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质体基因工程在植物育种中的应用研究进展

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摘要:与传统的核转化相比,质体遗传转化作为外源基因表达更精确、安全和高效的新一代转基因技术对作物品质改良和产量提高做出了极大的贡献,也给人们提供了植物育种的新思路。综述了质体遗传转化技术、筛选标记(体系)及其在植物抗性性状改良、产量提高、品质改良、杂种优势利用中的应用研究进展,以期为质体基因工程在植物遗传改良,尤其是在单子叶植物遗传改良中的应用提供理论依据。

关键词:质体;叶绿体;遗传转化;基因工程
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Progress on Application of Plastid Genetic Engineering in Plant Breeding

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Abstract: Compared with traditional nuclear transformation system, plastid genetic engineering that performs much safer, more precise and efficient expression of foreign genes has made great achievements in crop quality and yield improvement, even offers new ideas for plant breeding. This paper reviewed the plastid transformation techniques, screening markers(systems) and their applications in the improvement of plant resistance traits, yield and quality and heterosis utilization, so as to provide theoretical reference for the application of plastid genetic engineering in plant breeding, especially in monocotyledonous plants.

Key words: plastid; chloroplast; genetic transformation; genetic engineering

1988 年,首次在单细胞真核生物衣藻中实现的叶绿体遗传转化[1]使人们意识到:作为植物体内除细胞核以外含遗传信息的细胞器之一的叶绿体,不仅是植物体进行光合作用的重要场所,同时也具有作为植物遗传转化新工具的潜力。1990 年,烟草(*Nicotiana tabacum* L.)质体转化成功[2],标志着高等植物质体基因工程的开始。由于传统核转化所创制的转基因植物存在潜在的环境安全性问题[3-4],质体基因工程以其外源基因表达量高[5]、基因表达无位置效应和基因沉默现象[5-7]、可避免核转化系统中由于花粉逃逸所带来的环境安全性问题[8]及单次转化事件中可实现多个基因同时转化与表达[9-11]等特点而备受关注。近 20 a 来,人们已成功应用质体遗传转化手段将抗除草剂[12]、抗病[13]、抗虫[5,14]、抗旱[7]、耐盐渍[15]及综合抗性[16]等相关外源基因导入到烟草[13,16-18]、拟南芥(*Arabidopsis thaliana* L.)[19]、矮牵牛(*Petunia hybrida* L.)[20]、花椰菜(*Brassica oleracea* L.)[21]、结球甘蓝(*Brassica oleracea* L.)[22]、马铃薯(*Solanum tuberosum* L.)[23]、莴苣(*Lactuca sativa* L.)[24-27]、番茄(*Solanum lycopersicum* L.)[28-30]、胡萝卜(*Daucus carota*

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L.)[15]、棉花(*Gossypium hirsutum* L.)[31]、油菜(*Brassica napus* L.)[32]、大豆(*Glycine max* L.)[33]、水稻(*Oryza sativa* L.)[34-38]和野甘草(*Scoparia dulcis* L.)[39]等 20 多种植物中,并在作物农艺性状改良和生物反应器应用方面取得了一些进展,对作物品质改良和产量提高做出了极大的贡献,也给人们提供了植物育种的新思路。综述了质体遗传转化技术、筛选标记(体系)及其在植物抗性性状改良、产量提高、品质改良、杂种优势利用中的应用研究进展,以期为质体基因工程在植物遗传改良,尤其是在单子叶植物遗传改良中的应用提供理论依据。

1 质体的特点及其遗传转化技术

作为质体基因工程研究主体的质体是植物细胞内半自主的、原核生物起源的内共生细胞器,是由双层膜包裹,与碳水化合物的合成、贮藏密切相关的一类细胞器的总称,由前质体分化而来,根据其所含色素的不同分为叶绿体、有色体和白色体(造粉体、造蛋白体、造油体)。通常认为光合真核生物质体(叶绿体)中含有 120 ~ 220 kb 的环状双链 DNA 分子,其基因组只含 100 ~ 250 个基因(种子植物中约含 130 个基因),具有高度多倍性,结构高度保守,有自己特有的核酸和蛋白质合成机制[40-43]。大多数陆生高等植物都具有 20 ~ 30 kb 序列相同的反向重复区域 A 和 B(IR_A和IR_B),将质体基因组的大拷贝区(LSC)和小拷贝区(SSC)隔开,这决定了质体,尤其是作为植物光合作用重要场所的叶绿体,可以被用于植物遗传转化研究。

质体基因工程是利用携带外源基因的载体的侧翼序列与质体 DNA 同源区段之间发生同源重组[44-45]而实现的,转化过程中外源 DNA 首先整合到 1 个或多个质体基因组中,再在适合的选择压下进行 20 ~ 30 次细胞分裂,以去除未转化的质体来实现质体的同质化[44,46]。目前,人们常采用基因枪法[13,17,24]、PEG 介导法[47-49]、农杆菌介导法[50]、显微注射法[51]、激光注射法[52]、花粉管导入法[53]和转运肽介导的叶绿体间接转化法[54]等将外源 DNA 导入质体基因组中。由于外源 DNA 进入叶绿体必须突破细胞壁、原生质体膜和叶绿体膜的障碍,目前,质体基因工程中常采用基因枪法和 PEG 介导法进行遗传转化,PEG 介导法相对基因枪法更为经济,但 PEG 介导法中原生质体的分离和培养难度较大[55]。因此,目前质体遗传转化仍以基因枪法为主。

2 质体转化筛选标记(体系)

质体基因组的高拷贝数决定了质体转化过程中外源基因不可能同时转化所有的质体基因组。因此,为保证整合到受体植物质体基因组中的外源基因的遗传稳定性,必须利用筛选标记基因并结合相应的筛选底物在选择培养过程中逐步去除未转化的质体基因组,以实现质体基因组的同质化。与核转化中所使用的筛选标记类似,目前,用于质体转化的筛选标记主要包括 2 类,“正向”选择标记:可选择性地将转化细胞从非转化细胞中筛选出来并促进其生长;“负向”选择标记:可抑制转化细胞的生长[56]。

目前,在双子叶植物质体遗传转化中所使用的选择标记多是基于抗生素抗性基础的,包括:带壮观霉性和链霉素抗性的基因,如叶绿体基因组 16S rDNA 和 23S rDNA 不同位点的点突变基因[2,57-58]、编码氨基糖苷 -3′- 腺苷酸转移酶的 *aadA* 基因[59];编码新霉素磷酸转移酶、具有新霉素和卡那霉素抗性的 *npt*Ⅱ(*neo*)基因[60];编码氨基糖苷 -3′- 转移酶、具有卡那霉素抗性的 *aphA -6* 基因[31,61];编码氨基糖苷乙酰转移酶(6′)-Ie/氨基糖苷磷酸转移酶(2″)-Ia、具有氨基糖苷类抗生素广谱抗性的 *aac6 - aph2* 双功能抗性基因[62-63]。其中,壮观霉素具有高度特异性且对植物细胞无毒害作用,在双子叶植物质体遗传转化中常作为筛选剂使用,*aadA* 基因也因此成为最常用的标记基因。研究表明,高等植物尤其是禾谷类单子叶植物虽然对链霉素具有一定的敏感性[64],但其天然具有壮观霉素抗性[65],所以 *aadA* 标记对部分具抗生素抗性的双子叶植物和单子叶植物选择效率低[66],在一定程度上限制了该标记的应用。最近,研究发现,编码氯霉素乙酰转移酶、具氯霉素抗性的 *cat* 基因在使用过程中不会使植物产生自发的抗生素抗性突变,可扩大质体转化禾谷类作物的范围[67]。Maliga[46]认为,潮霉素 B 也可用于叶绿体转化筛选,筛选过程中应注意剂量效应:不适合只含少量整合有 *hpt* 基因拷贝的叶绿体基因组的转化细胞的筛选;当转化细胞中含大量整合有 *hpt* 基因拷贝的叶绿体基因组时,筛选才会有效,但最终未见其成功报道。李丁等[35-37]以 *hpt* 基因作为筛选标记进行了水稻叶绿体转化研究,在 T_0 代转化植株中检测到了 *hpt* 基因的表达,后续试验中采用 TALENs 辅助手段将转化效率提高了 2 倍,但仍未获得同质化植株。

为了避免抗生素抗性基因使用过程中存在的潜在危险,一些关于非抗生素筛选标记的研究已获得

一定进展。其中一类是基于除草剂抗性建立的筛选标记,包括 *psbA* 突变基因[68]、*AHAS* 突变基因[69]、编码草丁膦乙酰转移酶的 *bar* 基因[70-71]、编码 EPSP 合成酶的 *EPSPS* 基因[12,72]、编码 4 - 羟基苯丙酮酸双加氧酶的 *HPPD* 基因[73-74]、编码乙酰乳酸合成酶的 *ALS* 基因[75],但同 *hpt* 基因类似,这些标记基因也不适合初期筛选,当转化细胞中含大量整合有这些标记基因拷贝的叶绿体基因组时,筛选才会有效。另一类是基于代谢途径相关基因建立的,包括编码甜菜碱醛脱氢酶的 *badh* 基因[15,76-78]、编码邻氨基苯甲酸合成酶的 *ASA2* 基因[79-80]、编码 D - 氨基酸氧化酶的 *dao* 基因[81]、编码 D - 丝氨酸脱氨酶的 *dsdA* 基因[82],这在一定程度上促进了天然对壮观霉素有抗性的一些重要经济作物,尤其是禾谷类作物的质体基因工程的发展。同时,基于编码胞嘧啶脱氨酶的 *codA* 基因的"负向"选择体系也已建立[83-84],现多用于验证生长于添加 5 - FC 的培养基上的带 *codA* 基因叶绿体转化植株是否通过 P1 噬菌体位点特异性重组酶 CRE - *lox* 作用删除了 *codA* 基因[85]。

为方便在利用选择压筛选转化细胞之前,对转化组织进行可视化人工筛选含转化细胞的组织,一些可视化的选择标记和报告基因也得到相应开发,如编码 PSI 主要亚基的基因的突变体 *psaA*、*psaB* 和 *rbcL* 等[86-87]、来自大肠杆菌的编码葡萄糖苷酶的 *uidA* 基因[88-89]、编码绿色荧光蛋白的 *gfp* 基因[25,90-92]、编码荧光素酶的 *lux* 基因[93-94],但这些可视化的标记大多不能单独使用,必须结合抗生素或除草剂抗性基因才能发挥作用。

虽然大部分作物的质体遗传遵循母系遗传规律,但选择标记基因向野生型植物[95]或微生物[96]转移的可能性也不能够完全排除[97]。基于此,同核转化一样,从转化的质体基因组中去除标记基因是避免选择标记基因向野生型植物或微生物转移的最简单易行的一种方法。当前,正向重复序列介导的同源重组删除体系[71,74,86,98]、噬菌体位点特异性重组酶介导的删除体系[99-102]、标记基因的瞬时共整合体系[98]和共转化分离体系[70,72,103-104]已经在质体基因工程中得到发展。

3　质体基因工程在植物育种中的应用

目前,传统的杂交育种和现代的分子育种在作物育种中都起着举足轻重的作用,尤其是分子育种大大加快了育种的速度,但基于对传统核转化产品的安全性考虑,质体遗传转化也已用于作物育种并取得了一定成效。

3.1　抗性性状改良

植物在生长发育过程中,随时都面临着各种各样的生物和非生物胁迫,严重影响着植物的生长发育,尤其是作物的产量和品质。然而,通过传统的核转化将抗性基因导入植物具有潜在的风险:抗性基因可通过花粉逃逸,与杂草远缘杂交而使其获得相应抗性,从而产生"超级杂草"使得农药和杀虫剂失效。虽然通过基因沉默可显著降低这种风险,但其增加了运行成本。因此,通过无远缘杂交风险的质体基因工程提高植物对常见胁迫因子的抗性成为研究热点,进而提高作物产量、减少农药和杀虫剂的使用量、提高作物在逆境下的生存潜力。

3.1.1　*生物胁迫抗性*　通过质体遗传转化提供除草剂抗性最早见于 Daniell 等[12]将牵牛花的 *EPSPS* 基因成功整合到烟草叶绿体基因组中并表达草甘膦抗性。Ye 等[103]在后续的烟草叶绿体转化试验中证明,来自原核生物的 *EPSPS* 基因也可用于叶绿体遗传转化,但其产物的积累量和草甘膦抗性强弱无相关性。随后,相关学者通过在叶绿体中表达 *bar* 基因[70-71]、*HPPD* 基因[73-74]、*ALS* 基因[75]和编码 D - 氨基酸氧化酶的 *dao* 基因[81]陆续获得了带草铵膦、磺草酮、异恶唑草酮、咪唑啉酮、磺脲和 D - 氨基酸抗性的植株。

病虫害是影响作物产量的主要因素之一,其化学防治会增加环境危害和作物生产成本。虽然通过核转化引入相关抗性基因能够降低风险,但叶绿体的多拷贝性更大程度地提高了外源基因在叶绿体中的表达量,特别是在某些抗性强弱与抗性蛋白在细胞内的表达水平直接相关的时候。McBride 等[105]将苏云金芽孢杆菌(*Bacillus thuringiensis*)Bt 蛋白的编码基因 *crylAc* 导入烟草叶绿体基因组中,得到了能抗谷实夜蛾(*Helicoverpa zea*)、烟芽夜蛾(*Heliothis virescens*)和甜菜夜蛾(*Spodoptera exigua*)幼虫的转化植株,最早实现了质体抗虫基因工程。随后,Cry2Aa2[5]、Cry1Ab[106-107]、Cry1Ia5[108]和 Cry9Aa2[109]等 Bt 毒性蛋白分别通过叶绿体遗传转化应用于烟草[106,108]、大豆[5]、结球甘蓝[107]和马铃薯[109]等作物,Cry2Aa2 蛋白在烟草叶绿体转化株成熟叶片中的表达量最高可达到细胞总可溶性蛋白的 45%。DeGray 等[110]通过向烟草叶绿体基因组中导入抗菌肽 MSI - 99 的编码序列,最早证实了质体抗病基因工程的可行性,转化植株能显著抑制黄曲霉(*Aspergillus flavus*)、串珠镰刀菌(*Fusarium moniliforme*)、大丽轮枝菌(*Verticillium dahliae*)、烟草假单胞杆菌(*Pseudomonas syringae* pv. *tabaci*)的生长,最近发现

其也可抑制稻瘟病的发生[111]。Retrocyclin－101 和 Protegrin－1 抗菌多肽在烟草叶绿体中的高效表达(Retrocyclin－101 在烟草叶绿体转化株中的表达量可以达到细胞总可溶性蛋白的 32% ~38%),同样使转化植株能够抵御细菌或病毒感染[112]。为了培育具有抗病和抗虫复合性状的转基因植物材料,利用多基因表达元件,Chen 等[16]在烟草中表达了来自芋头(*Colocasia esculenta*)的半胱氨酸蛋白酶抑制剂、甘薯的贮藏蛋白和爪哇拟青霉的几丁质酶编码基因 *cystatin*、*sporamin* 和 *chitinase*,结果表明,外源基因的表达能够减轻胡萝卜软腐果胶杆菌胡萝卜亚种(*Pectobacterium carotovorum* subsp. *carotovorum*)导致的软腐病和烟草赤星病菌(*Alternaria alternata*)导致的叶斑病,并能导致甜菜夜蛾和斜纹夜蛾(*Spodoptera litura*)幼虫摄入叶片后生长迟缓和死亡,同时也提高了转化植株对渗透胁迫的抗性;Jin 等[113]在烟草叶绿体中表达具有复合抗性的半夏凝集素(pinellia ternata agglutinin,*pta*)基因,获得的转化植株不仅能够有效抗鳞翅目和同翅目害虫,而且具有明显的抗菌和抗病毒活性。

3.1.2 非生物胁迫抗性 随着人类的开发利用和极端天气出现频次的增加,为了保障植物的生长和产量,对植物耐旱、耐极端温度和耐盐能力的要求越来越高。渗透保护剂通过在渗透调节过程中稳定膜和蛋白质能有效提高植物的耐盐和耐旱能力,但大多数植物不能直接利用,在植物体内引入编码渗透保护剂的相关基因是一个可行的办法[114]。Khan 等[114]通过质体遗传转化在烟草叶绿体基因组中导入编码阿拉伯糖醇脱氢酶的 *ArDH* 基因,获得了能正常生长于含 350 mmol/L NaCl 土壤中的转化植株。Kumar 等[15]利用质体转化技术在胡萝卜中引入并表达了编码甜菜碱脱氢酶的 *BADH* 基因,发现在含 100 mmol/L NaCl 的培养基上,转化植株 *BADH* 基因的表达量比非转化植株提高了 50 ~54 倍,有效提高了转化株的耐盐能力。另外,通过增加不饱和脂肪酸含量来增强植物的抗氧化防御能力,或利用大肠杆菌 *panD* 基因编码的 L－天冬氨酸脱羧酶将 L－天冬氨酸分解为巴拉宁和 CO_2,可有效提高植物对高温胁迫的耐受性[115]。

3.2 产量提高

随着世界人口的快速增加,为了保障国家粮食安全,对作物产量的提高要求越来越迫切。目前,质体转基因作物产量的提高都是基于改进光合作用机制来提高光合作用效率实现的。叶绿体基质上的 1,5－二磷酸核酮糖羧化酶/加氧酶(RuBisco)能通过参与 CO_2 固定和初级生产进而提高作物产量,它包括叶绿体基因编码的大亚基(rbcL)和核基因编码并定向进入叶绿体的小亚基(rbcS),其催化活性缓慢且低效,常受到 CO_2 和 O_2 浓度及光照强度等因素的影响,在不同的植物之间也存在差异。因此,通过基因工程对 RuBisco 的表达进行调节有可能提高作物光合作用和生产率,进而提高作物产量,尤其是在 CO_2 浓度、温度和水氮供给等条件动态变化的环境中[116]。但研究发现,将 *rbcS* 基因导入并整合到叶绿体基因组中并不能提高植物的光合效率[117],核转化反而会限制 RuBisco 的活性[118];用其他物种来源的 *rbcL* 基因替换烟草叶绿体基因组中的 *rbcL* 基因可能会导致 RuBisco 功能缺失[119],而特殊设计的载体(含 *rbcL* 和 *aadA*)用于质体遗传转化却不会影响 RuBisco 的活性[120]。将来源于 C4 植物的 *rbcL* 基因导入并整合到 C3 植物叶绿体基因组中[121],或将从不同物种中筛选得到的光合作用效率较高的 RuBisco 的编码基因引入植物叶绿体基因组中[122],都有可能提高植物的光合作用效率。

因此,深入了解 RuBisco 的装配和活性调节因子、与其他生物技术协同作用提高光合作用的碳同化和开发新技术才能通过基因工程进一步实现 RuBisco 活性的提高并增加作物的产量。在此基础上,Lin 等[123]将蓝藻中 RuBisco 的大亚基(Se－rbcL)、小亚基(Se－rbcS)和组装分子伴侣(Se－RbcX)的编码基因导入烟草的叶绿体基因组中,有效提高了转化株的 CO_2 固定效率。Whitney 等[124]在烟草叶绿体中共表达拟南芥 RuBisco 的大亚基编码基因 *AtL* 和 RuBisco 累积因子 1 基因(*AtRAF1*)获得了高 CO_2 固定效率的转化株,其 CO_2 固定效率比单独表达 *AtL* 的转化株高 2 倍,而且生长速度明显加快,这进一步说明通过质体遗传转化增强 RuBisco 活性对作物产量的提高是有效的。

3.3 品质改良

随着人类生活水平的不断提高,人们对作物品质提出了更高的要求,功能性食品的开发利用也得到发展。作物品质改良可通过加强农业生产过程管理、常规育种或用包括质体遗传转化在内的不同基因工程方法改变植物代谢实现。与健康问题相关的最重要的饮食成分是食品中的大量元素和微量元素,然而,这些组分被认为有可能会导致某些不耐受症、存在潜在毒性或致敏性或随着营养物质的吸收而被干扰,但可以对其进行修改以提高食品的功能性[125]。一些以作物为基础的植物营养组分或含量经过改良的转基因植物已经被用于减轻与饮食相关

的疾病,如增加马铃薯中蛋白质含量、增加几种作物中氨基酸(玉米和水稻中的赖氨酸、苜蓿中的蛋氨酸)、胆碱、叶酸、黄酮、花青素、维生素 E、胡萝卜素、铁和锌的含量[126]。当然,这些也可以通过质体基因工程实现。研究表明,抗坏血酸在叶绿体基质中浓度达到 300 mmol/L 时能参与抗氧化保护[127-128];除了增加对胁迫条件的耐受性以外,抗坏血酸代谢经过改良的叶绿体转化植物中维生素 C 的含量也可能会增加[15]。被认为是一种前瞻性甜味剂的 Monellin 是一种甜味蛋白,通过分子药物开发技术从根本上增强了 Monellin 的表达,可以用于改进质体转化植株产品的味道[129]。基于植物体内异戊二烯生物合成的细胞质中甲羟戊酸和质体中丙酮酸磷酸甘油醛(MEP)代谢途径,人们发展了提高相关物质生物合成前体的利用率、修饰生物合成途径相关酶或调节机制和代谢途径向合成新物质成分转移等改进作物品质的方法。Hasunuma 等[130]在烟草叶绿体基因组中过表达生物合成异戊二烯前体物质异戊烯基焦磷酸(IPP)的 1 - 脱氧木酮糖 - 5 - 还原异构酶(DXR)发现,其下游产物叶绿素 a、β - 胡萝卜素、叶黄素、玉米黄素、茄尼醇和 β - 谷甾醇的含量得到有效提高。Kumar 等[131]利用多基因策略将整个细胞质的甲羟戊酸途径(MEV)成功导入烟草叶绿体基因组中,不仅增加了转化株体内类胡萝卜素的含量,而且也提高了甲羟戊酸、甾醇、角鲨烯和三酰基甘油酯的含量,进一步证实了异戊二烯在植物代谢中的中枢作用及合成的复杂性。在番茄的质体遗传转化技术成功建立之后[132],人们将编码番茄红素 β - 环化酶的 *Lyc*(来自水仙)[133]和 *crtY*(来自草生欧文菌)[134]基因导入番茄质体基因组中,成功促进了番茄红素转化为 β - 胡萝卜素并进一步提高了维生素 A 含量,极大程度地提高了番茄果实的营养价值。

Hasunuma 等[135]将海洋短波单胞菌属(*Brevundimonas*)细菌的 β - 胡萝卜素酮酶(crtW)和 β - 胡萝卜素环化酶(crtZ)的编码基因导入烟草叶绿体基因组中,转化株叶片中类胡萝卜素的总量提高了 2.1倍,而且合成了一种新的类胡萝卜素 4 - 茴香酸。随后,Harada 等[24]将 *crtW*、*crtZ* 和来自海洋副球菌属(*Paracoccus*)细菌的异戊烯基焦磷酸异构酶编码基因(*idi*)导入并整合到生菜的叶绿体基因组中,转化株以消耗本体类胡萝卜素为代价,在其叶片中富集了大量的游离虾青素和不同的虾青素脂肪酸酯等氧化类胡萝卜素。利用多顺反子间基因表达元件(IIEE)[136],Lu 等[137]在番茄质体基因组中整合并表达了维生素 E 合成途径中的 3 个关键酶尿黑酸叶绿基转移酶(HPT)、生育酚环化酶(TCY)和生育酚甲基转移酶(TMT)的编码基因,有效提高了番茄果实中维生素 E 的含量。

Madoka 等[138]利用改进的质体 *accD* 操纵子在烟草中过表达了乙酰辅酶 A 羧化酶(ACCase),延缓了转化株的叶片衰老,降低了淀粉含量,提高了脂肪酸和脂质含量,并提高了脂肪酸中不饱和脂肪酸的含量,对种子中脂肪酸和脂质含量及组成无影响,种子的产量提高了 2 倍,间接提高了其单位产油量。Craig 等[139]在烟草叶绿体基因组中导入来自美洲野生马铃薯(*Solanum commersonii*)或蓝藻(*Anacystis nidulans*)的 Δ^9 去饱和酶编码基因 *des*,有效改变了转化株叶片和种子中脂肪酸的组成,提高了不饱和脂肪酸含量,并提高了其耐寒性。Dunne 等[140]利用细菌来源的异戊烯转移酶编码基因 *ipt* 建立了基于细胞分裂素选择的质体转化系统,在烟草中表达了负责直链和支链脂肪酸生物合成起始的 3 - 酮脂酰 - 酰基载体蛋白合成酶Ⅲ(KasⅢ),改变了转化株叶片中脂肪酸的组成,不饱和脂肪酸含量得到一定提高。

3.4　杂种优势利用

一直以来,杂种优势利用是提高作物产量的重要途径。高纯度 F_1 代杂种的制备通常需要消耗大量的人力物力,随着对植物雄性不育的深入研究,人们发现利用植物的雄性不育可大幅度降低杂交制种的成本。因此,雄性不育资源的获得是杂种优势利用的关键环节。但长期以来,雄性不育系的传统选育周期过长、操作不便且不育基因过于单一,不能很好地满足育种的需要,极大程度地影响了杂种优势的利用。

雄性不育主要分为传统的雄性核不育和细胞质核互作雄性不育 2 种类型。传统的雄性核不育多由 1 对隐性基因控制,能够满足植物雄性不育系的最佳选育要求,但目前很难通过传统的杂交育种方式获得 100% 不育的水稻等作物的普通雄性不育系[141]。细胞质核互作雄性不育是由细胞质基因和细胞核基因互作控制的雄性不育,核质互作不育系是目前水稻等作物杂种优势利用的主要工具,但受遗传背景影响,可供利用的水稻优良核质互作不育系十分有限,微效恢复基因的存在增加了核质互作不育系选育的难度。伴随着生物技术的发展,通过植物基因工程手段,利用细胞毒素基因 *barnase* 和 *TA29* 启动子的特异表达[142]、反义技术和扰乱线粒体等细胞器[143-144]的正常功能来获得植物雄性不育

系极大地加快了植物雄性不育系选育的进程,也已成为当前利用基因工程手段创制植物雄性不育系的主要方法。在此基础之上,从转基因安全角度考虑,也有学者提出可利用质体转化将普通雄性不育基因或可育基因导入质体基因组来获得理想的植物杂种优势株系的方案[145],但目前尚未见报道。Ruiz 等[145]在烟草叶绿体基因组中表达编码 β-酮硫解酶的 *phaA* 基因,研究了其光调节效应,并评估了其在不同光周期下的表达,除正常可育的转基因株系外,还发现了缺少花粉的雄性不育表型,而且雄性不育表型可通过改变光照条件恢复其育性,证实了通过质体基因工程手段创制细胞质雄性不育系的可行性。随后,李丁等[146]提出了利用叶绿体转基因技术机械化生产非转基因杂交稻种子的策略,进一步丰富了质体基因工程在杂种优势利用上的应用。

4 展望

质体转化较核转化具有更大的优势,20 多年来被广泛应用于药物、疫苗、抗原和酶的生产及作物农艺性状改良等方面。虽然在实验室研究中已经取得了显著的成就,也有部分转化材料进入了田间试验阶段,但基于质体遗传转化技术的转基因材料的商业化应用仍未获得成功。目前,众多的研究在利用高通量克隆方法构建叶绿体表达载体[147-148]和寻找新的选择标记[67,149]上做出了较大的贡献,转基因植物的蛋白质纯化[150]和在绝对安全的转基因控制条件下生物反应器中植物组织的生长[151-152]等下游技术也得到了相应发展,但叶绿体表达载体的构建、外源基因在叶绿体基因组中的整合和同质化株系的获得仍是一个相对简单但漫长的过程。

另外,通过基因工程手段来增加主要粮食作物,尤其是提高单子叶植物光合作用效率,长期以来都被认为是提高作物产量的一种行之有效的改良策略。但截至目前,在单子叶植物中,尤其是水稻和玉米等主要粮食作物的质体转化技术仍不成熟,仅在水稻中有少量报道[34-38],同质化问题仍是限制其发展的主要问题。因此,在双子叶植物质体基因工程的框架基础上,开发可用于单子叶植物质体转化的有效方法,真正建立起成熟的单子叶植物质体基因工程体系,才能进一步解决传统核遗传转化的弊端,一旦获得突破性的进展,将推动质体基因工程的新发展,进而开启植物分子育种的新篇章。

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