrcene synthase genes of floral scent biosynthesis in snapdragon: Function and expression of three terpene synthase genes of a new TPS-subfamily. Plant Cell 2003; 15(5): 1227-41.

25   D'Auria JC, Chen F, Pichersky E. Characterization of an acyltransferase capable of synthesizing benzylbenzoate and other volatile esters in flowers and damaged leaves of *Clarkia breweri*. Plant Physiol 2002; 130(1): 466-76.

26   Dudareva N, Murfitt LM, Mann CJ, Gorenstein N, Kolosova N, Kish CM, *et al*. Developmental regulation of methyl benzoate biosynthesis and emission in snapdragon flowers. Plant Cell

2000; 12(6): 949-61.

27   Effmert U, Saschenbrecker S, Ross J, Negre F, Fraser CM, Noel JP, *et al*. Floral benzenoid carboxyl methyltransferases: From *in vitro* to in planta function. Phytochemistry 2005; 66(11): 1211-30.

28   Shalit M, Guterman I, Volpin H, Bar E, Tamari T, Menda N, *et al*. Volatile ester formation in roses: Identification of an acetyl-CoA: Geraniol/Citronellol acetyltransferase in developing rose petals. Plant Physiol 2003; 131(4): 1868-76.

29   Wu SQ, Watanabe N, Mita S, Dohra H, Ueda Y, Shibuya M, *et al*. The key role of phloroglucinol *O*-methyltransferase in the biosynthesis of *Rosa chinensis* volatile 1,3,5-trimethoxybenzene. Plant Physiol 2004; 135(1): 95-102.

30   Lavid N, Wang JH, Shalit M, Guterman I, Bar E, Beuerle T, *et al*. O-methyltransferases involved in the biosynthesis of volatile phenolic derivatives in rose petals. Plant Physiol 2002; 129(4): 1899-907.

31   Dudareva N, Raguso RA, Wang JH, Ross JR, Pichersky E. Floral scent production in *Clarkia breweri*. III. Enzymatic synthesis and emission of benzenoid esters. Plant Physiol 1998; 116(2): 599-604.

32   Wang J, Dudareva N, Bhakta S, Raguso RA, Pichersky E. Floral scent production in *Clarkia breweri* (Onagraceae) II. Localization and developmental modulation of the enzyme SAM: (Iso) Eugenol O-methyltransferase and phenylpropanoid emission. Plant Physiol 1997; 114(1): 213-21.

33   Pott MB, Hippauf F, Saschenbrecker S, Chen F, Ross J, Kiefer I, *et al*. Biochemical and structural characterization of benzenoid carboxyl methyltransferases involved in floral scent production in *Stephanotis floribunda* and *Nicotiana suaveolens*. Plant Physiol 2004; 135(4): 1946-55.

34   Pott MB, Pichersky E, Piechulla B. Evening specific oscillations of scent emission, SAMT enzyme activity, and SAMT mRNA in flowers of *Stephanotis floribunda*. J Plant Physiol 2002; 159(8): 925-34.

35   Rohrbeck D, Buss D, Effmert U, Piechulla B. Localization of methyl benzoate synthesis and emission in *Stephanotis floribunda* and *Nicotiana suaveolens* flowers. Plant Biol 2006; 8(5): 615-26.

36   Nagegowda DA, Gutensohn M, Wilkerson CG, Dudareva N. Two nearly identical terpene synthases catalyze the formation of nerolidol and linalool in snapdragon flowers. Plant J 2008; 55(2): 224-39.

37   Negre F, Kish CM, Boatright J, Underwood B, Shibuya K, Wagner C, *et al*. Regulation of methylbenzoate emission after pollination in snapdragon and petunia flowers. Plant Cell 2003; 15(12): 2992-3006.

38   Verdonk JC, Haring MA, van Tunen AJ, Schuurink RC. *ODORANT1* regulates fragrance biosynthesis in petunia flowers. Plant Cell 2005; 17(5): 1612-24.

39   Effmert U, Dinse C, Piechulla B. Influence of green leaf her-

bivory by Manduca sexta on floral volatiles emission by *Nicotiana suaveolens*. Plant Physiol 2008; 146(4): 1996-2007.

40 Kolosova N, Gorenstein N, Kish CM, Dudareva N. Regulation of circadian methyl benzoate emission in diurnally and nocturnally emitting plants. Plant Cell 2001; 13(10): 2333-47.

41 Aranovich D, Lewinsohn E, Zaccai M. Post-harvest enhancement of aroma in transgenic lisianthus (*Eustoma grandiflorum*) using the *Clarkia breweri* benzyl alcohol acetyltransferase (BEAT) gene. Postharvest Biol Technol 2007; 43(2): 255-60.

42 Hendel-Rahmanim K, Masci T, Vainstein A, Weiss D. Diurnal regulation of scent emission in rose flowers. Planta 2007; 226(6): 1491-9.

43 Majetic CJ, Rausher MD, Raguso RA. The pigment-scent connection: Do mutations in regulatory vs. structural anthocyanin genes differentially alter floral scent production in *Ipomoea purpurea*? S Afr J Bot 2010; 76(4): 632-42.

44 Ben Zvi MM, Negre-Zakharov F, Masci T, Ovadis M, Shklarman E, Ben-Meir H, *et al*. Interlinking showy traits: Co-engineering of scent and colour biosynthesis in flowers. Plant Biotechnol J 2008; 6 (4): 403-15.

45 Azuma A, Kobayashi S, Mitani N, Shiraishi M, Yamada M, Ueno T, *et al*. Genomic and genetic analysis of Myb-related genes that regulate anthocyanin biosynthesis in grape berry skin. Theor App1 Genet 2008; 117(6): 1009-19.

46 Xiang L, Milc JA, Pecchioni N, Chen LQ. Genetic aspects of floral fragrance in plants. Biochemistry-Moscow 2007; 72(4): 351-8.

47 Zuker A, Tzfira T, Ben-Meir H, Ovadis M, Shklarman E, Itzhaki H, *et al*. Modification of flower color and fragrance by antisense suppression of the flavanone 3-hydroxylase gene. Mol Breeding 2002; 9(1): 33-41.

48 Underwood BA, Tieman DM, Shibuya K, Dexter RJ, Loucas HM, Simkin AJ, *et al*. Ethylene-regulated floral volatile synthesis in petunia corollas. Plant Physiol 2005; 138(1): 255-66.

49 Dexter R, Qualley A, Kish DM, Ma CJ, Koeduka T, Nagegowda DA, *et al*. Characterization of a petunia acetyltransferase involved in the biosynthesis of the floral volatile isoeugenol. Plant J 2007; 49(2): 265-75.

50 Jones ML, Woodson WR. Interorgan signaling following pollination in carnations. J Am Soc Hort Sci 1999; 124(6): 598-604.

51 Orlova I, Marshall-Colon A, Schnepp J, Wood B, Varbanova M, Fridman E, *et al*. Reduction of benzenoid synthesis in petunia flowers reveals multiple pathways to benzoic acid and enhancement in auxin transport. Plant Cell 2006; 18(12): 3458-75.

52 Aharoni A, Giri AP, Deuerlein S, Griepink F, de Kogel WJ, Verstappen FW, *et al*. Terpenoid metabolism in wild-type and transgenic Arabidopsis plants. Plant Cell 2003; 15(12): 2866-84.

53 Lücker J, Schwab W, van Hautum B, Blaas J, van der Plas LH, Bouwmeester HJ, *et al*. Increased and altered fragrance of tobacco plants after metabolic engineering using three monoterpene synthases from lemon. Plant Physiol 2004; 134(1): 510-9.

54 Lücker J, Schwab W, Franssen MC, van der Plas LHW, Bouwmeester HJ, Verhoeven HA. Metabolic engineering of monoterpene biosynthesis: Two-step production of (+)-trans-isopiperitenol by tobacco. Plant J 2004; 39(1): 135-45.

55 Spitzer B, Ben Zvi MM, Ovadis M, Marhevka E, Barkai O, Edelbaum O, *et al*. Reverse genetics of floral scent: Application of tobacco rattle virus-based gene silencing in petunia. Plant Physiol 2007; 145(4): 1241-50.

56 Kaminaga Y, Schnepp J, Peel G, Kish CM, Ben-Nissan G, Weiss D, *et al*. Phenylacetaldehyde synthase from *Petunia hybrida* is a biofunctional enzyme that catalyzes the efficient coupling of phenylalanine decarboxylation to phenylalanine oxidation. J Biol Chem 2006; 281(33): 23357-66.

57 向　林, 陈龙清. 花香的基因工程研究进展. 中国农业科学 2009; 42(6): 2076-84.

58 Spitzer-Rimon B, Marhevka E, Barkai O, Marton I, Edelbaum O, Masci T, *et al*. *EOBII*, a gene encoding a flower-specific regulator of phenylpropanoid volatiles' biosynthesis in petunia. Plant Cell 2010; 22(6): 1961-76.

59 Vainstein A, Lewinsohn E, Pichersky E, Weiss D. Floral fragrance. New inroads into an old commodity. Plant Physiol 2001; 127(4): 1383-9.

60 Wang CL, Xing JS, Chin CK, Ho CT, Martin CE. Modification of fatty acid changes the flavor volatiles in tomato leaves. Phytochemistry 2001; 58(2): 227-32.

61 Chen F, Tholl D, D'Auria JC, Farooq A, Pichersky E, Gershenzon J. Biosynthesis and emission of terpenoid volatiles from Arabidopsis flowers. Plant Cell 2003; 15(2): 481-94.

62 Guterman I, Shalit M, Menda N, Piestun D, Dafny-Yelin M, Shalev G, *et al*. Rose scent: Genomics approach to discovering novel floral fragrance-related genes. Plant Cell 2002; 14(10): 2325-38.

63 Ross JR, Nam KH, D'Auria JC, Pichersky E. *S*-adenosyl-l-methionine: Salicylic acid carboxyl methyltransferase, an enzyme involved in floral scent production and plant defense, represents a new class of plant methyltransferases. Arch Biochem Biophys 1999; 367(1): 9-16.

64 Murfitt LM, Kolosova N, Mann CJ, Dudareva N. Purification and characterization of S-adenosyl-l-methionine: Benzoic acid carboxyl methyltransferase, the enzyme responsible for biosynthesis of the volatile ester methyl benzoate in flowers of *Antirrhinum majus*. Arch Biochem Biophys 2000; 382(1): 145-51.

65 喻　麟, 淳　泽, 张海英, 杨　涛, 王立洪, 刘世贵, 等. 矮牵牛 花香基因BSMT3的cDNA克隆与表达特征分析. 应用与环境 生物学报 2007; 13(2): 176-9.

66 Chen F, D'Auria JC, Tholl D, Ross JR, Gershenzon J, Noel JP, *et al*. An *Arabidopsis thaliana* gene for methylsalicylate biosynthesis, identified by a biochemical genomics approach, has a role in defense. Plant J 2003; 36(5): 577-88.

67 Dudareva N, D'Auria JC, Nam KH, Raguso RA, Pichersky E. Acetyl-CoA:benzylalcohol acetyltransferase-an enzyme involved in floral scent production in *Clarkia breweri*. Plant J 1998; 14(3):

297-304.

68 Koeduka T, Orlova I, Baiga TJ, Noel JP, Dudareva N, Pichersky E. The lack of floral synthesis and emission of isoeugenol in *Petunia axillaris* subsp. *parodii* is due to a mutation in the isoeugenol synthase gene. Plant J 2009; 58(6): 961-9.

69 Koeduka T, Fridman E, Gang DR, Vassao DG, Jackson BL, Kish CM, *et al*. Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of coniferyl alcohol esters. Proc Natl Acad Sci USA 2006; 103(26): 10128-33.

70 Koeduka T, Louie GV, Orlova I, Kish CM, Ibdah M, Wilkerson CG, *et al*. The multiple phenylpropene synthases in both *Clarkia breweri* and *Petunia hybrida* represent two distinct protein lineages. Plant J 2008; 54(3): 362-74.

71 Chen XM, Kobayashi H, Sakai M, Hirata H, Asai T, Ohnishi T, *et al*. Functional characterization of rose phenylacetaldehyde reductase (PAR), an enzyme involved in the biosynthesis of the scent compound 2-phenylethanol. J Plant Physiol 2011; 168(2): 88-95.

Adavances in Biosynthesis, Regulation and Genetic Engineering of Floral Scent

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Abstract Floral scent can enhance the aesthetic properties of ornamental plants and play an important role in their reproductive processes. With rapid development of molecular bio-technology, the researches of floral scent at molecular level which show a quickly advancing tendency have been becoming a hot project in recent years. In order to provide reference for the floral scent improving, this review describes biosynthesis of floral volatiles, related enzymes/genes, molecular regulation and co-regulation, and strategies of genetic engineering.

Key words floral scent; biosynthesis; molecular regulation; co-regulation; genetic engineering