植物单萜合酶研究进展

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摘要:单萜广泛存在于植物树脂和挥发油中,在植物生长发育和进化过程中发挥着重要作用,且在医药和生态农业等方面有着重要应用。这类物质是由质体内的 5-磷酸脱氧木酮糖(1-deoxy-D-xylulose-5-phosphate,DXP)途径合成,单萜合酶(monoterpene synthases,mono-TPS)是单萜生物合成的关键酶,决定了单萜结构的多样性。综述了植物单萜合酶催化机理、系统发育与谱系分化、基因表达调控、基因克隆及代谢工程等方面的研究进展,探讨了其生态学研究意义和发展前景。

关键词:单萜;单萜合酶;催化机理;系统进化;表达调控;次生代谢;代谢工程
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The progress of the research on plant monoterpene synthases

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Abstract: Monoterpenes are common constituents of plant resins and essential oils that play a vital role in plant growth, development and evolvement, with valuable applications in the medicinal and ecological agricultural fields. They are all synthesized in the plastid through 1-deoxy-D-xylulose-5-phosphate (DXP) pathway. Monoterpene synthases are the key enzymes of monoterpenes biosynthesis, which to a large extent responsible for the diversity of monoterpene structures. This paper focuses on the progress of the research on the catalysis mechanism, phylogeny, genetic expression and regulation, molecular cloning and metabolic engineering of monoterpene synthases. The ecological significance and developmental prospect of monoterpene synthases are also discussed.

Key Words: monoterpenes; monoterpene synthases; catalysis mechanism; phylogenetic evolution; expression and regulation; secondary metabolism; metabolic engineering

单萜是 C_{10}类萜类化合物,由两个异戊二烯结构单元组成,以链状、环状及其衍生物的形式广泛存在于植物挥发油和树脂中[1,2]。这类次生代谢产物在植物种群竞争、吸引昆虫传粉、防御植食性动物和控制病虫害发生等方面发挥着重要作用[3~5]。它们多具有较强的香气和生物活性,在工业、农业、医药和生态保护等方面有着广泛用途,如柠檬烯和松油醇等作为香料被广泛添加到食品和化妆品中[6,7],龙脑和桉树脑等对黏虫、小菜蛾和棉铃虫等有强烈的毒杀作用[6,8,9],长春花碱和香芹酮等单萜衍生物具有抗氧化和抗癌等功能[10~12]。

植物单萜是由质体内的 5-磷酸脱氧木酮糖(1-deoxy-D-xylulose-5-phosphate,DXP)途径,又称 4-磷酸-2-甲基赤藓糖(2-C-methyl-D-erythritol-4-phosphate,MEP)途径合成(图 1)[13~15]。单萜合酶(monoterpene synthases,mono-TPS),又称单萜环化酶(monoterpene cyclases),是单萜生物合成的关键酶,早已成为关注焦点[16]。本文综述了植物单萜合酶催化机理、系统发育与谱系分化、基因表达调控、基因克隆及代谢工程方面的研究进展,探讨了其生态学研究意义和发展前景。

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1　单萜合酶催化机理

单萜合酶采用亲电机理催化反应。在金属离子辅助下,前体 GPP 离子化,OPP 转移至 C3 上,C2-C3 键旋转,异构成能环化的顺式结构 LPP,接着再次离子化,C6-C1 衔接形成 terpinyl cation,由此继续重排、异构或环化形成各种环状碳阳离子。阳离子中间体去质子化或被亲核物质捕获便终止反应生成单萜(图 1)[16~19]。两类立体化学构象单萜,(+)-系列对映体和(-)-系列对映体,分别源于(3*R*)-LPP 和(3*S*)-LPP,由 GPP 与单萜合酶结合时的旋转状态决定[20,21]。一种单萜合酶通常只催化一类对映体,但往往有利用两种构象 LPP 的能力,只是催化效率上有差异[22]。

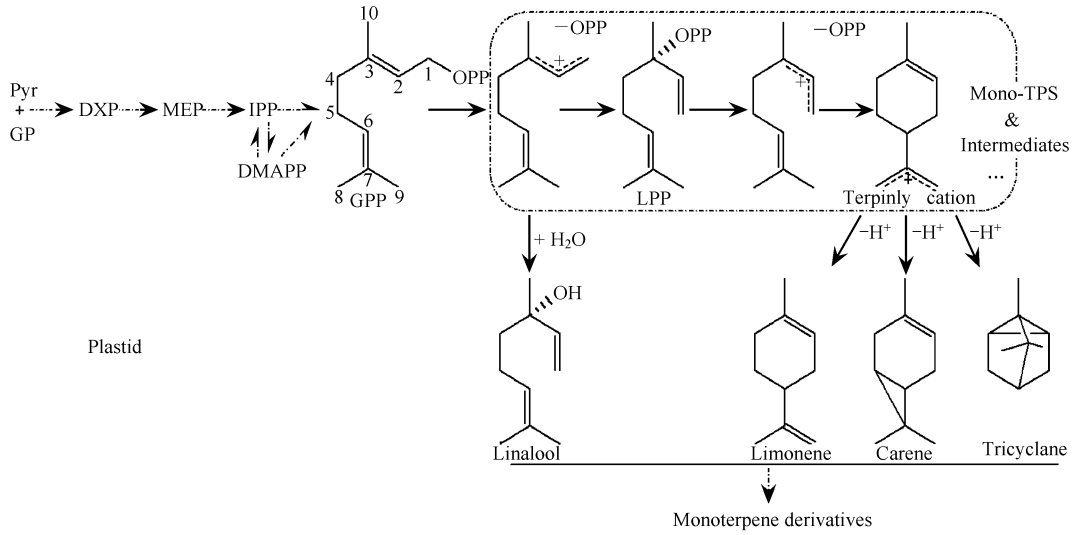

图 1　植物单萜生物合成路径

Fig. 1　The pathway of plant monoterpenes biosynthesis

实箭头表示单萜合酶催化的反应,虚箭头表示上游或下游相关酶催化的反应;环状单萜产物都需要 GPP 离子化和异构形成的萜品阳离子 terpinyl cation;链状单萜来源于 GPP 或 LPP 离子化形成的碳阳离子;Linalool,沉香醇;limonene,柠檬烯;carene,蒈烯;tricyclane,三环烷,分别代表链状、单环、双环和三环单萜产物;框内表示酶-底物共价复合体;忽略立体化学流程　Pry,pyruvate,丙酮酸;GP,D-glyceraldehyde-3-phosphate,磷酸甘油醛;DXP,1-deoxy-D-xylulose-5-phosphate,5-磷酸脱氧木酮糖;MEP,2-C-methyl-D-erythritol-4-phosphate,4-磷酸-2-甲基赤藓糖;IPP,isopentenyl diphosphate,异戊烯基焦磷酸;DMAPP,dimethylallyl diphosphate,二甲基烯丙基焦磷酸;GPP,geranyl diphosphate,香叶基焦磷酸;OPP,diphosphate moiety,焦磷酸残基;LPP,linalyl diphosphate,沉香基焦磷酸;intermediates,中间体　Solid arrows denote the reactions catalyzed by monoterpene synthases, broken arrows represent steps catalyzed by a series of upstream or downstream related enzymes; Formation of the cyclic products requires preliminary ionization and isomerizatoin of geranyl diphosphate to terpinyl caton via linalyl diphosphate; The acyclic proucts could be formed from either geranyl diphosphate or linalyl diphosphate via carbocations; The acyclic, monocyclic, bicyclic and tricyclic monoterpenes are represented by linalool, limonene, carene and tricyclane, respectively; Ligand conformations represented by the four intermediates are boxed; The stereochemical scheme was ignored

单萜合酶以单体或同型二聚体的形式催化反应。每个单体由 C 端和 N 端两个 α 螺旋区组成。C 端是活性区域,活性位点由其中 6 个 α 螺旋构成。其天冬氨酸保守区 DDxxD 首先结合一个 2 价金属离子(如 Mg^{2+}),并通过氢键络合 H_2O-Mg^{2+} 复合物和自由 H_2O 以维持活性位点的基本空间结构。当 GPP 进入后,DDxxD 再结合一个 Mg^{2+},旁侧的氨基酸也结合一个 Mg^{2+},焦磷酸基团(diphosphate,PP_i)也被其它氨基酸结合。此时,PP_i-Mg_3^{2+} 络合,诱导活性位点的 α 螺旋规则排列进入催化状态,并使 GPP 离子化。不稳定碳阳离子由 PP_i 离子补偿和芳香族氨基酸的 π 键作用稳定,继而在静电作用下发生异构、重排和环化等系列反应。PP_i、H_2O 和一些氨基酸共同影响着这个静电环境,对碳阳离子立体化学反应流向起着调节作用[17,21,23,24]。N 端在 PP_i-Mg_3^{2+} 诱导下会形成一个规则的“帽子”结构扣在 C 端活性位点裂口上,稳定其空间结构。这是通过二硫键、氢键和静电作用同 C 端氨基酸锚锭来间接实现的[23,24]。其中,N 端精氨酸保守区 RRx_8W 与 DDxxD

形成的 R-D 碳氢键等除了维持结构稳定外,还有重要的异构功能,即通过锚锭作用满足 GPP 到 LPP 及其它中间体的构象转换对活性位点空间结构的弹性需求[24]。Williams 等研究证明切除 RR 的柠檬烯合酶不能使 GPP 异构成 LPP,即不能催化产生环状单萜[25]。RRx_8W 野生缺失的仙女扇(*Clarkia breweri*)沉香醇合酶[26]、拟南芥(*Arabidopsis thaliana*)沉香醇合酶[27]、金鱼草(*Antirrhinum majus*)月桂烯合酶和罗勒烯合酶[28]也都没有环状产物。

单萜合酶催化产物的种类和比例受很多关键氨基酸调控。立体化学反应流程中的一系列碳阳离子中间体都可能去质子化或被亲核物质捕获而终止反应。因此,很多单萜合酶都能催化产生多种产物,以主产物命名。如紫苏(*Perilla frutescens*)月桂烯合酶的催化产物有月桂烯 53.8%、桧烯 20.9%、沉香醇 19.8%、柠檬烯 5.5%[18]。立体化学反应流程主要由 C 端序列决定,一些氨基酸影响到活性位点的尺寸、形状和动力学参数,涉及对中间体的异构方式、维持能力和终止方式,进而调控产物种类和比例。氨基酸序列交换研究表明,撒尔维亚(*Salvia officinalis*)桧烯合酶(去 H^+ 终止)、桉树脑合酶(H_2O 捕获终止)和龙脑焦磷酸合酶(PP_i 捕获终止)的 C 端一小段序列决定对(4*R*)-α-萜品阳离子终止方式的选择,从而导致产物不同[17];北美冷杉(*Abies grandis*)蒎烯合酶点突变研究发现活性位点内侧和外围的一些氨基酸都可以影响产物比例[29];薰衣草(*Lavandula angustifolia*)[30]、柠檬留兰香(*Mentha citrata*)[31]、罗勒(*Ocimum basilicum*)[32]、青蒿(*Artemisia annua*)[33]、仙女扇[26]、拟南芥[27]、草莓(*Fragaria ananassa*)[34]沉香醇合酶的催化产物都只有沉香醇,序列比较和模型分析发现,该类酶 C 末端附近缺少阻止亲核物质入侵的 3 个氨基酸导致 GPP 首次离子化时即被H_2O捕获而终止反应。

2 单萜合酶系统发育与谱系分化

2.1 氨基酸序列分化

萜类合酶(terpeniod synthase,TPS)均起源于古萜类合酶,以氨基酸序列相似度 40% 为基准将其分为 TPS-a→TPS-g 7 个亚家族(图 2A)[16,28]。单萜合酶分布于 TPS-b、TPS-d、TPS-f 和 TPS-g 4 个亚家族。裸子植物单萜合酶分化较小,和裸子植物其它萜类合酶一起归属于 TPS-d。被子植物单萜合酶变异很大,分化为 3 个独立的亚家族。TPS-f 可能是最古老的一个分枝。被子植物单萜合酶进化较快,谱系分化较大,推测其还有新亚家族存在。事实上,TPS-g 是近来在金鱼草里分离到的[28]。而且新发现的草莓沉香醇/橙花叔醇合酶[34]和金鱼草沉香醇/橙花叔醇合酶[35]的 N 端质体转运信号肽也部分退化,还有催化产生单萜和倍半萜双重功能。

被子植物单萜合酶系统发育和谱系分化与植物自然分类系统有较紧密联系,和产物划分没有必然关系。相同物种来源的单萜合酶相似性远高于功能相同而物种来源不同的单萜合酶,尽管前者催化产生不同产物[18,30]。同科属植物的单萜合酶几乎都聚于同一个小分枝上,如唇型科(*Lamiaceae*)紫苏属、薄荷属和鼠尾草属等(图 2A)。基于序列相似性仅可以粗略推测其是否是单萜合酶,却不能预测产物。如紫苏月桂烯合酶和罗勒月桂烯合酶序列相似性仅 51%,而前者和紫苏柠檬烯合酶序列相似度高达 80%。

2.2 基因结构进化

古萜类合酶基因的内含子和外显子十分丰富,在进化过程不断丢失。根据变化情况将萜类合酶基因归为 3 类:11 ~ 14 个内含子和 12 ~ 15 个外显子(Ⅰ类)、9 个内含子和 10 个外显子(Ⅱ类)以及和 6 个内含子(Ⅲ类)(图 2B)[36]。Ⅰ类保存了松类二萜合酶间隔序列(conifer diterpene internal sequence,CDIS),属此类的单萜合酶仅在仙女扇[37]、拟南芥[38]等少数物种里有发现。裸子植物单萜合酶基因属Ⅱ类,CDIS 已丢失。绝大多数被子植物单萜合酶基因属Ⅲ类,CDIS 也丢失[36]。拟南芥基因组除有 29 个Ⅲ类基因外,还保存有 3 个完整的Ⅰ类基因和 8 个残缺的基因[38],从而佐证了萜类合酶基因内含子丢失过程。先前认为Ⅲ类的 6 个内含子在所有萜类合酶中都是保守的[36],而新发现的草莓沉香醇/橙花叔醇合酶基因仅有 5 个内含子[34],进一步暗示了其基因结构的多样性。

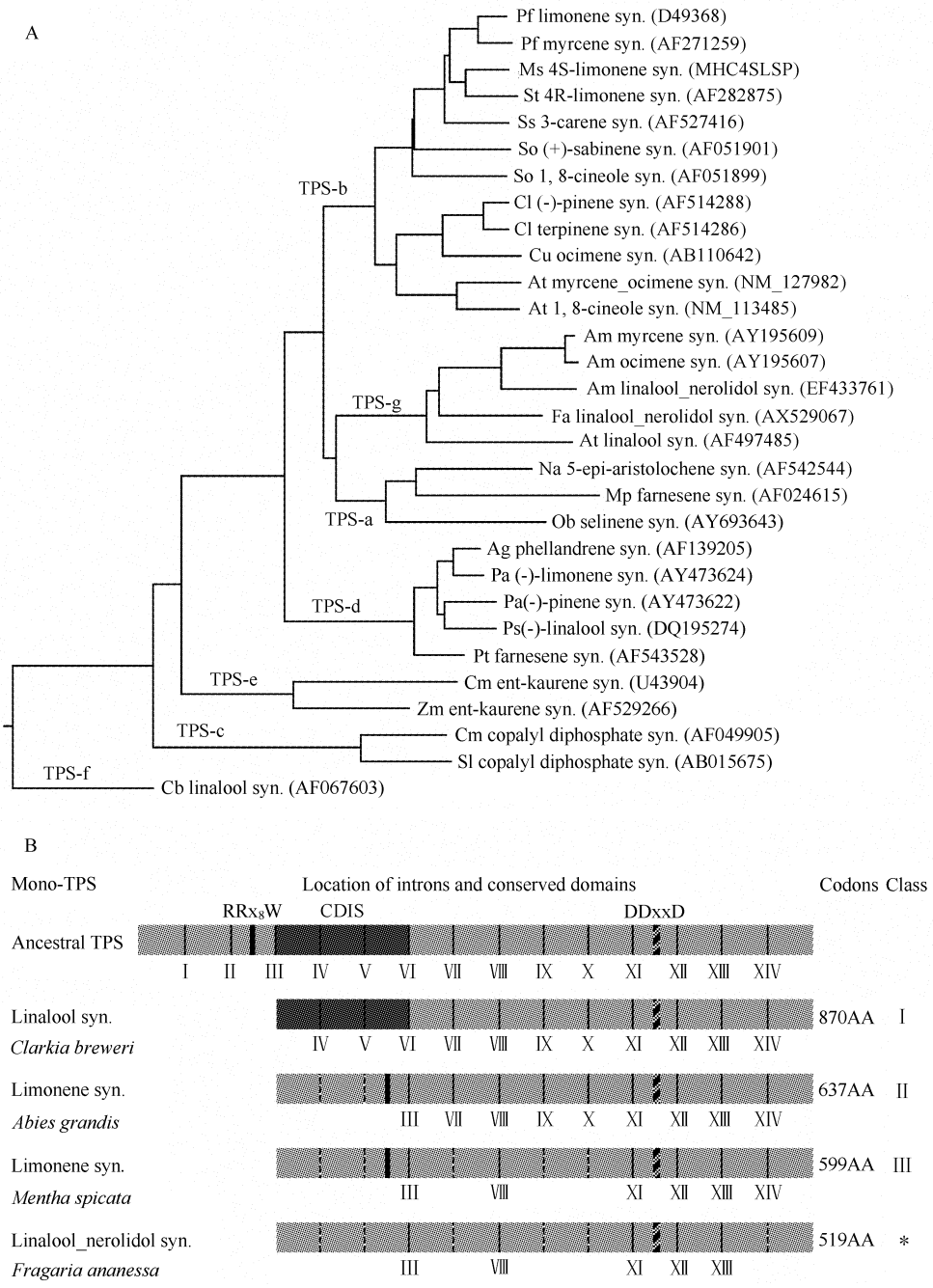

图 2　植物单萜合酶亚家族谱系分化 A 和基因结构 B

Fig. 2　Phylogenetic tree (A) and gene structures(B) of the plant mono-TPS subfamilies

A:植物萜类合酶聚类关系;基于 DNAStar 软件 ClustalV 法分析,所列仅为部分代表,括弧内为 GenBank 号　Phylogenetic analysis of the represented plant TPS based on ClustalV alignment of Lasergene DNAStar programs; GenBank accession numbers are shown in brackets

syn. :synthase,合酶;Pf:*Perilla frutescens*,紫苏;Ms:*Mentha spicata*,留兰香;Mp:*Mentha x piperita*,胡椒薄荷;St:*Schizonepeta tenuifolia*,荆芥;So:*Salvia officinalis*,撒尔维亚;Ss:*Salvia stenophylla*,狭叶鼠尾草;Cl:*Citrus limon*,柠檬;Cu:*Citrus unshiu*,温州蜜柑;At:*Arabidopsis thaliana*,拟南芥;Am:*Antirrhinum majus*,金鱼草;Fa:*Fragaria x ananassa*,草莓;Na:*Nicotiana attenuata*,烟草;Ob:*Ocimum basilicum*,罗勒; Ag:*Abies grandis*,北美冷杉;Pa:*Picea abies*,欧洲云杉;Pt:*Pinus taeda*,火炬松;Ps:*Picea sitchensis*,北美云杉;Cm:*Cucurbita maxima*,南瓜;Zm:*Zea mays*,玉米;Sl:*Solanum lycopersicum*,番茄;Cb:*Clarkia breweri*,仙女扇;Limonene,柠檬烯;myrcene,月桂烯;carene,蒈烯;sabinene,桧烯;cineole,桉树脑;pinene,蒎烯;terpinene,萜品烯;ocimene,罗勒烯;linalool,沉香醇;phellandrene,水芹烯;nerolidol,橙花叔醇;aristolochene,马兜铃烯;farnesene,法呢烯;selinene,芹子烯;ent-kaurene,贝壳杉烯;copalyl diphosphate,柯巴基焦磷酸

B. 单萜合酶基因结构模型;方框和竖线分别表示外显子和内含子(Ⅰ—ⅩⅣ),忽略长度;虚竖线表示内含子丢失;CDIS,松类二萜合酶间隔序列;RRx_8W 和 DDxxD 为保守序列;* 为新发现的结构类型　Models for the gene structures of plant mono-TPS, with lengths being ignored; Boxes and vertical lines represent exons and introns(1 ~ 14, Roman numerals), respectively; Broken vertical lines illustrate sequential loss of introns; CDIS region represents the conifer diterpene internal sequence domain; RRx_8W and DDxxD denote the highest conserved sequences. Asterisk indicates exception to the otherwise conserved architecture

3 单萜合酶基因表达调控

3.1 时空表达规律

单萜合酶基因表达是满足植物生长发育需要。单萜合酶多基因家族的不同成员往往在特定器官、组织和发育阶段表达,以合成通讯和防御所需的化感物质[27,39]。如温州蜜柑(*Citrus unshiu*)花里桉树脑和罗勒烯含量十分丰富,前者主要用于植物间通信和预防真菌病害,后者是作为花香物质吸引传粉昆虫;蒎烯和柠檬烯则主要出现在果实发育初期的果皮里,以驱赶害虫[6,9,40]。单萜合酶基因表达水平是影响单萜产量的主要因素,在植物器官和组织的发育期内呈类抛物线变化趋势[40~42]。

单萜合酶基因表达受生物钟调节。表达量以日为周期有节律的交替变换。如金鱼草月桂烯合酶和罗勒烯合酶[28]、青蒿蒎烯合酶[41]和拟南芥月桂烯/罗勒烯合酶[27]基因的表达量和产物都呈现午后高、夜间低的昼夜交替规律,甚至外源沉香醇合酶基因转入拟南芥后表达规律亦是如此[42]。基因表达量和单萜产物变化趋势趋于一致,其节律几乎不因持续光照或黑暗处理而改变[28]。

单萜合酶基因会因环境刺激而迅速表达。很多单萜是植物防御系统的重要组成部分。生物胁迫能激活基因表达来合成若干单萜,以对付草食动物、害虫和病原菌等[4,5,43]。如刺伤或昆虫诱导的青蒿沉香醇合酶基因[33]和云杉蒎烯合酶基因[44]随即过量表达,并维持高水平长达几天,释放大量相应单萜以抵抗细菌、霉菌或蚜虫等;百脉根(*Lotus japonicus*)受二斑叶螨等侵扰后 6 ~ 24h 便能释放大量罗勒烯以吸引瓢虫等天敌[45]。单萜还参与抵制高温、氧化等非生物胁迫[46~48]。如高温环境下冬青栎(*Quercus ilex*)利用单萜清除细胞内的自由基团、活性氧等,并释放大量挥发性单萜来降低体温[49,50]。随着环境日益恶化,研究非生物胁迫下单萜防御反应模式和机理对作物改良、生态保护等有着重要意义。

3.2 信号传导与调控

单萜合酶基因表达由诱导因子、信号传导和转录因子三环节综合调控。诱导因子包括真菌多糖、昆虫分泌物、茉莉酸甲酯(methyl jasmonate, MeJA)以及其它胁迫,起着开关作用[45,51]。信号经 G 蛋白、Ca^{2+}、H_2O_2、IP_3、茉莉酮酸(jasmonate, JA)和乙烯等信号分子传递给转录因子,作用于启动子区的顺式元件,增强或抑制单萜合酶基因表达,进而催化产生相应单萜[52~55]。

不同的单萜合酶基因有专一的顺式元件序列。因此,不同转录因子往往只调控一种或一类基因[54,56]。信号可同时由几个信号途径并行传递给不同的转录因子,但不同途径的传递速度差异较大。如真菌诱导因子的信号传导至少包含 H_2O_2、G 蛋白、Ca^{2+} 和 IP_3 4 个信号路径,前两个在 24h 内便发挥最佳作用,而 Ca^{2+} 路径需要约 48h[57]。因此,墨西哥柏(*Cupressus lusitanica*)感病后表现出两级单萜防御模式,即 8 ~ 24h 内释放大量强效杀菌剂扁柏素(一级),36h 后以释放一些防预性单萜为主(二级)[52,53];北美冷杉受机械损伤初期和 7d 后所释放的单萜种类也明显不同[43]。不同诱导因子所选择的信号途径有差异,因而调节速度也不同。一方面,MeJA 等是植物发育相关的信号物质,选择 Ca^{2+} 等传递速度较慢的信号分子更适合植物生长的长效需要[57];另一方面,环境刺激下的单萜防御通常分两阶段,即应急处理和长效保护,所以往往选择快速与慢速信号分子相结合的传递策略[58]。

4 单萜合酶基因克隆

单萜合酶基因克隆主要策略有反向推测法、相似性克隆和随机克隆。反向推测法是根据氨基酸序列设计探针从 cDNA 文库中钓取基因。此法能有针对性的获得某种单萜合酶基因,但植物体内萜类合酶量少,分离纯化难度系数高,仅在早期有一些应用[59]。相似性克隆是利用简并引物 PCR 扩增出部分基因序列,再设计探针筛选 cDNA 文库或通过 RACE-PCR 获得全长 cDNA。此法操作简单,发展非常迅速,但不太适用于远缘物种,获取单萜合酶基因数量有限[60]。随机克隆是指利用 cDNA 文库获得 EST 序列,再用生物信息学方法筛选目的 EST,从而获取目标克隆。此法能获得较全面的单萜合酶基因,还适用于远缘物种[9,61]。克隆到的基因需要原核表达重组酶来鉴别并分析酶学性质和产物特征。构建表达载体时需去掉编码 N 端质体转运肽的碱基序列。

自 Cobly 等从留兰香(*Mentha spicata*)中克隆到第一个 4S-柠檬烯合酶基因以来[59],现已获得几十个物种的近百个单萜合酶基因,主要集中在被子植物唇型科植物、拟南芥等和部分裸子植物的研究上。这些成果主要以美国、德国、加拿大、日本等发达国家为主,而国内则仅在青蒿[33,41]、细毛樟(*Melaleuca alternifolia*)[62]等物种上有一些研究。

5　单萜代谢工程

单萜代谢工程可应用于改善植物气味、提高植物抗性和生产有用单萜等方面。常在微生物或植物里构建新单萜代谢途径、诱导目的基因过量表达、抑制旁侧代谢和转基因等操作来实现。

5.1　微生物寄主的代谢工程

微生物是生产有益单萜的重要宿主。但其异戊二烯代谢缺乏,内源 DXP 途径供给的 IPP 和 DMAPP 几乎无法满足代谢工程的需要。因此,Carter 等向大肠杆菌导入香芹酮合成相关酶的基因却未能获得最终产物[12]。通过构建并行的甲羟戊酸途径(mevalonate pathway,MVA)(细胞质里的异戊二烯代谢途径)有效克服了 IPP 和 DMAPP 供应限制[63~65]。但仍有很多并发问题需进一步研究,如 ATP 等辅助因子短缺、产物毒害寄主、膜上的羟基化和氧化还原修饰等[12]。

5.2　植物寄主的代谢工程

诱导植物内源单萜合酶基因过量表达来获取单萜不失为良策。单萜构筑了植物与植物、植物与环境的通信系统和防御系统,受植物发育和外界刺激调控。因此,可利用诱导因子刺激、添加信号分子或抑制旁路信号传导来增产目的单萜。如真菌、蜂毒素、MeJA、JA、H_2O_2、Ca^{2+}、乙烯等诱导因子和信号分子都可以促进墨西哥柏悬浮细胞合成扁柏素[57]。植物悬浮细胞生长快、操作方便、可控性强,适宜规模化生产,是理想的操作对象。掌握目标单萜代谢的分子调控机理和信号传导途径是应用该策略的前提。

植物异戊二烯代谢十分旺盛,适合外源单萜合酶的转基因操作。虽然细胞质和质体都能提供 IPP 和 DMAPP,但是将外源单萜合酶定位于质体更利于发挥其催化活性[66]。首个用于转基因的仙女扇沉香醇合酶曾引入番茄(*Solanum lycopersicum*)、矮牵牛花(*Petunia hybrida*)和石竹(*Dianthus chinensis*)3 种植物。仅前者大量合成目标产物,而后两者分别因 GPP 供应不足和产物被糖苷修饰导致产物释放量极少[67]。因此,选择合适的宿主植物很重要。一些单萜合酶基因已在模式植物里成功表达,如草莓沉香醇/橙花叔醇合酶转基因拟南芥[42]、紫苏柠檬烯合酶转基因烟草[66]。目前,单萜合酶转基因研究主要存在两方面问题。一是植株矮小,叶片黄化,发育迟缓。主要由于外源酶与宿主本身的异戊二烯代谢网竞争底物,导致赤霉素、脱落酸、类胡罗卜素、叶绿素和醌类等植物生长和发育相关物质的合成受到影响[42,66];其次,过多的单萜物质释放到腺体外也会毒害植物细胞。二是单萜产量低。植物萜类代谢网络庞大,对 IPP 和 GPP 流向有较严紧的分配和控制,因此如金鱼草月桂烯合酶基因表达水平虽是罗勒烯合酶的 1.5 倍,但后者的产物量却是前者的 5 倍[28],而外源酶更不易争取到较多资源;其次,宿主内的氧化、还原等修饰酶将单萜修饰成衍生物而影响单萜积累,如薄荷柠檬烯易被羟化酶和脱氢酶修饰成薄荷醇、胡薄荷酮、胡椒烯酮和香芹酮[68,69];蒎烯是草莓香味物质桃金娘烯醇和乙酸桃金娘烯酯的前体[34];紫苏月桂烯被转化成其它物质而不在细胞内积累[18]。

6　讨论与展望

单萜合酶进化频率高,而目前分离到的基因涉及物种少。需要克隆更多基因以全面认识单萜合酶系统发育和功能进化。单萜合酶虽然都采用亲电机理催化反应,但立体化学反应流程和调控机制还十分模糊。仅有留兰香柠檬烯合酶[21,24]、撒尔维亚龙脑焦磷酸合酶[23]和北美冷杉蒎烯合酶[19,29]等极少数酶有较深入的晶体结构和氨基酸序列分析。氨基酸序列与功能和空间结构与功能的关系还有待进一步研究。双重功能的单萜/倍半萜合酶的发现又为这方面研究提供了新机遇和挑战[34,35]。

单萜次生代谢的分子调控机理研究不够深入。植物正常发育[6,9,28,34,40~42]、机械损伤[33,43,44]和病虫感染[44,45]等状态下单萜合酶基因表达规律的报道较多。而不同诱导因子选择哪些信号传递途径和转录因子的分子调控机理仍不太清楚。植物悬浮细胞为诱导或抑制信号传导和转录因子的操作创造了条件[57],还利于

调查下游修饰反应和衍生去向,为数量庞大的单萜及衍生物代谢研究和应用打下基础。最终开发诱导因子和信号抑制剂调整单萜合成量,改善植物香味、增强物种抗生物或非生物胁迫能力及控制生物入侵等,用于农业生产和生态建设。

单萜代谢工程需要权衡宿主生长发育所付出的代价。近来发现 MVA 和 DXP 两个异戊二烯代谢途径可以互相增补底物 IPP 和 GPP[15,70~74]。植物腺体涉及到产物积累,避免正常细胞遭毒害,保护单萜不被下游酶分解或修饰[75]。增加底物供应和腺体贮藏能力是改善植物宿主的研究目标。系统调查腺体结构和萜类代谢网络是该研究的基础和前提[76]。微生物和植物悬浮细胞则需要通过基因突变等方式改善细胞膜通透性和去除不必要的修饰酶,或通过连续发酵等工程技术手段降低产物积累量,以维持细胞正常生长,提高单萜产量。

单萜合酶转基因和病毒诱导基因沉默(virus-induced gene silencing,VIGS)操作为研究单萜次生代谢的生态学意义提供了新方法。通过这些操作调整植物挥发性成分,跟踪病虫害发生和周围的植物生长发育情况,调查草食动物、寄生昆虫和授粉昆虫的行为变化,研究抗氧化和抗热胁迫能力,从而准确把握其所扮演的生态学角色,最终应用于改善花卉香味、提高作物抗性和生态保护等方面。Page 等用烟草脆裂病毒(tobacco rattle virus,TRV)成功敲除了烟草一些酶基因,实现了对异戊二烯代谢途径的控制[77]。Lucker 等向内源单萜代谢较微弱的烟草引入蒎烯、柠檬烯和萜品烯,利于研究昆虫行为[78]。Aharoni 等将拟南芥转入外源基因过量释放沉香醇后大大减少了感蚜虫的机率[42]。首先在拟南芥和烟草等内源萜类代谢认识较透彻的模式植物里进行这些基因操作十分重要,以评价其可行性和安全性。

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