• 综 述 •

[反]-β-法尼烯合成酶基因在植物抗蚜分子育种中的应用

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摘 要: 蚜虫是重要的农业害虫，每年造成数以亿计的经济损失。[反]-β-法尼烯 [(*E*)-β-farnesene，EβF] 是绝大多数蚜虫报警信息素的主要成分，可使蚜虫产生骚动、从植株上脱落，并吸引蚜虫天敌，有效控制蚜虫危害。EβF 合成酶是催化合成 EβF 的关键酶，目前该基因已从薄荷、香橙、花旗松、黄花蒿、洋甘菊等植物中得到分离鉴定。植物中表达 EβF 合成酶基因以催化法呢基焦磷酸 (Farnesyl diphosphate，FPP) 合成 EβF 是控制蚜虫危害的重要策略。文中概括了当前植物抗蚜转基因研究现状，综述了植物 EβF 合成酶基因及其在植物抗蚜分子育种中的应用。针对当前转基因植物的 EβF 生成量较低等问题，展望了 EβF 合成酶基因在植物抗蚜分子育种中的应用前景和研究策略。

关键词: 蚜虫，蚜虫报警信息素，[反]-β-法尼烯，EβF 合成酶基因，植物分子育种

Metabolic engineering of (*E*)-β-farnesene synthase genes for aphid-resistant genetically modified plants

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Abstract: Aphids are major agricultural pests that cause significant yield losses of crops each year. (*E*)-β-farnesene (EβF), as the main component of the aphid alarm pheromones, can interrupt aphid feeding and cause other conspecies in the vicinity

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to become agitated or disperse from their host plant. Furthermore, EβF can function as a kairomone in attracting aphid predators. EβF synthase genes, which encode enzymes that convert farnesyl diphosphate (FPP) to the acyclic sesquiterpene EβF, have been isolated and characterized from peppermint (*Mentha* × *piperita* and *Mentha asiatica*), Yuzu (*Citrus junos*), Douglas fir (*Pseudotsuga menziesii*), sweet wormwood (*Artemisia annua*) and chamomile (*Matricaria recutita*), respectively. Transgenic plant overexpressing EβF synthase genes has been one of the most efficient strategies for aphid management. In this review, the current statuses of transgenic plants engineered for aphid resistance were summarized. The plant-derived EβF synthase genes with their potential roles in aphid management via genetic-modified (GM) approaches were reviewed. The existing problem in GM plants with EβF synthase gene, such as low EβF emission was usually detected in the transgenic plant, was discussed and the development direction in this area was proposed.

Keywords: aphid, aphid alarm pheromone, (*E*)-β-farnesene, EβF synthase gene, molecular plant breeding

蚜虫是危害农作物生产的重要害虫，蚜虫取食可使植株营养恶化，蚜虫分泌的蜜露附着在叶片表面影响植物的光合作用，并促进霉菌的滋生，诱发植物黑霉病害[1-2]。蚜虫还是植物病毒病的重要传播载体，蚜虫传播的病毒约占所有虫传病毒种类的 45%[3-4]。近年来因全球气候变暖、耕作制度变化等因素影响，蚜虫繁殖能力和适应性显著增强，危害日趋严重[5-6]。据统计，2010–2011 年间我国小麦、玉米、棉花、油菜、大豆等主要作物的蚜虫危害面积分别占当年种植面积的 62.5%、14%、90%、32%和 23%[7]。培育抗虫品种是防治蚜虫的最有效途径，现有农作物种质资源中缺乏有效的抗蚜基因，常规抗虫育种难以奏效[1,4]。因此，利用转基因技术培育抗蚜新种质，对于保障我国粮食安全具有重要意义。

[反]-β-法尼烯 [(*E*)-β-farnesene，EβF] 是绝大多数蚜虫类型报警信息素的主要甚至唯一成分，可使蚜虫产生骚动而从植株上脱落，并吸引蚜虫天敌[8-10]。在植物中表达 EβF 合成酶基因以获得释放 EβF 的转基因植株已成为蚜虫防治的重要策略之一。本文概述了植物抗蚜转基因的研究现状，并对当前 EβF 合成酶基因在植物抗蚜转基因研究中的应用及存在问题进行了讨论。

1 植物抗蚜转基因研究现状

作物抗虫转基因育种已持续开展 20 多年，多种表达苏云金芽孢杆菌 (*Bacillus thuringiensis*，*Bt*) 毒素蛋白的转基因作物，如棉花、玉米、大豆等已商业化种植[11]。*Bt* 毒素蛋白对鳞翅目和鞘翅目害虫有很强的毒杀作用，但对蚜虫等同翅目害虫防治效果不明显，制约了 *Bt* 基因在植物抗蚜分子育种中的应用。目前，用于抗蚜分子育种的基因和技术主要有植物凝集素基因、植物介导的 RNA 干扰技术等[1-2,12]。

1.1 植物凝集素

植物凝集素是一类保守性糖结合蛋白，可与昆虫消化道上皮细胞的糖蛋白结合，降低膜透性，并可直接降低虫体消化酶活性，影响昆虫对营养物质的吸收和消化。此外，凝集素可在昆虫消化道内诱发病灶，促进消化道内的细菌繁殖，影响害虫生长发育[13]。自 1988 年发现蓖麻凝集素以来，人们已经从豆科、茄科、禾本科和石蒜科等众多植物中分离鉴定出上千种植物凝集素基因，已有 10 余种不同植物来源的凝集素基因用于转基因抗蚜研究[1,13]。其中，雪花莲凝集素(*Galanthus nivalis* agglutinin，*gna*)、半夏凝集素(*Pinellia ternate* agglutinin，*pta*) 等基因对哺乳动物的毒性较小，是抗虫转基因研究的热点。转 *gna* 基因的小麦、玉米、马铃薯、烟草等植物能抑制蚜虫生长、降低蚜虫生殖力[14-17]。*pta* 基因与 *gna* 的序列相似性很高，转 *pta* 基因小麦株系上蚜虫的存活率可降为对照的 54%[18]。然而，植物凝集素抗虫

具有广谱性，对蚜虫天敌、食草动物等可能具有毒害作用，如二星瓢虫取食转 *gna* 基因马铃薯上的蚜虫后，其产卵力、卵的生存力和寿命明显降低[19-20]。转凝集素基因植物对生态环境的影响引起了人们的担忧。

1.2 植物介导的 RNA 干扰

RNA 干扰 (RNA interference，RNAi) 是由双链 RNA (Double-stranded RNA，dsRNA) 介导的一种序列特异性转录后基因沉默机制。dsRNA 进入生物体后被宿主细胞中的 Dicer 酶切割成 21–23 nt 的小干扰 RNA (Small interfering RNA，siRNA)；siRNA 在 RNA 解旋酶的作用下解链成正义链和反义链，反义 siRNA 与体内一些酶 (包括内切酶、外切酶、解旋酶等) 结合形成 RNA 诱导的沉默复合物 (RNA-induced silencing complex，RISC)，随后 RISC 以序列互补的方式与靶标 mRNA 结合并使之降解[12,21]。研究发现，在植物中表达 dsRNA 能抑制昆虫特定基因的表达，使昆虫生长发育受阻或致死，有效控制害虫[12,21]。RNAi 技术在蚜虫防治方面展示出很好的应用潜力[12]，植物介导的 RNAi 技术已用于大麦、烟草和拟南芥等植物的抗蚜研究[4,12,22-24]。大麦中表达 *shp* 基因的 dsRNA 能显著降低麦长管蚜的繁殖力，且 *shp* 基因的沉默效应可以遗传至第 7 代子蚜[22]。烟草和拟南芥中表达桃蚜 *MpC002*、*Rack-1*、*Mphb* 及 *MySP* 基因的 dsRNA，可显著降低蚜虫的繁殖率、减轻蚜虫危害[4,23-24]。RNAi 靶标基因的筛选是植物介导的 RNAi 抗蚜应用的前提，目前鉴定出的能显著致死或抑制蚜虫生长的靶标基因较少。其次，植物和蚜虫体内的核酸酶可降解外源 dsRNA，降低蚜虫目标基因的沉默效率，影响了该技术在转基因植物抗蚜上的应用。同时，植物介导的 RNAi 存在潜在的安全风险，如 RNAi 的脱靶效应，亦有待深入研究和解决[12,25]。

此外，研究者尝试在植物中表达抗性基因、蛋白酶抑制剂等基因来控制蚜虫危害[26-29]。抗性基因 (*R* 基因) 通常介导的是垂直抗性，表现出一定的物种特异性，如番茄 *R* 基因 *Mi-1.2* 转入茄子后，不能提高茄子对蚜虫的抗性[30]。蛋白酶抑制剂是抑制蛋白水解酶活性的一种小分子蛋白，昆虫摄食蛋白酶抑制剂后，其肠道内的蛋白水解受阻，进而扰乱昆虫的营养代谢[31]。然而，害虫可以通过合成同工酶或直接降解的方式，快速对外源蛋白酶抑制剂产生抗性[32-34]；并且，植物中表达蛋白酶抑制剂对非靶标害虫如蜜蜂有害[35]。因此，挖掘新的更加安全有效的抗蚜基因尤为重要。

2 蚜虫报警信息素及 EβF

蚜虫报警信息素是蚜虫遇到天敌等威胁时从腹管分泌的一种粘稠液滴，释放到体外具有挥发性，能引起同类其他个体骚动并从栖息地迅速逃散或从植株上脱落，并能作为天敌捕食蚜虫的重要线索[8]。EβF 是绝大多数蚜虫类型报警信息素的主要甚至唯一成分，包括桃蚜、玉米蚜、棉蚜、麦长管蚜、禾谷缢管蚜、麦无网长管蚜、大豆蚜等常见作物害虫[1,7,36]。EβF 利于蚜虫防控的作用特点如下：1) 蚜虫通过气味结合蛋白、昆虫化学感受蛋白等基因感应外界 EβF[37-39]，产生诸如骚动、停止取食，甚至从植物上脱落等警戒反应[7,9,36,40]。2) EβF 能够吸引多种蚜虫天敌，如瓢虫[10,41-42]、食蚜蝇[42-44]、草蛉[45-47]、蚜茧蜂等[42,47-48]，作为天敌的捕食信号。3) EβF 能显著提高产生有翅蚜的比率，使蚜虫主动离开寄主植物[49-50]。4) EβF 能产生类似保幼激素Ⅲ的作用，影响蚜虫形态类型和生长发育。如一龄棉蚜受 EβF 诱导后，蚜虫的发育期延长、产卵力下降、体重减轻[51]。5) EβF 与杀虫剂混用，可增加蚜虫的活动频率，提高杀虫效果[52-53]。

然而，EβF 在大田条件下不稳定，易氧化分解，制约了 EβF 在田间的抗蚜应用[54]。随后，为

提高 EβF 的稳定性，研究者尝试了人工改造及化学合成 EβF，并取得一定进展[55-56]。蚜虫报警信息素的专属性表明 EβF 与受体之间具有特定的作用部位，受体对信息素的结构和性质有严格的要求，导致化学合成的 EβF 对蚜虫防治效率较低。

3 植物体内 EβF 的代谢合成

EβF 作为一种无色无味的倍半萜类化合物，还是茼蒿、野生马铃薯、菊花、薄荷、黄花蒿、花旗松、香橙、洋甘菊等多种植物精油的主要组分[7,57-58]。植物来源的 EβF 亦可趋避蚜虫和吸引天敌，如与栽培品种相比，野生马铃薯叶片挥发物中存在高量 EβF，进而对蚜虫有很强的驱避作用[58]；海灰翅夜蛾取食后，玉米会释放含有 EβF 的挥发物，减少蚜虫取食[59]。此外，菊花来源的 EβF 可以吸引天敌瓢虫和蚜茧蜂减轻蚜虫对白菜的危害[10]；洋甘菊来源的 EβF 可有效降低马铃薯和小麦的田间蚜虫数量[42,47]。EβF 合成酶是催化生成 EβF 的关键酶，但蚜虫体内的 EβF 合成酶基因尚未分离鉴定[60]。

目前，研究者已对植物体内的萜类化合物及 EβF 生物合成机制进行了较为深入的研究。萜类化合物是植物次生代谢产物中最大的一个家族，根据所含碳原子数目不同，可分为单萜 (C_{10})、倍半萜 (C_{15}) 和二萜 (C_{20}) 等。植物萜类化合物通过两个独立途径合成，即位于质体中的 2-C-甲基-D-赤藓糖醇-4-磷酸 (2-C-Methyl-D-Erythritol-4-Phosphate，MEP) 途径和位于细胞质中的甲羟戊酸 (Mevalonate，MVA) 途径(图 1)[61]。在植物质体中，1 分子异戊烯焦磷酸 (Isopentenyl diphosphate，IPP) 和 1 分子二甲丙烯焦磷酸 (Dimethylallyl diphosphate，DMAPP) 在香叶基焦磷酸合成酶 (Geranyl diphosphate synthase，GPS) 的作用下，经头尾相连生成单萜合成的底物香叶基焦磷酸 (Geranyl diphosphate，GPP)；3 分子 IPP 和 1 分子 DMAPP 在香叶基香叶基焦磷酸合成酶 (Geranylgeranyl diphosphate synthase，GGPS) 催化下形成二萜合成的底物香叶基香叶基焦磷酸 (Geranylgeranyl diphosphate，GGPP)。在细胞质 MVA 途径中，2 分子 IPP 和 1 分子 DMAPP 在法呢基焦磷酸合成酶(Farnesyl diphosphate synthase，FPS) 催化下形成倍半萜化合物的底物法呢基焦磷酸 (Farnesyl diphosphate，FPP) (图 1)。EβF 合成酶基因已先后从欧洲薄荷[62]、亚洲薄荷[46]、香橙[63]、花旗松[64]、黄花蒿[65]和洋甘菊[66]等植物中得到分离鉴定。其中，欧洲薄荷与亚洲薄荷来源的 EβF 合成酶基因仅有 5 个核苷酸碱基的差异，编码的氨基酸序列完全一致[46]。植物 EβF 合成酶基因不含信号肽序列，主要位于细胞质中，催化 MVA 途径中的 FPP 生成 EβF，以挥发物的形式释放到植物体外。

欧洲薄荷、黄花蒿、香橙、洋甘菊及花旗松来源的 EβF 合成酶均含有 Terpene_synth (PFAM accession number: PF01397) 和 Terpene_synth_C (PFAM accession number: PF03936) 结构域，这两个结构域为植物萜类合成酶家族的典型特征(图 2)。将上述 5 种植物 EβF 合成酶与烟草表-马兜铃酸合成酶(5-*epi*-aristolochene synthase) 的氨基酸序列进行比对 (图 2)，发现不同物种来源的 EβF 合成酶序列差异较大，仅存在部分保守的氨基酸残基，如薄荷 EβF 合成酶 MpβFS “DDxxD” (301−305 位) 中的 Asp^{301}、Asp^{302} 和 Asp^{305}。“DDxxD”在植物萜类合成酶基因中普遍存在，参与催化反应中二价金属离子的螯合[67]。参照烟草表-马兜铃酸合成酶的晶体结构，保守氨基酸残基 Arg^{264} 和 Arg^{266} 位于 A-C loop 区，Asp^{528} 和 Lys^{537} 则位于 J-K loop 区；A-C loop 与 J-K loop 参与表-马兜铃酸合成酶与底物的结合[67]。植物萜类合成酶的活性中心一般位于羧基端 (C 端)，研究表明活性中心的半胱氨酸、组氨酸及精氨酸残基是维

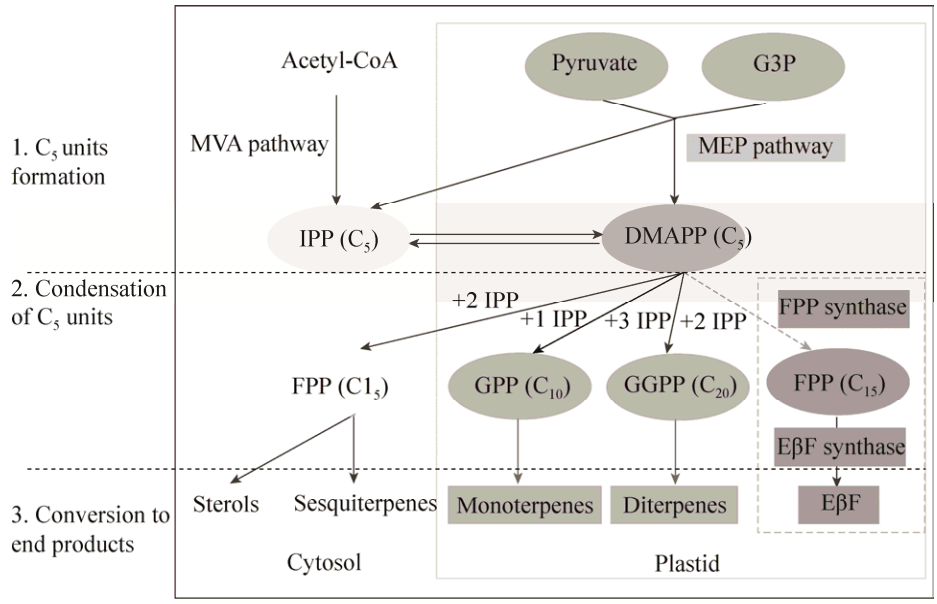

图1 植物萜类化合物的代谢途径

Fig. 1 Terpene biosynthesis pathway in plants. MVA: the mevalonate pathway; MEP: 2-C-methyl-D-erythritol 4-phosphate pathway; IPP: isopentenyl diphosphate; DMADP: dimethylallyl diphosphate; GPP: geranyl diphosphate; FPP: farnesyl diphosphate; GGDP: geranyl geranyl diphosphate. Former researches overexpressed exogenous EβF synthase gene in the cytosol of plants, but low EβF production was observed. One strategy indicated by the gray dotted box is redirecting sesquiterpene biosynthetic pathway into plastids, that is, simultaneously overexpressing the exogenous FPP synthase and EβF synthase in the plastid of plants.

持酶生物活性的关键[68-69]。序列比对发现，MpβFS 有 5 个保守精氨酸 ($Arg^{112, 115, 264, 266, 441}$) 及 1 个保守组氨酸残基 ($His^{82}$)，其中 $Arg^{264, 266, 441}$ 位于 C 端的 Terpene_synth_C 结构域内 (图 2)。

4 EβF 合成酶基因在植物抗蚜分子育种中的应用

倍半萜类化合物是植物萜类化合物中最大的一类，约为单萜类化合物的 7 倍，常以挥发物的形式存在于植物中[70-71]。研究发现，倍半萜化合物参与植物对害虫的直接与间接防御反应。直接防御反应中，倍半萜化合物作为毒素及害虫取食或产卵的干扰素；间接防御反应中，植物受到害虫取食所释放的挥发性萜类可吸引天敌[72]。倍半萜合成酶基因在植物抗蚜分子育种中也展现出很好的应用前景，如玉米在受到海灰翅夜蛾取食后会高量表达 *TPS10* 基因，生成 EβF、[反]-α-香柑油烯 [(*E*)-α-bergamotene] 等多种挥发物吸引害虫天敌；将玉米 *TPS10* 基因转入拟南芥，转基因株系可吸引鳞翅目害虫天敌寄生蜂[73]。作为 *TPS10* 在水稻中的同源基因，*TPS46* 参与 EβF 和柠檬烯 (Limonene) 等挥发物的生成；水稻中过表达 *TPS46* 基因可增强转基因植株对禾谷缢管蚜的抗性[74]。

体外表达试验表明，欧洲薄荷、花旗松、黄花蒿、洋甘菊等 4 种植物的 EβF 合成酶基因的产物均以 EβF 为主，纯度均达到 95%以上 (表 1)[62,64-66]。在植物中过量表达外源 EβF 合成酶基因，借助细胞质中的 FPP 为底物，可以获得持续释放 EβF 的转基因植株 (表 1)[1,7,45-46,75-80]。如将欧洲薄

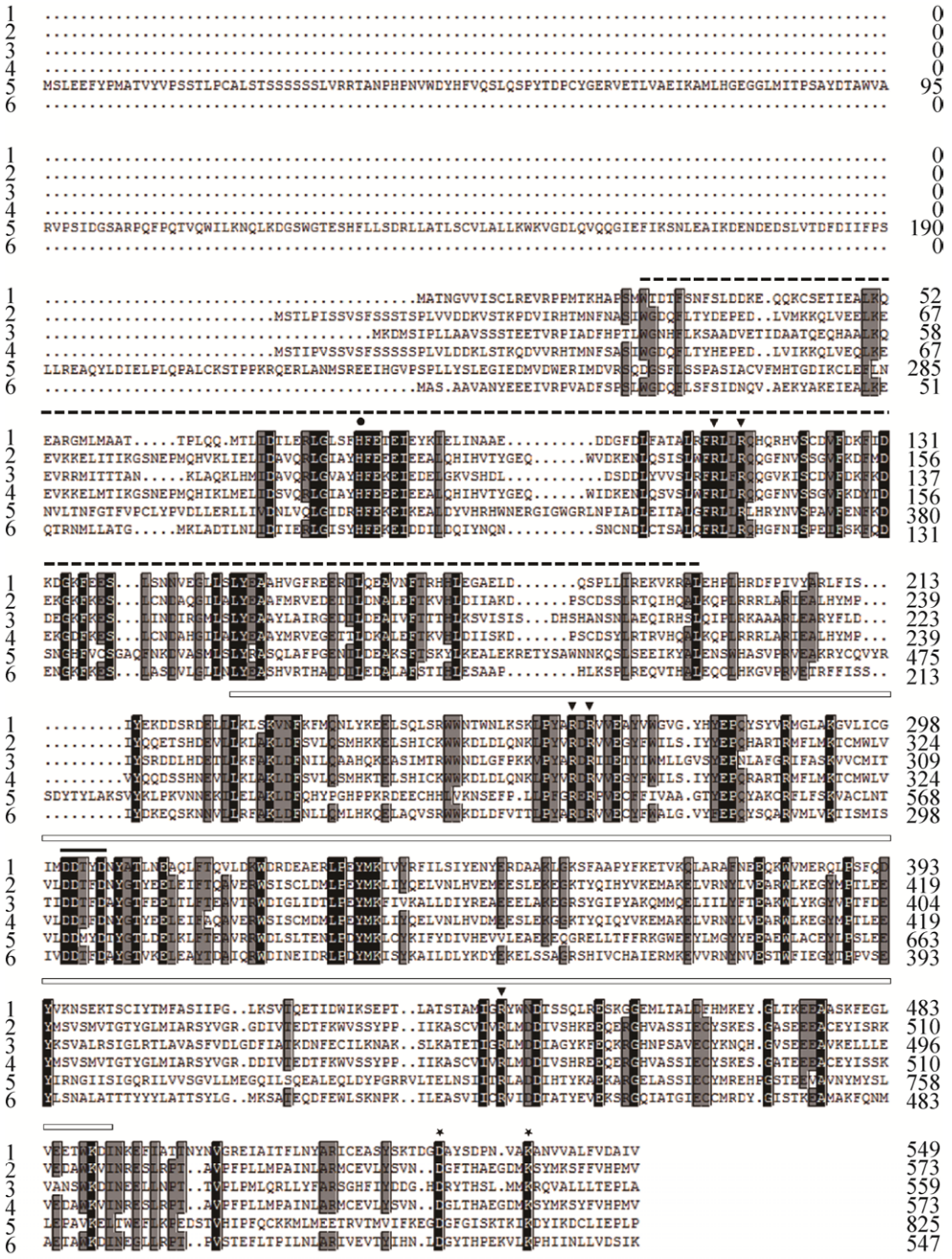

图 2　植物 EβF 合成酶基因的多序列比对

Fig. 2　Multiple sequence alignment of the isolated plant-derived EβF synthases. 1–5: EβF synthase from *M. piperita*, *A. annua*, *C. junos*, *M. recutita* and *P. menziesii*, respectively; 6: 5-*epi*-aristolochene synthase from tobacco with known crystal structure. Amino acids identical in all six proteins are marked in black. Amino acids identical in five proteins are marked in gray. Terpene_synth domain is indicated by a dashed line and Terpene_synth_C is indicated by an open box. The highly conserved DDxxD region is marked with a bar, solid circles indicate highly conserved His, triangles indicate highly conserved Arg, and pentagrams indicate highly conserved amino acid residues in J-K loop.

表 1 分离鉴定的 EβF 合成酶基因及转基因抗蚜研究

Table 1 The isolated EβF synthase genes and their application on molecular plant breeding

Gene name	Plant species	GenBank Accession No.	*In vitro* product purity (%)	Transgenic plants
MpβFS	Black peppermint *M. piperita*	AF024615	98[62]	*Arabidopsis thaliana*[75], rice[76] and wheat[77]
MaβFS1	Asian peppermint *M. asiatica*	HQ337896	–	Tobacco[46], mustard[78] and wheat[79]
PmβFS	Douglas fir *P. menziesii*	AY906867	100[64]	Tobacco[79]
CjβFS	Yuzu *C. junos*	AF374462	–[63]	–
AaβFS	Sweet wormwood *A. annua*	AY835398	100[65]	Tobacco[45,80]
MrβFS	Chamomile *M. recutita*	KM586847	100[66]	–

荷 EβF 合成酶基因 *MpβFS* 转入拟南芥，转基因植株能够释放 EβF，驱避蚜虫并吸引蚜虫寄生性天敌——蚜茧蜂[75]。笔者等分别将黄花蒿和亚洲薄荷来源的 EβF 合成酶基因 (*AaβFS* 和 *MaβFS*) 转入烟草，转基因烟草可以通过吸引大草蛉减轻蚜虫危害，与对照植株相比，有两个株系上的蚜虫数量分别减少 23.6%和 29.5%[45-46]。此外，薄荷来源的 EβF 合成酶基因亦转入小麦、水稻、芥菜等植物来减轻蚜虫危害[76-79]。以上研究表明，EβF 合成酶基因在作物转基因抗蚜虫应用上具有重要价值。蚜虫长时间处于高浓度 EβF 环境中，会对 EβF 产生适应性[81]；蚜虫连续取食释放 EβF 的转基因拟南芥后，其第三代子蚜对 EβF 的警戒反应降低，但显著增强了瓢虫对适应性子蚜的捕食[82]。

5 存在问题及展望

在植物倍半萜代谢改良过程中，目标倍半萜类化合物的生成量往往较低[1,83]，如烟草中表达紫穗槐-4,11-二烯合成酶基因 (Amorpha-4,11-diene synthase)，倍半萜化合物紫穗槐-4,11-二烯 (Amorpha-4,11-diene) 的生成量只有 0.2–1.7 ng/(d·g)[84]。转薄荷 *MaβFS* 和黄花蒿 *AaβFS* 基因烟草植株的 EβF 释放量只有 1.55–4.85 ng/(d·g)[45-46]，而转 *MpβFS* 基因水稻的 EβF 释放量仅为 4.89–5.03 ng/d(d·g)[76]。600 ng/μL 以上浓度的 EβF 方可显著趋避蚜虫[9]，EβF 释放量偏低严重影响了转基因植株对蚜虫的最佳防治效果。植物细胞质中底物 FPP 供应量不足是制约倍半萜化合物代谢改良的关键[85-86]。FPP 是植物倍半萜和甾醇的共同合成底物，甾醇为植物细胞膜的组成部分，对维持细胞结构具有重要作用。鉴于甾醇对植物细胞功能的重要性，FPP 优先供应于甾醇的合成[85,87]。同时，植物中 FPP 的供应量因物种差异而有所不同，相比青蒿、大冷杉等植物，小麦、水稻、烟草等植物内源倍半萜的量较低，可用于生成倍半萜的 FPP 较少[88]。

相比倍半萜类化合物的分子代谢改良，转基因植物单萜的生成量很高，不受底物供应的影响，表明植物质体中拥有足够的前体 IPP 和 DMAPP 合成 GPP (图 1)[83]。早期认为 FPP 合成酶仅存在于植物细胞质中，随着研究的深入，在拟南芥线粒体及水稻、小麦、烟草的叶绿体中均发现了 FPP 合成酶异构体[89-90]。据此，笔者等推测这些植物叶绿体中有可能合成 FPP，随后利用叶绿体转导肽在烟草中表达黄花蒿 EβF 合成酶基因 *AaβFS1*。转基因植株的 EβF 释放量达到 4.33–19.25 ng/(d·g)，

与在烟草细胞质中表达 *AaβFS1* 基因的植株相比提高 4−12 倍[80]。尽管叶绿体拥有足够的前体 IPP 和 DMAPP，但由于叶绿体中 FPP 合成酶异构体表达量或催化效率低，导致 FPP 合成量不足，转基因烟草在温室半自然条件下不能显著趋避蚜虫[80]。

笔者在前期研究的基础上，提出通过以下两种策略提高转基因植株的 EβF 释放量：1) 实施 EβF 代谢改良过程中的多基因协同转化，加强对 FPP 代谢流的调控。FPP 合成酶是 MVA 途径中的关键酶，同时表达 FPP 合成酶可增加植物细胞质中 FPP 的供应量，提高转基因植物的 EβF 释放量。2) 改变倍半萜代谢的细胞分区，即在质体中同时表达 FPP 合成酶和 EβF 合成酶基因，将合成 EβF 的代谢途径由细胞质转至质体 (图 1)。利用质体中充足的 IPP 和 DMAPP，催化形成足量的 FPP，提高转基因植株的 EβF 释放量。Wu 等[91]在烟草叶绿体中共表达 FPP 合成酶及紫穗槐-4,11-二烯合成酶基因，使烟草倍半萜紫穗槐-4,11-二烯的产量提高 1 000 多倍。同时，为增强 EβF 合成酶转基因植株的抗蚜效果，可实施 EβF 合成酶基因与其他抗蚜基因/技术的分子聚合育种。如在释放 EβF 的转基因植株中表达蚜虫气味结合蛋白基因的 dsRNA，转基因植株通过释放 EβF 吸引蚜虫天敌，而蚜虫气味结合蛋白基因的沉默则降低了对天敌的警戒反应，进而增强天敌对蚜虫的捕食。

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