生物安全型选择标记基因及其应用研究进展

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摘要: 在植物基因工程中, 选择标记基因(selectable marker genes, SMGs)的应用使“真”转化子得以辨认, 但目前常用的抗生素及除草剂类SMGs可能对人类及家畜健康、生态平衡等带来潜在的危害, 这也是影响转基因植物商业化的最重要因素之一。鉴于此, 基于可视化、非生物胁迫、代谢关键酶的安全SMGs迅速崛起, 并成功用于转化子筛选, 且筛选精度与效率明显提高。本文旨在对近年来生物安全型SMGs的有效性及适用性的研究进展进行分析与综述, 以期为转基因植物的广泛推广奠定基础。

关键词: 转基因植物; 安全标记; 生物安全性

早在20世纪80年代初, 外源基因导入植物基因组的尝试开启了植物育种的新时代, 陆续成功培育了大批抗旱、耐盐碱、抗冻、抗病虫害转基因植物; 在这些转基因植物中, 与外源优异基因相串联的选择标记基因(selectable marker genes, SMGs)多数是抗生素(如*NPT*II、*hpt*)和除草剂类基因(*BAR*、*AROA*、*BXN*、*CSRL*), 其编码的蛋白对抗生素、除草剂或其类似物具有解毒作用, 转化细胞由于含有该类解毒基因而得以生长(Miki和McHugh 2004)。然而, 有些植物如硅藻(*Phaeodactylum tricornutum*)对抗生素及除草剂有着天然的抗性(Apt等1996), 且许多海洋藻类生活在高盐环境, 降低了抗生素的活性(Allnutt等2000)。此外, 抗生素SMGs可能存在的潜在风险引起人们的广泛关注, 如: (1)当食用转基因食品时, SMGs有可能会发生水平转移致使病原微生物的抗药性增强, 引起抗生素类药品失效, 威胁人类健康; (2)转基因植物进行环境释放时, 可能会由于基因漂移造成物种间的优势关系发生质的改变, 破坏生态环境平衡; (3)在植物生长发育过程中, SMGs的存在可能导致原有基因或外源基因的沉默, 甚至影响二次转化, 如有学者发现用同一个SMG标记几个基因或重复使用同一个启动子驱动不同的SMG, 均有可能导致基因的沉默, 因此存在技术风险(Ebinuma等1997)。鉴于此, 众多研究者将视点转向了基于可视化、非生物胁迫、代谢关键酶的生物安全型SMGs (表1~3), 相较于“第一代标记物”抗生素类及除草剂类基因, 其拥有高安全性、遗传转化高效性及公众认可度高等优势。本文主要对近年来生物安全型SMGs在植物基因工程中的应用进行概述。

1 基于可视化的SMGs

绿色荧光蛋白(green fluorescent protein, GFP)基因(Franklin等2002; Li等2009; Lauersen等2013)、色蛋白基因(Chiang等2014)和生物荧光素酶基因(张秀娟等2013; Lerche等2014)是目前可视化SMGs的代表, 无需施加任何选择压便可筛选出转化细胞(Miki和McHugh 2004) (表1)。GFP是海洋动物维多利亚水母(*Aequorea victoria*)体内的一种光蛋白, 作为视觉标记, 有利于转化细胞特别是活性组织的实时监控(Chalfie等1994)及定位(韩晓敏等2016; 梁会超等2016), 且与“第一代标记物”相比更为精确、高效和安全。Zhu等(2003)将含*GFP-crylAc*融合基因及*GNA* (glanthus nivalis agglutinin)基因的双价抗虫表达载体导入烟草(*Nicotiana tabacum*)中, 发现外源基因高效表达, 转基因植株绿色荧光表型与抗虫性密切相关, 简化了抗虫转基因植物筛选程序。*GFP*同抗生素类SMGs筛选效率比较实验显示, *GFP*拥有更为稳定的遗传转化事件(Leclercq等2010), 有力的证据表明用*GFP*鉴定嵌合转基因胡桃(*Juglans regia*)体胚, 其鉴定效率为100% (Escobar等2000)。Gao等(2005)以*GFP*作为SMGs、*TLP* (thaumatin-like protein)为目标基因转化高粱(*Sorghum bicolor*), 通过Southern blot分析获得320株GFP表达植株, 后期证明GFP的表达与TLP存在的相关性为100%。Saika和Toki (2009)发现转化水稻(*Oryza sativa*)时以*GFP*为标记的筛选效率

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表1 基于可视化的安全SMGs在植物遗传转化中的应用

Table 1 The application of visual makers in genetic transformation

标记基因	基因来源	筛选剂	应用物种	参考文献
绿色荧光蛋白基因*GFP*	维多利亚水母(*victoria*)	无	高粱(*S. bicolor*) 胡萝卜(*D. carota*) 水稻(*O. sativa*) 橡胶树(*Hevea brasiliensis*) 拟南芥(*A. thaliana*) 三角褐指藻(*P. tricornutum*) 莱茵衣藻(*C. reinhardtii*)	Gao等2005 Baranski等2006 Saika和Toki 2009 Leclerc等2010 韩晓敏等2016 Zhang等2014 Noor-Mohammadi等2014; Ahmad等2015; Rasala等2012; Oey等2014
紫色色素蛋白基因*shCP*	地毯海葵(*S. haddoni*)	无	眼点拟微绿球藻(*Nannochloropsisoculata*)	Shih等2015
*Gaussia*分泌型萤光素酶基因*GLUC*	海洋桡足类动物(*Gaussia princeps*)	无	实球藻(*Pandorina morum*)	Lerche等2014
海肾荧光素酶基因*RLUC*	海肾(*Renilla reniformis*)	无	莱茵衣藻(*C. reinhardtii*)	Fuhrmann等2004; Eichler-Stahlberg等2009; Park等2013

高于*hpt*。类似情况亦在胡萝卜(*Daucus carota*) (Baranski等2006)、莱茵衣藻(*Chlamydomonas reinhardtii*)、三角褐指藻(*Phaeodactylum tricornutum*)等植物中出现。此外, *GFP*作为SMG不会导致植物形态变异, 对细胞无毒害作用, 其无需底物诱导表达的优点尤为突出。基于此, GFP是目前广泛用于研究胞间蛋白质运输和体内病毒运动的荧光蛋白(Howard等2004), 但其受宿主叶绿体内源性荧光的影响, 而且需要相应的仪器辅助。Chiang等(2014)发现了一种来源于地毯海葵(*Stichodactyla haddoni*)的GFP类似色蛋白shCP, 该蛋白可被生物降解, 在保障环境与食品安全的同时无需荧光显微技术的辅助, 显色肉眼可见; Shih等(2015)针对其作为遗传转化中的选择标记效率分析表明, 其作为SMGs可有效降低假阳性率。此外, 生物荧光素酶如Gaussia分泌型萤光素酶(gaussia luciferase, GLUC)、海蜃萤光素酶(renilla luciferase, RLUC)及萤火虫萤光素酶(firefly luciferase, FLUC)基因作为SMGs的灵敏性要明显高于*GFP*, 拥有更广的发展潜力(Shah等2003; Zhang等2013)。

2 基于非生物胁迫的SMGs

非生物胁迫如干旱、盐碱、高温等对植物有着致命的破坏。拥有高效抗逆基因的植物可在逆境中生存, 这就预示着其可作为SMGs的可能性(Zhou等2013)。目前, 拟南芥(*Arabidopsis thaliana*) *AtSOS1* (质膜Na^+/H^+逆向转运蛋白编码基因)、水稻(*O. sativa*) *OsDREB2A*转录因子、*rstB* (糖基转移酶编码基因)及*MsrB* (蛋氨酸亚砜还原酶B基因)等抗逆基因用于植物遗传转化的可能性相继被证明(表2)。依据高盐诱导*AtSOS1*及干旱、高盐诱导DREB转录因子表达的原理, Zhu等(2008)首次以其为SMGs分别对水稻进行了遗传转化, 发现200 mmol·L^{-1} NaCl可成功分离得到转化子。Zhang等(2009)将*rstB*作为SMG用于烟草遗传转化, 并以170 mmol·L^{-1} NaCl 为筛选剂, 筛选率高达83.3%。随后*rstB*亦用于紫花苜蓿(*Medicago sativa*)的遗传转化筛选(Zhang等2015)。近期, 有研究发现氧化胁迫基因*MsrB*的超表达在提高植物抗氧化能力的同时亦可用于转化子的筛选, 用甲基紫精(methyl viologen, MV)为筛选剂成功获得了转基因拟南芥和西红柿(*Solanum lycopersicum*) (Li等2012, 2013; Lee等2014, 2015)。本课题组发现共转化霸王(*Zygophyllum xanthoxylum*) *ZxNHX-ZxVP1-1*的百脉根(*Lotus corniculatus*)在200 mmol·L^{-1} NaCl和缺水状态下生长良好(Bao等2014); 200 mmol·L^{-1} NaCl和干旱处理下的共转化*ZxNHX-ZxVP1-1*紫花苜蓿生长状况远远优于野生型(Bao等2016; Kang等2016)。由此可见, 抗逆相关基因如*ZxVP1-1*、*ZxNHX*、*ZxHKT1;1*和*ZxSOS1* (Wu等2011; Ma等2014)均有作为筛选标记的潜能, 可用于植物的遗传转化。

表2 基于非生物胁迫的安全SMGs在植物遗传转化中的应用

Table 2 The application of safety SMGs based on abiotic stress in genetic transformation

标记基因	基因来源	筛选剂	应用物种	参考文献
Na^+/H^+逆向转运蛋白基因*AtSOS1*	拟南芥(*A. thaliana*)	NaCl	拟南芥(*A. thaliana*)	Shi等2002
			水稻(*O. sativa*)	Zhu等2008
DREB转录因子*OsDREB*	水稻(*O. sativa*)	NaCl	水稻(*O. sativa*)	Zhu等2008
糖基转移酶基因*rstB*	大豆根瘤菌RT19	NaCl	烟草(*N. tabacum*)	Zhang等2009
	(*Sinorhizobium fredii* strain RT19)		苜蓿(*M. sativa*)	Zhang等2009, 2015
蛋氨酸亚砜还原酶B基因*MsrB*	拟南芥(*A. thaliana*)	甲基紫精	拟南芥(*A. thaliana*)	Li等2012
			西红柿(*S. lycopersicum*)	Li等2013

3 基于代谢关键酶的SMGs

生化代谢是每个生物体必不可少的物质与能量的来源。筛选调控代谢活动的关键酶基因作为转基因工程SMGs已成为许多研究者的首选, 因为它们为生物体内固有代谢途径, 属绿色选择标记, 可有效提高转基因植物为人们所接纳的可能性, 具有良好的应用前景。目前, 基于糖类代谢、激素代谢、氨基酸代谢途径的SMGs已广泛应用于基因工程(表3)。

3.1 基于糖类代谢的SMGs

植物组织培养中愈伤组织的正常生长分化需要诸如蔗糖、甘露糖、葡萄糖、麦芽糖和糖醇等碳源提供能量。其中甘露糖、木糖、核糖醇和阿拉伯糖醇等不能直接为植物细胞所利用, 需要关键酶的代谢转化。采用糖代谢的关键酶基因作为SMGs、相应碳源作为筛选剂具有双馨效果: 一是可为转化细胞提供能量获得优势生长, 而非转化细胞处于饥饿状态; 二是其纯化蛋白没有毒性和致敏性, 保证食品安全。

来源于大肠杆菌(*Escherichia coli*)的*manA/pmi*基因作为糖类代谢关键酶基因之一, 编码的6-磷酸甘露糖异构酶(6-phosphomannose isomerase, PMI), 可将不能直接利用的6-磷酸甘露糖转化为可进入糖酵解途经提供能量的6-磷酸果糖。基于此原理, *manA/pmi*基因标记的离体细胞或组织可转化D-甘露糖从而获得优势增殖, 而非转化细胞会因“饥饿”停止生长(Joersbo等1996; Reed等2001)。该体系已成功应用于诸多单子叶和双子叶植物如小麦、水稻、玉米(*Zea may*)和高粱等重要农作物与经济作物以及豆科牧草中(表3), 均获得了较高的筛选效率。*pmi*作为SMGs, 在转基因玉米中其低拷贝率增加了43%~60% (Sivamani等2015); 在甘薯(*Ipomoea batatas*)和甘蔗(*Saccharum officinarum*)中应用的效果尤为明显(Mbinda等2015; Zhang等2015)。本课题组以*pmi*为SMGs成功获得了转基因拟南芥和紫花苜蓿, 且对紫花苜蓿的遗传转化效率要明显高于*hpt* (未发表数据)。此外, 有报告显示, PMI的纯化蛋白没有毒性和致敏性、且转*manA/pmi*基因植株的农艺性状与野生植物相比较没有显著差异(Reed等2001; 卓晓蕾等2007)。当然, *pmi*虽具有以上优点, 但仍存在一定短板。有研究显示葡萄(*Vitis vinifera*)胚若长时间在含甘露糖的培养基中生长, 其萌发会受到损害(Vaccari和Martinelli 2009); 其次, 甘露糖并不适用于所有植物如肉桂(*Cinnamomum cassia*)等含内源*pmi*的物种及烟草等对甘露糖不敏感植物(郭倩倩等2011)。

与甘露糖一样, 木糖只有在被木糖异构酶(xylose isomerase, XYL)催化为D-木桐糖时才能被植物细胞所利用。Morawala等(2007)将*xylA*作为标记成功筛选了油菜(*Brassica napus*)及向日葵(*Helianthus annuus*)的转化体, 效率为12%~15%。Chen等(2015)为验证几丁质酶基因启动子的活性将*xylA*作为SMG、*GUS*为报告基因, 成功完成启动子的验证。且有学者发现在对马铃薯的筛选中*xylA*的筛选效率是*npt*II的10倍(Haldrup等1998)。由上可知, 木糖异构酶基因*xylA*作为SMG潜在能力不可小觑, 加之木糖广泛用于食品加工及淀粉工业, 其安全性无可非议, 相信该类标记基因会越来越实用。此外, 也有报道将甘露糖-6-磷酸还原酶(mannose-6-phosphate reductase, M6PR)基因*M6PR*、海藻糖-6-磷酸合成酶1 (trehalose 6 phosphate synthase 1, TPS1)基因*TPS1*、2-DOG-6-磷酸酶(2-DOG-6-P phosphatase,

表3 基于代谢关键酶基因的安全SMGs在植物遗传转化中的应用

Table 3 The application of safety SMGs based on key enzyme of metabolism in genetic transformation

标记分类	标记基因	基因来源	筛选剂	应用物种	参考文献
基于糖类代谢	6-磷酸甘露糖异构酶基因*pmi*	大肠杆菌(*Escherichia coli*)	D-甘露糖	大麦(*H.vulgare*)	Reed等2001
				玉米(*Zea may*)	Sivamani等2015
				硬质小麦(*Triticum turgidum*)	Gadaleta等2006
				高粱(*S. bicolor*)	Gao等2005
				狗尾草(*Pennisetum glaucum*)	O'Kennedy等2004
				百脉根(*L. corniculatus*)	Guo等2015
				鹰嘴豆(*Cicer arietinu*m)	Patil等2009
				豇豆(*Vigna unguiculata*)	Bakshi等2012
				李子(*Prunus domestica*)	Wang等2013a, b
				黄瓜(*Cucumis sativus*)	He等2006
				水稻(*O. sativa*)	Hoa等2003; Paine等2005; Duan等2012; Gui等2014; Hu等2016; Rong等2016
	木糖异构酶基因*XylA*	梭状芽孢杆菌(*Clostridium thermosulfurogenes*)	鼠李糖或木糖	玉米(*Z. mays*)	Guo等2007
				向日葵(*H. annuus*)	Morawala等2007
				油菜(*B. napus*)	Morawala等2007
				花生(*Arachis hypogaea*)	Chen等2015
		罗伊氏乳杆菌(*Lactobacillus reuteri*)		酿酒酵母(*Saccharomyces cerevisiae*)	Stovicek等2015
		链霉菌属(*Streptomyces rubiginosus*)		植物乳杆菌(*Lactobacillus plantarum*)	Staudigl等2014
	甘露糖-6-磷酸还原酶*M6PR*	芹菜(*Apium graveolens*)	D-甘露糖	拟南芥(*A. thaliana*)	Gao和Loescher 2003; Sickler等2007 Song等2010
				烟草(*N. tabacum*)	
	海藻糖-6-磷酸合成酶1At*TPS1*	拟南芥(*A. thaliana*)	高浓度(6%~7%)葡萄糖	拟南芥(*A. thaliana*)	Avonce等 2004
				烟草(*N. tabacum*)	Leyman等2006
	2-DOG-6-磷酸酶基因*DOG^R*1	酵母(*Saccharomyces cerevisiae*)	脱氧葡萄糖	烟草(*N. tabacum*)	Kunze等2001
				马铃薯(*S. tuberosum*)	Kunze等2001
	阿拉伯糖脱氢酶基因*atlD*	大肠杆菌(*E. coli* strain C)	阿拉伯糖醇	水稻(*O. sativa*)	LaFayette等2005
基于激素代谢	异戊烯转移酶基因*ipt*	农杆菌(*Agrobacterium tumefaciens*)	无	烟草(*N. tabacum*)	Endo等2000
				水稻(*O. sativa*)	Endo等2002
				白杨(*Populus tomentosa*)	Ebinuma等1997
	β-葡萄糖醛酸酶基因*uidA*/*gusA*	大肠杆菌(*E. coli*)	N-3-葡糖苷酸	烟草(*N. Tabacum*)	Joersbo等1996
				甜菜(*Beta vulgaris*)	Snyder等1999
	发状根基因*Rol*	发根农杆菌(*Agrobacterium rhizogenes*)	无	烟草(*N. tabacum*)	Komarnytsky等2004
				矮牵牛(*Petunia hybrida*)、长寿花(*Kalanchoe blossfeldiana*)	Khan等2010 Thirukkumarana等2010; Christensen等2012
	组氨酸激酶基因*CKII*	拟南芥(*A. thaliana*)	无	拟南芥(*A. thaliana*)	Zuo等2002
	促芽转录因子基因*ESR1*	拟南芥(*A. thaliana*)	无	拟南芥(*A. thaliana*)	Banno等2002
基于氨基酸代谢	天冬氨酸激酶基因*AK*	大肠杆菌(*E. coli*)	赖氨酸和苏氨酸	马铃薯(*S. tuberosum*)	Perl等1993
				大麦(*H. vulgave*)	Brinch等1999
	邻氨基苯甲酸合成酶*AS*	烟草(*N. tabacum*)	4-甲基吲哚 7-甲基DL-色氨酸	烟草(*N. tabacum*)	Barone和Widholm 2008; Barone等2009;

表3 (续)

标记分类	标记基因	基因来源	筛选剂	应用物种	参考文献
					Tsai等2005; Zhang等2015
				水稻(*O. sativa*)	Yamada等2004
				大豆(*G. max*)	Inaba等2007; Cho等2004
				紫云英(*Astragalus sinicus*)	Cho等2004
	色氨酸合酶基因*AtTSB1*	拟南芥(*A. thaliana*)	5-甲基-色氨酸	拟南芥(*A. thaliana*)	Daniell等2001; Hsiao等2007
	D-型氨基酸氧化酶基因*DAO1*	股博肌红酵母(*Rhodotorula gracilis*)	D-丝氨酸和D-丙氨酸	拟南芥(*A. thaliana*)、苹果(*M. borkh*)	Erikson等2004 Hattasch等2009

2-DOG-6-P)基因DOG^R1以及阿拉伯糖脱氢酶(arabitol dehydrogenase)基因*atD*作为SMGs的成功案例(表3)。

3.2 基于激素代谢的SMGs

异戊烯基转移酶(isopentenyl transferase, IPT)是细胞分裂素生物合成第一步的催化酶, 也是限速酶, 主要催化异戊烯基腺苷-5-单磷酸酯的合成(吴吉林等2005)。基于此, 导入*ipt*基因的转化子可在没有外源激素(分裂素、生长素)的培养基上正常增殖分化形成不定芽。在莴苣(*Lactuca sativa*)及烟草转化系统中, 其筛选效率明显优于*npt*II基因(Endo等2000)。但*ipt*基因的持续过表达可能会造成植物形态上的畸形(Gan和Amasino 1997; Kang等1999; Mori等2001; Ouwerkerk等2001), 通过*ipt*基因融合乙醇诱导型启动子, 可有效解决这一缺点并能提高转化效率, 该方法已成功的应用于许多物种(Caddick等1998; Roslan等2001)。如Lu等(2010)构建了*AlcA*::*ipt*和*35S*::*ipt* 载体并转化烟草, 结果显示乙醇诱导系统可获得健康叶、花和种子且转化效率提高。另外, 这种局限亦可通过SMGs剔除技术(转座子系统、结合位点特异性重组酶系统)克服(Peng等2015; Darwish等2014; Zou等2013)。负性β-葡萄糖醛酸酶(β-glucuronidase)基因*gusA*/*uidA*、发状根基因*rol*、组氨酸激酶(histidine kinase)基因*CKI1*和促芽转录因子*ESR1*等作为SMGs亦得到了广泛应用(表3)。

3.3 基于氨基酸代谢的SMGs

在氨基酸合成代谢中, 末端产物会对关键酶的活性进行反馈抑制或反馈阻遏。以反馈抑制或阻遏不敏感基因为SMGs, 加以相应氨基酸为筛选剂进行转化子筛选的策略, 目前已得到了广泛的应用。其中天冬氨酸激酶(aspartate kinase, AK)、邻氨基苯甲酸合成酶(anthranilate synthase, AS)、色氨酸合酶(tryptophansynthase β1, TSB1)和D-氨基酸氧化酶(D-amino acidoxidase, DAAO)基因最为科学家们青睐(表3).

在天冬氨酸代谢途径中, 调控关键酶将天冬氨酸盐通过不同支链转换为赖氨酸(lysine, Lys)、苏氨酸(threonine, Thr)、蛋氨酸(methionine, Met)和异亮氨酸(isoleucine, Ile)。有报道指明, 毫摩尔级的Lys和Thr便可强烈抑制植物的生长(Arruda等1984; Miao等1988; Rognes等1983), 其原因是它们会抑制AK酶的活性从而造成植物Met饥饿。此外, 细菌中的AK的敏感性弱于植物中的, 所以作为标记的*AK*主要从植物中提取, 具有安全性, 已成功应用于烟草(Shaul等1992; Karchi等1993)、马铃薯(*Solanum tuberosum*)和大麦(*Hordeum vulgare*) (Brinch等1999)的遗传转化中。

*AS*是烟草的内源基因, 合成必需氨基酸色氨酸(tryptophan, Trp), 它是许多化合物诸如生长素、植保素和次生代谢产物的前体。在莽草酸途径中, 分支酸是重要的枢纽物质, Trp就是由分支酸代谢而来。AS是该代谢中的一个关键酶, 其将分支酸分解成邻氨基苯甲酸并受Trp的反馈抑制(Singh和Widholm 1974)。*AS*基因作为SMGs已被用于多种植物(表3)。Barone和Widholm (2008)以烟草为材料, *AS*为标记, 4-甲基吲哚(4MI)或7-甲基DL-色氨酸(7MT)为选择剂, 结果发现4MI的选择效率是

7MT的2倍。*AS*基因除了用于核转化, 其亦可用于质体转化(Barone等2009)。但由于该基因与转化植物的原生基因可能有同源性, 造成的转基因沉默会影响转化效率, 这在烟草(Barone和Widholm 2008; Tsai等2005)和大豆(*Glycine max*) (Inaba等2007)中均有报道。因此, *AS*的部署需要进一步改进, 以克服基因沉默。此外, 色氨酸合酶(tryptophansynthase β1, TSB1)对Trp的合成亦十分重要(Last等1991; Pruitt和Last 1993; Zhao等2001), 可作为SMG。Hsiao等(2007)构建了*35S*::*AtTSB1*载体用于转化拟南芥, 并以75 μmol·L^{-1} 5-甲基-色氨酸(5-methyl-tryptophan, 5MT)为筛选剂进行筛选获得了阳性植株。

*DAO 1*基因作为安全SMG的原理在于植物体内少量D-丙氨酸或D-丝氨酸的存在便可毒害细胞, 而*DAO 1*基因编码的DAAO可将其氧化为无毒物质。Erikson等(2004)将从股博肌红酵母(*Rhodotorula gracilis*)中得到的*DAO 1*基因和CaMV *35S*启动子整合入拟南芥中, 并且在不同底物如D-丙氨酸或D-丝氨酸下筛选出转基因植株, 同时, 也在转基因烟草、大麦、玉米、云杉上对其进行了测试, 结果与拟南芥一致。同样地, Hattasch等(2009)以*DAO 1*基因为标记获得了转基因苹果。

总之, 生物途径及生物来源SMGs的成功应用, 极大地降低了转基因植株的潜在安全风险, 能够有效安抚公众的消极情绪, 为转基因植物商业化及广泛应用奠定了基础。然而, 随着公众对其安全意识的不断提高, 开发新型生态友好植物来源的SMGs依然是未来生物技术发展的研究重点。

4 展望

生物技术能将异源或同源物种的优良基因快速、准确地整合到植物中, 并通过部署SMGs间接地对性状进行选择。转基因技术中, SMGs的部署十分必要, 利于高效地筛选出目标植株。但抗生素和除草剂类SMGs的使用对人类健康及自然生态系统的潜在不利影响, 迫使科学家们不断挖掘易检测、高效性、多功能的新型生态友好型标记。即便如此, 目前安全SMGs的选择范围依然很狭小, 且存在诸多问题。如*ASA2*的表达可能造成基因的沉默; *pmi*基因的筛选剂D-甘露糖很昂贵; *ipt*基因持续过表达可能会造成植物形态上的畸形等。开发新的安全SMGs, 尤其是抗逆相关标记迫在眉睫, 它们在作为抗逆基因的同时, 亦可作为标记进行转化子的筛选, 同时这类SMGs来源于植物, 可不必担心引起的基因漂移问题, 通过这种转化一次便可实现多种目的, 是研究人员一直梦寐以求的目标。目前, 具有开发为标记潜能的抗逆基因有DREB转录因子、*rstB*、*MsrB*、*NHX*和*SOS1*。此外, 与“第一代标记物”抗生素类及除草剂类基因相比, 安全SMGs拥有遗传转化高效性, 因为在非转化细胞被抗生素及除草剂杀死的过程中会产生大量的醌类、酚类等毒素, 抑制了转化子的正常生长; 但安全标记SMGs仅是使非转化细胞处于饥饿状态、不会产生其它代谢产物降低转化率。因此, 根据遗传转化及转基因作物商品化发展的趋势, 理想的标记基因研究应当具备安全性、易检测、高效性和多功能性等特点。随着研究的不断深入和完善, 安全SMGs的应用前景十分广阔, 转基因植物的大规模商业化将指日可待。

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Research progress of biosafety selectable marker genes

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Abstract: In genetic engineering, selectable marker genes (SMGs) are generally deployed to identify 'true' transformants. The frequently-used SMGs such as antibiotics or herbicides generally obtained from prokaryotes, posing risks due to their lethal nature. In this regard, some safety SMGs based on visual, abiotic stress and metabolism are more attractiveand successfully used for screening of transformants with high selection efficiency. This review mainly focus on their availability and applicable range which contributes to breeding biosafety transgenic plants.

Key words: transgenic plants; safety maker; biosafety

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